Electric fields and biological cells
Numerical insight into possible interaction mechanisms

Juan Carlos Vanegas-Acosta
1. The interaction mechanisms depend on both the attributes of the biological cells such as location, shape, number and size, and the conditions of the exposure such as magnitude, frequency, and orientation of the incident electric field (this thesis).

2. The biological effects that an electric field with frequency below 1-10 MHz causes to a cell are likely due to interaction mechanisms occurring in the cell membrane. For frequencies above 1-10 MHz, the effects are likely due to interaction mechanisms occurring in the intracellular compartment (this thesis).

3. Increasing the magnitude of an incident electric field increases the cell migration speed and the rate of new tissue formation, but also the temperature of the cells (this thesis).

4. Computational models assist experimentalists to explore the right windows to look at the cells and identify possible biological effects.

5. The predictions provided by computer simulations are insufficient to conclude on the existence of biological effects if they are not accompanied by an adequate experimental validation.

6. After decades of searching for possible non-thermal harmful effects, more attention should be paid to the development of medical applications that take advantage of the well-identified beneficial effects.

7. The exposure to electric fields may be a harmless situation that causes harm because you believe it is harmful.

8. Time will tell the truth about the existence of non-thermal detrimental effects to health caused by the exposure to modern sources of electromagnetic radiation. The same applies to genetically modified food, inadequately tested medications, and environmental degradation.

9. Dat het dal in zicht is betekent niet dat het dal bereikt is.

10. Scientific results that prove the existence of interaction mechanisms and related biological effects should be in accordance with the ABCDE-rule: Accurate, Brief, Clear, Decisive and Effective.

Juan Carlos Vanegas-Acosta
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Electric fields and biological cells: numerical insight into possible interaction mechanisms
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PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven, op gezag van de rector magnificus, prof.dr.ir. F.P.T. Baaijens, voor een commissie aangewezen door het College voor Promoties in het openbaar te verdedigen op donderdag 17 december 2015 om 16.00 uur

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Het onderzoek of ontwerp dat in dit proefschrift wordt beschreven is uitgevoerd in overeenstemming met de TU/e Gedragscode Wetenschapsbeoefening.
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Exposure of cells and tissues to electric fields can lead to a variety of physiological changes. These changes are caused by the interactions between the incident field and biological, chemical and electric (magnetic) processes. Evaluating whether the physiological changes are beneficial or harmful to the functionality of cells and tissues requires understanding the nature of the interactions, which can be associated with either thermal or non-thermal (bio)-effects. Although a considerable amount of research worldwide has shed light on these effects and their influence in the biological response, much more is left to say about the possible interaction mechanisms and the underlying biological processes modified by the incident field. In fact, the adequate conditions of exposure (dosimetry) needed for cells and tissues to trigger a certain positive response and avoid possible damage are still a matter of debate. Moreover, concerns coming from social sectors about the hazards of the exposure have motivated multiple studies that have yielded controversial results.

In view of the lack of knowledge on the biological cues suitable for explaining the effects, and in the light of the social interest in clarifying possible risks derived from the exposure, the aim of this thesis is to identify and assess possible interaction mechanisms between an incident electric field and biological cells and tissues. These mechanisms are elucidated upon determination of the electrical response of biological cells exposed to electric fields, which is obtained by means of the numerical implementation and solution of mathematical models formulated from a biophysics standpoint which combines the fundamentals of biological cells and tissues with the electromagnetic theory. In a first group of models, a numerical approach suitable to model multiple cells based on the quasi-static approximation and the equivalent dipole moment of a dielectric sphere is presented. This approach indicates that cell-to-cell interactions depend on the number and position of the cells. Another formulation, based on a symbolic scattering-matrix approach, turns out useful to investigate the response of the cell membrane and the
proximity effects during exposure to the incident electric field. Finally, in order to study the influence of the shape of both the cell and the organelles in the overall electrical response, a full-wave formulation based on the electric field volume integral equation is introduced. Altogether, these approaches provide insight into feasible interaction mechanisms which take place at different cell structures and that might be related with the biological effects, especially those of non-thermal nature.

Contrary to common belief, the effects at the cell or tissue level due to exposure to electric (magnetic) fields, especially those of thermal nature, may not necessarily represent a health hazard. In fact, several novel therapeutic techniques and clinical procedures for the treatment of injuries and diseases exploit the benefits derived from the exposure to properly chosen incident fields. Therefore, a second group of models is devoted to the numerical simulation of clinical (in-vitro) scenarios in which the use of electric fields is known for improving the biological response of cell populations and tissues under formation. Upon the introduction of biological processes described by reaction-diffusion equations such as cell migration and tissue formation, a mathematical model describing the effect of an external electric field during cell migration is derived. The numerical results therein demonstrate the suitability of the electric stimulus to promote tissue formation and reveal a threshold to the intensity of the electric field in order to avoid cell damage. Following this limitation, the model is extended to analyze the effect of the thermal heating generated during electrostimulation on cell viability and bone formation. The results from this analysis are the basis for a final mathematical formulation used to simulate the effect of electrostimulation in bone tissue formation around a biomaterial device, in this case, a dental implant.

All in all, the numerical results obtained in this thesis provide additional support to the open discussion about the plausibility of an electric field to interfere with the homeostasis of the cells, the dosimetry of the exposure for this interference to take place, the feasibility of an electric field to promote tissue formation, the limitations and possible risks derived from the exposure. The thesis presents an approach to investigate perspectives for improving health care and medical practice by means of exposure to electric fields.
Part I

General concepts in bioelectromagnetics
1.1 The concept of bioelectromagnetics

Bioelectromagnetics is the area of study dedicated to exploring and understanding the interactions between electromagnetic fields and biological systems. Regarding the human body, these interactions are related to the possible effects that external electromagnetic sources might have on the temperature distribution and biological, chemical and electric (magnetic) internal processes. Therefore, bioelectromagnetics is an interdisciplinary subject that combines the knowledge from biology and medicine with the understanding of electromagnetics coming from mathematics, physics and engineering.

Understanding the biological impact of an external electromagnetic field is strongly related to the assessment of possible changes in the metabolism and behaviour of the body constituents, namely, organs, tissues and cells. These constituents also depend on the performance of a series of natural electromagnetic events occurring, in most of the cases, in an aqueous medium [1]. Since the cells are living organisms and the elementary units of the human body, changes in their metabolism and behaviour due to the presence of the external electromagnetic field represent changes in both tissues and organs. Contrary to usual belief, effects at the cell or tissue level due to exposure to electromagnetic fields may not necessarily represent a health hazard. In fact, several novel therapeutic techniques and clinical procedures for the treatment of injuries and deceases are based on the benefits deriving from the exposure to properly chosen electromagnetic fields [1, 2, 3].

In the light of the electrical nature of biological cells and tissues, these approaches take advantage, for instance, of the effect of electromagnetic fields on the synthesis (upregulation)/inhibition (downregulation) of specific signalling pathways (growth factors) causing increased tissue formation and reduced wound healing times [4]. Likewise,
dielectric thermal heating induced during exposure to electromagnetic radiation leads to temperature increases that are suitable to ablate malfunctioning tissues and treat tumors [5, 6]. Besides, the mechanism of cell membrane poration is stimulated after exposure to certain types of electromagnetic fields, improving both gene delivery (transfection) [7] and drug delivery (electrochemotherapy) [8], two therapies used to treat cancer and other diseases.

Despite the current applications and evident benefits of using electromagnetic fields in medicine [2, 3], much is left to say about the nature of (possible) interaction mechanisms that can be manipulated in order to obtain an expected biological response. Additional pressure coming from social sectors concerned about hazards caused by electromagnetic exposure has also motivated studies that have yielded controversial results [1, 9]. Moreover, the adequate amount of energy (dosimetry) needed for cells, tissues and organs to trigger a certain positive response and avoid any possible damage is still a matter of debate [10, 11]. A quick look at the history of bioelectromagnetics will set the appropriate background to introduce the main reasons for both the public concerns and the unclear interaction mechanisms.

1.2 A bit of history

Biological systems can communicate and interact with the environment using several mechanisms defined at different scales. The most known mechanisms are of chemical, mechanical and electric nature. Transmission of electrical signals is important for many cellular processes. The study of this kind of signals dates back to the mid-1700s with the experiments carried out in frog legs by the Italian physician, physicist and philosopher Luigi Galvani. These experiments set the basis for today’s electrophysiology and bioelectricity [12, 13]. Roughly speaking, electrophysiology refers to the study of the electrical properties of cells and tissues, whereas bioelectricity is the study of the electric (magnetic) fields produced by the living matter [14]. Galvani observed that the contraction of a frog’s leg is stimulated by touching the leg with the sciatic nerve from the opposite one. Additionally, he showed that the application of the electrical discharge of the recently invented Leyden jar also induced the contraction of a frog’s leg. With this observation, Galvani also was a pioneer in the field of electrical stimulation of biological systems [12].

By 1831, the idea of animal electricity evidenced after Galvani’s work was improved by the also Italian scientist Carlo Matteucci, who described the frog’s leg contractions observed by Galvani in terms of potential differences between the injured and the intact nerves. These observations, repeated and improved with new instrumentation by the German physiologist Emil Du-Bois Reymond, led him in 1848 to the discovery of the action potential [12, 13]. An action potential is the difference in the electric potential
across the cell membrane caused by the flow of ions through the ionic channels in the membrane [15]. Action potentials are transmitted from cell to cell and play a role in the activation of intracellular processes. The progress in neuroscience and electrophysiology have improved the understanding of action potentials [12], providing a better identification of both the interactions between electric fields (charges), the bio-chemical (molecular) nature of cell membranes and the propagation of electrical signals within the body. By 1850, electrochemical cells and discharge shocks coming from induction coils were widely used to study the interaction between electric fields, currents and human tissues [13].

By the beginning of 1900’s, the French physician and physicist Jacques d’Arsonval initiated the era of thermal applications by using high frequency electromagnetic fields and observing the heat and current generated in the body [13]. This type of thermal applications resulted, by the time of the World War II, in concerns about possible health hazards caused by the exposure to electromagnetic fields generated by military devices such as the radar. This led to the first exposure standard initially proposed in 1953 by the German biomedical engineer Herman Schwan: an incident power density of 100 W/m$^2$ as the maximal value to prevent cell damage [16]. Simultaneously, additional research was conducted to determine the electrical properties of tissues and cells in suspensions as well as the mechanisms of tissue heating and propagation of microwaves and ultrasound into tissues [16].

At this point, attempts to explain observations derived from the use of electromagnetic waves in tissues and cells led to the formulation of the first mechanisms of interaction. Accordingly, the auditory effect, a series of buzzes heard when the head was exposed to short microwave pulses, found its biophysical explanation. The small amount of energy absorbed from the pulses was enough to launch an acoustic wave that induced the hearing sensation [17]. Likewise, the pearl-chain effect, an enhanced attraction between cells which leads to their aggregation or lining up when in close proximity, was explained in terms of attractive electric forces and dipole interactions [18]. Other mechanisms of interaction are the ionizing potential only present at extreme high frequencies (above $2 \times 10^{16}$ Hz) [18, 19], the rotation and orientation of polar molecules (relaxation) creating nodes of charge accumulation, and the thermal elevation due to dielectric heating [18, 20].

Concomitantly, between 1930 and 1950, the American researcher Harold Burr conducted experiments to determine differences in the electric potential between the hands of a human being. His results showed that there is a difference of 2-10 mV between the left and the right forefinger, which remains constant in time among males but showed increases over a period of 24 h among females due to the menstrual cycle [14]. These results led to the discovery of the so-called endogenous electric fields, controlling cellular signalling processes of great importance during morphogenesis, tissue formation and
repair [12, 21]. These fields are created by the flow of ions through cells and embryos, and range between 10 and 100 V/m. They are present throughout the human body, especially between the layers of the dermis, and vary depending on the location in the body where they are measured [12].

By 1950 the electrical nature of the human body was evident. However, it was in 1952 with the work by the English physiologists Alan Hodgkin and Andrew Huxley on the action potentials in the axon of the giant squid that the electrical nature began to be understood directly in its source, i.e., the cell membrane and its permeability to the flow of ions. The cell membrane has an intrinsic electrical nature. It is in charge of controlling the interchange of (charged) molecules between the intracellular and the extracellular environment. Due to the difference in ions and molecules concentrations, a so-called transmembrane potential appears between the intra- and extracellular compartments [15]. In their work, Hodgkin and Huxley developed a mathematical model suitable to predict the generation of an action potential due to the changes in the flow of ions through the membrane, leading to variations in the membrane potential. This model has been the basis for the development of several other biophysical models aimed at describing the electrical nature of cells and tissues, especially neurons, the nervous system and the heart. It has also inspired novel approaches to understand other interaction mechanisms between electromagnetic fields and cells such as electrofusion and electroporation [22].

Two main issues have been historically creating debate in bioelectromagnetic research. The first one deals with public concerns about possible harmful health effects of exposure to electromagnetic fields. The second issue refers to the lack of consensus in the scientific community about interaction mechanisms between cells and electromagnetic exposure. In this regard, the presence of both contradictory results and results which cannot be explained in terms of known biophysical mechanisms, contributes to what can be called the mechanisms paradox [9].

1.3 Public concerns about the exposure to electromagnetic fields

Life on Earth has evolved in coexistence with Earth’s natural electric and magnetic fields [1, 19], and there is a permanent exposure to a natural background electromagnetic noise coming from ubiquitous thunderstorms and high-energy radiation particles entering Earth’s atmosphere [23]. Nevertheless, since this natural electromagnetic radiation is almost static and of low magnitude, its interaction with biological systems is considered negligible [23]. However, with the expansion of communication networks, the levels of man-made electromagnetic radiation have increased. Thus, the fear of a possible negative health impact due to the casual exposure to this radiation, as well
1.3 Public concerns about the exposure to electromagnetic fields

as to that coming from common devices such as microwave ovens, resulted in defining the term *electrosmog*, or contamination of the environment by electromagnetic radiation [11].

Already in the 80’s and 90’s public concerns about the implications of living near power transmission lines and their possible incidence in cases of childhood leukemia fueled a vast amount of research. Results from research on this matter suggest a weak association between exposure to residential power transmission lines and health risks, although the lack of an adequate animal model and the presence of inconsistent results does not completely rule out a possible causal relation [1, 24]. Furthermore, the possible incidence of electromagnetic exposure in reported cases of several different types of cancer, together with its possible relation in DNA fragmentation, cell apoptosis and genotoxicity [3, 20], paved the way for the definition of guidelines and standards defining safety limits for use and exposure to electromagnetic fields. These guidelines and standards are, however, surrounded by a mantle of precaution since there is still much uncertainty and contradiction about demonstrated health risks possibly caused by electromagnetic exposure [25]. Moreover, there is additional debate over the safe exposure limits defined in these documents provided that some of them have been proposed on the grounds of either incomplete or not yet well understood biophysical mechanisms [10], or of pragmatical yet not purely scientific (political) arguments [1, 26].

Additionally, the proliferation of electronic devices such as mobile phones and wireless transmitters has raised extra public concern about the possible negative implications of the exposure to these ubiquitous man-made wireless communication networks. To this respect, scientific research suggests possible associations between cancer development and both the usage of mobile phones and exposure to related electromagnetic radiation [27]. However, further research is needed to clarify the possible interaction mechanisms between mobile phone-related radiation and cells, especially regarding possible interferences of signal modulation with biological processes [11, 28]. Furthermore, current guidelines and standards fall short in their appreciations related to the influence of mobile telephony in biological processes, especially regarding the use by children [27].

Consequently, the International Agency for Research on Cancer (IARC), affiliated to the World Health Organization (WHO), appointed in 2011 a working group of experts to conclude about the possible adverse health effects resulting from exposure to electromagnetic fields in the radiofrequency (RF) part of the spectrum, which are emitted by wireless communication devices. After reviewing a vast amount of literature, the working group was unable to identify a indisputable causal relation between RF fields and the incidence of cancer, and in view of the limited evidence, it opted for a cautious conclusion: to classify the RF electromagnetic fields as *possibly carcinogenic to humans* [29] within the so-called *group B* that encompasses other potentially carcinogenic agents, mixtures or exposure circumstances.
Given this mantle of precaution advised by the WHO working group, and despite the fact that experimental evidence does not suggest children to be more susceptible to RF radiation than adults [20], it is not surprising that in 2013 the Belgian government prohibited the selling of mobile phones specially designed for the use of children below the age of seven, banning also the broadcasting of mobile phone advertisements targeting this same age group [26].

Additional source of the public concern comes from a sector of the population which attributes diverse health symptoms to the presence of electromagnetic fields. This condition, known as electromagnetic (hyper)-sensitivity, refers to the possible infliction of electromagnetic fields in the well-being of a person by means of headaches, concentration problems, sleep disturbances, dizziness, nervousness, among others [30]. Although studies reveal that these health alterations might be related to other conditions such as age, sex, occupation, use of electronic devices and underlying diseases, current research is does not suffice to completely exclude a possible causal relation with electromagnetic fields [31].

Therefore, there is a need for methodologies to quantify the coupling between electromagnetic fields and biological systems, in order to better elucidate the (possible) biophysical mechanisms underlying the (apparent) interaction causing biological effects. However, considering the limitations in our ability to obtain specific experimental data such as intracellular electric fields or micro-heating variations, it might be possible that some of the biophysical mechanisms responsible for the interaction with electromagnetic fields are not fully understood, or are still awaiting to be discovered or identified [20].

1.4 The mechanisms paradox

There are many proposed mechanisms to explain the interaction between electromagnetic fields and the human body [10, 11, 32]. It is usually accepted that these mechanisms can be divided into thermal and non-thermal effects [1, 9]. Furthermore, an electromagnetic field can be classified according to its frequency, which in turn determines the way it interacts with cells and tissues. Following the International Telecommunication Union (ITU), the electromagnetic spectrum is divided in a series of frequency bands (Fig. 1.1). Hence, the low frequency (LF) range is defined for electromagnetic fields with frequency lower than 300 kHz [24]. Fields with frequencies below 300 Hz, which is the maximum frequency of many biological processes [12, 14], are part of the so-called extremely low frequency (ELF) and super low frequency (SLF) ranges. In the radio-frequency (RF) range, the lower frequency is 300 kHz and the maximal 300 GHz [20]. Frequencies between 300 MHz and 300 GHz are also referred to as microwave frequencies. In the terahertz frequency (THF) range, the frequency is between 300 GHz and 3 THz, the latter defining the lower boundary of the infrared range. Frequencies
1.4 The mechanisms paradox

Figure 1.1: Electromagnetic spectrum, classification of a wave depending on its frequency, and the scale of the corresponding wavelengths. Created using elements taken from http://www.freepik.com/

between 400 and 790 THz are within the spectrum of the visible light, and further frequencies correspond to ultraviolet, x-ray and gamma-ray radiation.

Despite the vast amount of literature available on both experimental and numerical approaches aimed at elucidating the mechanisms by which electromagnetic fields interact with biological systems and the dependence that this interaction may have in terms of characteristics of the electromagnetic field such as magnitude and frequency [2], there is still a lack of basic knowledge and therefore consensus to come up with solid explanations to all observations [3, 11, 18]. The main reason is the apparent contradiction between multiple results obtained by using the same type of electromagnetic field exposure and in many cases, the lack of reproducibility of the findings.

There are three main reasons for this apparent mechanisms paradox [9, 19, 33]. One way of considering the exposure to a given electromagnetic field strong enough to induce an effect in a biological system is by applying certain biophysical rules which in principle indicate whether the exposure is sufficient to trigger a response. Good examples of these rules are the thermal energy criterion [18], the mobility and velocity principles applied to a simple ion in an aqueous medium [9] and the average SAR [10]. However, several observations of alleged electromagnetic effects are not easily explicable in terms of the biophysical rules. For example, the thermal energy criterion imposes a threshold value for the electromagnetic field exposure to induce a change in the temperature of the biological system above the thermal noise [18, 19]. However, this criterion is limited to systems thermally coupled with the surrounding medium and therefore is not applicable to exposures that induce free radicals formation [18]. Free radicals are molecular species that behave as either oxidants or reductants and are associated with multiple diseases including cancer [18, 34]. Therefore, the first reason for the mechanisms paradox is that biophysical theory might not be complete and current knowledge of biophysical mechanisms can be insufficient. As science evolves, new theories may appear allowing us to better characterize a given effect.

The second reason is that much of the experimental evidence is often unclear. In
many cases, experimental results lack reproducibility, and even after repeating the same conditions for a given experiment (parameters of exposure, type of cells, culture protocol, among others), results from two separated laboratories happen to be different. In this regard, it is important to highlight that different cells from different cell types and lines have different responses. Moreover, both the differences in the population density due to culture conditions varying between laboratories and in the cell cycle of the sets of exposed cells might also lead to variations in the results obtained from two similar assays [1, 33].

In other cases, results are inconsistent due to inadequate experimental techniques and/or failure in the interpretation of the results [17]. There are studies in which the authors claim that they investigated non-thermal exposure levels and effects. However, due to inappropriate experimental setups and dosimetry, it has been later demonstrated that the results in fact correspond to the application of an exposure level that induces thermal effects [25]. In consequence, the constant interplay between experiments and observations is thus far the most reliable source of basic knowledge about the matter, and possible sources of speculation and debate have to be addressed properly to avoid reaching (wrong) conclusions.

Probably the most striking reason to consider the mechanisms paradox is the existence of the so-called biological windows. These “windows” correspond to combinations of parameters at which a biological system responds to electromagnetic field exposure. The most used parameters are magnitude, frequency, exposure time, waveform and duty-cycle [1, 35]. Interestingly, biological systems respond and react to a given impinging field within a biological window, but are not affected for other ranges of parameters [1]. This explains why there is little consensus about the set of parameters used in one or another application or therapy [2, 36]. Furthermore, in addition to being determined by performing systematic research, dosimetry parameters are in some cases still determined by pure intuition, past experience and the patient response [1].

In other cases, the conception adopted is to use parameters similar to those of the processes taking place naturally in biological systems [2]. Whichever the rationale for the adoption of parameters, it happens that a given combination of parameters may be enough to trigger a response in a given scenario, but insufficient to achieve a positive effect in other. It might also be the case that the same stimulus triggers different responses. This means that while in one condition a given stimulus is high enough to induce damage in the system, in another condition this same stimulus can result in beneficial effects [35].

Despite the inconsistencies between multiple observations, the lack of consensus about the consequences in health of such observations, and the public concern about the health hazards of electromagnetic radiation, several possible interaction mechanisms between electromagnetic fields and biological systems have been identified. Although
these mechanisms are mostly supported by experimental research, in recent years numerical simulations have started to shed additional light. The advance in computer power has provided extraordinary capabilities for the progress of numerical methods and tools which are being applied to the solution of complex computational simulations and in-silico experimentation setups [10], otherwise difficult or even not feasible to address by using current experimental methods [23, 37]. Furthermore, numerical results are inciting experimentalists to design their experiments based on hypothesis motivated by numerical findings [10, 38] and to perform assays to better determinate numerical parameters needed to validate computational results [39].

The need for better clarifying the mechanisms of interaction between electromagnetic fields and biological systems, together with the availability of numerical tools suitable to investigate the matter, sets the main motivation of this thesis. Accordingly, the question for this research reads: is it feasible to characterize the possible causal relations between a biological system and a electromagnetic field to which it is exposed by using a physical (numerical) approach? In order to answer this question, based on a set of conditions and assumptions defining the scope of the thesis, special attention has been paid to the identification of specific dosimetry parameters able to trigger a biological response, due to either thermal or non-thermal interaction mechanisms between the electromagnetic field and the biological system.

1.5 Scope of the thesis

The results presented in this thesis are based on two main simplifications. The first one refers to the nature of the biological system. The second one is related to the nature of the electromagnetic field exposure. In principle, a biological system can be any anatomical structure or network of complex biological entities. However, it is important to define the scale of the biological system accordingly in order to establish the level of complexity of the biological, chemical, mechanical and electrical processes related [10].

Therefore, the human body is considered as a general macro-scale biological system (Fig. 1.2), which can be divided first into meso-scale networks dedicated to specific functions such as the respiratory, vascular and nervous systems, but also into organs. The specific functions carried out by organs are mainly due to the presence of different types of tissues and cells. Therefore, organs can be considered as biological systems made up of tissues, while tissues are micro-scale biological systems with a specific function dictated by the type of constitutive cells. Moreover, the complexity of cells as living organisms on their own allows them to be considered as individual biological systems. A deeper look into the composition and structure of cells reveals that the complex network of biochemical processes related to protein synthesis and conformation carried out internally can individually be considered as a nano-scale biological system, with molecules
1 Introduction

Accordingly, in this work the concept of biological systems refers first to a single cell and then to a group (density) of cells. In practice, there are many different types of cells in the human body with many different types of functions and behaviors. However, conducting simulations to deal with the plethora of genotypes and phenotypes might be a daunting if not cumbersome task. Therefore, the exploration of the effects of electromagnetic fields in cells is limited to the effects in generalized cells. The advantage of using this approach is the possibility of modifying certain parameters such as size and material properties in order to adjust the representation to one or another specific cell type. It is important to emphasize, however, that the description for a generalized cell is given from the electromagnetic point of view only, and biochemical and molecular mechanisms naturally involved are outside such a representation.

The second important simplification is the assumption that the possible biological effects due to exposure of the general cell to an external electromagnetic field are mostly due to the electric field component. This assumption is not intended to completely rule out the possibility of magnetic field-induced biological effects, but to assume them negligible compared to those due to the electric field. A natural restriction to the
possible interference of magnetic fields with cells is the 50-\(\mu\)T magnetic field of the earth [14, 19]. Therefore, it would not be surprising if magnetic fields below this value cause no effect on cells. However, since the magnetic field of the earth is static, such consideration may not apply to varying magnetic fields. In fact, due to Faraday’s law, a varying magnetic field causes a varying electric field. These magnetically induced electric fields are able to directly affect cell structures which are otherwise shielded by the presence of membranes [19]. Since everything inside a cell, from atoms and molecules to the cell itself is always in movement [14], it is reasonable to hypothesize that any possible effect on the cell is due to (only) the induced electric field [19].

Furthermore, a typical human cell in suspension can be fairly approximated by an sphere of radius 5-20 \(\mu\)m [40]. Exceptions are made for elongated cells such as muscle cells, star-shaped cells such as neurons, or disc-like cells such as erythrocytes, for which the use of geometries close to ellipsoids, rods or cylinders is more adequate [41]. Despite the differences in shape, electromagnetic field theory states that when the wavelength of an impinging wave is far large than the dimension of the object subject to the wave, the electromagnetic solution can be approximated as quasi-static [42, 43]. This means that the decoupled electric and magnetic fields can be derived from a set of two independent field solutions.

It can be assumed that any possible cell effect due to exposure to a magnetic field is in fact the consequence of the induced electric field [10, 14]. Moreover, we can assume that any possible effect still caused by the magnetic field is negligible as compared to the possible effect caused by an electric field [23]. Hence, in view of the quasi-static condition, the exposure of a cell to an electric field would be enough to study the cell response. Therefore, for the cases studied in this thesis, the incident electromagnetic field is reduced to an incident electric field. Despite this simplification, it is important to emphasize that since the magnetic properties of biological materials and cells are similar to those of free space, magnetic fields are not attenuated by the cells and in consequence they might be able to affect signalling mechanisms and chemical reactions, even in a subtle way [14, 19]. In fact, there are realistic ways for a magnetic field to influence a cell, such as rotational motion, translational force, Larmor precession, interaction with magnetic particles such as magnetite, and formation of radical pairs [14, 18], the biological effects of which are in consequence considered outside the scope of this work.

Based on these two simplifications, the focus of this thesis is on elucidating possible interaction mechanisms between cells and electric fields, describing both the cells and the electric fields in terms of mathematical formulations implemented and solved through numerical methods. Among the plausible changes in cells due to exposure to electric fields, and considering that the mathematical description used for the cells does not include molecular movement or the biochemistry of reactions, special attention is paid
to variations in the cytoplasmatic and nuclear electric fields, the transmembrane electric field and the membrane electric potential. Additionally, criteria such as material properties, frequency dispersion, size, shape, position and number of cells are used to extend the approach to the study of proximity effects due to electric field interactions between neighboring cells.

The working hypothesis is that any perturbation in the biological system triggered by exposure to an electric field must be somehow related to a noticeable variability in the physicals variables above mentioned. Such variability leads to possible interaction mechanisms between a cell and the electric field. However, the presence of variability is only a necessary condition for an effect to take place. A sufficient condition would be related to how this variability induces molecular and biochemical changes above biophysical thresholds which might then trigger an effective cell response due to the electric field exposure [44]. This confirmation, however, is outside the scope of this thesis and left as a matter of future research aimed at combining the models presented here with a suitable molecular and/or bio-chemical description for cellular processes.

1.6 Outline of the thesis

The goal of this thesis is to identify possible interaction mechanisms between electric fields and biological cells and tissues using mathematical formulations and numerical simulations. In order to provide the proper conceptual background, and to tackle individual cells and cell-cell interactions separately from cell populations and tissue response, the thesis is divided in three parts.

The first part, General concepts in bioelectromagnetics, begins with this introduction chapter and unfolds towards the fundamentals of biological cells, biological tissues, the exposure to electric fields, and electromagnetic theory. Accordingly, Chapter 2 presents the basics of cell biology and the aggregation of cells into body tissues. A review of the interaction mechanisms between electric fields and cells is also presented. Additional attention is paid to the exposure limits defined by international standards and to current medical applications of electric fields. Chapter 3 is devoted to an overview of the electromagnetic theory and basic concepts useful in the analysis of the electrical response of biological cells exposed to electric fields.

The second part, Investigating the interaction mechanisms, is dedicated to introduce mathematical models suitable to represent single cells and identify cell-to-cell interactions. Therefore, Chapter 4 presents a numerical approach to model multiple cells based on the quasi-static approximation and the equivalent dipole moment of a dielectric sphere. Chapter 5 is dedicated to an additional formulation based on a symbolic scattering-matrix approach useful to investigate the cell membrane response and the proximity effects generated during the exposure to an incident electric field. Finally,
in order to study the influence of the shape of both the cell and the organelles in the overall electrical response, a full-wave approximation based on the electric field volume integral equation is introduced in Chapter 6.

The third part, Getting advantage of the electric field, is devoted to present the numerical simulation of clinical (in-vitro) scenarios in which the use of electric fields is known for modifying the biological response of cell conglomerates and tissues under formation. Accordingly, Chapter 7 introduces the mathematical modelling of biological systems, with an overview of reaction-diffusion equations and its suitability for describing cell behaviour and tissue formation. Based on ideas from biologically-inspired models, Chapter 8 presents a mathematical model to describe the effect of an external electric field during cell migration, an effect known as electrotaxis [45]. The numerical results demonstrate the suitability of the electric stimulus to promote tissue formation and evidences a limitation in the intensity of the electric field needed to avoid cell damage. Following this limitation, Chapter 9 is dedicated to present an extended electrotaxis model to analyze the effect that thermal heating generated during electrostimulation has on cell viability and bone formation [5, 46, 47]. Considering the results from the thermal model on the limited electric field intensity, and based on the reliability of the reaction-diffusion-based models for representing tissue formation, Chapter 10 presents a mathematical formulation used to simulate the effect of electrostimulation in bone tissue formation around a biomaterial device, in this case, a dental implant. The biological (clinical) problem concerning the insertion of a dental implant, the simplifications adopted for the conception of the model, and the results of the implementation against verifiable experimental evidence are discussed.

Finally, Chapter 11 is devoted to present the concluding remarks that the numerical results provide on the interaction mechanisms between an electric field and both biological cells and tissues. The plausibility of beneficial/hazardous cell effects, the suitability of an electric field promoting favorable tissue responses, the limitations of the usage thereof, and the perspectives for future work, are also discussed.
In order to study possible interaction mechanisms between electromagnetic fields, cells and tissues, it is important to first define a conceptual framework. This is needed considering the vast amount of physical and mathematical concepts used in electromagnetic field theory, on the one hand, and the complexity of the biological and biochemical terminology describing cell physiology, metabolism and behaviour, on the other. Accordingly, this chapter is dedicated to first introduce some general concepts about biological cells and tissues, and then to review the effects of their exposure to electromagnetic fields. The discussion is focused on those effects originated from the exposure to electric fields and the possible (intracellular) mechanisms of interaction. Next, the guidelines and standards that define (propose) limits to electromagnetic fields exposure are reviewed. Finally an overview is given of medical applications in which the use of electromagnetic fields is accepted as mediator for improved biological outcomes.

2.1 The cell

A cell is the basic unit of life. It is the result of an evolutionary process that provided the cells with a such a rich structure and functional complexity that still current research is performed to further unravel the bio-chemical, mechanical and electrical mechanisms it involves [48]. Human cells are structures surrounded by a cell membrane which separates the intracellular compartment or cytoplasm from the extracellular medium. The internal structure of a cell depends on its functional nature and might contain diverse and complex organelles. A general cell is shown in Fig. 2.1 [49]. The size and shape of a cell greatly depends on the type and function of the cell. For instance,
neuron cells are stellated cells, whereas muscle cells are spindle-shaped cells. Other cells like stem cells have a rounded spherical-like shape, while others like erythrocytes have disc-like shapes.

The intra- and extracellular compartments are mostly aqueous saline solutions. These saline solutions, also referred to as body water, correspond in total to about 45-75 % of the total body weight of a human being. Variations are mostly due to the amount of adipose tissue in the body. About two thirds of body water correspond to cellular water (cytosol), which is the aqueous solution in the cytoplasm. The remaining 30 % of body water correspond to extracellular fluid, which is further divided into interstitial fluid and plasma [49]. The interstitial fluid bathes all cells and serves as an interface between blood and cells necessary to carry out nutrients and waste materials interchange. Plasma in contrast corresponds to the liquid content of blood [50]. The saline content of these solutions is specified by the presence of inorganic salts and charge ions, the most important of which are sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$) and calcium ($Ca^{2+}$) [15].

2.1.1 The cell membrane

The cell membrane is a 5-10 nm thick structure surrounding and enclosing the cell. It is composed by lipid molecules and proteins. The most abundant membrane lipids are called phospholipids. These molecules are amphiphilic, which means that they have
2.1 The cell

Figure 2.2: Sketch of the phospholipid bilayered cell membrane. Modified from http://commons.wikimedia.org/

an *hydrophilic* polar end and a *hydrophobic* non-polar end [51]. The hydrophilic end is characterized by its ability to be attracted to water molecules. In contrast, the hydrophobic end repels from water. Therefore, phospholipids must aggregate in such a way that their hydrophilic ends are exposed to water while the hydrophobic ends are not. One way for the phospholipids to accomplish this is by forming a *bilayered* structure where the hydrophobic ends are sandwiched by the hydrophilic ends (Fig. 2.2). Due to the presence of exposed hydrophobic ends at the borders, an initial planar bilayer tends to curve forming a sealed compartment, the cell itself. Membrane proteins can be classified into *integral* and *peripheral*. Integral proteins are embedded and attached to the phospholipid bilayer and interact with the hydrophobic heads, whereas peripheral proteins are externally attached to the cell and interact only with the hydrophophilic heads [51]. Another important type of membrane proteins is the *transport* protein. This type of protein facilitates the movement of ions, molecules and other proteins across the membrane by creating a channel that communicates the extracellular medium with the cytoplasm. Other important membrane proteins are the glycoproteins which play a role in cell-cell interactions and globular proteins, which are involved in signalling and regulatory processes [51].

The cell membrane not only delimits the cell, but plays an important role in almost all internal metabolic processes due to its permeability to both water and charged ions [15, 50]. Water transport through the membrane is carried out by specific membrane transport proteins called *aquaporines*. By means of them, the cell membrane regulates
the osmolarity of the cell, which is an indicator of the flow of water across the membrane due to difference in concentration at its both sides. It also regulates the tonicity of the cell, which is a measure of the movement of water across the membrane due to differences in the concentration of impermeable particles at both the intra- and extracellular compartments. Accordingly, cells might change their volume and either swell or shrink because of the flow of water across the membrane needed to balance out the particle concentration differences [50]. These volume changes lead to specific changes in localized ion concentrations which might represent a risk for pathological conditions [52]. Therefore, most saline solutions used as extracellular medium for in-vitro cell cultivation are both isosmotic and isotonic, meaning that both the flow of water across the membrane and the particle concentrations are in equilibrium at its both sides. Such solution allows the study of metabolic processes which are not related to water ingestion or excretion [50].

Furthermore, the cell membrane regulates the pH of the cytoplasm by controlled activation of specific channel proteins or ion pumps which regulate the concentration of hydrogen ions (H\(^+\)). This regulation maintains the pH between 7.0 and 7.5, characteristic for the biochemical functions [53]. Changes in intracellular pH are known to influence cell metabolic functions and behaviour [14]. Additional regulation of charged ions is also performed by controlled opening and closure of specific ion pumps. Assuming a cell whose cell membrane only contains Cl\(^-\) ion pumps, if the extracellular medium is enriched with sodium-chloride (NaCl), the difference in the salt concentration between the intra- and extracellular media creates a diffusion force. This force pulls Cl\(^-\) ions into the intracellular medium, which will pass through the membrane due to its permeability. As this happens, the intracellular Cl\(^-\) concentration increases balancing out with the extracellular medium and creating a net negative intracellular charge. Because of this negative charge and the excess of positive charge at the extracellular medium due to the excess of Na\(^+\), an electric force starts to act upon the Cl\(^-\) ions which counteracts the diffusion force. When both forces neutralize each other, the resulting equilibrium condition is called Donnan equilibrium.

### 2.1.2 The membrane potential

For a cell at rest, the balance between the diffusion and electric force leads to an unbalance in electrical charge between the intra- and the extracellular media. Accordingly, a so-called membrane potential appears, which for most biological cells ranges between -35 mV and -90 mV [4, 49, 54]. The negative sign is a convention used to indicate that the intracellular medium is negatively charged. Such a negative charge is the consequence of the activation at rest of much more chloride and potassium (negative) ion pumps than sodium or calcium (positive) ones [15]. As the membrane potential becomes less negative, a cell is said to be depolarized. In contrast, as the membrane potential be-
comes more negative a cell is said to be hyperpolarized [4, 49]. The controlled activation of ion pumps is of paramount importance in excitable cells like neurons and muscle cells because many metabolic functions and behaviour depend on the adequate formation and transmission of action potentials [15].

Furthermore, changes in the value of the resting potential due to depolarization caused by variations in ions concentrations but also due to changes in the surface charge at the cell membrane [55] are responsible for the activation of so-called voltage-gated ion channels. The resulting variations in intracellular ion concentrations cause membrane repolarization and channels closure. The temporary flow of ions down their concentrations gradients generate the action potentials and cause variations in signalling transduction pathways [49]. Such a variation is especially important in the case of $Ca^{2+}$, for which variations in the intracellular ion concentration modify cellular processes such as migration, proliferation, cell attachment, necrosis and apoptosis [4, 14, 56].

## 2.2 The body tissues

Cells with similar structure and function aggregate to form tissues. Tissues constitute the basic unit of organs, which carry out most of the biological functions of the human body. There are four basic types of tissues: epithelial, connective, muscle and nervous [49].

The epithelial tissue is the type of tissue covering the surface of organs, separating one organ from the other. It consists of cells aggregated side by side and one over the other, providing it with a stratified appearance. Although it has no vascular irrigation, it does have regeneration ability due to the continuous replacement of cells.

The connective tissue is dedicated to protect, support and bind organs together. It encompasses different types of specialized cells typically arranged into fibers. This type of tissue is very vascular and in most cases has regeneration ability. Cells can be found dispersed within an extensive extracellular matrix. Adipose reservoirs, cartilage and bone belong to this classification.

The muscular tissue is dedicated to generate movement. Cells in this type of tissue are elongated and aggregate into fiber-like arrangements which are covered by connective tissue and supplied with blood vessels. There are three types of muscle tissue: smooth, dedicated to generate involuntary movement as in the intestines, cardiac, dedicated to the involuntary pumping activity of the heart, and skeletal, which has direct contact with the skeleton and provides the voluntary movement of the body parts.

Finally, the nervous tissue is dedicated to the conduction of electrical impulses across the network of interconnected neurons, lay a determinant role in the control of both voluntary and involuntary functions. This network is divided into the central nervous
system consisting of the brain and the spinal cord, and the peripheral nervous system referring to the connections between individual neurons. Nervous tissue has a very limited natural regeneration ability, restricted to small injuries, is well provided with blood vessels and is protected with layers of connective tissue [49].

2.3 Evidence of interaction mechanisms

Effects of exposure to an electric (magnetic) field can be understood as a chain of interrelated multiscale responses in the biological system [10]. This chain of events is initiated with the interaction between the impinging wave and the atomic structure (charge) of biomolecules (ions), especially outside the organism (cell) [55]. This interaction leads to changes in the chemical composition and charge distribution of proteins and other macromolecules which transduce into changes in biochemical signalling pathways [14]. In cells, these conformational changes lead to alterations in both metabolism and behaviour with the consequent alteration of biological processes such as proliferation, differentiation and apoptosis [1, 3, 10].

Cells and tissues exposed to an impinging wave are ultimately exposed to a source of energy. At the atomic level, the interaction between the wave and the biological system can be quantified in terms of the energy needed to remove orbital electrons from atoms of the biological material through which the wave propagates, also known as ionization energy [20]. The energy $E$ of a photon of an electromagnetic wave is given by the relation $E = hf$, where $h$ is Planck’s constant ($6.625 \times 10^{-34} \text{ J s}$) and $f$ is the frequency. The ionization energy for biological materials compounding living organisms is about $10 \text{ eV}$ ($1 \text{ eV} = 1.602 \times 10^{-19} \text{ J}$) [18, 20]. Hence, an impinging wave with a frequency above $2.418 \times 10^{15} \text{ Hz}$ is able to ionize molecules and atoms, breaking chemical bonds and potentially causing cell and DNA damage, while a wave with a frequency below this value is unable to damage biological molecules [11].

In addition to ionization, and depending on their atomic structure, certain molecules present natural vibration frequencies, which can be modified by the application of the right amount of energy in order to induce resonance (excitation) effects [18, 57]. Considering the principle of energy conservation, the excitation of a molecule would occur the moment the energy of a photon coming from the external source matches the energy of a photon in vibration state. Since the energy of both photons must be equal to $hv$, the excitation is only accomplished when the frequency is the same, i.e., when the frequency of the external source matches the vibration frequency of the molecule [18]. Typical vibration frequencies are within the range $300 \text{ GHz} - 1.2 \text{ THz}$. Therefore, it might not be possible for signals with frequencies below $300 \text{ GHz}$ to effectively affect vibration modes. Nevertheless, increments in the membrane potential caused by activation of $K^+$ channels correspond with a resonance response to the electric field at frequencies in the
2.3 Evidence of interaction mechanisms

10-100 Hz range. This resonance might increase the sensitivity of the cell array to the external field with frequency components in the mentioned low-frequency range, but might not be significant for a single cell [57].

Other interaction mechanisms in which impinging waves with frequencies below the ionization threshold are known to cause effects in the biological system which can be divided into thermal and non-thermal effects. On the one hand, thermal effects are related to the absorption of energy transported by the impinging wave as it propagates through the biological material, interacting with the electrical conductivity, increasing molecular motion in the material that induce dielectric heating and a consequent rise of temperature [18]. On the other hand, non-thermal effects correspond to alterations in the biological system which appear after exposure to the impinging wave and could not be explained from the standpoint of temperature increase. These alterations are associated with molecular vibrational states, changes in protein conformation, DNA fragmentation, changes in cell morphology, proliferation and differentiation, free radical formation, among others [3, 4, 18, 33]. Table 2.1 summarizes some non-thermal effects found in the literature that have significant experimental evidence for its existence [20, 24, 58] not only at the cellular (molecular) level but also at tissue and whole-body level.

Additional evidence for the existence of thermal effects other than due to dielectric heating comes from the differences in the dielectric properties between cell compartments and the extracellular medium. These differences may cause temperature increases during exposure to electric fields which might be able to initiate biological responses [10]. Therefore, at the interface between cell compartments, the difference in dielectric properties causes gradients of electric field which might trigger micro-thermal heating in structures of molecular size. Although it is accepted that micro-heating is negligible [10, 20], there is evidence suggesting that changes in temperature as small as 0.1-0.2 °C cause biological effects [17, 20, 59], possibly due to the presence of small temperature gradients and thermal accumulation inducing overproduction of harmful reactive oxygen species (ROS) and changes in both cell morphology and protein synthesis [10, 18, 20]. These considerations, however, may also depend on the frequency of the exposure.

Besides, the question behind non-thermal effects is whether the interaction between the impinging electric field and the biological tissue is limited to the transfer of energy causing dielectric heating, or whether there are other bio-chemical or molecular pathways that are activated by the presence of the electric field [10]. Charge particles in suspension present a random movement caused by the collisions with their neighbor particles. This movement, called Brownian movement, introduces thermal fluctuations in the suspension medium, which can be quantified in terms of thermal energy, or in this case, thermal noise. The thermal noise is given by $W_T = k_B T$, where $k_B$ is Boltzmann’s constant ($1.3806x10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$) and $T$ is temperature. At room temperature of
In the presence of an electric field with magnitude $E$ and frequency $f$, charged particles experience an electric force and a consequent oscillatory movement which can be quantified in terms of the amount of the kinetic energy transferred to the particle by the electric field. The kinetic energy for a particle is expressed in terms of its mobility by $W_K = m \mu^2 E^2 / 2$, where $m$ and $\mu$ are the particle’s mass and mobility respectively. For a chloride ion subject to a 200-V/m electric field, $m = 5.88 \times 10^{-26}$ kg, $\mu = 7.9 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ and $W_K$ is $4.5814 \times 10^{-17}$ eV ($7.3394 \times 10^{-36}$ J). The difference of 15 orders of magnitude between $W_T$ and $W_K$ suggests that the transfer of energy from the electric field to (chloride) ions in suspensions is below the energy corresponding to thermal noise. Hence, by virtue of the thermal energy principle, low intensity electric fields might not be enough to elicit oscillatory movement in ions that can possibly cause biological effects [18, 19].

Interestingly, non-thermal effects are considered to exist even when the calculated energy induced by the field is lower than the thermal noise. The thermal energy principle is based on the thermodynamic equilibrium, meaning that the energy provided by the field always creates heat. However, there are other physical means of interaction between the field and charged particles, such as rotation, translation and mechanical vibrations, which might be also transduced into a biochemical signal with a correspondent biological effect [17, 18, 60]. Moreover, uncharged particles may also experiment electric forces through the induction of dipole moments [9], and it is possible for fields inducing energies below the thermal threshold to change the thermoregulatory activity and cause biological effects [1]. In addition, due to frequency dispersion in the electrical properties, the cell, cell membrane and cell internal structures may absorb more energy than the extracellular medium, especially at RF frequencies [38, 61]. This difference in absorbed energy might be related with non-thermal interaction mechanisms such as the effects of GSM-900 MHz fields on intracellular molecular pathways reported by [38], and the cytoskeleton (microtubules) damage reported by [11].

In order to quantify the amount of energy absorption the specific absorption rate (SAR) is used [1]. This quantity is defined in the IEEE standard C95.3 of 2002 as the time derivative (rate) of the incremental energy $dW$ absorbed by (dissipated in) an incremental mass $dm$ contained in a volume element $dV$ of given density $\rho$ [62]. Symbolically:

$$SAR = \frac{d}{dt} \left( \frac{dW}{\rho dV} \right), \quad (2.1)$$

The SAR is expressed in W/kg, and can be related to the electric field at a given point through

$$SAR = \frac{\sigma |E|^2}{2\rho}, \quad (2.2)$$
### 2.3 Evidence of Interaction Mechanisms

<table>
<thead>
<tr>
<th>Effect</th>
<th>System</th>
<th>Frequency</th>
<th>Magnitude / SAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic development</td>
<td>Embryonic cells</td>
<td>DC</td>
<td>100 - 500 V/m</td>
</tr>
<tr>
<td>Redistributed (surfaces) ions</td>
<td>Embryonic cells</td>
<td>DC</td>
<td>100 - 500 V/m</td>
</tr>
<tr>
<td>Cell elongation, drift of integrins</td>
<td>Embryonic cells</td>
<td>DC</td>
<td>500 - 1200 V/m</td>
</tr>
<tr>
<td>Cell differentiation, surface change</td>
<td>Embryonic cells</td>
<td>DC</td>
<td>500 - 1200 V/m</td>
</tr>
<tr>
<td>Direct field perception</td>
<td>Whole body</td>
<td>DC</td>
<td>2 - 5 KV/m</td>
</tr>
<tr>
<td>Calcium upregulation</td>
<td>Embryonic cells</td>
<td>50 Hz</td>
<td>0.45 mV/m</td>
</tr>
<tr>
<td>Electrotaxis (guided cell migration)</td>
<td>Neuronal cells</td>
<td>50 Hz</td>
<td>0.45 mV/m</td>
</tr>
<tr>
<td>Electrical polarization - cell excitability</td>
<td>Neuronal cells</td>
<td>≈ 100 Hz</td>
<td>100 mV/m</td>
</tr>
<tr>
<td>Enhanced fracture healing</td>
<td>Bone</td>
<td>&lt;10 Hz</td>
<td>1 - 10 V/m</td>
</tr>
<tr>
<td>Cell proliferation, upregulation of growth</td>
<td>Osteoblastic cells</td>
<td>&lt;50 Hz</td>
<td>2 - 5 KV/m</td>
</tr>
<tr>
<td>Protein conformation</td>
<td>Bovine serum albumin</td>
<td>&lt;1 MHz</td>
<td>2 KV/m</td>
</tr>
<tr>
<td>Cell attraction (peptide-chain effect)</td>
<td>Cells and colloidal particles</td>
<td>&lt;100 MHz</td>
<td>1 - 10 V/m</td>
</tr>
<tr>
<td>Decrease Ca²⁺ spikes per cell</td>
<td>Neuronal cells</td>
<td>70-1100 MHz</td>
<td>0.5 - 1 W/kg</td>
</tr>
<tr>
<td>Expression of apoptotic genes</td>
<td>Drosophila</td>
<td>50-1000 MHz</td>
<td>1 W/kg</td>
</tr>
<tr>
<td>Cell apoptosis and effects in the regulatory</td>
<td>Human neuroblastoma cells</td>
<td>835 MHz</td>
<td>4 W/kg</td>
</tr>
<tr>
<td>function</td>
<td></td>
<td>900 MHz</td>
<td>1 W/kg</td>
</tr>
<tr>
<td>Suspected hypersensitivity (field perception)</td>
<td>Whole body</td>
<td>900 MHz, 1.8 GHz</td>
<td>1.6, 0.7 W/kg</td>
</tr>
<tr>
<td>Protein conformation</td>
<td>Bovine serum albumin</td>
<td>1.8 GHz</td>
<td>15-20 mW/kg</td>
</tr>
<tr>
<td>Modulations in gene expression</td>
<td>Neuronal cultures</td>
<td>1.8 GHz</td>
<td>2 W/kg</td>
</tr>
<tr>
<td>ROS increase - DNA damage</td>
<td>Human epithelial cells</td>
<td>1.8 GHz</td>
<td>3-4 W/kg</td>
</tr>
</tbody>
</table>

Table 2.1: Some identified (possible) non-thermal effects reported in the literature.
where $\sigma$ is the (effective) electrical conductivity of the material at that point, and $\mathbf{E}$ is the (time-harmonic) electric field.

Additionally, the SAR can be used to determine the increase of temperature at a point by means of

$$SAR = c \frac{dT}{dt},$$

where $c$ is the specific heat capacity given in $\text{J K}^{-1} \text{C}^{-1}$, $dT$ is the change in temperature expressed in $\text{C}$ and $dt$ is the duration of the exposure in $\text{s}$.

The SAR is accepted as the standard to define thermal exposure doses. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the exposure limit for whole-body adverse effects, assumed subject to an increase in temperature of more than 1 $\text{C}$, is 4 W/kg [70]. Thus, the safe exposure level is 2.0 W/Kg for frequencies ranging from 100 kHz to 10 GHz [10, 20, 70]. At these same frequencies, the SAR level for safe occupational exposure is adjusted using a safety factor of 10 to 0.4 W/kg, while for public exposure the safety factor is 50 and SAR is 0.08 W/kg [62, 70]. Higher SAR values of 20 W/kg can be expected in exposed body parts (limbs), due to heat removal by blood circulation [71].

Furthermore, the lack of a thermal mapping of the cell during in-vitro experimentation makes it difficult to specify, based solely on the SAR value, which exposure range might induce a thermal effect in cells and which not [1]. In this regard, activation of the cellular protective mechanisms to stress, the heat-shock proteins (HSP), can be achieved by both temperature increase and exposure to an electric (magnetic) field [72]. Accordingly, thermally induced HSP’s activation is obtained by applying an equivalent SAR of $\approx 10^{-12}$ W/kg while non-thermal activation is obtained, assuming public safety exposure, for a SAR of 0.08 W/kg at most. The difference of 9 orders of magnitude between the values serves to detractors of SAR to state that it should not be used as a criterion for the evaluation of safety during exposure to electromagnetic fields, at least not for assessment of possible effects in cells [1, 73].

Numerical estimations show that the exposure of cell cultures to GSM 900 MHz waves leads to SAR of 0.18 W/kg in the extracellular medium [74], a SAR of 9.3 W/kg in the cytoplasm and a SAR of 23.3 W/kg in the nucleoplasm. Additional estimations indicate that the high value at the cell interior may be enough to induce a significant temperature gradient capable of triggering thermal-mediated interaction mechanisms at the cell interior [38]. However, if this is the case, both temperature variations in the culture medium and activation of HSP’s should be monitored concomitantly with SAR in order to reach conclusions about possible interaction mechanisms [1, 17, 72].

Nevertheless, monitoring the temperature fluctuations in a point (or set of points) does not completely mean that the temperature at all other locations is not fluctuating away
from the measurement value(s), especially if the cell thermoregulation process is taken into account. This poses an extra problem for the assessment of possible non-thermal effects. Besides, in view of the complexity to measure temperature during cell exposure, the lack of adequate (measured) parameters, and the variability that temperature has according to the initial and exposure conditions, predictions based solely on temperature are insufficiently reliable in the sense that it seems unlikely that using temperature as the dosimetry quantity instead of SAR can help forward in the discussion about non-thermal effects [71].

Despite the evidence of effects, methods and experimental techniques from data extraction and reproducibility of experiments must be improved in order to better conclude on interaction mechanisms, especially regarding non-thermal effects. In fact, ensuring adequate dosimetry and temperature control are mayor difficulties to completely exclude the presence of heating accounting for the observed effects [20]. Moreover, the definition of standards has focused on identifying exposure values that prevent damage and adverse effects in the whole-body [24], defining basic restrictions but not minimum dosimetry. Research is still ongoing to identify the distribution of absorbed energy at the cellular and subcellular levels (microdosimetry) needed to clarify the possible influence that electric fields might have on biochemical processes, especially regarding non-thermal interaction mechanisms [20].

### 2.4 Evaluation of possible health effects: exposure guidelines

The determination of plausible interaction mechanism between electric fields and cells depends on both the conditions of the experimental setup and the dosimetry parameters. Experimental conditions must ensure reproducibility and correct interpretation of the results. Furthermore, a single showing evidence of an interaction mechanism provides at most an hypothesis for that mechanism to exist, which must be confirmed through replication and additional supportive studies [25]. Different groups of experts in recent years have carried out the evaluation of published literature in order to find sufficient evidence to support the acceptance of interaction mechanisms and effects due to exposure to electromagnetic radiation. Most of these evaluation have focused on the assessment of possible adverse health effect such as cancer, defining safety exposure parameters.

In 1998, ICNIRP published the first recommendations for exposure to RF fields, which were reviewed in 2009 and 2010. In these documents, the committee considered the evidence in favour of non-thermal effects very weak, although not enough to completely rule out their existence [20, 24, 25]. The same conclusion was presented regarding possible non-thermal effects derived from the use of mobile phones. Regarding hypersensitivity, the committee concluded that headaches and other symptoms are not related to elec-
tromagnetic radiation. Concerning cancer-related effects, the evaluation of consistent studies indicated that such effects appear at SAR levels above 4 W/kg [20].

In the 1998 document, the ICNIRP defined guidelines for limiting exposure to electric and magnetic fields with frequency up to 300 GHz [70]. These guidelines establish the basic restrictions needed to prevent induced currents able to activate functions in the nervous system. Using mathematical modeling and extrapolation from the experimental results at certain frequencies, these basic restrictions are translated into reference levels for whole-body (occupational and general public) exposure.

A complementary ICNIRP evaluation presented in a document released in 2010 introduced additional basic restrictions limiting whole-body exposure to electric fields with intensities below those inducing internal electric fields able to stimulate the nervous system [24]. Accordingly, the internal electric field due to exposure to external fields in the range 1 Hz - 3 kHz should be lower than 0.8 V/m for occupational exposure and than 0.4 V/m for general public. For the frequencies in the range 3 kHz - 10 MHz the maximal induced electric field in V/m follows the relation $2.7 \times 10^{-4} f$ for occupational exposure, and $1.35 \times 10^{-4} f$ for general public, with $f$ the frequency in Hz. These basic restrictions are also translated into reference levels, mainly by means of mathematical modelling.

Table 2.2 summarizes the electric field reference levels for both occupational and general public exposure in the range 1 Hz - 300 GHz. Ranges are adjusted to the lowest exposure values reported among the ICNIRP guidelines [24, 70]. An additional set of permissible exposure values can be found in IEEE standards C95.1 and C95.6 regarding the safety of exposure to electromagnetic fields in the range 0 - 300 GHz. This set provides less conservative limits than those provided by ICNIRP, especially below 1 MHz.

<table>
<thead>
<tr>
<th>Frequency range</th>
<th>Occupational (V/m RMS)</th>
<th>General public (V/m RMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 25 Hz</td>
<td>20000</td>
<td>5000</td>
</tr>
<tr>
<td>25 Hz - 50 Hz</td>
<td>$5 \times 10^5 / f$</td>
<td>5000</td>
</tr>
<tr>
<td>50 Hz - 820 Hz</td>
<td>$5 \times 10^5 / f$</td>
<td>$2.5 \times 10^5 / f$</td>
</tr>
<tr>
<td>820 Hz - 3 kHz</td>
<td>610</td>
<td>$2.5 \times 10^5 / f$</td>
</tr>
<tr>
<td>3 kHz - 1 MHz</td>
<td>610</td>
<td>87</td>
</tr>
<tr>
<td>1 - 10 MHz</td>
<td>$6.1 \times 10^8 / f$</td>
<td>$8.7 \times 10^4 / f^{1/2}$</td>
</tr>
<tr>
<td>10 - 400 MHz</td>
<td>61</td>
<td>28</td>
</tr>
<tr>
<td>400 MHz - 2 GHz</td>
<td>$3 \times 10^{-3} f^{1/2}$</td>
<td>$1.375 \times 10^{-3} f^{1/2}$</td>
</tr>
<tr>
<td>2 - 300 GHz</td>
<td>137</td>
<td>61</td>
</tr>
</tbody>
</table>

$f$ is frequency in Hz.
Other evaluation committees have pronounced about the possible effects and incidence of electromagnetic fields in health. The Health Council of the Netherlands published in 2009 a report focused on the possible incidence of electromagnetic radiation following mobile phone usage and power transmission lines on brain function and electromagnetic hypersensitivity. Conclusions are that current evidence is not enough to indicate clear causal relationships between symptoms and electromagnetic radiation [31]. Likewise, the Scientific Committee on Emerging and newly Identified Health Risks (SCENIHR) published in 2009 a report according to which evidence is insufficient to conclude on electromagnetic hypersensitivity and cognitive effects following exposure to electromagnetic fields [25].

Moreover, the European Health Risk Assessment Network on Electromagnetic Fields Exposure (EFHRAN) presented in 2010 a report on the risk associated with exposure to electromagnetic fields. Due to the inadequate and limited in-vitro and in-vivo evidence, there are not strong indications of effects on health derived from the electromagnetic field radiation [25]. Despite the conclusions of these reports, the International Agency for Research on Cancer (IARC) presented in 2013 an evaluation of carcinogenic risks of RF electromagnetic fields, including mobile phones, microwaves and radar. According to this evaluation, RF-radiation was classified as possibly carcinogenic in humans [58]. A previous report from the same agency published in 2002 also classifies extreme low frequency radiation as possibly carcinogenic to humans, although limited and inadequate evidence was found to establish clear causal relationships. Additionally, since no relevant data was found regarding carcinogenesis due to exposure to static electric fields, this type of fields could not be classified as to its carcinogenicity to humans [75].

Despite the lack of consensus about the possibility of interaction mechanisms, it is widely accepted that clinical application of the properly chosen electric field exposure is beneficial in the treatment of various diseases and injuries, especially in the cases where other traditional medical treatments tend to fail [1, 18]. In addition, medical applications of electric fields have a significant impact in physical therapy and wound healing (pain relief, muscle activation, neuromotor activity, tissue formation). Therefore, the use of electric fields in medicine has gained an important role for the development of advanced therapeutic and clinical applications [1, 14].

2.5 Medical applications

Nowadays there are several medical techniques based on the application of therapeutic doses of electric fields. Since these treatments successfully exploit the biological windows, a set of dosimetry parameters might be suitable for the treatment of a given medical condition but might not have any effect in another [35]. Furthermore, the adjustment of therapeutic doses is also conditioned to tolerance thresholds to both heat or
tingle induced by the impinging field which are defined by the patient at the beginning and during the treatment. Therefore, the dose is subjectively conditioned in order to avoid detrimental or unwanted side-effects such as pain or skin burns [35]. Despite the clinical benefits, and in view of the specificity of the dosimetry [10], the underlying biological effects caused by the application of therapeutic doses of electric fields are still a matter of research [35, 36, 76]. Nevertheless, medical application of electric fields is called to revolutionize the concepts of medicine and treatment [1, 2], with techniques dealing with wound healing, tumor treatment, drug delivery, among others.

2.5.1 Electrotherapy

Electrotherapy is a concept that unifies three different types of therapeutic applications: 1) those used for pain relief and treatment during the inflammatory response, 2) those aimed at improving wound healing and tissue repair, also known as electrostimulation modalities, and 3) those inducing dielectric heating in cells and tissues, also known as thermotherapies.

The techniques most used for pain relief are transcutaneous electrical nerve stimulation TENS, interferential therapy and ultrasound, the latter generating a more electromechanical stimulus rather than a purely electrical [35]. These techniques stimulate excitable cells (muscle cells and neurons) by acting on the membrane potentials and affecting the electrical activity that controls the formation of action potentials. Frequencies vary from tens of hertz in the case of TENS up to megahertz in the case of ultrasound [35]. Intensities are determined by the comfort response of the patient to tingle.

Electrostimulation modalities are intended to stimulate cell behaviour, especially cell proliferation and cell biochemical signalling, in order to speed up the wound healing response to injury and reduce the time for tissue repair [45, 67]. It has proven effects on different types of tissues, ranging from bone, cartilage and ligament, to skin and cardiovascular system (endothelium) [2, 36]. The beneficial electrostimulation effects have been observed for electric fields as low as 1 mV/m [12, 14] up to about 1000 V/m [45, 77], with frequencies as low as 1 Hz up to tents of kilohertz [2, 76]. Although higher intensity fields are used, intensities above 1200-1400 V/m have been observed to induce cell death [77], possibly due to an increase in the temperature of the culture above the maximum level bearable by cells [78].

Thermotherapies are a group of techniques in which the electrical stimulus interacts with the dielectric properties of both cell and tissues to induce heating. An extensively used modality of thermotherapy is hyperthermia. Other modalities are related to the use of ultrasound (mechanical stimulation) and infrared radiation (light stimulation). During hyperthermia, an increase in the physiological temperature (around 37 °C) influences
thermo-regulated biochemical reactions that control cell behaviour [73, 79]. Temperature increases of 38-42 °C are considered safe for the viability of cells and tissues, and enough to trigger physiological responses such as an increase in cell differentiation and proliferation [47, 78].

The physiological response for temperatures above 42 °C is mediated by the activation heat-shock proteins (HSP). HSP’s are a set of protective proteins upregulated when cells undergo temperature elevation. Their activation is a cellular attempt to preserve the stability of the internal conditions in the presence of external stimulus, a process also called homeostasis [47, 73]. Mild activation of HSP’s at temperatures around 45 °C has the ability to increase cell differentiation and repair cell damage [78]. However, activation of HSP’s due to increases in temperature around 48 °C is a primary response for cell apoptosis and cell necrosis, especially due to protein denaturation. Increments in temperature above 50 °C are related to tissue ablation, a way to burn tissue for the treatment of clinical conditions such as heart arrhythmia and (carcinogenic) tumors by localizing the high temperature [5, 80].

The intensity of the electric field applied during hyperthermia varies according to the expected temperature in the tissue, being as low as 2 V/m for mild-HSP’s upregulation [47], and from 500 V/m to several KV/m to induce cell death and tissue ablation [80, 81]. Frequencies in turn range from DC to ELF, triggering thermoregulatory responses due to HSP’s activation [14, 72], up to RF (≈ 500 kHz) for tissue ablation and cell damage [5, 80].

2.5.2 Electroporation

In addition to Joule heating, the application of an external electric field may cause cell membrane electroporation. Electroporation is the electrically driven formation of pores in the cell membrane which allows the flux of ions and macromolecules that otherwise are not allowed to reach the cytoplasm [8]. The formation of these pores is mediated by the application of high-intensity electric fields (tenths of KV/m) [82] which increase the membrane potential from its resting value of about 100 mV up to the breakdown membrane potential, which depending of the cell membrane ranges from 200 mV to 1 V [8, 83]. Hence, a 5-nm thick cell membrane might support an electric field of 40-200 MV/m, a field strong enough to compress the lipid bilayer and reorientate the phospholipid charge groups, mechanically disrupting the membrane structure [84]. Because of both the intensity of the transmembrane electric field and the possibility of increasing the temperature beyond the viability range, exposure to the high-intensity electric fields causing electroporation is limited to short-duration pulses, lasting from tens of nanoseconds to hundreds of milliseconds [8, 82]. The most frequently used pulse-shapes are exponential and square pulses [8].
There are two types of electroporation. During *reversible* electroporation the intensity of applied electric field is such that the membrane potential is below the breakdown value, allowing the resealing of the pores and recovery of the cell membrane integrity by switching off the field. The purpose of this technique is purely therapeutic, aimed at opening pores in the cell membrane needed to insert specific molecules such as drug delivery vectors for cancer treatment (electrochemotherapy) and the penetration of molecules carrying genetic information (transfection) [8]. For example, liver tissue cells can be reversible electroporated when exposed to a train of eight 100-µs pulses at a frequency of 1 Hz with electric field intensity up to 36 KV/m, a field strong enough to porate the membrane but not enough to break it down [80].

The second type of electroporation is *irreversible* electroporation. This modality consists in the application of an electric field strong enough to effectively break down (destroy) the cell membrane, and consequently, kill the cell [8]. This technique is suggested as an alternative to chemotherapy drugs used for cancer treatment aimed at killing specific and localized groups of cells. Irreversible electroporation of liver tissue cells can be reached during exposure to electric fields above 64 KV/m [80].

An additional mechanism is the excitation of molecular vibrations to allow transfection. In this technique, an electric field delivered to the cells provides additional kinetic energy to exogenous molecules and surface proteins. When this energy is higher than the hydrophobic bonding energy of the phospholipids in the cell membrane, the exogenous molecules gain specific modes of vibration which allow them to pass through the otherwise impenetrable cell membrane [7]. The delivered discharge corresponds to hundreds of kV at frequencies between 20 and 200 Hz during 10s, which is a set of parameters similar to those used for electroporation. In consequence, molecular vibrations needed for cell transfection might be related to increases in the membrane potential near the threshold activation value (≈ 1 V), increasing the permeability of the cell membrane to exogenous molecules.
In the previous chapter, general concepts about biological cells and tissues were introduced, together with general remarks about the (possible) interaction mechanisms with electromagnetic fields. Since the focus of this work is on the biological effects due to the exposure to electric fields, this chapter is dedicated to presenting the fundamentals of electromagnetic fields. These notations will be used throughout this thesis to describe the electric nature of cells and tissues.

3.1 Maxwell’s equations

An electromagnetic field is a physical entity created by electric charges and electric currents, referred to as sources, and takes on a specific value to any point in space at any time [42, 43]. Although the existence of electromagnetic fields was demonstrated since the 1700’s by means of pure observation [13], it was not until 1864 that a complete theoretical description summarizing all the known electromagnetic laws was obtained. In that year, the Scottish physicist and mathematician James Clerk Maxwell presented his famous memoir to the Royal Society in which he introduced a system of first-order differential equations that describe the macroscopic behaviour of electromagnetic fields, the so-called Maxwell’s equations [42]. In modern vector notation, Maxwell’s equations

\[ \begin{align*}
\n\n\end{align*} \]
read

\[\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}, \quad (3.1a)\]

\[\nabla \times \mathbf{H} = \frac{\partial \mathbf{D}}{\partial t} + \mathbf{J}, \quad (3.1b)\]

\[\nabla \cdot \mathbf{D} = \rho, \quad (3.1c)\]

\[\nabla \cdot \mathbf{B} = 0, \quad (3.1d)\]

where the quantities \(\mathbf{E}, \mathbf{H}, \mathbf{D}, \mathbf{B}, \mathbf{J}\) are vector fields, and the vector quantities and unit are as follows [42, 43]:

- \(\mathbf{E}(\mathbf{r}, t)\) is the electric field intensity [V/m],
- \(\mathbf{H}(\mathbf{r}, t)\) is the magnetic field intensity [A/m],
- \(\mathbf{D}(\mathbf{r}, t)\) is the electric flux density [A \cdot s/m²],
- \(\mathbf{B}(\mathbf{r}, t)\) is the magnetic flux density [V \cdot s/m²],
- \(\mathbf{J}(\mathbf{r}, t)\) is the electric current density [A/m²],
- \(\rho(\mathbf{r}, t)\) is the electric charge density [A \cdot s/m³],

with \(\mathbf{r}\) the position vector defined from the origin to the observation point.

Many applications related to electromagnetic fields involve solutions having a periodic time variation [85]. Such time dependence can be represented by the term \(e^{j\omega t}\), where \(\omega\) is the angular frequency. Accordingly, it is customary to write

\[\mathbf{E}(\mathbf{r}, t) = \Re \left[ \mathbf{E}(\mathbf{r})e^{j\omega t} \right], \quad (3.2a)\]

\[\mathbf{H}(\mathbf{r}, t) = \Re \left[ \mathbf{H}(\mathbf{r})e^{j\omega t} \right], \quad (3.2b)\]

\[\mathbf{D}(\mathbf{r}, t) = \Re \left[ \mathbf{D}(\mathbf{r})e^{j\omega t} \right], \quad (3.2c)\]

\[\mathbf{B}(\mathbf{r}, t) = \Re \left[ \mathbf{B}(\mathbf{r})e^{j\omega t} \right], \quad (3.2d)\]

\[\mathbf{J}(\mathbf{r}, t) = \Re \left[ \mathbf{J}(\mathbf{r})e^{j\omega t} \right], \quad (3.2e)\]

\[\rho(\mathbf{r}, t) = \Re \left[ \rho(\mathbf{r})e^{j\omega t} \right], \quad (3.2f)\]

where \(\mathbf{E}, \mathbf{H}, \mathbf{D}, \mathbf{B}, \mathbf{J}\), and \(\rho\) are complex functions that depend on position only. In addition, \(\mathbf{J} = \mathbf{J}_c + \mathbf{J}_i\), where \(\mathbf{J}_c\) is the conduction current and \(\mathbf{J}_i\) is the impressed (source) current [42]. By invoking the linearity of the operators and by implying the
3.2 Boundary conditions

Maxwell’s equations can be expressed in complex (time-harmonic) equivalent form as

\[ \nabla \times \vec{E} = -j\omega \vec{B}, \]  
\[ \nabla \times \vec{H} = j\omega \vec{D} + \vec{J}, \]  
\[ \nabla \cdot \vec{D} = \rho, \]  
\[ \nabla \cdot \vec{B} = 0. \]  

(3.3a) (3.3b) (3.3c) (3.3d)

The importance of Maxwell’s equations is that they allow to express in mathematical terms the physical laws that govern (time-varying) electromagnetic fields. Specifically, (3.3a) is the generalization of Faraday’s Law and states that a spatial-varying electric field is always accompanied by a time-varying magnetic field. Similarly, (3.3b) or Maxwell-Ampere’s Law states that either an electric current density or a time varying electric flux density gives rise to a magnetic field. In addition, (3.3c) indicates that charge densities are the sources of the electric field and that electric monopoles exist. This equation is also known as the Gauss’s Law. Finally, (3.3d) states that the magnetic field is always solenoidal, or equivalently magnetic monopoles are non-physical. This equation is also known as the Gauss’s Law for magnetism [42, 86].

The set of Maxwell’s equations is supplemented by the continuity equation that couples the charges and current densities. Taking the divergence of (3.3b), combining it with (3.3c), and since the divergence of the curl of any vector field is equal to zero, it follows that

\[ \nabla \cdot \vec{J} + j\omega \rho = 0. \]  

(3.4)

The continuity equation states that there is no difference between the time variation (decrease) in the amount of charge contained in an infinitesimal volume element with respect to the divergence (flow) of charge away from this same volume element [43].

3.2 Boundary conditions

Maxwell’s equations written in differential form are complemented by a set of boundary conditions governing the behaviour of the electromagnetic fields at an interface between different materials. At the interface separating materials 1 and 2 as shown in Fig. 3.1, the boundary conditions derived from Maxwell’s equations are [43, 86]
Figure 3.1: Sketch of a material interface [42].

\[
\mathbf{n} \times (\mathbf{E}_2 - \mathbf{E}_1) = 0, \quad (3.5a)
\]
\[
\mathbf{n} \times (\mathbf{H}_2 - \mathbf{H}_1) = \mathbf{J}_s, \quad (3.5b)
\]
\[
\mathbf{n} \cdot (\mathbf{D}_2 - \mathbf{D}_1) = \rho_s, \quad (3.5c)
\]
\[
\mathbf{n} \cdot (\mathbf{B}_2 - \mathbf{B}_1) = 0, \quad (3.5d)
\]

where \(\mathbf{n}\) is a vector normal to the interface pointing into material 2 from material 1, \(\mathbf{J}_s\) is the surface current and \(\rho_s\) is the surface charge. The latter two are present when one of the materials is a perfect conductor [42, 43, 86].

In contrast, at the interface between two dielectric materials which have finite conductivity and no conduction charges as in the case of biological cells and tissues [87, 88], the boundary conditions read

\[
\mathbf{n} \times (\mathbf{E}_2 - \mathbf{E}_1) = 0, \quad (3.6a)
\]
\[
\mathbf{n} \times (\mathbf{H}_2 - \mathbf{H}_1) = 0, \quad (3.6b)
\]
\[
\mathbf{n} \cdot (\mathbf{D}_2 - \mathbf{D}_1) = 0, \quad (3.6c)
\]
\[
\mathbf{n} \cdot (\mathbf{B}_2 - \mathbf{B}_1) = 0, \quad (3.6d)
\]

### 3.3 Constitutive parameters and relations

Every linear, isotropic, instantaneously reacting material can be characterized in terms of three constitutive parameters, namely, the permittivity \(\varepsilon\), the permeability \(\mu\) and
the conductivity $\sigma$. Linear materials are those for which the constitutive parameters are independent of $E$ and $H$. Isotropic materials are those for which the constitutive parameters are independent of direction [86]. The applicability of Maxwell’s equations relies on the constitutive relations, which are a set of relations between fields and fluxes that depend on how the electromagnetic fields interact with the charged particles within materials. Recurring to these constitutive parameter, the constitutive relations for a linear, isotropic material are given by [42]

$$D = \varepsilon E, \quad (3.7a)$$
$$B = \mu H, \quad (3.7b)$$
$$J_c = \sigma E, \quad (3.7c)$$

where $\varepsilon = \varepsilon_0 \varepsilon_r$, $\mu = \mu_0 \mu_r$. In addition, $\varepsilon_0$ is the permittivity of free space equal to $8.857 \times 10^{-12}$ F/m, $\mu_0$ is the permeability of free space equal to $4\pi \times 10^{-7}$ H/m, and both $\varepsilon_r$ and $\mu_r$ are the permittivity and permeability relative to the material, respectively. In free space, since $\sigma = 0$ S/m, the constitutive relations reduce to

$$D = \varepsilon_0 E, \quad (3.8a)$$
$$B = \mu_0 H, \quad (3.8b)$$

Due to the electric and magnetic properties of matter, the constitutive relations in (3.7a), (3.7b) are sometimes written as

$$D = \varepsilon_0 E + \varepsilon_0 \chi_e E, \quad (3.9a)$$
$$B = \mu_0 H + \mu_0 \chi_m H, \quad (3.9b)$$

where the dimensionless quantities $\chi_e$ and $\chi_m$ are the electric and magnetic susceptibilities of the material [43]. Introducing the polarization and magnetization vectors $\{P, M\}$ [42, 43]

$$P = \varepsilon_0 \chi_e E, \quad (3.10a)$$
$$M = \mu_0 \chi_m H, \quad (3.10b)$$

the constitutive relations take the form

$$D = \varepsilon_0 E + P, \quad (3.11a)$$
$$B = \mu_0 H + M \quad (3.11b)$$
and the material permittivity and permeability are given by

\[
\varepsilon_r = 1 + \chi_e, \quad (3.12a)
\]
\[
\mu_r = 1 + \chi_m. \quad (3.12b)
\]

For linear isotropic materials, \(\varepsilon_r\) and \(\mu_r\) are scalars that may also depend on position as is the case of inhomogeneous materials. For anisotropic materials the expressions for the constitutive relations turn into

\[
D = \bar{\varepsilon} \cdot \mathbf{E}, \quad (3.13a)
\]
\[
B = \bar{\mu} \cdot \mathbf{H}, \quad (3.13b)
\]
\[
J_c = \bar{\sigma} \cdot \mathbf{E}, \quad (3.13c)
\]

where \(\bar{\varepsilon}, \bar{\mu}\) and \(\bar{\sigma}\) are tensors representing the generalized constitutive parameters [43].

### 3.4 The wave equation

Similarly to waves, time-varying electromagnetic fields are described by means of functions of both time and space. In fact, one special way to obtain such functions is by solving the so-called wave equation or Helmholtz’s equation, an equation that is obtained from Maxwell’s equations and corresponds to a decoupling strategy in which the first-order differential Maxwell’s equations are turned into a second-order differential equation. Accordingly, taking the curl at both sides of (3.3a) and assuming a medium simple enough to be described by the constitutive relation in (3.7b) with constant \(\mu_0\), yields [42]

\[
\nabla \times (\nabla \times \mathbf{E}) = -j\omega \mu (\nabla \times \mathbf{H}). \quad (3.14)
\]

Combining with (3.3b) and resorting to the vector Laplacian

\[
\nabla \times (\nabla \times \mathbf{A}) = \nabla (\nabla \cdot \mathbf{A}) - \nabla^2 \mathbf{A}, \quad (3.15)
\]

yields the Helmholtz’s equation for the electric field

\[
\nabla^2 \mathbf{E} + \omega^2 \mu \varepsilon \mathbf{E} - j\omega \mu \sigma \mathbf{E} + j\omega \mu \mathbf{J}_i = \frac{\nabla \rho}{\varepsilon}. \quad (3.16)
\]

For a homogeneous, source-free, non-conductive \((\sigma = 0)\) medium, the Helmholtz’s equation simplifies into

\[
\nabla^2 \mathbf{E} + k^2 \mathbf{E} = 0, \quad (3.17)
\]

where \(k\) is the wavenumber of the medium given by [42, 86]

\[
k = \omega \sqrt{\mu \varepsilon}. \quad (3.18)
\]
3.5 Plane waves

Plane waves correspond to the simplest set of solutions for both Maxwell’s equations and Helmholtz’s equation. Plane waves are good approximations to real waves in several practical cases such as the radio waves at a very large distance from the transmitter [42, 86]. Consider a homogeneous, source-free, non-conductive ($\sigma = 0$) medium in which the electric field is assumed to have only $x$-component which in turn depends only on $z$. Accordingly, (3.17) yields

$$\frac{d^2 E_x}{dz^2} + k^2 E_x = 0,$$

which is the one-dimensional Helmholtz’s equation [85, 86]. The solution to this expression is

$$E_x = Ae^{-jkz} + Be^{jkz},$$

where $k$ is the wavenumber as in (3.18), and $A$ and $B$ are the magnitudes of forward and backward waves respectively, which propagate (travel) in the $z$-direction. Since the field $E_x$ presents no variations in the plane perpendicular to the direction of propagation, the wave is called a plane wave [85].

Furthermore, the plane wave

$$E_x = E_0 e^{-jkz},$$

where $E_0$ is the magnitude of the electric field, satisfies the source-free equivalent of (3.3c), i.e., $\nabla \cdot E = 0$ and can be considered as a possible electromagnetic field. Solving (3.3a) using both (3.7b) and (3.18) yields

$$E_x = \sqrt{\frac{\mu}{\varepsilon}} H_y = \eta H_y,$$

where $\eta$ is the intrinsic impedance of the medium [85, 89]. Both $E_x$ and $H_y$ are then divergence-free plane waves which serve as solutions of Maxwell’s equations. Generalizing the plane-wave solution to three-dimensional fields means that

$$E = E_0 e^{-jk \cdot r},$$
$$H = H_0 e^{-jk \cdot r},$$

where $k$ is the wave vector, and $E_0$, $H_0$ are the field intensity vectors [89]. Solving (3.3a), (3.3b) for a source-free medium yields

$$E = \frac{-1}{\omega \varepsilon} (k \times H),$$
$$H = \frac{1}{\omega \mu} (k \times E)$$

(3.24a)
(3.24b)
3.6 Frequency dispersions

In certain materials such as in biological tissues and cells, the constitutive parameters vary with respect to the frequency of the impinging electromagnetic field [61, 88]. In order to deal with these so-called frequency dispersive materials, the complex permittivity and complex permeability are introduced, viz.,

\[
\tilde{\varepsilon}(\omega) = \varepsilon'(\omega) - j\varepsilon''(\omega),
\]

\[
\tilde{\mu}(\omega) = \mu'(\omega) - j\mu''(\omega),
\]

where the real parts account for energy stored in the field (acting like \(\varepsilon, \mu\) in simple matter), and the imaginary parts are indicative of power dissipation, i.e., the conversion of electromagnetic energy into heat [85].

The complex permittivity and permeability can be written as [42]

\[
\tilde{\varepsilon}(\omega) = \varepsilon_0 \varepsilon_r(\omega) - j\frac{\sigma_e}{\omega},
\]

\[
\tilde{\mu}(\omega) = \mu_0 \mu_r(\omega) - j\frac{\sigma_m}{\omega},
\]

where \(\sigma_e\) and \(\sigma_m\) are the equivalent electric and magnetic conductivities. While the electric conductivity measures the ability of the material to transport electric charge in the presence of an electric field, giving rise to the conduction current \(J_c = \sigma_e \mathbf{E}\), there is no evidence of the existence of the magnetic conductivity and therefore the concept is just a mathematical expedient to preserve symmetry in the solutions [42, 85].

In the case of dielectric dispersive materials such as those encountered in biological systems, the relative permittivity exhibits an \(n\)th-order frequency-dependent relaxation process that is described by the Debye dispersion relation [39]. Accordingly, (3.26a) is rewritten for the relative values as [90]

\[
\varepsilon(\omega) = \left[\varepsilon(\infty) + \sum_{k=1}^{n} \frac{\Delta\varepsilon_k}{1 + j\omega\tau_k}\varepsilon_r(\omega)\right] - j\frac{\sigma_e}{\omega\varepsilon_0},
\]

where \(\varepsilon(\infty)\) is the permittivity at very high frequency, \(n\) is the number of dielectric relaxation steps, \(\Delta\varepsilon_k\) is the relaxation amplitude, and \(\tau_k\) is the relaxation time. Separating (3.27) into its real and imaginary parts and solving for \(\omega = 0\) (or \(\omega\tau \ll 1\)) yields
3.6 Frequency dispersions

\[ \varepsilon(\omega) = \varepsilon(0) - \sum_{k=1}^{n} \frac{\Delta \varepsilon_k \omega^2 \tau_k^2}{1 + \omega^2 \tau_k^2}, \]  
\( (3.28a) \)

\[ \sigma(\omega) = \sigma(0) + \sum_{k=1}^{n} \frac{\Delta \varepsilon_k \omega^2 \tau_k^2}{1 + \omega^2 \tau_k^2}, \]  
\( (3.28b) \)

where \( \varepsilon(0) \) and \( \sigma(0) \) are the static permittivity and conductivity, respectively.

A similar relaxation model can be used to approximate the corresponding magnetic quantities [91]. However, since this work is restricted to the interactions between electric fields and cells, additional details about magnetic properties are not provided. Moreover, in what follows the focus is on presenting details associated with electric fields only.
Part II

Investigating the interaction mechanisms
Chapter four

Modelling the electrical response of biological cells

The numerical simulation of the exposure of biological cells and tissues to an electric field is a multiscale problem in which different entities ranging from atoms, ions and molecules of nanometer size, up to clusters of cells, organs and anatomical structures above the centimeter scale are involved. Therefore, the mathematical formulations used to shed light onto the interaction mechanisms between cell and electric fields are mostly devised to tackle problems at one scale only.

Some of the mathematical formulations rely on analytical descriptions available for specific geometries such as spheres and spheroids which fairly approximate particles and cells. These descriptions allow for a fast and cost-effective computation of the influence of the electric field in cell adhesion forces [60], the dynamics of charge particles dissolved in the extracellular matrix and within cell compartments [53, 92] and (intracellular) electric field distributions [93], which can be studied in terms of specific parameters such as sizes, material properties and concentrations [39, 54, 63]. Other numerical approaches based on techniques such as the finite elements method, are suitable to calculate transport mechanisms in charge molecules [94], inhomogeneous electric field distributions due to the use of realistic cell shapes [95], and to determine the electric field distribution in cell assemblies [96, 97].

This chapter begins with a discussion of the reasons why biological cells and tissues are a multiscale modelling problem. Then, the importance of devising models capable of providing information about the distribution of the induced electric fields and the energy absorbed by either groups of cells, individual cells or cellular structures is elucidated. Considering that the scope of this thesis is limited to studying possible interaction mechanisms between electric fields and cells (tissues) as a whole, the chapter
then unfolds towards the introduction of a mathematical formulation suitable to tackle individual cells and groups of cells. To this end, the fundamentals of the quasi-static approximation for the electric field are presented, together with the application thereof to a (multi-layered) spherical object immersed in an electric field. Next, an application example in which the quasi-static approximation is used to numerically simulate the electrical response of a group of spherically-shaped cells is presented. The results from this implementation show that the intracellular electric field depends on the number and position of neighbor cells, the frequency of the incident field, and the material properties of the cell compartments.

4.1 Biological cells and tissues are a multiscale modelling problem

In recent years, the traditional experimentally-oriented study of the possible interaction mechanisms of electric fields in cells and tissues has received additional support from the formulation and implementation of mathematical models. These models are based on bio-(physical) rules aimed at describing the behaviour of a biological system when exposed to an electric field. However, since tackling the possible interaction between an incident electric field and biological cells implies dealing not only with cells as a whole but also with ions, proteins, organelles, cell compartments and groups of cells, the formulation of an appropriate mathematical model must take into account the multiscale nature of the problem. Therefore, and depending on the level of complexity of the interaction to be studied, the mathematical description might represent atomic (molecular) structures, macromolecules, cell compartments, cells and groups of cells, chunks of tissue, organs and anatomical structures (whole body) [10].

Atomic and molecular models aim at describing the dynamic of charged particles to investigate how the impinging electric modifies their behaviour. These models have been used to investigate the pH regulation in cells [53] and molecular dynamics at the cell membrane level, especially in relation to the formation of pores, the interchange of ions, and membrane integrity during reversible and irreversible electroporation [98, 99]. Macromolecular models are dedicated to investigate both the possible conformational changes that the electric field might induce in proteins such as enzymes, ionic channels and ion pumps [10], and the impact of the drift of (surface) charge caused by the presence of the electric field [92].

Cell compartment models are dedicated to analyze possible interaction mechanisms with structural units of a cell, especially the cell membrane. Thus, accurate analytical descriptions based on partial differential equations in which cells are represented by simple spheres and ellipsoids have been used [100], even though the geometry of many human cells is far from spherical [51]. Moreover, using circuit theory, biological cells including
the cell membrane have been modelled in terms of networks of resistors and capacitors [101, 102]. These approaches have provided relevant information about the behavior of the cell membrane at different frequencies [22, 102], and have contributed to elucidate the importance of the cell membrane for both the cell bioelectricity and homeostasis [15, 22]. Nevertheless, these representations fail to evaluate localized changes in the intracellular electric field, especially within the cell membrane.

Therefore, additional spatially-dependent representations have been devised to analyze changes in the intracellular electric field and membrane integrity [93, 94]. These representations are based on spherical-shell structures with different material properties that are suitable to include (concentric) cell organelles such as the cell membrane. These models have provided insight into the effect of the incident electric field in both the transmembrane potential and the transmembrane electric field of single cells. Changes in these variables are directly related to the activation of ion channels, the ion flow (ion currents) through the cell membrane, and the maintenance of the cell membrane integrity (electroporation and dielectric breakdown) [54, 82]. Although in most of the cases this formulation has been used to analyze a single cell, additional efforts have shed light onto how these changes depend on the density and position of well-organized cell arrangements [41, 97].

Furthermore, numerical methods using finite elements have taken the representation of cells as spheres or ellipsoids a step further by allowing the study of the electrical response of realistic irregular cell shapes extracted from images taken directly from cell cultures [96], or approximated using analytical geometric formulations such as a toroid to represent an erythrocyte [95]. Although the results of these efforts have provided insights into how the cell shape and the presence of neighbors affects the electrical response of a single cell, the difference of about three orders of magnitude between the thickness of the cell membrane and the diameter of the cell poses a problem for the spatial discretization required during the finite elements implementation. Therefore, and especially due to the computational cost, these approaches have been implemented to study one or two cells only.

Contrary to the cell compartment models, the models for chunks of tissue rely mostly on the differences in electrical properties between the different tissues rather than on the geometry of the specimen to obtain the electric field distribution. Accordingly, layered models employing estimations of the electrical properties of biological tissues have been used to approximate the absorbed energy and electric field distribution in skin sections and brain tissue [37, 87]. Moreover, models for electrically-driven tissue formation and remodelling have been proposed [103], and applications in tissue ablation and irreversible electroproportion have also been presented as alternatives for therapy (exposure) planning [5, 80].

The modelling of organs and anatomical structures (whole body) has focused on cal-
culating the SAR to limit exposure to comply with safety standards, avoiding hazards such as extreme currents, temperature elevations and excessively high induced electric fields [20]. These models have been used to propose safety guidelines for the exposure dosimetry in applications such as the treatment of tumors during hyperthermia [104, 105], and the design of implants using wireless communication for real-time data transfer [106] and consumer devices such as mobile phones [37].

4.2 Microdosimetry

The solution of the mathematical models described above provides information about the microdosimetry needed for the electric field to induce a (beneficial or hazardous) response, especially at the microscale level. The microdosimetry corresponds to the quantitative study of the distribution of induced electric fields and energy absorbed by either groups of cells, individual cells or cellular structures, and their relationship to biological effects observed at both the cellular and subcellular level [20, 75]. From a general viewpoint, macrodosimetry refers to the induced electric fields and energy absorbed by organs and tissues, which can be computed numerically using mathematically formulations. These formulations can be refined to deal with subcellular structures and establish relations to better understand biophysical interactions between the macro- and the micro-scale level [10, 20].

Thus, equivalences between macro and microdosimetry are useful to define restrictions limiting the whole-body exposure to electric fields based on the internal electric field capable to stimulate, for instance, the nervous system [58, 75]. The opposite is also plausible, namely, the identification of intracellular electric fields and signalling pathways which upon scaling are equal to the whole-body restrictions [107]. These equivalences, however, are still a matter of current research [10, 11], and are likely to assist the future design of safer consumer gadgets such as mobile phones, which are nowadays believed to pose risks for the brain and its functions [27, 28].

In addition, the significance of the effects observed during both the macro and microdosimetry is associated with the metrics of the exposure [75]. These metrics correspond to parameters defining the exposure such as the intensity (strength) of the electric field -given in terms of the root-mean-square (RMS) value, the mean value or the peak value, the duration of the exposure at a given intensity, the frequency and harmonic content, the orientation and polarization of the incident field, among others [59, 75]. Furthermore, numerical simulations have suggested that the induced electric fields and energy absorbed by cells depend on the cell geometry [41, 95] and cell-to-cell interactions [96]. These dependences pose an additional complication in determining the microdosimetry of the exposure since the possible interaction mechanisms might not be due to the incident field alone, but might also be related to the shape (elongation) of the cells [107],
their culture (proliferation) conditions \[1\], and the current phase of their cell cycle \[33\]. These facts, combined with the existence of the biological windows for some of the metrics, pose serious complications in identifying (quantifying) the possible biological effects induced by one or another electric field.

Despite the difficulty to determine which metrics are critical parameters for a given biological interaction \[75\], several reports have provided information needed to identify the minimal values associated with possible biological responses. Accordingly, simple biophysics laws applied to the scenario of cells exposed to electric field indicate that it seems unlikely for electric fields with powers below 100 W/m\(^2\) to cause any effect that might not be overridden by thermal noise or cellular metabolic processes \[19, 44\]. Similar approaches demonstrate that electric fields with intensities lower than 200 V/m might not be enough to cause any effective biological responses \[18, 19\]. However, it has been observed that electric fields as low as 0.25 V/m are enough to direct cell migration \[45\] while intensities in the order of \(10^{-5}-10^{-3}\) V/m might be sensed by cells and excite responses depending, among others, on the frequency of the applied field \[23, 44, 107\].

Although for fields at frequencies lower than about 50 MHz the low conductivity of the cell membrane shields the cell interior \[22, 61, 102\], fields at the ELF range might intervene with surface charges, ionic concentrations and chemical signalling mechanisms and thus affect biological processes \[44, 59, 108\]. Furthermore, triangular and rectangular waves have potential to cause the cell membrane damage \[109\] and might perform well in pulling back and forth calcium ions from the cell membrane that end up affecting molecular signalling processes even at low frequencies and at the intracellular level \[110\]. However, many other responses including morphological changes, effects in protein synthesis and induced intracellular fields have been observed using sine-wave electric fields in both experimental and theoretical approaches \[10, 37, 107\]. In fact, the sine-wave electric fields are preferable because non-sinusoidal waves can be represented in terms of sinusoidal components of different frequency, phase and magnitude by applying the Fourier transformation \[24\]. Thus, any possible effect of non-sinusoidal waves might be related to specific components within the transformation.

Finally, the diameter of a single cell which is approximated by a sphere will be in all cases several orders of magnitude smaller than the wavelength of the incident electric field for frequencies up to hundreds of gigahertz. Therefore, it can be safely assumed that the phase of the electric field wave in all points of the cell is constant and hence the field is referred to as quasi-static. Accordingly, a mathematical description suited for computing the intracellular electric field can be obtained by using a quasi-static approximation for the incident electric field, as described in the following sections.
4.3 Quasi-static approximation for the electric field

In the case of spherical-shaped cells exposed to an incident electric field with frequency up to the gigahertz range, it is convenient to consider that the electric field is time-invariant. Accordingly, the set of Maxwell’s equations in (3.1) can be rewritten as [43]

\[
\begin{align*}
\nabla \times \mathbf{E} &\approx 0 \\
\nabla \times \mathbf{H} &= j\omega \mathbf{D} + \mathbf{J} \\
\nabla \cdot \mathbf{D} &= \rho \\
\nabla \cdot \mathbf{B} &= 0.
\end{align*}
\]

(4.1a-d)

Note than in (4.1) the electric and magnetic fields are decoupled. This means that the electric quantities can be obtained first and do not depend on the magnetic quantities. Such form of the Maxwell’s equations is approximately valid when the wavelength \( \lambda \) of the impinging electromagnetic wave is much greater than (about 100 times) the size of the object illuminated by the wave, also known as the scatterer [42].

To put it in numbers, most of the human cells can be fairly represented by a sphere of radius 10-100 \( \mu m \) [4, 40]. The wavelength \( \lambda \) of a wave is expressed in terms of its frequency \( f \) as \( \lambda = c/f \), where \( c \) is the speed of light (\( \approx 3x10^8 \) m/s). Hence, the size of a human cell and the wavelength of an impinging electromagnetic wave are comparable when the frequency is about 3-30 THz, and the quasi-static approximation applies as long as the frequency is below 30-300 GHz.

Accordingly, a solution for electric field (independent of the magnetic field) can be obtained after solving (4.1a) and (4.1c), because a vector field can be specified once its curl and divergence are known [89]. Hence, considering that \( \nabla \times \nabla \psi = 0 \) for all \( \psi \) [43], it follows from (4.1a) that the electric field can be written in terms of a scalar potential \( \Phi(\mathbf{r}) \) as [89]

\[
\mathbf{E}(\mathbf{r}) = -\nabla \Phi(\mathbf{r}).
\]

(4.2)

By substituting both (4.2) and the constitutive relation (3.7a) \( \mathbf{D} = \varepsilon \mathbf{E} \) into (4.1c) with \( \nabla \varepsilon = 0 \) (homogeneous material), yields Poisson’s equation [43]

\[
\nabla^2 \Phi(\mathbf{r}) = -\frac{\rho}{\varepsilon}.
\]

(4.3)

Assuming that the density of charge is equal to zero \( (\rho = 0) \), which applies to dielectric (biological) materials [4], (4.3) becomes Laplace’s equation [43]

\[
\nabla^2 \Phi(\mathbf{r}) = 0.
\]

(4.4)
4.4 Solution to Laplace’s equation

Consider a dielectric sphere of radius $a$ centered in the origin of the coordinates and immersed in a constant and uniform electric field $E_0 = E_0 \hat{z}$, as shown in Fig. 4.1a. The analytical solution to Laplace’s equation is obtained by writing the divergence operator in spherical coordinates $(r, \theta, \phi)$

$$
\frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \Phi \right) + \frac{1}{r \sin \theta} \frac{\partial}{\partial \theta} \left( \sin \theta \frac{\partial \Phi}{\partial \theta} \right) + \frac{1}{r^2 \sin^2 \theta} \frac{\partial^2 \Phi}{\partial \phi^2} = 0,
$$

and searching for a solution complying with the form [89],

$$
\Phi(r, \theta, \phi) = \frac{U(r)}{r} P(\theta)Q(\phi).
$$

Such a solution is obtained by recurring to separation of variables and considering a problem with symmetry around $\hat{z}$, as is the case for spheres representing biological cells. Accordingly, the solution is given by [89]

$$
\Phi(r, \theta) = \sum_{n=0}^{\infty} \left[ A_n r^n + B_n r^{-(n+1)} \right] P_n(\cos \theta),
$$

where $A$ and $B$ are coefficients to be determined from the boundary conditions and $P_n(\cos \theta)$ are the Legendre polynomials of order $n$. 

---

**Figure 4.1**: a) Graphical representation of a spherical cell or radius $a$ exposed to a homogeneous electric field $E_0$. b) The exposed cell gets polarized, creating electric field lines similar to those produced by an electric dipole.
In the case of biological cells exposed to an electric field, it is also useful to assume that
the distance between the source of the applied field and the cells is large enough for the
cells not to alter the applied electric field \[43\]. Therefore, the total solution to the electric
problem is given in terms of the applied incident field and a secondary scattered field
satisfying Laplace’s equation subject to the boundary conditions. According to (4.2),
the incident potential is given by \( \Phi_0(r) = -E_0z \). In spherical coordinates \( \Phi_0(r) =
-E_0 r \cos \theta = -E_0 rP_1(\cos \theta) \), where \( P_1(\cos \theta) \) is the Legrende polynomial of order
\( n = 1 \) \[89\]. Hence, the total potential outside the sphere is
\[
\Phi(r, \theta) = \Phi_0(r, \theta) + \Phi_s(r, \theta), \tag{4.8}
\]
where \( \Phi_s(r, \theta) \) is the scattered potential which also satisfies Laplace’s equation.
The solution of (4.8) requires the electric potential at infinity to be the incident potential.
Moreover, since inside the sphere there are no free charges, the electric potential
at the origin must be bounded. Therefore, it can be written that \[89\]
\[
\Phi_{in}(r, \theta) = \sum_{n=0}^{\infty} A_n r^n P_n(\cos \theta), \tag{4.9a}
\]
\[
\Phi_{out}(r, \theta) = \sum_{n=0}^{\infty} B_n r^{-(n+1)} P_n(\cos \theta) - E_0 rP_1(\cos \theta). \tag{4.9b}
\]
Accordingly, from (4.9a) the electric potential at the center of the sphere is finite, and
from (4.9b) the first term decays at large distances, leaving the solution in terms of
the incident potential. Applying boundary conditions for the continuity of the electric
potential at the surface of the sphere \( \Phi_{in}(r = a, \theta) = \Phi_{out}(r = a, \theta) \), and enforcing the
continuity of the normal component of the flux density (3.6c), yields
\[
-E_0 aP_1(\cos \theta) = \sum_{n=0}^{\infty} \left[ A_n a^n - B_n a^{-(n+1)} \right] P_n(\cos \theta), \tag{4.10a}
\]
\[
-\varepsilon_2 E_0 a P_1(\cos \theta) = \sum_{n=0}^{\infty} \left[ \varepsilon_1 A_n a^{n-1} + \varepsilon_2 B_n (n + 1)a^{-(n+2)} \right] P_n(\cos \theta). \tag{4.10b}
\]
By invoking the orthogonality of Legrende polynomials, the terms in the right-hand side
of (4.10) vanish except for \( n = 1 \). Accordingly, a little algebra leads to \[89\]
\[
\Phi_{in}(r, \theta) = -\frac{3\varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} E_0 r \cos \theta \tag{4.11a}
\]
\[
\Phi_{out}(r, \theta) = \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} E_0 \frac{a^3}{r^2} \cos \theta - E_0 r \cos \theta. \tag{4.11b}
\]
4.4 Solution to Laplace’s equation

Using (4.2) to obtain the electric field inside the sphere from (4.11a) and transforming from spherical coordinates into rectangular yields a constant electric field parallel to the applied field given by

\[ E_{in}(z) = \frac{3\varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} E_0 \hat{z}, \quad (4.12) \]

As for the electric field outside the sphere, (4.11b) can be rewritten in terms of the moment of an equivalent electric dipole, namely,

\[ p_{eq}(z) = 4\pi\varepsilon_1 \left( \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} \right) a^3 E_0 \hat{z}, \quad (4.13) \]

with the term between brackets known as the Clausius-Mossotti factor [89]. Therefore, the potential outside the sphere reads

\[ \Phi_{out}(r, \theta) = \frac{p_{eq} \cdot \hat{r}}{4\pi\varepsilon_1 r^2} - E_0 r \cos \theta. \quad (4.14) \]

Since \( \hat{z} \cdot \hat{r} = \cos \theta \), (4.2) and (4.14) lead to

\[ E_{out} = E_0 - \nabla \frac{p_{eq} \cos \theta}{4\pi\varepsilon_1 r^2}, \quad (4.15) \]

In conclusion, the electric field outside the sphere can be interpreted as the incident electric field plus the field produced by an equivalent electric dipole located at the origin with moment \( p_{eq} \) [89]. Solving for the gradient in spherical coordinates results in

\[ E_{out} = E_0 + \frac{p_{eq}}{4\pi\varepsilon_1} \cdot \left( \frac{2\cos \theta}{r^3} \hat{r} + \frac{\sin \theta}{r^3} \hat{\theta} \right), \quad (4.16) \]

where \( p_{eq} \) is a constant with respect to differentiation. Since \( \hat{\theta} \cdot \hat{z} = -\sin \theta \), and \( \hat{\phi} \cdot \hat{z} = 0 \), the factorization of \( \hat{z} \) yields

\[ E_{out} = E_0 + \frac{2\hat{r} \hat{r} - \hat{\theta} \hat{\theta}}{4\pi\varepsilon_1 r^3} \cdot p_{eq}, \quad (4.17) \]

which can be rewritten as

\[ E_{out} = E_0 + \frac{3\hat{r} \hat{r} - \hat{I}}{4\pi\varepsilon_1 r^3} \cdot p_{eq}, \quad (4.18) \]

with \( \hat{I} \) the identity dyadic given by \( \hat{I} = \hat{r} \hat{r} + \hat{\theta} \hat{\theta} + \hat{\phi} \hat{\phi} \). Notice that, similarly to (4.8), (4.18) can be written in the form

\[ E_{out} = E_0 + E_s, \quad (4.19) \]

where the contribution due to the equivalent dipole moment constitutes the scattered electric field.
4.5 The spherical shell

A similar approach to obtain the solution to Laplace’s equation can be followed for cells modelled as (multi-)layered dielectric spherical shells. This representation allows including an internal organelle such as the cell nucleus, and considering conduction losses by representing the material regions in terms of their complex permittivity. Accordingly, a cell with its nucleus can be represented as a two-layered spherical shell with three material regions, namely, the extracellular matrix (1), the cytoplasm (2), and the nucleus (3), as shown in Fig. 4.1b. Hence, applying (4.7) to each material region of the two-layered spherical cell, and reducing for \( n = 1 \) as is the case due to the orthogonality of Legendre polynomials, yields [111]

\[
\Phi_1 = \left( -A_1 r + \frac{B_1}{r^2} \right) \cos \theta, \quad r > R_1
\]  

(4.20a)

\[
\Phi_2 = \left( -A_2 r + \frac{B_2}{r^2} \right) \cos \theta, \quad R_1 > r > R_2
\]  

(4.20b)

\[
\Phi_3 = \left( -A_3 r + \frac{B_3}{r^2} \right) \cos \theta, \quad r < R_2,
\]  

(4.20c)

The solution of (4.20) can be obtained by applying boundary conditions for both the continuity of the electric potential at the interfaces between the material regions, and the continuity of the normal component of the flux density. These boundary conditions are given by [111]

\[
\Phi_1 = \Phi_2 \quad \text{and} \quad \varepsilon_1 \frac{\partial \Phi_1}{\partial r} = \varepsilon_2 \frac{\partial \Phi_2}{\partial r}, \quad \text{at} \quad r = R_1
\]  

(4.21a)

\[
\Phi_2 = \Phi_3 \quad \text{and} \quad \varepsilon_2 \frac{\partial \Phi_2}{\partial r} = \varepsilon_3 \frac{\partial \Phi_3}{\partial r}, \quad \text{at} \quad r = R_2,
\]  

(4.21b)

where \( R_1 \) and \( R_2 \) are the radii of the cytoplasm and the nucleus, respectively (Fig. 4.1b). Besides, \( A_1 = E_0 \) because the electric potential at infinity equals the incident potential. Similarly, the electric potential at the inner-most region must be bounded, hence \( B_3 = 0 \). These simplifications, together with the boundary conditions, allow expressing (4.20) as a system of four equations with four unknowns. Solving this system gives the remaining coefficients \( A_i, B_i \), where \( i \) is the number of material regions. These coefficients depend linearly on the incident electric field \( E_0 \), and are given in terms of the radii \( R_1 \) and \( R_2 \), and the complex permittivity of the material regions, i.e., \( \varepsilon_1, \varepsilon_2 \) and \( \varepsilon_3 \).

Once that the electric potential is known, the electric field in every material region \( k = 1, 2, 3 \), can be calculated using (4.2) to write the equivalent dipole moment (4.13)
4.6 Multiple spherical shells: equivalent dipole moments

as

\[ p_k = 4\pi \varepsilon_k B_k \hat{z}. \quad (4.22) \]

Similarly to (4.15), it follows that

\[ E_k = A_k \hat{z} - \nabla \frac{p_k \cos \theta}{4\pi \varepsilon_k r^2}, \quad (4.23) \]

which in the same way as (4.18) turns into

\[ E_k = A_k \hat{z} + \frac{3\hat{r} - \hat{I}}{4\pi \varepsilon_k r^3} \cdot p_k, \quad (4.24) \]

This method can be extended to spheres (cells) described by multiple material regions, as long as the regions are all considered concentric. Furthermore, the solution provides both the intracellular and extracellular electric potential and electric field, which are paramount to assess possible interactions between the cell and the impinging field and define microdosimetry parameters. The following section shows that this method is also suited to calculate the electric field distribution in a scenario in which more than one cell is exposed to the electric field.

4.6 Multiple spherical shells: equivalent dipole moments

The problem of investigating the possible interaction mechanisms between an electric field and biological cells has been addressed typically by calculating the electrical response of one single cell. However, it has been suggested that the presence of neighbor cells modifies this response and that such “interference” might be enough to trigger (or alter) molecular mechanisms, especially at the interface between the cell and the extracellular medium [14, 41, 96, 97]. Moreover, numerical simulations show that despite the cells being fairly approximated by spheres, a regular spatial distribution of cells leads to homogeneous (intracellular) electric fields which might not be realistic for a cell-culture setup exposed to an incident electric field where the cells are arbitrarily distributed while immersed in the culture medium [97].

Therefore, it is possible to adapt the method to calculate the electric field distribution obtained for a single multi-layered cell exposed to an incident electric field to compute the electric field distribution due to multiple non-intersecting spherical cells arbitrarily positioned in a given region. Let \( N \) be the number of cells, each one represented as a dielectric two-layered spherical structure, as shown in Fig. 4.1b. Due to the dielectric properties, the application of an incident electric field causes in every cell a redistribution
of charge which results in a non-zero dipole moment \[54\]. Each dipole moment produces an electric field that corresponds with the scattered electric field in (4.19). Therefore, resorting to the principle of superposition, the total incident electric field \( E_{TO} \) to which a cell is exposed corresponds to the incident electric field \( E_0 \) plus the summation of the \( N - 1 \) scattered electric fields \( E_s \) of (4.18), (4.19) generated by the \( N - 1 \) neighbor cells. In symbols, this reads

\[
E_{TO} = E_0 + \sum_{j=1,j\neq i}^{N} \frac{3\hat{r}_j \hat{r}_j - \vec{I}}{4\pi\varepsilon_0 r_j^3} \cdot p_{k,j}, \tag{4.25}
\]

where \( \hat{r}_j \) is the unitary vector between the observation point and the center of the neighboring cells, \( r_j \) the distance between these two points, and the sub-index \( k \) denotes the material region. It is important to emphasize that the total incident electric field to which the \( i \)-th cell is exposed corresponds to the summation of the equivalent dipole moments observed from a point external to all the cells and therefore located in the material region 1. Thus, \( k = 1 \) and \( p_{k,j} \) from (4.25) rewrites into \( p_{1,j} \). Likewise, \( \varepsilon_k \) is actually \( \varepsilon_1 \).

Considering the linear dependence of both \( A_k \) and \( B_k \) on \( E_0 \), \( A_k = A'_k E_0 \) and \( B_k = B'_k E_0 \). Therefore, from (4.22), the dipole moment \( p_{k,i} \) of the \( i \)-th (bi-layered spherical) cell observed from the material region \( k \) is calculated from the resultant electric field in (4.25) as follows

\[
p_{k,i} = 4\pi\varepsilon_k B_k' \left[ \left( E_0 + \sum_{j=1,j\neq i}^{N} \frac{3\hat{r}_j \hat{r}_j - \vec{I}}{4\pi\varepsilon_1 r_j^3} \cdot p_{1,j} \right) + \frac{3\hat{r}_i \hat{r}_i - \vec{I}}{4\pi\varepsilon_1 r_i^3} \cdot p_{1,i} \right], \tag{4.26}
\]

Notice that for the case of a single cell (\( N = 1 \), \( j = i \) and (4.26) reduces to (4.22).

Using (4.24) in combination with (4.25), the electric field at a point located in the material region 1 or extracellular matrix (Fig. 4.1b) is given by

\[
E_{1,i} = A'_1 \left[ E_0 + \sum_{j=1,j\neq i}^{N} \frac{3\hat{r}_j \hat{r}_j - \vec{I}}{4\pi\varepsilon_1 r_j^3} \cdot p_{1,j} \right] + \frac{3\hat{r}_i \hat{r}_i - \vec{I}}{4\pi\varepsilon_1 r_i^3} \cdot p_{1,i}. \tag{4.27}
\]

Since in region 1 it holds that \( A_1 = E_0, A'_1 = 1 \), rearranging like terms yields

\[
E_{1,i} = E_0 + \sum_{i=1}^{N} \frac{3\hat{r}_i \hat{r}_i - \vec{I}}{4\pi\varepsilon_1 r_i^3} \cdot p_{1,i}. \tag{4.28}
\]

Similarly, the electric field at a point located in the material region 2 or cytoplasm is given by

\[
E_{2,i} = A'_2 \left[ E_0 + \sum_{j=1,j\neq i}^{N} \frac{3\hat{r}_j \hat{r}_j - \vec{I}}{4\pi\varepsilon_1 r_j^3} \cdot p_{1,j} \right] + \frac{3\hat{r}_i \hat{r}_i - \vec{I}}{4\pi\varepsilon_2 r_i^3} \cdot p_{2,i}. \tag{4.29}
\]
where \( p_{2,i} \) corresponds to the equivalent dipole moment observed from the material region 2 of cell \( i \), corresponding to the dipole moment due to its nucleus. This dipole moment can be obtained using (4.26) for \( k = 2 \). By doing so, (4.29) turns into

\[
E_{2,i} = A'_{2} \left[ E_{0} + \sum_{j=1,j \neq i}^{N} \frac{3\hat{r}_{j} - \vec{I}}{4\pi \varepsilon_{r} r_{j}^{3}} \cdot p_{1,j} \right] + 4\pi \varepsilon_{2} B'_{2} \frac{3\hat{r}_{i} - \vec{I}}{4\pi \varepsilon_{2} r_{i}^{3}} \cdot \left[ E_{0} + \frac{3\hat{r}_{i} - \vec{I}}{4\pi \varepsilon_{2} r_{i}^{3}} \cdot p_{1,i} \right].
\]

Finally, the electric field at a point located in the material region 3 or nucleus is given by

\[
E_{3,i} = A'_{3} \left[ E_{0} + \sum_{j=1,j \neq i}^{N} \frac{3\hat{r}_{j} - \vec{I}}{4\pi \varepsilon_{r} r_{j}^{3}} \cdot p_{1,j} \right] + \frac{3\hat{r}_{i} - \vec{I}}{4\pi \varepsilon_{3} r_{i}^{3}} \cdot p_{3,i}.
\]

However, since \( E_{0} \neq 0 \) and the electric field at the inner-most layer of a cell must be bounded, it holds that \( B_{3} = B'_{3} = 0 \). Therefore, from (4.26), \( p_{3,i} = 0 \) and (4.31) simplifies into

\[
E_{3,i} = A'_{3} \left[ E_{0} + \sum_{j=1,j \neq i}^{N} \frac{3\hat{r}_{j} - \vec{I}}{4\pi \varepsilon_{r} r_{j}^{3}} \cdot p_{1,j} \right].
\]

### 4.7 Numerical implementation

The total electric field at every point within a domain \( \Omega \) containing \( N \) arbitrarily positioned layered-spheres that represent biological cells which are exposed to an incident electric field can be computed using (4.28), (4.30) or (4.32), depending on whether the point lies in the interior of a cell or at the extracellular matrix. In any case, the first step in the solution is to compute the equivalent dipole moments using (4.26) when observed externally to all the cells, i.e., for \( k = 1 \). To illustrate this, consider the case in which only three cells are exposed to the incident electric field. According to (4.26), the equivalent dipoles moments are

\[
p_{1,1} = 4\pi \varepsilon_{1} B'_{1} E_{0} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{1} - \vec{I}}{4\pi \varepsilon_{1} r_{1}^{3}} \cdot p_{1,1} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{3} - \vec{I}}{4\pi \varepsilon_{1} r_{3}^{3}} \cdot p_{1,3}
\]

\[
p_{1,2} = 4\pi \varepsilon_{1} B'_{1} E_{0} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{1} - \vec{I}}{4\pi \varepsilon_{1} r_{1}^{3}} \cdot p_{1,2} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{3} - \vec{I}}{4\pi \varepsilon_{1} r_{3}^{3}} \cdot p_{1,3}
\]

\[
p_{1,3} = 4\pi \varepsilon_{1} B'_{1} E_{0} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{1} - \vec{I}}{4\pi \varepsilon_{1} r_{1}^{3}} \cdot p_{1,3} + 4\pi \varepsilon_{1} B'_{1} \frac{3\hat{r}_{2} - \vec{I}}{4\pi \varepsilon_{1} r_{2}^{3}} \cdot p_{1,2}.
\]
which can be rewritten as

\[
\begin{bmatrix}
    p_{1,1} \\
p_{1,2} \\
p_{1,3}
\end{bmatrix} = \begin{bmatrix}
    \alpha E_0 \\
    \alpha E_0 \\
    \alpha E_0
\end{bmatrix} + \alpha \begin{bmatrix}
    0 & \tilde{T}_{12} & \tilde{T}_{13} \\
    \tilde{T}_{21} & 0 & \tilde{T}_{23} \\
    \tilde{T}_{31} & \tilde{T}_{32} & 0
\end{bmatrix} \cdot \begin{bmatrix}
    p_{1,1} \\
p_{1,2} \\
p_{1,3}
\end{bmatrix},
\]

(4.34)

where \( \tilde{T}_j \) is a dyadic defined for the interaction between the cells \( i \) and \( j \). Since this interaction only depends on the source and the observation point, \( \tilde{T}_j = \tilde{T}_i \). Therefore, the matrix in (4.34) results symmetric. Reorganizing and grouping like terms leads to a matrix system of the form \( A \cdot x = b \) given by

\[
\begin{bmatrix}
    1 & \alpha \tilde{T}_{12} & \alpha \tilde{T}_{13} \\
    \alpha \tilde{T}_{12} & 1 & \alpha \tilde{T}_{23} \\
    \alpha \tilde{T}_{13} & \alpha \tilde{T}_{23} & 1
\end{bmatrix} \cdot \begin{bmatrix}
    p_{1,1} \\
p_{1,2} \\
p_{1,3}
\end{bmatrix} = \alpha E_0 \begin{bmatrix}
    1 \\
    1 \\
    1
\end{bmatrix},
\]

(4.35)

the solution of which yields the equivalent electric dipole moments of the three cells exposed to an incident electric field. The same procedure can be followed to compute the equivalent electric dipole moments of \( N \) exposed cells.

Once the dipole moments are known, the calculation of the electric field at a given point \( p_r \) is obtained by first determining in which material region this point lies on. To this end, the distance \( r_{pc} \) from \( p_r \) to the center of each cell is calculated. If it holds true that \( r_{pc} \geq R_1 \) for all the \( N \) cells, then the point \( p_r \) lies somewhere within the extracellular matrix. In such case, the electric field is calculated through (4.28). However, if for any \( i \) cell it holds that \( R_2 < r_{pc} < R_1 \), then the point \( p_r \) lies within the cytoplasm of this \( i \)-th cell and the electric field is computed through (4.30). Similarly, if for any \( i \) cell \( R_2 \geq r_{pc} \), then the point \( p_r \) lies within the nucleus of this \( i \)-th cell and the electric field is obtained through (4.32).

### 4.8 Results

#### 4.8.1 Single-layered spherical cell

The numerical implementation of the model described in this chapter is initially tested for the case of one single-layered spherical cell of radius \( R_1 = 10 \mu m \) centered in an square domain of 50-\( \mu m \) side. This domain is divided into a two-dimensional grid of 250000 equidistant \( p_r \) points where the electric field is computed. The relative permittivity of the extracellular matrix and the cytoplasm are assumed as 80 and 60, respectively [54, 93, 102]. In both materials the conductivity is assumed zero. The incident electric field is a 1-V/m static field oriented in the \( x \)-direction.

The numerical result for the distribution of the electric field throughout the domain is shown in Fig. 4.2a. Inside the cell, the electric field is uniform as expected from (4.12),
Figure 4.2: Magnitude [V/m] of the electric field distribution in a cell without nucleus exposed to a static electric field of 1 V/m a) throughout the domain, b) along the x axis of the cell.

whereas the electric field outside the cell corresponds to that of an equivalent electric dipole moment as expected from (4.18). Thus, the higher electric field magnitude is found at the polar sides of the cell, while the lower magnitude lies at the cell equator. Besides, the electric field magnitude is not much different from that of the incident field, especially outside the cell. This is explained by the fact that the electric field both inside and outside the cell depends linearly on the incident electric field, and the dielectric contrast between the two material regions is not high. In addition, the electric field at the domain boundary is nearly the same as the incident field. This is in accordance with (4.18) since the contribution of the equivalent dipole moment decreases with the cube of the distance away from the cell.

In addition, computing the electric field along a line running parallel to the x-axis and intersecting the center of the cell leads to the plot shown in Fig. 4.2b. Accordingly, the magnitude of the electric field at the cell surface observed from outside is lower than that observed from inside. This is explained by the fact that the permittivity is higher in the extracellular matrix as compared to that of the cytoplasm. In fact, substituting (4.13) into (4.18), using complex permittivities, and solving for a point on the cell surface with no y-component, i.e., $\mathbf{r} = R\hat{x}$, yields an expression for the electric field at the cell surface observed from outside, viz.,

$$E_{\text{out}} = \frac{3\hat{\varepsilon}_2}{\hat{\varepsilon}_2 + 2\hat{\varepsilon}_1} E_0 \hat{z}. \quad (4.36)$$

Using both (4.12) and (4.36) to write the ratio of the electric field from outside the cell surface to the field inside and writing in terms of the magnitude, yields

$$\frac{E_{\text{out}}}{E_{\text{in}}} = \left| \frac{\hat{\varepsilon}_2}{\hat{\varepsilon}_1} \right|. \quad (4.37)$$
Accordingly, when the magnitude of the complex permittivity outside the cell is higher than inside (|\tilde{\varepsilon}_1| > |\tilde{\varepsilon}_2|), the ratio in (4.37) is less than one. In this case, the electric field at the surface of the cell observed from the outside is lower than that observed from the inside, as is the case in the inset of Fig. 4.2a. Contrarily, when the magnitude of the complex permittivity outside the cell is lower than inside (|\tilde{\varepsilon}_1| < |\tilde{\varepsilon}_2|), the relation in (4.37) is greater than one. In this case, the electric field at the surface of the cell observed from the outside is higher than that observed from the inside, which leads to values of electric field at the surface of the cell higher than elsewhere in the domain.

Notice that both (4.12) and (4.37) are subject to the conservation of the electric flux density at the boundary of a dielectric sphere, and hence \( \tilde{\varepsilon}_1 E_{\text{out}} = \tilde{\varepsilon}_2 E_{\text{in}} \) as expected.

### 4.8.2 Two two-layered spherical cells

A second simulation case is performed using two two-layered spherical cells of radius \( R_1 = 10 \, \mu m, R_2 = 2.5 \, \mu m \) that are positioned alongside the center of a square domain of 60-\( \mu m \) side and separated a distance equal to \( R_1 \). The domain is divided into a grid of 250000 equidistant points, which are necessary to plot the field. The relative permittivity of the extracellular matrix, the cytoplasm and the nucleus are assumed as 80, 60 and 50, respectively [54, 93, 102]. The conductivity in all the material regions is assumed zero. The incident electric field is a 1-V/m static field oriented in the \( x \)-direction.

Numerical results for the magnitude of the electric field are shown in Fig. 4.3a. The electric field distribution inside each cell corresponds to the electric dipole moment due to the cell nucleus plus the contribution to the field produced by the equivalent dipole moment of the other cell. Outside the cells, the electric field is the interaction of the two equivalent dipole moments. Fig. 4.3b corresponds to the electric field computed along a line running parallel to the \( x \)-axis and through the center of the cells. The results indicate that due to the presence of the neighboring cell, the electric field inside one cell is 1.8 % lower at the side facing the neighbor cell than at the opposite side. Furthermore, the electric field inside the nucleus is not completely homogeneous as could be assumed from Fig. 4.3a. Instead, it might be approximated to a straight line with slope different from zero. The fact that this slope is different from zero is an additional indication of the interaction coming from the additional cell, which accounts to a difference of 0.35 % between the extreme points of the nucleus.

For comparison, the same simulation case is implemented in CST Studio Suite 2013 (CST AG, Darmstadt, Germany), a commercial software suitable to solve numerically problems in electromagnetism. The results for both for the electric field distribution across the domain and for the electric field along the \( x \)-axis of the cells, shown in Fig. 4.3c-d, respectively, indicate that the solution provided by CST is analogous to
4.8 Results

Figure 4.3: Magnitude [V/m] of the electric field distribution for two cells with nucleus exposed to a static electric field of 1 V/m solved by using (a-b) current approach and (c-d) the commercial software CST. The electrical conductivity is zero for the three materials (extracellular matrix, cytoplasm and nucleus). The same two cells exposed to a 1-V/m, 5 GHz electric field solved by using (e-f) current approach and (g-h) an the CST software.
that obtained using the equivalent dipole moments approach. In fact, the intracellular electric fields obtained using the two approaches have an average difference of less than 0.1 %, which corresponds to an electric field of approximately 10 mV/m.

A third simulation case also considers two two-layered spherical cells of radius $R_1 = 10 \mu m$, $R_2 = 2.5 \mu m$ positioned alongside the center of an square domain of 60-\mu m side and separated a distance equal to $R_1$. In this case, the incident electric field is a 1-V/m 5 GHz plane wave propagating in the $z$-direction and polarized in the $x$-direction. The relative permittivity (conductivity) of the extracellular matrix, the cytoplasm and the nucleus are 80 (1.2), 60 (0.3) and 50 (0.3), respectively [54, 93, 102]. The domain is divided into a square grid of 250000 equidistant points.

Numerical results for the magnitude of the electric field are shown in Fig. 4.3e. The electric field along the line parallel to the $x$-axis and intersecting the center of the cells is shown in Fig. 4.3f. Similarly to the case shown in Fig. 4.3a-b, the electric field distribution outside the cells corresponds to the interaction between the two equivalent dipole moments representing the cells, while the intracellular electric field features the equivalent dipole moment of the cell nucleus. Despite the similarities, a reduction in magnitude especially at the extracellular matrix is observed. This reduction is due to the introduction of the material conductivities (conduction losses).

This solution compares favourably with the solution obtained using CST, shown in Fig. 4.3g-h. However, there are differences in magnitude that account for an average error of 3.5 % between the electric field shown in Fig. 4.3f and 4.3h. This error might be related to the different numerical approximations used by the current approach and CST to obtain the intracellular electric field, but also to the differences in the grid of points used to obtain the solution. Despite this (small) difference in magnitude, the similarities in the electric field distribution demonstrate that the current model is reliable to deal with bi-layered spherical shells representing biological cells, with material regions described by a complex permittivity and exposed to time-harmonic electric fields in the quasi-static regime [111].

### 4.8.3 Five two-layered spherical cells

The results in Fig. 4.3 indicate that the electric field distribution inside a cell is modified by the presence of a neighboring cell. However, during an in-vitro cell culture, more than two cells are exposed simultaneously to an electric field. In order to assess how the presence of more than one cell affects the electric field distribution in a single cell, a fourth simulation case was devised. Accordingly, five two-layered spherical cells of radius $R_1 = 10 \mu m$, $R_2 = 2.5 \mu m$ are positioned in a square domain of 100-\mu m side and exposed to a 1-V/m, 5 GHz incident electric field (plane wave, $z$-propagating, $x$-polarized). The five cells are arranged in a symmetric configuration in which one cell
sits in the center of the domain, whilst the other four are centered in the corners of a square that shares its center with the domain. Accordingly, the minimal intercellular distances, measured as the shortest distance between the surface of these four cells and the surface of the cell centered in the domain takes one of the following three values: $0.83 \times R_1$ (close), $1.54 \times R_1$ (intermediate), or $2.95 \times R_1$ (far).

Numerical results for the magnitude of the electric field in the three configurations (close, intermediate, far) are shown in Fig. 4.4 (upper row). In the close configuration (Fig. 4.4a), the intracellular electric field presents variations similar to those observed in Fig. 4.3 (left column). These variations are due to the interactions between neighboring cells and the separation between two cells, which in this case is maximal between the surfaces of the cells located at diametrically opposite corners of the square and amounts to $3.66 \times R_1$. Furthermore, these variations are symmetrical with respect to the center of the domain, as expected from the symmetry of the configuration. This symmetry leads to noticeable magnitude variations in both the extracellular matrix close to the cells, especially between cells number 1 (3) and 2 (4), and in the cytoplasm of the five cells, especially in cell 5 in which the overall intracellular field magnitude is lower than in the other cells.

Similarly, in the intermediate configuration (Fig. 4.4b), the intracellular field variations are present but lesser in magnitude as compared to the close configuration. However, the electric field in the central cell still shows more variations than in the other four cells. Distinctively, the extracellular interactions between cells number 1 (3) and 2 (4) are practically unnoticeable. This is related to the fact that there is more separation between two adjacent cells, which according to (4.18) reduces the magnitude of the electric field associated with the equivalent dipole moments and thereby the interactions between the cells. In this configuration, the maximal separation between the surface of two cells is $5.08 \times R_1$, which is 1.38 times larger than the separation in the close configuration.

In contrast, the intracellular electric field of the cells arranged in the far configuration shows no significant variations due to the presence of the neighbors (Fig. 4.4c). This result includes the central cell, in which the intracellular electric field is essentially the same as in the other four cells. In addition, the electric field interactions in the extracellular matrix between cells number 1 (3) and 2 (4), as observed from the color lines between these cells, are less strong than in the previous configurations. This result is consistent with the larger separation between the surface of two cells, which for this configuration extends up to $7.9 \times R_1$. Therefore, it is observed that the variations in the electric field distribution, especially in the cytoplasm and in the extracellular matrix close to the cells, depends not only on the presence of neighboring cell but also on the distance between them.

The dependence on the distance between the cells is also observed in the magnitude
Figure 4.4: Magnitude [V/m] (upper row) of the electric field distribution in five cells with nucleus that are exposed to a 1-V/m, 5 GHz electric field. The cells are positioned in the a) close, b) intermediate, and c) far configuration. See text for details. The magnitude of the electric field is plotted along the x-axis of each cell (middle row). A detail of the electric field in the nucleus of two cells (3 and 4) is also plotted (lower row).

of the electric field (normalized to the cell diameter) plotted along the x-axis of each one of the five cells, as is shown in Fig. 4.4 (middle row). Due to the symmetrical distribution of the cells, the intracellular electric field in cell number 1 (3) has the same magnitude as in cell number 2 (4). Therefore, for the close configuration, the electric field in the nucleus (center) of cell number 5 is reduced by 5 mV/m (a 0.43 %) as compared to the other four cells. In addition, the cytoplasmic electric field in cell 5 is also increased by 6 mV/m (a 0.46 %) with respect to the cytoplasmic electric field in the other four cells, especially in the region close to the cell surface. For the intermediate configuration, the electric field inside the nucleus of cell 5 is reduced by 3 mV/m (a 0.26 %) as compared to the other four cells, whereas the cytoplasmatic field is increased by about 1 mV/m (a 0.1 %) near the cell surface. The values for the same comparisons in the far configuration are 1 mV/m and 0.3 mV/m, respectively.
Finally, a close view to the magnitude of the electric field across the nucleus of cell number 3 and 4 is shown in Fig. 4.4 (lower row). These results indicate that the magnitude of the electric field inside the cell nucleus also depends on the position of the cells. Accordingly, for the close configuration, the maximal difference in the nuclear electric field between cells number 3 and 4 (or similarly, cells number 1 and 2) is about 300 $\mu$V/m. For the intermediate configuration, the maximal difference is about 130 $\mu$V/m, and for the far configuration the maximal difference amounts to approximately 60 $\mu$V/m. Although the magnitude of these differences is low as compared to the magnitude of the overall electric field distribution, the presence of gradients in the electric field at the cell nucleus might determine the extent to which an incident electric field influences DNA replication and cell division [33].

### 4.8.4 Multiple two-layered spherical cells arbitrarily positioned

The numerical results obtained using specific cell configurations, indicate that the intracellular electric field depends on both the number and position of the cells. In order to obtain further details about this dependence, the cell configuration is extended to deal with multiple cells arbitrarily positioned within the simulation domain. Accordingly, fifteen (forty-five) two-layered non-intersecting spherical cells of radius $R_1 = 10 \mu$m, $R_2 = 2.5 \mu$m are arbitrarily positioned in a 100-(200-)μm side square domain and exposed to a 1-V/m, 5-GHz incident electric field (plane wave, $z$-propagating, $x$-polarized). Figs. 4.5a-b show the magnitude of the electric field obtained along the $x$-axis of all the cells, normalized to the cell diameter. In all cases, the intracellular electric field follows a pattern similar to that shown in Fig. 4.2b for the case of a single two-layered cell.

However, due to the presence of neighbors, in some cells the cytoplasmatic electric field near the surface is either increased or reduced in magnitude. Moreover, the shape of the electric field is also affected by the presence of neighbor cells. In the case of fifteen cells, the variations in the cytoplasmatic electric field range from 0.93 V/m to 1.09 V/m approximately (Fig. 4.5a), whereas for forty-five cells the variations range from 0.97 V/m to 1.12 V/m approximately (Fig. 4.5b). A similar situation applies to the nucleus, where variations in both the magnitude and shape of the field curve are also observed. Thus, in the case of fifteen cells, the electric field in the nucleus varies between 1.11 V/m and 1.15 V/m, whilst in the forty-five-cell case this variation ranges between 1.12 V/m and 1.17 V/m.

Additionally, the variations in magnitude are evident from the electric field distribution shown in Fig. 4.5c, which corresponds to the forty-five-cell case. Even though the dipole-like distribution at each cell resembles somewhat that of the single cell shown in Fig. 4.2b, with the higher extracellular electric field magnitude towards the poles of the cells and the lowest magnitude towards the equator, the presence of the neighbor
cells induces changes in both the intracellular and extracellular fields. These changes correspond to regions of higher electric field (hot spots) in the cytoplasm of cells nearly touching each other’s poles, and to regions of lower electric field (cold spots) in the extracellular matrix between cells nearly touching each other’s equators. Despite the formation of these hot and cold spots, and in view of the low dielectric contrast between the material regions, the variations in the magnitude of the electric field distribution anywhere in the domain are restricted to (virtually) the same range as in the case of the single cell shown in Fig. 4.2b and the five cells shown in Fig. 4.4 (upper row). Therefore, for two-layered spherical cells with the material properties as described, the presence of multiple neighbor cells leads to variations in the magnitude of the electric field that range in an interval bounded by 0.78 and 1.12 times the magnitude of the incident electric field.

4.8.5 Frequency response of multiple two-layered cells

In order to assess the influence of the frequency of the incident field in the intracellular electric field, the current modelling approach is used to compute the electric field distribution in forty-five two-layered spherical cells of radius $R_1 = 10 \, \mu m$, $R_2 = 2.5 \, \mu m$ which are arbitrarily positioned in a 200-\(\mu\)m side square domain exposed to a 1-V/m incident electric field (\(z\)-propagating, \(x\)-polarized) with frequency varying in the range 5 MHz - 5 GHz. This range of frequency is chosen considering that the spherical representation
4.8 Results

Figure 4.6: Magnitude of the electric field plotted along the $x$-axis of forty-five two-layered arbitrarily-positioned two-layered spherical cells exposed to a 1-V/m incident electric field with frequency a) 5 MHz, b) 50 MHz, c) 500 MHz, d) 5 GHz.

of the cells includes the cell nucleus but disregards the cell membrane. This assumption is adopted to restrict the cell-to-cell interactions to those caused by the exposure of an incident electric field with a frequency above which the electrical shielding of the cell membrane starts to vanish, which is also known as the cut-off frequency [102]. Above this frequency, the intracellular electric field variations are evident, especially inside the nucleus of the cell [93, 102]. Numerical results for the magnitude of the electric field along the $x$-axis of all the cells (normalized to the cell diameter) are shown in Fig. 4.6 for four different frequencies, namely, 5 MHz, 50 MHz, 500 MHz, and 5 GHz.

It is observed from Fig. 4.6a that for the 5-MHz incident field the intracellular electric field does not exhibit a discernible pattern related to the presence of the cell nucleus. Instead, the intracellular electric field in some cells is almost linear, while in others there is a preferential side at which the magnitude is higher and decays exponentially towards the opposite side. In addition, the magnitude varies between 0.86 V/m and 1.42 V/m, indicating that the incident electric field is already affecting the cytoplasm.
Similar magnitudes are observed for the 50-MHz incident electric (Fig. 4.6b), in which case the cell nuclei are evident. In this case, the electric field in the nucleus varies between 1.18 V/m and 1.36 V/m.

Additional results for the incidents electric fields at 500 MHz and 5 GHz (Fig. 4.6c-d) show that the intracellular electric fields have, on the one hand, noticeable features indicating the presence of the cell nuclei, and on the other, variations in the magnitude across the cytoplasm. These variations in the cytoplasmatic electric field are maximal at the cell surface and range from 0.97 V/m to 1.17 V/m for the 500-MHz incident field, and from 0.97 V/m to 1.12 V/m for the 5-GHz case. Moreover, the electric field curve at the cytoplasm resembles, in most of the cases, a logarithmic behaviour that initiates at the nucleus and moves upwards all the way toward the cell surface.

In some other cases, especially for those cells in which the magnitude of the electric field close to the cell surface is lower than 1.1 V/m, the logarithmic variation in the cytoplasm changes into a decaying exponential. This variation in the cytoplasm contrasts with the apparent linear variation observed in the nuclear electric field. This linear variation accounts for differences in the magnitude of the nuclear electric field among the forty-five cells that range from 1.14 V/m to 1.21 V/m for the 500-MHz incident field, and from 1.12 V/m to 1.17 V/m for the 5-GHz case.

### 4.8.6 Influence of the cell density in the intracellular electric field

The simulation cases presented until now are intended to study the influence of neighbor cells in the intracellular electric field in a single cell in terms of the frequency of the incident electric field and number (position) of the cells. An additional metric to assess the changes in the intracellular electric fields is to evaluate the effect of the cell density, i.e., how the variations in the closeness (aggregation) of neighbor cells might affect the overall results.

To this end, a number $N$ of two-layered spherical cells of radius $R_1 = 10 \mu m$, $R_2 = 2.5 \mu m$ is arbitrarily positioned in a 200-\mu m side square domain exposed to a 1-V/m incident electric field ($z$-propagating, $x$-polarized) with frequency varying in the range 5 MHz - 50 GHz. The number of cells $N$ is arbitrarily generated within an interval limited by a fixed number of maximal and minimal cell numbers, namely, 10-20 cells, 20-30 cells, 30-40 cells, and 40-50 cells. These four intervals define four different cell densities, related to four ranges of cell aggregation. The magnitude of the intracellular electric field is computed along the $x$-axis of all the cells, normalized to the cell diameter, and then averaged among the $N$ number of cells. The resultant average intracellular electric field is then compared against the intracellular electric field of a single two-layered spherical cell exposed to the same incident field, as shown in Fig. 4.2b. The differences between these two curves are then calculated in terms of the relative difference. The
4.8 Results

resultant relative difference is averaged among ten repetitions to account for different (arbitrarily-generated) numbers of cells.

The numerical results of this analysis are shown in Fig. 4.7. Accordingly, for the four cell densities considered, the relative difference between the mean intracellular electric field and the intracellular electric field of a single cell is described by a parabolic curve opening up with the vertex a positive number (parabola without roots). The overall relative difference is maximal for the 5-MHz frequency and is located at the proximity of the cell surface. The overall minimal difference is equally obtained at both 5 GHz and 50 GHz, since the results at these two frequencies overlap each other. This minimal difference is obtained at the mid-way of the normalized length, about the center of the cells. Specifically, for the case in which \( N \) takes values in the interval 10-20 as shown in Fig. 4.7a, the maximal difference is 1.6 % whereas the minimal amounts to 0.3 %. For the case in which \( N \) takes values in the interval 20-30 as shown in Fig. 4.7b, the maximal difference is 4.5 % and the minimal is 0.7 %. The same values are 6.5 % and 1.1 % for \( N \) varying in the interval 30-40 (Fig. 4.7c), and 7.9 % and 1.5 % for \( N \) varying in the interval 40-50 (Fig. 4.7d).

It must be added that the relative difference conveys information about the variation of the intracellular field compared to the intracellular electric field of a single cell. Although this comparison results useful in determining how the average response of multiple cells differ from the response of a single cell, it conceals the information related to the interaction that one cell may have on the response of its neighbors, and vice versa. In order to circumvent this hurdle, the \( N \) equivalent dipole moments obtained at each iteration and at each interval of \( N \) are used to calculate the coefficient of variability (CV). The CV shows the extent of variability between the equivalent dipole moments in relation to its mean value, and is given by the expression

\[
CV = \frac{100 \times \sigma}{\bar{p}},
\]

where \( \sigma \) is the standard deviation between the dipole moments given by

\[
\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (p_i - \bar{p})^2},
\]

with \( p_i \) the magnitude of the equivalent dipole moment at the \( i \)-th cell and \( \bar{p} \) the mean equivalent dipole moment given by

\[
\bar{p} = \frac{1}{N} \sum_{i=1}^{N} p_i.
\]

The mean equivalent dipole moment \( \bar{p} \) and the \( CV \) obtained are calculated at each iteration and then averaged for the total number of iterations. The results of this process are summarized in Table 4.1.
On the one hand, these results indicate that the average equivalent dipole moment is inversely proportional to both the frequency of the incident electric field and the cell density. Accordingly, the average dipole moment reaches a maximum of $3.66 \times 10^{-26}$ Coulombs-meter (Cm) for the case in which the number of cells $N$ varies in the interval 10-20 and the incident field has a frequency of 5 MHz. This value contrasts with the minimum of $1.03 \times 10^{-26}$ Cm obtained when $N$ varies in the interval 40-50 and the frequency of the incident field is 5 GHz.

On the other hand, the results in Table 4.1 indicate that the CV is inversely proportional to the frequency of the incident electric field but directly proportional to the cell density. Thus, for $N$ varying in the interval 10-20, the CV amounts up to 1.85 % for an incident field at 5 MHz, while at 5 GHz the average variation is 0.43 %. In contrast, for $N$ varying in the interval 40-50, the CV reaches an average of 2.89 % for a 5-MHz incident
### 4.8 Results

**Table 4.1:** Magnitude of the mean equivalent dipole moment \( p_\alpha \) and the coefficient of variability \( CV_\alpha \) for different frequencies and \( N \) cells. The values of \( \alpha \) correspond to the maximal value of the intervals defined for the variation of \( N \), as detailed in the text. The dipole moments \( p_\alpha \) are normalized to \( 1 \times 10^{-26} \) and expressed in Coulombs-meter (Cm).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>( p_{10-20} )</th>
<th>( CV_{10-20} )</th>
<th>( p_{20-30} )</th>
<th>( CV_{20-30} )</th>
<th>( p_{30-40} )</th>
<th>( CV_{30-40} )</th>
<th>( p_{40-50} )</th>
<th>( CV_{40-50} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 MHz</td>
<td>3.66</td>
<td>1.85</td>
<td>3.61</td>
<td>2.53</td>
<td>3.56</td>
<td>2.74</td>
<td>3.54</td>
<td>2.89</td>
</tr>
<tr>
<td>50 MHz</td>
<td>3.57</td>
<td>1.82</td>
<td>3.55</td>
<td>2.08</td>
<td>3.49</td>
<td>2.66</td>
<td>3.46</td>
<td>2.74</td>
</tr>
<tr>
<td>500 MHz</td>
<td>1.76</td>
<td>0.71</td>
<td>1.75</td>
<td>0.94</td>
<td>1.74</td>
<td>1.09</td>
<td>1.74</td>
<td>1.17</td>
</tr>
<tr>
<td>5 GHz</td>
<td>1.04</td>
<td>0.43</td>
<td>1.03</td>
<td>0.64</td>
<td>1.03</td>
<td>0.72</td>
<td>1.03</td>
<td>0.85</td>
</tr>
</tbody>
</table>

field, and an average of 0.85 % for an incident field with frequency 5 GHz.

### 4.8.7 Influence of the material properties on the intracellular electric field

In the previous simulation cases, the material properties of the cell compartments have been kept fixed. However, in the interest of studying how changes in the value of these material properties influence the intracellular electric field of the exposed cells, a final simulation case is proposed. For this case, the current modelling approach is used to compute the electric field distribution of forty-five two-layered spherical cells of radii \( R_1 = 10 \ \mu m \), \( R_2 = 2.5 \ \mu m \) which are arbitrarily positioned in a 200-\( \mu m \) side square domain exposed to a 1-V/m incident electric field (plane wave, \( z \)-propagating, \( x \)-polarized) with frequency varying in the range 5 MHz - 50 GHz. These forty-five cells are positioned in the same locations as those of the cells used to obtain the solution presented in Fig. 4.6.

Unlike as for the patterns shown in Fig. 4.6, the material properties for this last simulation case are given by the combination of parameters that maximize the mean intracellular electric field of a single two-layered cell exposed to the incident field. To this end, both the relative permittivity and the conductivity at the cell compartments are let to vary within given intervals. Accordingly, the relative permittivity in the extracellular matrix varies in the interval 70-90, whereas the conductivity thereof (expressed in S/m) varies in the interval 0.9-1.5. Similarly, both the permittivity and the conductivity in the cytoplasm vary in the intervals 50-70 and 0.1-0.5, whereas in the nucleus the variations are restricted to the intervals 40-60 and 0.1-0.5, respectively. The variations are set to steps of 1 for the permittivity and of 0.1 for the conductivity. The iterative process of combining the parameters is repeated for different frequencies, yielding the set of material properties summarized in Table 4.2.

The numerical results for the magnitude of the electric field along the \( x \)-axis of all the cells (normalized to the cell diameter), obtained using the parameters in Table 4.2, are
Figure 4.8: Magnitude of the electric field plotted along the x-axis of forty-five two-layered arbitrarily-positioned two-layered spherical cells with material properties that maximize the mean intracellular electric field. The incident field has a magnitude of 1 V/m and a frequency of a) 5 MHz, b) 50 MHz, c) 500 MHz, and d) 5 GHz.

shown in Fig. 4.8. Therein, the curves for the incident field with frequency 5 MHz, 500 MHz and 5 GHz are similar in shape to the curves shown in Fig. 4.7. However, in the case of the 50-MHz frequency, the curves appear reversed as compared to those shown in Fig. 4.7b. This is because at this frequency the maximization process leads to a complex permittivity that is larger in the cell nucleus than in the cytoplasm.

Therefore, the electric field at the nucleus surface observed from the cytoplasm is higher than the field observed from inside the nucleus, as expected from the findings related to (4.37). In consequence, the electric field at the nucleus surface observed from the cytoplasm is maximal at this frequency, and amounts to 2.5 V/m. Conversely, for the other three frequencies analyzed, the complex permittivity in the cytoplasm is higher than in the nucleus. Hence, the electric field at the nucleus surface is lower than inside the nucleus, and amounts to 1.2 V/m, 1.04 V/m, and 0.95 V/m for an incident electric
4.8 Results

Table 4.2: Material properties per cell compartment that maximize the mean intracellular electric field given by $\overline{E}_{\text{intra}}$.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>ECM</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>$\overline{E}_{\text{intra}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 MHz</td>
<td>70</td>
<td>1.5</td>
<td>70</td>
<td>0.1</td>
</tr>
<tr>
<td>50 MHz</td>
<td>70</td>
<td>1.5</td>
<td>70</td>
<td>0.1</td>
</tr>
<tr>
<td>500 MHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>5 GHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>50 GHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 4.3: Material properties per cell compartment that maximize the hotspot of the intracellular electric field given by max($E_{\text{intra}}$).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>ECM</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>max($E_{\text{intra}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 MHz</td>
<td>70</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>50 MHz</td>
<td>70</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>500 MHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>5 GHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>50 GHz</td>
<td>90</td>
<td>1.5</td>
<td>50</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Despite the difference in shape observed at 50 MHz, the intracellular electric field exhibits variations in both the cytoplasm and the nucleus that are associated with the interactions between the cells. In the cytoplasm, these variations range from 0.78 V/m to 1.58 V/m for the 5-MHz case, from 0.81 V/m to 1.58 V/m for the 50-MHz case, from 0.92 V/m to 1.28 V/m for the 500-MHz case, and from 0.94 V/m to 1.21 V/m for the 5-GHz case. Similarly, the variations in the nucleus range in the intervals 1.20-1.49 V/m, 0.93-1.24 V/m, 1.19-1.31 V/m, and 1.17-1.27 V/m, for the four frequencies of interest, respectively.

In addition to the maximization of $\overline{E}_{\text{intra}}$, the same iterative process is used to obtain the combination of parameters that leads to the highest local value or hotspot in the intracellular electric field. Thus, using the same cell distribution and incident electric field as before, the maximal value among the intracellular electric fields is computed and stored. This value is then compared between consecutive iterations and updated accordingly as to preserve the highest value. The iterative process is carried out using the intervals for the material properties of the cell compartments as above mentioned, yielding the set of properties tabulated in Table 4.3.

The numerical results for the magnitude of the electric field along the $x$-axis of all the cells (normalized to the cell diameter) are shown in Fig. 4.9 for four different frequencies,
Modelling the electrical response of biological cells

Figure 4.9: Magnitude of the electric field plotted along the $x$-axis of forty-five two-layered arbitrarily-positioned two-layered spherical cells with material properties that maximize the hotspot of the intracellular electric field. The incident electric field has a magnitude of 1 V/m and a frequency of a) 5 MHz, b) 50 MHz, c) 500 MHz, d) 5 GHz.

namely, 5 MHz, 50 MHz, 500 MHz, and 5 GHz. Therein, the curves of the intracellular electric field for the forty-five cells are inverted as compared to those obtained using the initial set of material properties (Fig. 4.6b), and are similar to the pattern shown in Fig. 4.8b. This result is a consequence of the changing dielectric contrast considered during the variation of the material properties, which after the iterative process, leads to a complex permittivity that is higher in the nucleus than in the cytoplasm.

Therefore, and in accordance with (4.37), the electric field in the cell nucleus is lower than in the cytoplasm, and the maximal intracellular electric field magnitude is obtained at the nucleus surface when observed from the cytoplasm. For an incident electric field with frequency 5 MHz, this maximal magnitude amounts to 3.2 V/m, whereas for the 50-MHz incident field the maximum amounts to 2.8 V/m. The maximal values obtained for the incident fields with frequency 500 MHz and 5 GHz amount to 1.43 V/m and 1.33 V/m, respectively.
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Table 4.4: Magnitude of the mean equivalent dipole moment ($p_\alpha$) and the coefficient of variability ($CV_\alpha$) of forty-five cells exposed to a 1-V/m incident field at the frequencies of interest and with material properties as in the initial ($\alpha = ini$), the average ($\alpha = ave$), and the hotspot ($\alpha = hot$) cases. The dipole moments $p_\alpha$ are normalized to $1 \times 10^{-26}$ and expressed in Coulombs-meter (C m).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>$p_{ini}$</th>
<th>$CV_{ini}$</th>
<th>$p_{ave}$</th>
<th>$CV_{ave}$</th>
<th>$p_{hot}$</th>
<th>$CV_{hot}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 MHz</td>
<td>3.53</td>
<td>3.08</td>
<td>4.70</td>
<td>4.18</td>
<td>4.68</td>
<td>4.15</td>
</tr>
<tr>
<td>50 MHz</td>
<td>3.45</td>
<td>2.97</td>
<td>4.59</td>
<td>3.99</td>
<td>4.61</td>
<td>4.05</td>
</tr>
<tr>
<td>500 MHz</td>
<td>1.73</td>
<td>1.22</td>
<td>2.69</td>
<td>2.10</td>
<td>2.68</td>
<td>2.07</td>
</tr>
<tr>
<td>5 GHz</td>
<td>1.03</td>
<td>0.86</td>
<td>1.91</td>
<td>1.63</td>
<td>1.88</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Despite the changes in orientation, yet the variations in the intracellular electric field reveal the presence of interactions between neighboring cells. These variations cause differences in the electric field of both the cytoplasm and nucleus of the cells exposed to the 5-MHz incident electric field that range from 0.79 V/m to 1.61 V/m, and from 0.53 V/m to 0.65 V/m, respectively. For the 50-MHz case, these variations in the cytoplasm range from 0.8 V/m to 1.59 V/m, and from 0.78 V/m to 0.95 V/m in the nucleus. For the 500-MHz case, the corresponding intervals are 0.93-1.29 V/m, and 1.04-1.15 V/m, whereas for the 5-GHz case the intervals for the cytoplasm and for the nucleus are 0.95-1.22 V/m and 1.02-1.11 V/m, respectively.

In order to further quantify the differences in the intracellular electric field, the mean equivalent dipole moment $\bar{p}$ and the coefficient of variability $CV$ are calculated for the three sets of material properties previously used, namely, the initial set –used to obtain the results shown in Fig. 4.6, the average set –used to obtain the results shown in Fig. 4.8, and the hotspot set –used to obtain the results depicted in Fig. 4.9. The corresponding values for $\bar{p}$ and $CV$ are tabulated in Table 4.4 for the frequencies of interest.

Similarly to the results in Table 4.1, both the mean equivalent dipole moment and the coefficient of variability are inversely proportional to the frequency of the incident electric field. In addition, the values of $\bar{p}$ and $CV$ obtained using the initial set of material properties are lower than those obtained using the other two sets of material properties. In fact, the equivalent dipole moments are higher for the average set than for the hotspot set, except for the incident field at 50 MHz. Note that this is the same frequency at which the shape of the intracellular electric field differs from the pattern obtained at the other frequencies, as depicted in Fig. 4.8.
4.9 Discussion

This chapter is dedicated to introducing a mathematical framework suitable for computing the electric field distribution in a cluster of biological cells exposed to an incident electric field. The cells are represented by two-layered spherical shells that account for the presence of a concentric cell nucleus. The approach is based on the quasi-static approximation for the electric field and the solution of Laplace’s equation in spherical coordinates. This solution is written in terms of the incident electric field and the equivalent electric dipole moment that represents the polarization of the cell. The method is extended to explore the electric field distribution in multiple two-layered simultaneously-exposed biological cells by computing the individual equivalent dipoles and the associated electric fields which, together with the incident electric field, account for the existence of cell-to-cell interactions between neighbor cells.

4.9.1 Validation of the implementation: one and two cells

The implementation of the model is validated by solving for a single-layered dielectric sphere and comparing the results with the analytical solution given by (4.12) and (4.18). The numerical results shown in Fig. 4.2 indicate a homogeneous intracellular electric field and extracellular electric field equivalent to that caused by an electric dipole moment, with higher magnitude at the poles and lower magnitude at the equator. These results correspond to the analytical solution.

Additionally, a two two-layered cells approach is used to validate the presence of interactions between adjacent cells and account for the influence of the cell nucleus. The results in Fig. 4.3 show variations in the intracellular and extracellular electric field which are due to the cell-to-cell interactions, in response to the superposition of the individual electric fields generated by each equivalent dipole moment. Moreover, the presence of the nucleus accounts for an additional equivalent dipole moment that induce further modifications in the intracellular electric field.

The magnitude and distribution of the electric field are corroborated by solving the same two-cell configurations in the commercial software CST Studio. Aside from minor differences in magnitude, which are confined to errors below 5%, the comparison between the two approaches confirms that the solution based on equivalent dipole moments is reliable to compute the electric field distribution in two-layered spherical biological cells. The small differences in magnitude are presumably due to the different numerical implementations used to obtain the solution and the differences in the arrangement of points (mesh) used to compute the electric field.

Although the formulation applies to general three-dimensional problems, the test cases used in the current implementation correspond to two-dimensional (planar) compu-
4.9 Discussion

According to the numerical results in Figs. 4.2 and 4.3, this two-dimensional implementation is an accurate representation of a cutting-plain made to a three-dimensional geometry (sphere), which reduces the number of points in the domain (divisions of the mesh) and the computational cost. Future work tackling a test case defined in a three-dimensional domain would require the adaptation of the grid of points (pre-processing) and the modification of the visualization (post-processing) without making significant changes to the mathematical formulation (processing).

4.9.2 Effects due to the intracellular distances

In a multi-cell scenario, the number \( N \) of the cells that are distributed in the domain are all assumed to have the same geometry, size and material properties. Therefore, from (4.26), the \( N \) equivalent dipole moments differ from each other in terms of the distance between the cell centers. Hence, the contributions to the electric field in a given cell due to the equivalent dipole moment of the surrounding cells depend on the distances between the center of the given cell and the center of the neighbors. Since these contributions decay with the third power of the intracellular distances, the closer the cells are, the higher the interaction between them. Accordingly, the numerical results shown in Fig. 4.4 for five spherical cells indicate that higher interactions in the intracellular electric field between two cells can be obtained when the intercellular distance is shorter than (approximately) the cell radius. In this case, shown in Fig. 4.4a, an incident electric field of 1 V/m with frequency 5 GHz leads to the maximal variations in the intracellular electric fields (near the cell surface) of 6 mV/m, and of 5 mV/m in the nucleus.

These values rise to 150 mV/m and 50 mV/m respectively in the forty-five-arbitrarily-positioned cells shown in Fig. 4.5b. This is because the arbitrarily-generated cell arrangement leads to some the intercellular distances that are already shorter than the cell radius, as shown in Fig. 4.5c. Furthermore, it is observed from the forty five cells in Fig. 4.6b that an incident field with frequency 50 MHz results in electric field variations in the cytoplasm that amount up to 560 mV/m, and to 180 mV/m in the nucleus. Therefore, higher interactions between neighbor cells can be achieved by reducing the frequency of the field and shortening the intercellular distances, which can be accomplish by either increasing the number of cells or placing the cells close to each other.

In order to quantify the interactions due to the shortest intercellular distance, the same two spherical cells used to obtain the results shown in Fig. 4.3a are positioned as close as possible to each other without intersecting, i.e., with collinear centers separated by \( 2 \times R_1 \). In this configuration, the two cells share one surface point and have an intracellular distance equal to zero. By exposing these two cells to an incident electric field of 1 V/m with frequency 50 MHz, the intracellular electric fields vary from 1.31 V/m
to 1.19 V/m in the cytoplasm, and from 1.31 V/m to 1.29 V/m in the nucleus. These values correspond to maximal variations of 120 mV/m in the cytoplasm (near the cell surface) and 20 mV/m in the center of the nucleus, indicating that even with no intercellular distance, the cell-to-cell interactions that modify the intracellular electric field of two cells are not as high as those found for the forty-five cells shown in Fig. 4.6b. In consequence, and even though decreasing the distance between the cells heightens the interactions between neighbor cells, the highest variations in the intracellular electric field are obtained by increasing the number of exposed cells.

Moreover, moving the four external cells shown in Fig. 4.4 closer enough to the central cell so as to share one surface point, maximizes the intracellular electric field variation with respect to the results from Fig. 4.4, which are in all cases lower than 0.5 %. Accordingly, the exposure of the five touching cells to an incident field of 1 V/m with frequency 50 MHz results in an electric field in the cytoplasm of the central cell (at the cell surface) that amounts to 1.49 V/m, whereas in the other four cells the electric field at the same location amounts to 1.29 V/m. These values represent a maximal variation of 200 mV/m that account for a 13 % difference in the intracellular electric field between neighboring cells. In the cell nucleus, more exactly in the cell center, an electric field of 1.3 V/m is obtained for the four external cells, whereas in the central cell the electric field reaches 1.26 V/m. These values indicate a maximal variation of 40 mV/m that account for a variation of 3.1 %. Thus, higher cell-to-cell interactions are obtained in groups of cells close to each other and exposed to incident electric fields with frequency ranges from 5 MHz to 50 GHz, with maximal variations observed at the lower frequencies. Moreover, the fact that the sum of all the electric field contributions (dipole moments) of the surrounding cells modifies the intracellular electric field allows speculating that the influence of the electric field is not limited to the action on a single cell. In fact, the local field experienced by a cell is also enhanced by the interaction of the electric field with the surrounding cells.

4.9.3 Effects due to the cell density

According to the numerical results at a frequency of 5 MHz, the more the cells are packed into the domain, the shorter the intracellular distances and the higher the CV (Table 4.1). This observation is preserved even if the domain increases size. In fact, increasing the size of the domain up to 500 µm and allowing the number of cells N to vary within the interval 90-100, restricts the maximal CV at 5 MHz to 3.5 %. This value is the same value found for the 200-µm domain with a number of cells varying in the interval 40-50. Therefore, no matter how large is the area available for the cell distribution, the interactions between the cells are limited to those cells located within an intercellular distance shorter than about one cell radius.
As a result, the higher $CV$ values found at 5 MHz represent a higher variation between the equivalent dipole moments that denotes stronger interactions between nearby cells, and hence, higher intracellular electric field variations for the frequencies close to the cut-off frequency. Conversely, the lower $CV$ values found at 50 GHz correspond to the lower variation between the equivalent dipole moments leading to weaker cell-to-cell interactions. The lower variation in the dipoles corresponds once more to the fact that at this frequency the material properties of the cell compartments used to obtain the results shown in Figs. 4.4-4.7 are close to an unchanging, stable value. Hence, the intensity of the equivalent dipole moments is lower as compared to the low frequency case, and causes interactions that are more noticeable when the cells are more packed and closer to each other ($N$ varying in the interval 40-50) than otherwise ($N$ varying in the interval 10-20), as presented in Table 4.1.

Moreover, from the viewpoint of the mean dipole moment and the coefficient of variation tabulated in Table 4.1, the lower the cell density, the lower the interactions (lower $CV$), but also the higher the mean dipole moment. Accordingly, decreasing the cell density causes the magnitude of the dipole moments to approach the magnitude of the equivalent electric dipole moment of a single cell exposed to the incident field. In that case, the magnitudes of the single dipole moment amount to $3.71 \times 10^{-26}$ Cm, $3.62 \times 10^{-26}$ Cm, $1.77 \times 10^{-26}$ Cm, and $1.04 \times 10^{-26}$ Cm for an incident field of 1 V/m with frequencies 5 MHz, 50 MHz, 500 MHz and 5 GHz, respectively. Interestingly, the values for the equivalent dipole moments of a single cell are higher than the equivalent dipole moments ($\bar{p}_{ini}$) obtained for the forty-five cells presented in Table 4.4. This demonstrates that the higher interactions between neighbor cells observed in the forty-five cells are not only the consequence of the intensity of the equivalent dipole moments, but are the result of a combination of these intensities, the intercellular distances and the number of cells.

In addition, from the results in Fig. 4.6, the higher differences between the average intracellular electric field of forty-five cells and the response of a single cells are obtained when the cell density is given by a number of cells ranging in the interval 40-50 cells. In this case, the intercellular distances are shorter (cells are more aggregated), which results in higher magnitudes of the equivalent dipole moments that yield higher cell-to-cell interactions. Additionally, a maximum difference of 7.6 (7.9) % is obtained when the incident field has a frequency of 50 MHz (5 MHz, control case). This is consistent with the fact that in the MHz range of frequencies the conduction losses dominate and increase the complex permittivity, inducing stronger dipole moments. This dominance vanishes at the GHz range of frequencies because the material properties used reach the unchanged stable value and the equivalent dipole moments of all the cells are, despite the intercellular distances, virtually equal to the equivalent dipole moment of a single cell.

Interestingly, the shortest intercellular distance, obtained for cells positioned close
enough to each other as to share a surface point (results not shown), leads to differences lower than 4 % at the MHz range and lower than 1.25 % at the GHz range. Moreover, changing the material properties for those in Table 4.3 at both 5 MHz and 5 GHz leads to maximized differences lower than 5 % and 2.3 %, respectively. Therefore, and regardless of the cell distance, the exposure of multiple cells to a given incident electric field magnitude would not induce intracellular electric fields about 8 % higher than the intracellular electric field obtained in a single exposed cell. Such results may turn out useful to approximate the electrical response of multiple cells with the response of a single (equivalent) cell.

4.9.4 Effects of changing the material properties

The maximal difference between $p_{\text{ave}}$ and $p_{\text{hot}}$ as in Table 4.4 is only 1.78 %, whereas the maximal difference between $p_{\text{ini}}$ and $p_{\text{ave}}$ ($p_{\text{hot}}$) is 87.38 (84.04) %. These differences indicate that both the average and the hotspot sets of material properties lead to stronger dipole moments compared to the initial set. The cell-to-cell interactions product of these stronger dipole moments are responsible for the higher intracellular electric fields obtained in comparison with the initial set of material properties, as observed from Figs. 4.6, 4.8 and 4.9. These more intense interactions are attested by the higher values of the coefficient of variability obtained for both the average and hotspot cases as compared to the initial case. In consequence, both the average and the hotspot set of parameters result in higher variations in the intracellular electric fields that are concomitant with stronger equivalent dipole moments.

In addition, the values in Table 4.4 indicate that changing the material parameters is an effective way to increase the magnitude of the equivalent dipole moments. This yields stronger interactions between neighboring cells without reducing the separation between them. This fact is important to be highlighted considering that the exact values of the material properties in the cell compartments are still to be determined and significant (2,3-fold) disparities can be found in the literature for the values of the same property [93, 112, 113].

Therefore, further research is needed to draw solid conclusions on the extent to which (plausible and verifiable) variations in the material properties bring about (substantial) changes in the electrical response of cells that might be responsible for possible effects due to the exposure to the incident electric field. To this respect, additional considerations regarding the dependence of these material properties on temperature, ionic concentrations, pH levels, among others [39, 53, 88] are variables that, albeit outside the scope of the current approach, deserve to be analyzed.
4.9.5 Magnitude of the electric field needed to induce an effect

Regardless of the simulation parameters, the underlying question is whether the intracellular electric field and the variations generated by the surrounding cells are enough to trigger cellular responses resulting in biological effects caused by the incident electric field.

Therefore, and considering the values displayed in Figs. 4.3 - 4.6, variations in the intracellular electric field lower than 10% the magnitude of the incident electric field are most likely masked by both the thermal noise and other noise generated by regular cellular mechanisms [19, 114]. Moreover, variations from 10% to 20% are presumably insufficient to trigger responses in the cells and might be overridden by the metabolic activity (homeostasis) of the cells [73, 114], the protective stress-response [72, 73], the endogenous (intracellular) electric fields [10, 114] and changes in the electric (negative) charge of intracellular messenger proteins during phosphorylation [115]. In contrast, variations above 20% might be enough to change (to some extent) protein electroconformation mechanisms, ion (molecules) movement in both the intra- and extracellular compartments, and activate field-sensitive enzymes [101].

Quantitatively speaking, the presence of surrounding cells and the equivalent electric dipole moments therein bring about variations in the cytoplasmatic electric field from a few hundreds of mV/m as in the cases displayed in Fig. 4.5 and 4.9, to more than 600 mV/m for the case displayed in Fig. 4.8. These variations, which change with the frequency of the incident field, the cell density and the material properties, are associated with different gradients of intracellular electric field that, depending on the magnitude, may cause a particular effect inside a given cell. In fact, such gradients are presumed to be responsible for possible changes in intracellular molecular pathways, specifically the drift of charged ions (proteins), and certain transport mechanisms associated with electroosmotic flows [94, 115].

Furthermore, the electric field in the cell nucleus experiences variations due to the surrounding cells that range from hundreds of µV/m in the case displayed in Fig. 4.4, up to hundreds of mV/m as displayed in Figs. 4.6, 4.8 and 4.9. Therefore, the existence of additional gradients in the electric field inside (and at the surface of) the cell nucleus might determine the extent to which an incident electric field influences DNA replication and cell division [33], especially considering the variations induced by the surrounding cells.

Besides, in all the cases in which the material properties correspond to those in the initial set (Figs. 4.3 - 4.6), the maximal variation in the intracellular electric field is approximately 40% of the incident electric field, whereas the one in the results obtained using the average and hotspot set of parameters (Figs. 4.8 and 4.9), the maximal variation can be as high as 300% of the incident electric field. Interestingly, this sig-
significantly large variation is confined to the surface of the cell nucleus. Thus, it might be associated with experimental observations in which internal cell organelles such as the nucleus and the mitochondria are affected by the exposure to (pulsed) electric fields capable of inducing mitochondria-dependent apoptosis and cell electroporation without inflicting damage to the cell membrane [93].

4.9.6 Exposure to fields in the THz regime

There is evidence of specific effects on biological cells caused by exposure to field with frequencies above 100 GHz (sub-THz frequency range) [116]. Specifically, stimulation of cell proliferation, changes in cell morphology, increased membrane permeability, structural protein damage, activation of intracellular stress responses, and disruptions of organelle functions are associated with changes in the water dynamics such as slow relaxation of bulk molecules and vibrational modes, but also with the frequency dispersions of the material properties of the cell compartments [39, 61].

In an attempt to analyze the relation of cell-to-cell interactions in the effects observed during exposure to electric fields with frequency in the THz range, the same configuration of forty-five cells plotted in Fig. 4.5c was exposed to incident electric fields with frequencies up to 5 THz. No appreciable differences were found between the intracellular electric fields at these frequencies and the intracellular electric field at 5 GHz. Furthermore, the electric field distribution within the simulation domain obtained for the 5-THz incident field, compared to the distribution obtained for the 5-GHz incident field, has no significant differences. In fact, there are no significant differences between the results already for an incident field with frequency 50 GHz.

These findings are the consequence of using the complex permittivity to represent the material regions of the cells, and the lack of frequency dispersions. Hence, at 5 GHz the imaginary part of the complex permittivity in (3.26a), which is related to the conductivity (power dissipation), is almost negligible compared to the real part, related to the relative permittivity (energy storage). Therefore, since the permittivity in the material regions of the cell is independent on the frequency, and the positions of the cells are preserved unchanged, the frequency-dependent complex permittivity reaches an unchanged stable value at frequencies in the range of 5-50 GHz and the same dipole moments are obtained for all the frequencies above 5-50 GHz. Therefore, the use of frequency material dispersions would be needed to obtain more accurate results at this (high) frequencies. An additional simulation approach that includes frequency-dispersive material properties given by the Debye dispersion relations in (3.28), is discussed in Chapter 5.

Nevertheless, and despite the lack of frequency-dispersive materials, it must be recognized that the current approach is unsuited to tackle the exposure to incident fields with frequencies in the order of THz. At these frequencies the quasi-static approximation
is not applicable because the size of the cells is comparable to the wavelength of the incident field. The corresponding wavelength of a frequency of 50 GHz is approximately 6 mm, which is still large as compared to the diameter (major axis) of most cells (≈ 10-100 µm). However, when the incident electric field has a frequency of 500 GHz, the corresponding wavelength is approximately 600 µm. This value is comparable with the length of the major axis of some (elongated) cells such as muscle cells and neurones (dendrites) [52]. Therefore, and to guarantee the applicability of the quasi-static approximation to all types of human cells, it is not recommended to use the current model to compute the electric field distribution of cells exposed to fields with frequencies above 50 GHz. For frequencies above this value, instead of solving Laplace’s equation, the solution of the general (full-wave) Helmholtz’s equation must be obtained [42, 43]. A method to reach this solution is presented in Chapter 6.

4.10 Perspectives

Even though the model of the two-layered spherical shell is a fair approximation to biological cells with a nucleus [54, 97], such a representation leaves out the influence that the cell shape might have in the intracellular electric field. Thus, better insights into the possible effects of the incident field in a biological cell might be obtained by studying other geometries such as ellipsoids [41, 100], biconcave shapes [95], and even shapes derived from images taken from in-vitro cell cultures [96], which indicate that the cell shape affects the cell response and the cell-to-cell interactions. In fact, in dealing with specific cells that lack a cell nucleus such as platelets and erythrocytes [51, 95], for which a single-layered representation such as the one displayed in Fig. 4.2 results a good enough representation, an more realistic shape would possibly lead to more accurate results.

In addition to cell shape, a more accurate description of the cells should include the cell membrane, which unlike the nucleus, is present in all the cells of the human body [51]. Having a thickness of about 10 nm [51, 93], the cell membrane provides the cells with an electrical shielding that blocks an incident electric field with frequency below the cut-off frequency [61, 102, 112, 113].

Considering that the current implementation makes use of a squared-like grid of equidistant points to plot the electric field, with separations between adjacent points of 400 nm, the inclusion of a material layer thin enough to deal with the cell membrane requires scaling down the grid by a factor of 200 (400), which results in separations of 2 (1) nm. Such separations are sufficient to plot the electric field inside the cell membrane, preserving equal distances between the adjacent points of the grid. This scaling down entails using 1(2)x10^5 divisions per domain axis, and a total of 1(4)x10^10 grid points. Such a high number of points poses a significant increase in the computational cost
of the solution that can be easily avoided by recurring to an adaptive mesh scheme. The discussion of such an adaptive mesh, especially developed to overcome the problem of dealing with a regular grid that makes cumbersome to have points inside the cell membrane, is intentionally excluded from this chapter and postponed until in Chapter 5.

Nevertheless, it must be highlighted that the aim of the current approach is to analyze the presence of cell-to-cell interactions described in terms of the equivalent dipole moments. Furthermore, it is dedicated to identify the influence of these equivalent dipoles in the intracellular electric field once the electrical shielding of the cell membrane is vanishing, i.e., for frequencies above the cut-off frequency of the cell membrane. As is demonstrated by the numerical results, dealing with two-layered spherical cells exposed to incident fields with frequencies above 5-50 MHz results then plausible.

Besides, the exact value of the cut-off frequency of the cell membrane depends on the size of the cells and the material properties used to represent both the extracellular matrix and the cell compartments, especially the cell membrane [93, 102]. Thus, considering the material properties used to obtain the results shown in Fig. 4.3b, and assuming the cell membrane to have a relative permittivity of 4-5 and a conductivity of $\approx 3 \times 10^{-7}$ S/m [40, 54], the cut-off frequency is approximately 0.5-1 MHz [93]. As a result, the intracellular electric fields in two-layered spherical cells exposed to an incident electric field with frequency of 1 MHz and above (for which the cell membrane is negligible) should be expected to display patterns that reveal the presence of the cell nucleus.

Although at first sight the results shown in Fig. 4.6a for the exposure of forty-five cells to an incident electric field of 5 MHz lack of such patterns, zooming into the curves reveals the presence of patterns associated with the nuclei that correspond to variations in the order of 0.5-1.5 mV/m. The low magnitude of these variations is the reason why there are not clearly distinguishable in Fig. 4.6a. In contrast, the exposure of the same cells to an incident field of 1 V/m with a frequency of 1 MHz leads to intracellular electric fields in which no variations related to the cell nuclei can be accounted for.

Therefore, and even though the model initially applies to frequencies down to the cut-off frequency, the numerical results indicate that significant effects in the cell nucleus can only be observed when the frequency of the incident electric field is higher than 5 MHz, the effect being most noticeable at a frequency of 50 MHz. This result is related to the sigmoid-like behaviour of the cell membrane electrical response, which between approximately 500 kHz and 50 MHz varies from a state of complete shielding of the cell interior towards a state in which the cell membrane is virtually negligible [93, 102]. Accordingly, the numerical result for the 5-MHz incident electric field, similar to those shown in Fig. 4.6a, should be considered as a control (ideal) case suitable for comparing the variations at higher frequencies with those of an ideal cell without the electrical shielding.
Furthermore, the fact that for frequencies above 50 MHz the interactions in the cell nucleus are most evident, provides additional support to the existence of a biological window for the frequency of the exposure to influence (possible) effects related to variations in the intracellular electric field. Nevertheless, care must be taken in obtaining conclusive remarks on the possible biological effects of these interactions, especially because for frequencies in the range 5 MHz - 50 GHz frequency dispersions in the material properties should be considered [38, 93]. These dispersions modify the material properties, especially at frequencies in the GHz range, which in turn modify the intracellular electric field distributions. Although frequency-dispersive materials can be also addressed in combination with the current equivalent dipole moment approach, the inclusion thereof has been intentionally postponed to Chapter 5 to explore the effect of the changing frequency in the electric field of both the cytoplasm and the cell membrane.

### 4.11 Conclusion

The numerical results presented in this chapter demonstrate that the presence of neighbor cells cause cell-to-cell interactions that can be quantified by means of the equivalent dipole moments that describe the electrical polarization at each cell. These interactions cause variations in the intracellular electric field among the different cells that depend on the number and position of the cells, the frequency of the incident field, and the material properties of the cell compartments. These interactions may be related to some of the biological effects observed in cells exposed to incident electric fields in-vitro.

In addition, assuming the cells as spherical shells results useful to include a (concentric) nucleus and explore the influence it has on the intracellular electric field. Furthermore, the frequency of the exposure is assumed above the cut-off frequency of the cell membrane so that the dielectric shield it provides to the interior of the cell is negligible. Accordingly, the numerical results suggest that if there are biological effects associated with the exposure to an incident electric field with frequency above 5-50 MHz, they may be most likely related to the intracellular electric field, especially due to the presence of both the nucleus and the cell-to-cell interactions.

Moreover, the change of the material properties is suitable for maximizing the variations in the intracellular electric fields. The maximum magnitudes thereof may provide additional clues to effects that might be targeting the interior of the cell, especially the cell nucleus. However, additional work from both the experimental and the numerical sides is needed to reach solid conclusions about the magnitude of the incident electric field associated with plausible (non-thermal) effects in biological cells.
Chapter Five

Implications of the cell membrane in the cell-to-cell interactions*

As shown in the previous chapter, a mathematical approach based on the quasi-static approximation and the equivalent dipole moment is useful to identify local field distributions inside and outside an generalized biological cell with a spherical size and a spherical concentric nucleus. By adding up the contributions that the equivalent dipole moment from each cell generates, such an approach is suitable to compute the intracellular electric field in multiple cells exposed to a time-harmonic incident electric field. This approach is limited by the representation of the cells, which does not include the cell membrane and the frequency dispersions of the material properties describing the cell compartments.

However, the cell membrane and the membrane potential are paramount for the viability of a cell [15, 51]. Therefore, a more detailed model of the distortions in the intracellular electric field can be achieved if the electrical behaviour of the cell membrane, which elicits different cell responses with variations in the magnitude and frequency of the incident field [102], is considered. Thus, this chapter presents an alternative numerical framework to investigate the induced cell-to-cell electric field gradients at different regions of three-layered spherical cells that include both the cell nucleus and the cell membrane. This framework is based on a symbolic scattering-matrix approach that uses the equivalent electrical network of the three-layered sphere to obtain the induced electric field in a single cell. An adaptive meshing scheme is introduced to facilitate the

visualization of the fields in the thin cell membrane. In addition, the materials properties in the cell compartments are represented by the frequency-dependent complex permittivity obtained through the Debye dispersions relations in (3.28).

This framework is suitable for investigating possible electrical interactions between multiple cells positioned in arbitrary positions, focusing on the cell-to-cell interactions in the cell membrane and the variations in the membrane potential. Moreover, since each of the cell compartments is electrically described by means of a complex permittivity, the model is suitable to study changes in the electric field distribution with variations in the frequency of the incident field, from the static (DC) case up to the sub-terahertz range of frequencies.

5.1 Modelling the cell membrane

Biological cells have an inherent electrical nature [15]. This electrical nature is mostly controlled by the thin cell membrane surrounding the cells [51]. The cell membrane is a 5-10 nm thick structure composed by an arrangement of lipid molecules and proteins that surrounds and encloses the cell, separating the intracellular medium (cytoplasm) from the extracellular medium. The presence of different ionic concentrations at both sides of the cell membrane induces the so-called (trans)-membrane potential, which for most cells at rest ranges between -35 mV and -90 mV [4, 54]. The membrane potential plays a significant role during the transport mechanisms for the ionic interchange between the extracellular matrix and the cytoplasm. In fact, some cell types, like neurons and muscle cells, have specialized mechanisms for the transmission of electrical signals that rely on the activation of specific voltage-controlled membrane proteins that gate the flow of ions across the cell membrane [15].

Several numerical models describing the cell membrane or a cell surrounded by the cell membrane have provided explanations to the electrical signalling controlling the flux of ions [15], identifying threshold values necessary to keep cell viability and homeostasis, and shedding light on what happens to the membrane potential when exposed to an incident electric field [54, 60]. Accordingly, sets of parameters for the incident field have been identified to induce membrane permeabilization and electroporation [117], and analytical expressions have been proposed for the membrane potential in both spherical and deformed (prolated/oblated) spheroids [93, 100]. These findings, however, rely on simplifications to the cell structure, the electrical behaviour of the cell membrane, and the material properties of the cell compartments.

Despite the agreement between the numerical findings and experimental observations, these models are in most cases suitable to evaluate the distortions induced in a single cell. Such approach seems incomplete, given that neighboring cells (even when not in contact) affect the electric response of one another, as shown in the previous chapter. In
5.1 Modelling the cell membrane

In this regard, both Sebastian et al. [41] and Puchar et al. [96] devised different strategies by using a finite-elements based analysis to simulate the electrical interaction between two irregularly-shaped cells including the cell membrane. Moreover, Pavil et al. [97] simulated a cluster of cells in various geometrical arrangements.

However, these models are not well suited to study changes in the membrane potential for cases in which the frequency of the impinging field varies, since the conductivity/permittivity of the cell compartments is frequency-dependent [113]. Furthermore, the finite element approaches [41, 96] result in an excessive computational cost that limits the implementation to a pair of cells, whereas the findings by Pavil et al. [97] are limited with respect to the position of the cells, since the latter are constrained to well-defined cubic arrangements. In consequence, the interactions between cells are relevant to regular distributions, and thus they may not fully apply to the case of suspended cells arbitrarily positioned.

5.1.1 The theory of voltage inducement

A single cell is most commonly represented either as a homogeneous sphere [54], or as a prolated/oblated sphere [100], or as multi-layered body [93]. The homogeneous sphere approach is good enough when the interest is in the electrical response outside the cells or directly over the cell surface. The prolated/oblated geometries are used to better represent the non-spherical shape of cells in suspension. These models are also reliable to compute the fields in the exterior of the cells but are not suitable to evaluate the electric field inside the cytoplasm, determined by the influence of the internal organelles such as the cell nucleus and the cell membrane. Thus, a better approximation to the cell is a multi-layered body, with each layer representing a part of the cell. Since the spherical shape is a fair approximation to a biological cell, a three-layered sphere may be enough to represent a cell with membrane and nucleus [61, 93].

The electric field and the electric potential in the cell membrane of a three-layered cell can be obtained using the methods described in the previous chapter. Interestingly, a very simple (albeit approximated) estimation of the membrane potential can be obtained from the theory of voltage inducement. According to this theory, which was first described by the German biophysicist Herman P. Schwan [100], if the three-layered spherical cell model is reduced to a single-layered spherical cell (by for instance determining the equivalent permittivity and conductivity [111]), the membrane potential can be approximated by the expression [40, 100]

$$\Delta \Phi = \frac{3}{2} E_0 R \cos \theta,$$

where $E_0$ is the magnitude of the incident electric field, $R$ is the radius of the cell, and $\theta$ is the angle measured from the center of the cell with respect to the direction of
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The expression in (5.1) is referred to as the (steady-state) Schwan’s equation and holds true if the cell membrane is considered a non-conductive layer with negligible thickness. Since in the real physiological case both the conductivity and thickness of the cell membrane are different from zero, a more general approximation to the membrane potential, including both the intracellular and extracellular compartments, was obtained by the Slovenian biophysicist Tajed Kotnik as [100]

$$\Delta \Phi = f_s E_0 R \cos \theta,$$

with $f_s$ given by

$$f_s = \frac{3 \sigma_e [3dR^2 \sigma_i + (3d^2 R - d^3)(\sigma_m - \sigma_i)]}{2 \left[ R^3(\sigma_m + 2\sigma_e)(\sigma_m + \frac{3}{2}\sigma_i) - (R - d)^3(\sigma_e - \sigma_m)(\sigma_i - \sigma_m) \right]}.$$

where $\sigma_i$, $\sigma_m$ and $\sigma_e$ are the conductivities of the (equivalent) cell interior, the cell membrane and the extracellular matrix, respectively, and $d$ is the membrane thickness. It can be checked that for $\sigma_m = 0$, (5.2) reduces to (5.1).

An special feature of the cell membrane is that it provides an electrical shielding to the intracellular compartments when the incident electric field has a frequency below approximately 0.5-1 MHz [93]. This means that, electrically speaking, the cell membrane behaves like a low-pass filter when the cell is exposed to time-harmonic fields [102]. In order to account for this frequency-dependance, a more general expression for the membrane potential reads [93]

$$\Delta \Phi(\omega) = \frac{f_s E_0 R \cos \theta}{1 + j\omega \tau_m},$$

where $\omega$ is the angular frequency and $\tau_m$ is the time constant of the membrane, which can be approximated as

$$\tau_m = \frac{\varepsilon_m}{R(\sigma_i + 2\sigma_e) + \sigma_m},$$

with $\varepsilon_m$ the permittivity of the cell membrane.

Numerical approaches based on this theory of voltage inducement have provided valuable information about the variations in the membrane potential in spherical cells exposed to incident electric fields with different magnitudes, frequencies and shapes (rectangular, trapezoidal and sinusoidal pulses) [40, 93, 100]. These variations are associated with biochemical and physical changes in the cells regulating the permeability of the cell membrane and might under supra-physiological conditions lead to cell electroporation [22, 54, 82].

This theory has been already employed to determine the influence of regularly-positioned surrounding cells in the membrane potential of a given cell [97], to evaluate the power
dissipation in the cell membrane of cells exposed to time-harmonic electric field \([100]\) and to compute the membrane potential in ellipsoidal cells \([100]\). However, it is inadequate to obtain the variations in the electric field and the electric potential in cell compartments other than the cell membrane. Even though these variations can be obtained by resorting to the three-layered cell model and solving for multiple simultaneously exposed cells using the approach detailed in the previous chapter, an alternative method of solution, suitable to include a material region to represent the cell membrane and further details therein, is explained in the following section.

5.2 Electric field distribution in a single cell

The spatial distribution of the electric potential of a solitary cell exposed to a uniform electric field is obtained by solving the Laplace’s equation in spherical coordinates, as detailed in Chapter 4. Accordingly, the electric potential at each of the internal layers of the cell and in the extracellular matrix is described as

\[
\phi_i(r, \theta) = \left( A_i r + \frac{B_i}{r^2} \right) \cos \theta = \psi_i(r) \cos \theta, \quad (5.6)
\]

where \(r\) is the distance from the cell center, \(\theta\) is the angle between the position vector and the external uniform electric field, and \(A_i, B_i\) are constants that depend on the electrical properties of each material region \(i = 1, \ldots, N_a + 1\), with \(N_a\) the number of material interfaces \((N_a = 3\) in the three-layered spherical cell). Since the angular dependence (accounted by the factor \(\cos \theta\)) is the same for all regions, the auxiliary “wave-like” function \(\psi_i(r)\) accounts for the radial variation of the potential.

From an electrical circuit viewpoint, the three-layered sphere can be modelled as a cascade of concentric spherical regions of different materials as illustrated in Fig. 5.1a, where the nucleus, the cytoplasm, the cell membrane and the extracellular matrix are represented by the complex permittivities \(\tilde{\varepsilon}_1, \tilde{\varepsilon}_2, \tilde{\varepsilon}_3, \text{ and } \tilde{\varepsilon}_4\), respectively.

Within each region \(i\), the total electric potential (and hence, \(\psi_i\)) is the sum of an “incident” (positive) and a “reflected” (negative) partial wave, even though strictly speaking these are not actual waves. Thus,

\[
\psi_i(r) = \psi_i^{(+)}(r) + \psi_i^{(-)}(r), \quad (5.7)
\]

which has the same functional form of the term between brackets in (5.6). The incident wave \(\psi_i^{(+)}(r)\) is assumed equal to \(A_i r\), and the reflected wave \(\psi_i^{(-)}(r)\) equal to \(\frac{B_i}{r^2}\). As in transmission line theory \([118]\), the reflected wave can be expressed in terms of the reflection coefficient \(R_i\) of the material interface. Therefore,

\[
\psi_i(r_i) = (1 + R_i)\psi_i^{(+)}(r_i), \quad (5.8)
\]
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Figure 5.1: a) The three-layered spherical cell is a cascade of concentric spherical regions of different materials, which can be represented in terms of b) an equivalent electrical network.

where \( r_i \) corresponds to the radius of a sphere that encloses the material interface \( i \).

In addition to (5.7), the absence of free electric charges guarantees the continuity of both the electric potential \( \psi_i \) and the normal component of the flux density \( \tilde{\varepsilon}_i \partial \psi_i / \partial r \) at a material interface \( i \). Therefore, it is possible to model the multi-layered sphere as an equivalent electrical network in which \( \psi_i \) and \( \tilde{\varepsilon}_i \partial \psi_i / \partial r \) play the role of voltages and currents. In this network, the cell nucleus acts as a load \( R_1 \) and the subsequent spherical layers act as two-port components (Fig. 5.1b). Hence, from (5.6) - (5.8), and the requirement of finiteness imposed on the electric potential in the innermost layer \( i = 1 \), the reflection coefficient therein (i.e., at the cell nucleus) is given by

\[
R_1 = -\frac{\tilde{\varepsilon}_1 - \tilde{\varepsilon}_2}{\tilde{\varepsilon}_1 + 2\tilde{\varepsilon}_2}, \tag{5.9}
\]

which coincides with the Clausius-Mossotti factor introduced in (4.13) except for a minus sign. The evaluation of the outermost reflection coefficient \( R_3 \) requires first determining the equivalent scattering matrices \( S_2 \) and \( S_3 \). This is accomplished by using the expression

\[
S_i = \begin{bmatrix}
\frac{2(\tilde{\varepsilon}_i - \tilde{\varepsilon}_{i+1})}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{i+1}} & \left(\frac{r_{i-1}}{r_i}\right)^3 \frac{3\tilde{\varepsilon}_{i+1} r_{i-1}}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{i+1}} \\
-\frac{3\tilde{\varepsilon}_i}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{i+1}} & \left(\frac{r_{i-1}}{r_i}\right)^2 \frac{3\tilde{\varepsilon}_{i+1} r_{i-1}}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{i+1}}
\end{bmatrix}
\]

Next, the inward-looking reflection coefficients \( R_2 \), and ultimately \( R_3 \), can be computed by applying network theory [119],

\[
R_i = S_{22}^i + \frac{S_{12}^i S_{21}^i R_{i-1}}{1 - S_{11}^i R_{i-1}}, \quad i = 2, 3, \tag{5.11}
\]
where $S_{i11}$, $S_{i12}$, $S_{i21}$, $S_{i22}$ are the entries of $S_i$ in (5.10).

From the knowledge of the reflection coefficients in each material interface, the total $\psi_i$ at each location within a material region $i$ is determined by means of (5.7) and the appropriate translation of both the reflection coefficient $R_i$ and the incident wave $\psi_i^{(+)}(r_i)$ to positions other than the material interface. This translation yields

$$
\psi_{i+1}(r) = \frac{r - r_i^2}{1 + R_i} \psi_i(r_i), \quad 0 = 1, \ldots, N_a
$$

(5.12)

where $r_i \leq r < r_{i+1}$, and when $r = r_i$, (5.12) equals (5.8) as expected.

In the outermost material interface, $\psi_N^{(+)}(r_N) = -E_0r_N$, where $E_0$ the magnitude of the incident uniform electric field. This incident wave leads via (5.8) to $\psi_N(r_N)$, which by virtue of continuity of the electric potential at the material interfaces, is equal to the solution of (5.12) when $i = N_a - 1$ at $r = r_{i+1}$.

Therefore, the solution of (5.12) is carried out by solving first for $\psi_i(r_i)$ at $r = r_{i+1}$ in an inverse way, i.e., starting from $N_a$ towards $i = 0$. Notice that when $i = N_a$ (the outermost layer), $r_{i+1}$ in (5.12) refers to infinity. Furthermore, since there is no radius $r_0$ because the innermost layer is already defined by $r_1$, and because the finiteness of the electric potential requires $r_0 \neq 0$, it results convenient to set $r_0 = r_1$. Moreover, due to the absence of the reflected wave in the innermost layer, $R_0 = 0$ and $\psi_0(r_0) = \psi_1(r_1)$.

Using these definitions, the solution of (5.12) for $i = 0$ yields

$$
\psi_1(r) = \frac{r}{r_1^2} \psi_1(r_1),
$$

(5.13)

which complies with the finiteness of the solution at $r = 0$.

From (5.6) and (5.12), the electric potential in each material layer becomes

$$
\phi_{i+1}(r, \theta) = \frac{r - r_i^2}{1 + R_i} \psi_i(r_i) \cos \theta, \quad i = 0 \ldots N_a.
$$

(5.14)

At a closer look, it can be noticed that the second term of the right-hand side in (5.14) has the same functional form of the electric potential generated by a dipole. Hence, by using (5.8) and assuming a $z$-directed incident electric field, the equivalent dipole moment in each material interface is

$$
 p_{eq,i+1}(z) = 4\pi \varepsilon_i r_i^2 \psi_i^{(+)}(r_i) \hat{z}, \quad i = 0 \ldots N_a.
$$

(5.15)

Based on (4.2), the total electric field at every material interface is then given by

$$
 E_{i+1} = -\frac{\psi_i^{(+)}(r_i)}{r_i} \hat{z} - \frac{p_{eq,i+1} \cdot \hat{r}}{4\pi \varepsilon_i r_i^2}, \quad i = 0 \ldots N_a,
$$

(5.16)
which after a little algebra becomes

$$E_{i+1} = -\frac{\psi_i^{(+)}(r_i)}{r_i} \hat{z} + \left( \frac{3\hat{r}_i \hat{r}_i - I}{4\pi\varepsilon_{i+1} r_i^3} \right) \cdot p_{eq,i+1}, \quad i = 0 \ldots N_a. \quad (5.17)$$

Assuming $i = N_a$, from (5.15) the equivalent dipole moment in the extracellular matrix of a three-layered sphere becomes

$$p_{eq,N_a+1} = -4\pi\varepsilon_{N_a+1} R_{N_a} r_{N_a}^3 E_0 \hat{z}, \quad (5.18)$$

which is similar to (4.13). The negative sign is canceled out by the negative sign of the innermost reflection coefficient in (5.9), which is carried along for the calculation of $R_{N_a}$. Therefore, the total electric field from (5.18) becomes

$$E_{N_a+1} = E_0 + \left( \frac{3\hat{r}_{N_a} \hat{r}_{N_a} - I}{4\pi\varepsilon_{N_a+1} r_{N_a}^3} \right) \cdot p_{eq,N_a+1}, \quad (5.19)$$

which is the same expression in (4.18) and holds true for arbitrary-oriented uniform time-harmonic incident electric fields.

### 5.3 Electric field distribution in multiple cells

The generalization to the case of $N$ cells can be carried out in a similar way as described in the previous chapter, solving first for the equivalent dipole moments observed from outside all the cells and then solving for the electric field in each material region. However, since (5.17) provides the contribution to the electric field of a single cell in each material region, it results unnecessary to compute the externally observed equivalent dipole moments.

Instead, both (5.15) and (5.16) are solved repeatedly for each single cell and for each point within the solution domain $\Omega$, so that the individual cell contributions are added following the expression

$$E_{i,k} = -\frac{\psi_i^{(+)}(r_i)}{r_i} \hat{z} + \sum_{j \neq k}^N \left[ \frac{3\hat{r}_j \hat{r}_j - I}{4\pi\varepsilon_j r_j^3} \right] \cdot p_{eq,j}, \quad (5.20)$$

where the sub-index $k$ identifies the current cell, $\hat{r}_j$ is the unitary vector between the observation point and the center of the $j$-th cell, $\hat{r}_j$ the distance between these two points, and the sub-index $i$ denotes the material region. To address the differences in the electric field within each material layer, (5.20) is evaluated in terms of the location of each solution point with respect to the center of all the $N$ cells. Hence, the contribution due to the equivalent dipole moment of the $k$-th cell is added to (5.20) if the point in
which the solution is being computed is external to all the cells. By doing so, \((5.20)\) becomes \((4.28)\), and all the contributions are observed from the extracellular matrix. However, if the observation point lies inside cell \(k\), the procedure is first to compute the total electric field due to the other \(N-1\) cells using \((5.20)\), and then use this solution as the (equivalent) incident electric field observed by cell \(k\). Thus, the total electric field at the current solution point is obtained through \((5.15)\) and \((5.17)\). Notice that this approach holds true as long as the cells are assumed to be non-intersecting spherical shells, for which a point within the solution domain can only be either in the extracellular matrix or inside one unique cell.

### 5.4 Numerical implementation

The electric field distribution for an \(N\) number of three-layered spherical cells exposed to an incident electric field is computed for each point within a solution domain by means of \((5.20)\). Care is exercised to first determine whether the point lies within a cell or in the extracellular matrix. In contrast to Chapter 4, this approach does not provide the equivalent electric dipole moment representing each cell but leads to an expression suitable for computing the electric field at each point of the simulation domain. Hence, \((5.20)\) serves to replace the expressions per cell compartment \((4.28)\), \((4.30)\) and \((4.32)\) obtained using the equivalent dipole moments approach, and can be considered a generalized formula that applies to both the extracellular matrix and the cell compartments, regardless of the number of layers per cell. However, the solution also relies on a well-defined set of points located at each material region, especially within the cell membrane. As mentioned in Chapter 4, such an adequate set of points can be obtained by means of an adaptive mesh, especially designed to handle the thin cell membrane.

Hence, a cobweb-like mesh as shown in Fig. 5.2a is used to represent a single 10-µm-radius cell with a 2.5-µm-radius cell nucleus and a cell membrane 10-nm thickness [14, 93]. Each cell is defined within a so-called cell box (CB) that guarantees a minimum distance \(d_c = CB - (2 \times R_3)\) between adjacent cells and avoids intersections. The contour of the cell box is divided into equally spaced divisions in order to have a quadrilateral-like mesh that wraps the cobweb-like cell mesh.

The domain mesh is generated by first meshing with quadrilateral elements a so-called domain box (DB) of size \(h \times CB\), with \(h\) a parameter that ensures the domain box has enough space to hold the given number of cells. Care must be exercised to ensure the size of the quadrilateral elements in the domain box is equal to the size of the quadrilateral elements at the boundary of the cell box.

By doing so, the strategy for positioning a cell is based on the arbitrary selection of a point within the meshed domain box, distant enough for the contour to fit in. This
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Figure 5.2: a) The cobweb-like mesh used to evaluate the electric field inside and outside an three-layered spherical cell with nucleus and cell membrane. $CB = 22 \, \mu m$ and $d_c = 2 \, \mu m$. b) The square grid used as computational domain modified to include seven arbitrarily positioned cells. $DB = 4 \times CB \, \mu m$. c) Close-up of the mesh refinement employed in the interior of the cell membrane.

The point is then assumed to be the center of the new cell box, and all the mesh points within an area equivalent to a cell box centered in this point are removed and replaced by the mesh points defined by the cobweb-like mesh. This process is repeated for the given number of cells, taking care of determining a center point for which the current cell box is not intersecting the previous cell boxes. This ensures that at most (when $d_c$ approaches to zero) two adjacent cells share one surface point (touching cells). Such a meshing scheme leads to domain meshes similar to that displayed in Fig. 5.2b, in which seven cells are arbitrarily positioned.

Furthermore, the cobweb-like mesh is generated in such a way that the number of radial divisions corresponds with the number of external divisions in the cell box, whereas the number of azimuthal divisions varies for each material layer. This ensures an appropriate mesh refinement which yields divisions within the cell membrane as shown in Fig. 5.2c.
Figure 5.3: a) Membrane potential for different frequencies and around the cell circumference starting from the right-most point on the cell equator and moving clockwise. b) Membrane potential (maximum) as a function of frequency computed along the cell circumference. c) Membrane electric field (maximum) as a function of frequency computed along the cell equator ($\cos \theta = 1$) and across the right-most portion of the cell membrane.

5.5 Results

5.5.1 One three-layered spherical cell

The numerical implementation of the scattering-matrix approach described in this chapter is validated by considering the case of one three-layered spherical cell as in Fig. 5.1a defined by the radii $R_1 = 2.5 \, \mu m$, $R_2 = 9.99 \, \mu m$, and $R_3 = 10 \, \mu m$, which corresponds to a cell with nucleus and a cell membrane of 10-nm thickness. This cell is discretized into a set of points given by a cobweb-like mesh with $CB = 25 \, \mu m$, centered in a 50 $\mu m$-side domain box. Dividing the cell-box side into 10 equally-spaced segments, and using 10-20 azimuthal divisions for each material region, the number of points within the cell box amounts to 2361, and the final number of simulation points amounts to 2721. The material properties of the extracellular matrix are 80 and 1.2 S/m for the relative permittivity and the conductivity, respectively. Likewise, the cell membrane properties are 5 and $3 \times 10^{-7}$ S/m, the cytoplasm properties are 60 and 0.3 S/m, and the nucleus properties are 50 and 0.3 S/m [54, 93, 102]. This single cell is exposed to an incident electric field of 1 V/m, propagating in the z-direction and oriented in the x-direction, with frequency varying in the interval 1 kHz - 1 GHz.
Numerical results for the membrane potential, defined as the difference in the electric potential between $R_2$ and $R_3$ around the cell circumference, are shown in Fig. 5.3a. Regardless of the frequency, the membrane potential is represented by a cosinusoidal function. The maximum value of this function is obtained for the zero-frequency (static) case, and decreases with the frequency. By normalizing the results to the external radius of the cell $R_3$, the maximum value in the static case amounts to 1.5, which corresponds to the factor in (5.1). Thus, the low-frequency numerical results are in agreement with the values predicted by the analytical expression in (5.1). Moreover, the reduction in magnitude at high frequencies is in agreement with the analytically predicted response obtained from (5.4), since at high frequencies the denominator increases and hence the potential decreases.

Besides, the results for the membrane potential as a function of the frequency shown in Fig. 5.3b display a sigmoid-like response, with the maximum value at low frequencies and the minimum value at high frequencies. Such behaviour resembles that of a low-pass filter, and represents the electrical shielding that the cell membrane provides to the cytoplasm [4, 102]. In fact, it can be seen from Figs. 5.3a-b that the frequency at which this electrical shield starts to vanish (the cut-off frequency) is located between 0.1 MHz and 1 MHz.

A similar sigmoid-like low-pass filter response is observed for the (maximal) electric field in the cell membrane obtained for different frequencies, as shown in Fig. 5.3c. Actually, this curve displays the same decay profile and the same cut-off frequency as in Fig. 5.3b, which are both expected since the electric field at the thin cell membrane $E_m$ can be approximated as $E_m = \phi_m / R_2$, where $\phi_m$ the membrane electric potential [59, 93]. However, the values for the electric field are $10^3$ higher than the values plotted for the membrane potential Fig. 5.3b. This factor corresponds to $R_3 / R_2$ and comes from the de-normalization of the values shown in Fig. 5.3b prior to the calculation of the electric field plotted in Fig. 5.3c.

In both the membrane potential and the membrane electric field, the results obtained at 1 kHz apply for the lower frequencies down to the static (zero frequency) case, and due to the lack of frequency dispersions in the material properties, the results obtained at 1 GHz differ negligibly from the results at 10 GHz and further. Additional results for the distribution of the electric field at zero frequency are equal to the results obtained using the equivalent dipole moments, as shown in Fig. 4.2, which in turn correspond with the analytical solution for the electric field inside and outside the cell provided by (4.12) and (4.18), respectively.
5.5 Results

5.5.2 Five three-layered spherical cells

A second numerical scenario is aimed at investigating the possible variations in both the electric potential and the electric field in the cell membrane due to the presence of surrounding cells. Accordingly, five three-layered spherical cells with radii \( R_1, R_2, R_3 \) and material properties as in the previous case are exposed to a 1-V/m incident electric field \((z\text{-propagating, } x\text{-oriented})\) with frequency varying in the range 1 kHz - 100 GHz.

The set of points needed to compute the solution are obtained using a cobweb-like mesh with \( CB = 2.05 \mu m \) and a domain box of 10.25-\( \mu m \) side \((h = 5)\). Dividing the cell-box side into 30 equally-spaced segments, and using 10-20 azimuthal divisions for each material region, the number of points within each cell box amounts to 6781, and the final number of simulation points amounts to 52537. The five cells are arranged in a symmetric configuration with one cell centered in the middle point of the domain whilst the other four are centered in the corners of a square concentric with the domain.

Following this distribution, the position of the cells is determined by setting the intercellular distance –defined as the shortest distance between the surface of one of the four external cells and the surface of the center cell– to one of the following three values: \(0.03 \times R_3\) (touching), \(0.22 \times R_3\) (quarter-radius) and \(0.51 \times R_3\) (half-radius). The coefficients are the closest values to 0, 0.25 and 0.5 respectively so that the centers of the five cells coincide with points of the mesh.

Numerical results for the electric field distribution for the five-cell configurations exposed to a 1-V/m, 5 GHz incident electric field are shown in Fig. 5.4. Accordingly, the interactions in the intracellular electric field are inversely proportional to the intercellular distance, especially for the central cell. In this cell, the cytoplasm presents significant variations in the electric field, which increase with shorter intercellular distances and are maximal in the touching configuration. In this configuration, the cell-to-cell interactions induce variations in the cytoplasmatic electric field at the equatorial region of the central cell that are up to 12% higher than the magnitude of the incident field, whereas at the poles the magnitude is at most 5% lower than the incident electric field. In an additional configuration, the minimal intracellular distance is set to \(0.8 \times R_3\). The results obtained are virtually equal to those shown in Fig. 4.4a for an intracellular distance of \(0.83 \times R_3\) (close), as expected.

Moreover, computing the intracellular (cytoplasm and nucleus) electric field along the \(x\)-axis of five cells exposed to an incident electric field with frequency 5 GHz, arranged in each of the three configurations mentioned above, provides curves similar to those plotted in Fig. 4.4 (middle row), despite the slightly different intracellular distances. However, and in contrast to the results presented in Chapter 4, decreasing the frequency below the cut-off frequency of the cell membrane (i.e., below 1 MHz), also decreases the intracellular electric field. In fact, according to the results obtained using five three-layered cells exposed to an incident field with frequency below 1 kHz, the in-
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Additional numerical results for the membrane potential around the cell circumference of the five cells in each of the three configurations (touching, quarter-radius and half-radius) are shown in Fig. 5.5 for three different frequencies, namely, 5 kHz, 5 MHz and 5 GHz. Accordingly, the presence of the surrounding cells cause cell-to-cell interactions that induce perturbations in the otherwise sinusoidal shape of the membrane potential. These interactions are more intense at 5 kHz (low frequency) and decrease for the 5-MHz and 5-GHz frequencies. Furthermore, the intensity of these interactions is inversely proportional to the intercellular distance. Thus, stronger interactions are obtained for the touching cells distribution, as shown in Fig. 5.5a-c.

Interestingly, these interactions cause four marked deviations in the otherwise sinusoidal curve obtained for the central cell (cell 5), whereas in the other cells the deviations are present only once. As observed, these deviations appear along sections of the cell circumference which are close to the points at which the intercellular distances are minimal, for instance, at the touching points (Fig. 5.5a). This finding suggests that even though the interactions between surrounding cells depend on the intercellular distances, their intensity is also mediated by the location at which adjacent cells face one another.

Additional results indicate that the values obtained at 5 kHz are the same for the exposure to all other frequencies down to the static (zero-frequency) case. Similarly, and due to the lack of frequency-dispersive material properties, the results at 5 GHz apply to all upward frequencies.

Further results shown in Fig. 5.6 illustrate the frequency-dependent response of the membrane potential, the membrane electric field and the intracellular electric field of the five cells arranged in the touching configuration, which yields the highest cell-to-cell interactions.
5.5 Results

Figure 5.5: Membrane potential in five three-layered cells arranged in the touching (upper row), quarter-radius (middle row), and half-radius (lower row) configurations, exposed to a 1-V/m incident electric field with frequency 5 kHz (left column), 5 MHz (central column) and 5 GHz (right column). The potential is computed around the cell circumferences starting from the rightmost point on the cell equator and moving clockwise. The cell numbers are as shown in Fig. 5.4a.

Interactions as shown in Fig. 5.5a-c. Both the membrane potential and the membrane electric field are obtained as described in Fig. 5.3. The (maximum) electric field in the cytoplasm is computed along a line parallel to the $x$-axis intersecting the cell center, whereas the electric field in the nucleus is computed in the center of the cell.

Accordingly, and due to the symmetry of the cell arrangement, the membrane potential (Fig. 5.6a), the cytoplasmatic electric field (Fig. 5.6c) and the electric field in the cell nucleus (Fig. 5.6d) are equal among the four external cells (aquamarine-colored line), whereas the additional purple line corresponds to the central cell. The presence of an additional line for the central cell corresponds to higher cell-to-cell interactions therein.
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In the case of the membrane electric field (Fig. 5.6b), however, and additional red line (cell 3) running slightly away from the aquamarine line corresponds to the fact that the membrane electric field is computed at the right-most equatorial side of the cell membrane. The lack of equivalence between these two lines indicates a difference in the membrane electric field at both sides of the cells. Such difference is maximal for frequencies below 1 kHz and amounts to 11 V/m.

Besides, the curves for the membrane potential and the membrane electric field in Figs. 5.6a-b are characterized by maximum values at low frequencies and minimal values for frequencies above the cut-off frequency, as expected from the low-pass filter behaviour [61, 120]. In contrast, the shielding effect of the intracellular compartments caused by the cell membrane results in low intracellular electric fields at low frequencies as shown in Figs. 5.6c-d. Concomitantly with the sigmoid-like decrease in this shielding, at about 3 kHz the intracellular electric field begins to increase, reaching maximum values at frequencies near 10 MHz. Then, approximately at a frequency of
50 MHz, the intracellular fields experience a slight decrease, reaching stable values once the frequency is higher than 1 GHz. Such a decrease has been already noticed in the results presented in Fig. 4.6, and is related to the fact that at frequencies of 1 GHz and above the frequency-dependent imaginary part of the complex permittivity in (3.26a) becomes negligible as compared to the real part. Thus, since the real part is frequency-independent, the intracellular electric field at frequencies above 1 GHz is (practically) constant.

5.5.3 Frequency-dependent material properties

So far the material properties used in each of the material regions defined by the three-layered cells have been assumed constant. However, for most biological materials, the relative permittivity and/or the conductivity are only constant over a limited frequency range [88]. Actually, the rotation of molecular dipoles known as dielectric relaxation that occurs especially at high frequencies [61] causes a decrease in the relative permittivity concomitant with an increase in the conductivity [61, 113]. This dielectric relaxation results in frequency-dependent (dispersive) material properties that can be modelled using the Debye dispersion relations given in (3.28).

Accordingly, the material properties of the material regions correspond to first-order relaxations processes except for the cell membrane, in which a second-order relaxation process results more accurated [61, 113]. Table 5.1 summarizes the parameters of dielectric relaxation required in (3.28) for each material region. The parameters related to the static relative permittivity and conductivity correspond to the material properties used in the previous simulation case.

The frequency-dependent relative permittivity and conductivity for each material region (Fig. 5.7) are used to simulate the five three-layered cells arranged in the touching configuration mentioned above, which are exposed to an incident electric field of 1 V/m (z-propagating, x-directed), with frequency varying in the range 1 kHz - 100 GHz. Cell radii, cell box and domain dimensions are equal to those used in the previous case. Numerical results for the membrane potential and the intracellular (cytoplasm and nucleus) electric field, computed as in the previous implementation case, are shown in Fig. 5.8.

Although the relative permittivity of the cell membrane starts to deviate from the static values at frequencies above 10 MHz, while the conductivity already at 100 kHz, the electrical response of the cell membrane is only sensitive to the frequency dispersions for frequencies above 100 MHz, as observed by comparing the curves for the membrane potential (Fig. 5.8a) and the membrane electric field (Fig. 5.6b) with those obtained using non-dispersive materials (Figs. 5.6a and 5.8b, respectively). At this frequency, the differences in magnitude due to the inclusion of frequency dispersions account for
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Figure 5.7: Frequency dependence of the a) relative permittivity, and b) conductivity in each of the material regions defined by a three-layered cell. In b) the curves for the cytoplasm and the nucleus are overlapping.

Table 5.1: Parameters of dielectric relaxation of the material regions in a three-layered cell taken from [61, 113].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
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<tbody>
<tr>
<td>Dielectric relaxation of the extracellular matrix</td>
<td></td>
<td></td>
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<tr>
<td>First relaxation time</td>
<td>$\tau_1^e$</td>
<td>$6.2x10^{-12}$ s</td>
</tr>
<tr>
<td>First relaxation amplitude</td>
<td>$\Delta_1^e$</td>
<td>$5.9x10^{-10}$ As(Vm)$^{-1}$</td>
</tr>
<tr>
<td>Dielectric relaxation of the cytoplasm and the nucleus</td>
<td></td>
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</tr>
<tr>
<td>First relaxation time</td>
<td>$\tau_1^{c,n}$</td>
<td>$6.2x10^{-12}$ s</td>
</tr>
<tr>
<td>First relaxation amplitude</td>
<td>$\Delta_1^{c,n}$</td>
<td>$4.1x10^{-10}$ As(Vm)$^{-1}$ *</td>
</tr>
<tr>
<td>Dielectric relaxation of the cell membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First relaxation time</td>
<td>$\tau_1^m$</td>
<td>$3.0x10^{-9}$ s</td>
</tr>
<tr>
<td>Second relaxation time</td>
<td>$\tau_2^m$</td>
<td>$4.6x10^{-10}$ s</td>
</tr>
<tr>
<td>First relaxation amplitude</td>
<td>$\Delta_1^m$</td>
<td>$2.3x10^{-11}$ As(Vm)$^{-1}$</td>
</tr>
<tr>
<td>Second relaxation amplitude</td>
<td>$\Delta_2^m$</td>
<td>$7.4x10^{-12}$ As(Vm)$^{-1}$</td>
</tr>
</tbody>
</table>

* Subject to the high frequency permittivity in the cytoplasm $\varepsilon_\infty = 13.33$ [61].

a three-fold increase in both the membrane potential and the membrane electric field, reaching at 3.4 GHz a magnitude of 0.42 µV and of 41.7 V/m, respectively.

Similarly, as shown in Figs. 5.8c-d, the variations in the intracellular electric field due to the material frequency dispersions are only noticeable for frequencies above 1 GHz. At this frequency, the steady-state electric field magnitudes observed in the results without frequency dispersions (Fig. 5.6c-d) starts to decrease in the cytoplasm and to increase in the nucleus. Considering the central cell of the arrangement exposed to a 100-GHz incident electric field, in which the variations are greater, the increment in the cytoplasm amounts to 4.45 V/m and the reduction in the nucleus amounts to 6.75 V/m with respect to the results obtained in the non-dispersive case.

Additionally, since the influence of the frequency dispersions is evident above 1 GHz,
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Figure 5.8: Frequency-dependent a) membrane potential, b) membrane electric field, c) electric field in the cytoplasm, and d) electric field in the nucleus of five three-layered cells with frequency-dispersive material properties and arranged in the touching configuration. The membrane potential around the cell circumference of each cell, starting from the right-most point on the cell equator and moving clockwise, is plotted for the frequencies e) 5 MHz, f) 5 GHz, and g) 50 GHz.

the membrane potential at 5 MHz shows no variations due to the modifications of the material properties (Fig. 5.8a). However, the variations caused at frequencies above 1 GHz are responsible for increasing the maximal membrane potential in the central cell.
of the five-cell touching arrangement exposed to an incident field with frequency 5 GHz to approximately 0.4 μV (Fig. 5.8b), which represents an increase of 67% with respect to the non-dispersive case (Fig. 5.5c). A similar increment, but of 36%, is obtained for an incident electric field with frequency 50 GHz (Fig. 5.8c), in which case the maximal membrane potential amounts to approximately 0.2 μV. No other changes are induced in the cosinusoidal curves obtained for the 5- and 50-GHz cases, indicating that the sections of the cell circumference which are affected by the cell-to-cell interactions remain unchanged at these frequencies.

5.5.4 Multiple three-layered cells arbitrarily positioned

A way to compute the electrical response of multiple arbitrarily-positioned two-layered cells was presented in Chapter 4. The results thereof indicated that, due to the absence of the cell membrane, the intracellular electric field can only be obtained when the frequency of the incident electric field is above 5 MHz (Fig. 4.6a and discussion). Since the three-layered cell model accounts for the cell membrane, an additional simulation case is conducted to analyze multiple arbitrarily-positioned cells. For the sake of convenience and comparison of the results, forty-five three-layered cells positioned in the same arbitrary locations as in Fig. 4.5c are used. These cells are exposed to a z-propagating, x-directed incident electric field of 1 V/m with frequency varying from the static case (zero frequency) up to 100 GHz. The material properties correspond to the static (non-dispersive) values used in previous simulation cases. The points needed to plot the electric field are obtained using a cobweb-like mesh with \( CB = 2.05 \, \mu m \) and a domain box \( DB \) of 205 μm (\( h = 10 \)). Dividing the cell-box side into 20 equally-spaced segments, and using 10-20 azimuthal divisions for each material region, the number of points within each cell box amounts to 4521, and the final number of observation points amounts to 227625.

Numerical results for the intracellular (cytoplasm and nucleus) electric field computed along the x-axis of the forty-five three-layered cells at three different frequencies, namely, 5 kHz, 5 MHz and 5 GHz, are shown in Fig. 5.9a-c (normalized to the diameter of the cell). Accordingly, the exposure to an incident field of 5 kHz (Fig. 5.9a) leads to intracellular electric fields with magnitudes below 8 mV/m, which are repeatedly obtained for all other frequencies down to the static case. This result is expected considering the shielding mechanism that the cell membrane provides to the intracellular compartments so that incident electric fields with frequencies below the cut-off frequency (about 1 MHz) are blocked out. Despite the low magnitudes, the curves obtained at 5 kHz are almost linear for some cells, while in others there is a preferential side at which the magnitude is higher and decays exponentially towards the opposite side, evidencing the presence of variations among the cells due to cell-to-cell interactions. In all cases, there is a lack of a visible pattern indicating the presence of the cell nucleus, which also
comes from the shielding effect at the cell membrane.

Furthermore, increasing the frequency of the incident field up to 5 MHz leads to the intracellular electric fields shown in Fig. 5.9b. In this case, the curve distribution is similar to that obtained at 5 kHz, but with magnitudes in the same order of magnitude as the incident electric field. Such a result is expected since 5 MHz is above the cut-off frequency of the cell membrane and the electrical shielding is decaying. According to this, and since the positions of the forty-five cells are the same as those of the two-layered cells shown in Fig. 4.5c, the results for the three-layered cells at 5 MHz compare favourably with those obtained at the same frequency for the two-layered cells, as shown in Fig. 4.6a. In fact, increasing the frequency up to 5 GHz (Fig. 5.9c) leads to the results equivalent to those shown in Fig. 4.6c, which means that the influence of the cell membrane in the intracellular behaviour at this frequency is negligible.

The absence of cell membrane intervention can be also seen from the intracellular electric fields, which display a clear pattern revealing the presence of the cell nuclei. In addition to this pattern, the electric field in the cytoplasm of most of the cells resembles an increasing exponential that initiates at the nucleus surface and increases towards the cell surface. In some other cases, however, the increasing exponential variation in the cytoplasm turns into a decaying exponential. Moreover, the variations among the different cells evidences cell-to-cell interactions at the intracellular level, which are more visible at frequencies above the cut-off frequency. Additional results for frequencies above 5 GHz show virtually no difference as compared to the 5 GHz case, especially above 50 GHz.

Additional results for the membrane potential obtained when the frequency of the incident field is 5 kHz, 5 MHz and 5 GHz are shown in Fig. 5.9d-f. The membrane potential is computed around the circumference of each cell, starting from the right-most point over the cell equator and moving clockwise. Results indicate that for the three frequencies of interest the membrane potential in each cell tends to follow a cosinusoidal curve, as expected from (5.4). However, the presence of the surrounding cells causes cell-to-cell interactions that disrupt this cosinusoidal shape in a similar way as exemplified in Fig. 5.5, i.e., by deviating the curve along sections of the cell circumference which are close to the points at which the intercellular distance with any adjacent cell is minimal.

Also similarly to Fig. 5.5, numerical results for the forty-five cells indicate that the magnitude of these deviations is inversely proportional to the frequency of the incident field. Thus, for the 5-kHz case (Fig. 5.9d), most of the variations in the membrane potential take place within a pair of upper and lower cosinusoidal envelopes, the maximum values of which (computed at the arc length equal to zero) are 6.04 µV and 16.58 µV, respectively. In some cases, especially at arc lengths equal to 0 and π, the magnitude of the deviations reach values beyond the contour of these envelopes. Although these envelopes are still observed at the 5-MHz case (Fig. 5.9e), the maximum values are de-
Figure 5.9: a-c) The intracellular electric field, and d-f) the membrane potential in forty-five three-layered arbitrarily-positioned cells exposed to an incident electric field of 1 V/m oriented in the x-direction with frequency 5 kHz (left), 5 MHz (center), and 5 GHz (right). g-j) Frequency-dependent electrical response of the same forty-five three-layered cells. Each colored line corresponds to the response from one of the forty-five cells.

Further numerical results for the frequency-dependent membrane potential and intracellular electric field (cytoplasm and nucleus) in the forty-five cells are shown in Fig. 5.9g-j. Accordingly, for the frequency-dependent (maximal) membrane potential (Fig. 5.9g), the curves obtained per cell display the same sigmoid-like shape as obtained in both the single-cell case shown in Fig. 5.3b and the five-cell case shown in Fig. 5.6, with the point of inflection corresponding to the cut-off frequency of the cell membrane. In addition, the cell-to-cell-interactions due to the multiple surrounding cells lead to variations in the magnitude of these curves that in all cases are confined to contours of the envelopes defined by the same maximum and minimum values as from Fig. 5.9d. In this case, the
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The difference between the values of these envelopes derives in a maximal variation of 10.54 \( \mu \text{V} \) in the membrane potential between two simultaneously-exposed cells. A similar sigmoid-like behaviour is obtained for the (maximal) membrane electric field shown in Fig. 5.9h. In this case, the cell-to-cell interactions induce maximal magnitude variations at frequencies below the cut-off frequency that range from 604 V/m to 1658 V/m, so that the maximal difference between any two of the forty-five simultaneously-exposed cells amounts to 1054 V/m.

In contrast to the single-cell and the five-cell simulation cases, the frequency-dependent cytoplasmatic electric field of the forty-five arbitrarily-positioned cells is computed for each cell at the left-most point internal to the cytoplasm and located over the \( x \)-axis. By doing so, the values obtained do not completely correspond with the maximum (or minimum) magnitudes over the entire region of interest, but to the magnitudes of the cytoplasmatic electric field at the inner surface of the cell membrane, as is shown in Fig. 5.9i.

Numerical results indicate that these magnitudes are almost negligible at 1 kHz (and below). Above approximately 3 kHz, these magnitudes increase with the frequency, defining upper and lower envelopes for the variation of the cytoplasmatic electric field due to the cell-to-cell interactions. Accordingly, the maximum value for the upper envelope amounts to 1.4 V/m at a frequency of 17.6 MHz. At this same frequency, the value of the lower envelope amounts to 0.8 V/m, so that the maximal variation between two simultaneously-exposed cells amounts to 0.6 V/m. For frequencies above 17.6 MHz, the upper envelope decays whereas the lower envelope increases, reaching both a steady-state value at about 4 GHz. From this frequency onwards, the upper envelope amounts to 1.1 V/m whereas the lower envelope amounts to 0.96 V/m, hence registering a maintained difference between two simultaneously-exposed cells of 0.14 V/m.

A similar behaviour is obtained for the electric field in the nucleus (computed at the cell centers), with magnitudes almost negligible at 1 kHz that increase with frequencies up to approximately 80 MHz, for which the maximum value (upper envelope) amounts to 1.3 V/m and the minimum value (lower envelope) amounts to 1.2 V/m. Increasing the frequency up to approximately 2-3 GHz brings about a decay in the magnitudes, which reach a steady-state value in all frequencies above 2-3 GHz. For these frequencies, the upper envelope amounts to 1.15 V/m whereas the lower envelope amounts to 1.11 V/m, hence registering a maintained difference between two simultaneously-exposed cells of 0.04 V/m.
5.5.5 Effect of the orientation of the incident field

Besides of investigating the effects that cell-to-cell interactions may have on the intracellular electric field and the membrane potential, the role of the orientation of the electric field with respect to a given axis of the cell has been also explored. To this end, the same forty-five three-layered arbitrarily-positioned cells used in the previous simulation case are exposed to an incident electric field of 1 V/m oriented in the y-direction, with frequencies varying from 1 kHz to 100 GHz. The material properties, cell radii and meshing parameters are unchanged with respect to the previous case.

Numerical results for the intracellular electric field (cytoplasm and nucleus), computed along the x-axis of the forty-five cells, are shown in Fig. 5.10a-c (normalized to the cell diameter). Similarly to the results displayed in Fig. 5.9a-c, the magnitude of the intracellular electric field is proportional to the frequency of the incident field, being in the order of mV/m for frequencies of 5 kHz and below (Fig. 5.10a), and of the same order of magnitude as the incident electric field for frequencies above 5 MHz (Fig. 5.10b-c). However, the change in the orientation of the electric field changes the appearance of the curves from a concave-dominant pattern (x-orientation) into a convex-dominant pattern (y-orientation). These change in orientation also results in the maximum values for the intracellular electric fields increased approximately 1 mV/m at 5 kHz (Fig. 5.10a), 100 mV/m at 5 MHz (Fig. 5.10b), and 10 mV/m at 5 GHz (Fig. 5.10c), as compared to the equivalent cases shown in Fig. 5.9a-c.

Additional results for the membrane potential, computed around the cell circumference starting at the right-most point over the cell equator, are shown in Fig. 5.10d-f for incident fields with frequency 5 kHz, 5 MHz and 5 GHz, respectively. Accordingly, the curves obtained display a sinusoidal shape equivalent to a shift of π/2 in the phase of the curves obtained from the exposure to the x-oriented incident field (Fig. 5.9d-f). Thus, shifting the results π/2 to the left results in cosinusoidal-like curves which are modified by cell-to-cell interactions that, on the one hand, have magnitudes inversely proportional to the frequency of the incident field, and on the other hand, give rise to upper and lower envelopes the maximum and minimum values of which are also comparable to those obtained when the incident field is oriented along the x-direction (Figs. 5.9d-f). For the 5-kHz case, these envelopes are bounded to a minimum value of 8.58 μV and a maximum of 15.68 μV. For the 5-MHz, these values amount to 2.07 μV and 2.93 μV respectively, whereas at 5 GHz the envelopes are defined by 0.12 μV and 0.13 μV.

Moreover, results for the frequency-dependent membrane potential and intracellular electric field (cytoplasm and nucleus) indicate that for the frequency-dependent membrane potential (Fig. 5.10g), the curves obtained per cell display the same type of sigmoid-like shape obtained when the field is oriented in the x-direction, displaying maximum values at lower frequencies and minimum values at high frequencies, as shown
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Figure 5.10: a-c) The intracellular electric field, and d-f) the membrane potential in forty-five three-layered arbitrarily-positioned cells exposed to an incident electric field of 1 V/m oriented in the y-direction with frequency 5 kHz (left), 5 MHz (center), and 5 GHz (right). g-j) Frequency-dependent electrical response of the same forty-five three-layered cells.

in Fig. 5.9g. Important to notice is, however, that the values in this case do not correspond to the maximal possible values but to those computed at the point for which the arc length in Fig. 5.10d is equal to zero. In fact, this same point develops the maximal values of membrane potential and intracellular electric field for the cells exposed to an incident field oriented in the x-direction, and hence, the calculations have been so far carried out at the same location. Thus, due to the change in the orientation of the field, the frequency-dependent membrane potential at the location of interest varies from 97 nV to 4 µV, causing a maximal difference of approximately 3.9 µV.

Similarly, the membrane electric field shown in Fig. 5.10e displays a sigmoid-like behaviour with an inflection point at the cut-off frequency and maximum values at lower frequencies. Due to the cell-to-cell interactions among the forty-five cells, the membrane electric field ranges from 9.7 V/m to 401 V/m, causing a maximal difference of
391.3 V/m between two simultaneously-exposed cells. Moreover, the calculation of the cytoplasmatic electric field at the left-most point as in the previous simulation case, leads to almost negligible magnitudes at 1 kHz which increase with the frequency up to 17.6 MHz, as shown in Fig. 5.10i. At this frequency, the maximum and minimum values obtained are 1.5 V/m and 1.07 V/m respectively, decaying from 80 MHz down to about 4 GHz, above which the electric field in the cytoplasm of the forty-five cells reaches an steady-state value bounded by a maximum of 1.13 V/m and a minimum of 1.03 V/m, which correspond to a difference of 0.1 V/m.

Additional results for the frequency-dependent electric field in the nucleus are shown in Fig. 5.10j. The behaviour is similar to that obtained using the incident electric field oriented in the $x$-direction (Fig. 5.9j). In fact, a comparison between the frequencies of inflection and both the maximum and minimum values accounts for minor difference between the two plots.

### 5.5.6 Frequency dispersions in multiple three-layered cells

An additional simulation case is implemented to evaluate the effect of the dispersive materials (Fig. 5.7) in the same forty-five arbitrarily-positioned cells used as before. Therefore, the cells are assumed exposed to an $x$-directed incident electric field of 1 V/m with frequency varying in the interval 1 kHz - 1 THz. The cell radii and the mesh parameters are equal to those used for the previous simulation case. Numerical results are shown in Fig. 5.11.

Accordingly, the results obtained for the intracellular electric fields (normalized to the cell diameter) indicate a slight variation of approximately 1-3 % in the magnitudes obtained at both 5 GHz and 50 GHz (Fig. 5.11a-b), as compared to the non-dispersive case. No significant variations are found for the results at frequencies below 5 GHz. Although these values might represent an almost negligible difference, they introduce a frequency-dependent response which is more significant for frequencies above 100 GHz. In fact, the results at 500 GHz (Fig. 5.11c), display an increase of about 11.5 % in the values reached at the cell nucleus and a reduction of approximately 10.5 % in the values obtained at the inner surface of the cell membrane with respect to the non-dispersive case, which at this frequency is virtually equal to the 5-GHz case. In addition, for the 500-GHz frequency, the strength of the cell-to-cell interactions is reduced in such a way that the intracellular electric fields of the forty-five cells are practically overlapping, resembling as a whole the response obtained for a single cell (Fig. 4.3b).

In the case of the membrane potential, the frequency-dispersive materials modify the maximal value of the cosinusoidal (average) curve at 5 GHz, causing an increment of 67.5 % compared to the non-dispersive case and setting the maximum magnitude at 0.4 $\mu$V (Fig. 5.11d). Such an increment is less strong at 50 GHz, corresponding to
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Figure 5.11: Electrical response of forty-five three-layered arbitrarily-positioned cells with frequency-dispersive material properties exposed to 1-V/m $x$-directed incident electric field. Intracellular electric field for a frequency of a) 5 kHz, b) 5 MHz, and c) 5 GHz. Membrane potential for a frequency of d) 5 GHz, e) 50 GHz, and f) 500 GHz. g-j) Frequency-dependent electrical response.

an increase of 35% which sets the maximum magnitude at 0.2 $\mu$V (Fig. 5.11e). In contrast, for the 500-GHz case (Fig. 5.11f), the maximum membrane potential is 0.09 $\mu$V, corresponding with a reduction of 3% with respect to the non-dispersive case. In addition to the variations in magnitude, the material dispersions reduce the strength of the cell-to-cell interactions for frequencies above 5 GHz. In consequence, the membrane potential in the forty-five cells exposed to the 500-GHz incident electric field are virtually overlapping each other, resembling as a whole the response obtained for a single cell (Fig. 5.3a).

Additionally, the frequency-dependent membrane potential and membrane electric field shown in Figs. 5.11g-h features no variations for frequencies lower than 100 MHz with respect to the non-dispersive cases (Figs. 5.9g-h). Above this frequency, however, the response of the forty-five cells is equivalent to that of the five cells summarized in Figs. 5.8c-d, especially after considering that the cell-to-cell interactions become weaker.
once the frequency of the incident field is above the cut-off frequency of the cell membrane.

Further results for the frequency-dependent electric field in the cytoplasm (Figs. 5.11i) reveal that for frequencies ranging approximately from 4 GHz to 50 GHz, the magnitudes vary within 1-3 % with respect to the non-dispersive case. No differences are displayed for all other frequencies below this range. Above 50 GHz, however, the magnitudes of the distinctive curves for each cell begin to converge, reaching the single value of 0.98 V/m at 1 THz. This value represents a reduction of 11 % with respect to the maximum value obtained at 4 GHz and an increase of 2 % with respect to the minimum value. In contrast, the frequency-dependent electric field in the nucleus (Figs. 5.11j) has no variations compared to the non-dispersive case for frequencies below 4 GHz. For frequencies above 4 GHz, the magnitudes of the curves for each cell increase in a converging-like fashion, reaching a maximum of 1.3 V/m at 1 THz. This value represents an increase of 16.5 % with respect to the minimum value obtained at 4 GHz. Nevertheless these findings, and as mentioned in section 4.9.6, care must be taken prior to conclude on the interactions due to electric fields with frequencies in the THz range because frequencies this high are in the limit of applicability of the quasi-static approximation (5.20) and therefore may not be adequate to describe the electrical response of (spherical) cells.

5.5.7 Variations in the membrane electric field

The results for the membrane electric field presented in Figs. 5.3 - 5.11 correspond to the values obtained at an specific point $p_c$ over the cell surface, i.e., over the circumference with radius $R_3$. Particularly for the exposure to an incident electric field oriented along the $x$-direction, this point is also the right-most point over the cell equator, at which the membrane electric field reaches a maximum value, regardless of the frequency. This is an expected behaviour since the electric field at the membrane $E_m$ can be approximated as $E_m = \phi_m/R_3$, and, therefore, it inherits the cosinusoidal shape of the membrane electric potential $\phi_m$, which is also maximal at point $p_c$. Therefore, by computing the transmembrane electric field, given as the difference between the membrane electric field calculated along the circumference with radius $R_3$ and the circumference with radius $R_3$, an additional cosinusoidal-like behaviour is uncovered, as shown in Fig. 5.12. Notice that the double cosine is the expected cosine inherited from the membrane electric potential but plotted in absolute value as to be consistent with the magnitude of the membrane electric field.

Accordingly, the transmembrane electric field in a single cell exposed to an incident electric field of 1 V/m ($z$-propagating, $x$-directed) with frequency 5 kHz (Fig. 5.12a) is characterized by a cosinusoidal-like curve with a static (DC) component that amounts to 2.25 V/m. The maximum value of this curve is 3 V/m and the minimum value is 1.5
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V/m. The curve remains unchanged for all other frequencies down to the static (zero frequency) case. In contrast, increasing the frequency up to 5 MHz reduces the DC component down to 0.42 V/m, with a maximum value of 0.55 V/m and a minimum of 0.28 V/m (Fig. 5.12b). A further increase in the frequency up to 5 GHz results in a DC component that amounts to approximately 17.5 mV/m and maximum and minimum values that amount to about 23.5 mV/m and 11.5 mV/m, respectively (Fig. 5.12c). No further changes are observed for frequencies above 5 GHz.

In addition, the transmembrane electric field in five three-layered cells arranged in the touch configuration as described in previous simulation cases, indicates that the cosinusoidal-like behaviour is also subject to deviations due to cell-to-cell interactions (Fig. 5.12d-f). In fact, the exposure to an incident field of 1 V/m with frequency 5 kHz causes deviations which are particularly evident at the locations around the cell circumference closer to the adjacent cells (Fig. 5.12d), in a similar way to the results of the membrane potential shown in Fig. 5.5 (upper row). Accordingly, the mean DC component (computed using the curves for cells 3 and 4, as labeled in Figs. 5.5) amounts to 2.16 V/m, which represents a reduction of 4 % with respect to the value obtained for the single cell. Likewise to the results shown in Figs. 5.4-5.6, the cell-to-cell interactions have more influence in the transmembrane electric field of the central cell (cell 5). Therein, the induced deviations in the cosinusoidal-like shape reach a maximum magnitude of 3.6 V/m and minimum of 0.69 V/m. These values correspond to variations of 16.7 % and 54 % with respect to the values obtained in the single-cell case.

Further results for the five cells exposed to the same incident field but with frequencies of 5 MHz (Fig. 5.12e) and 5 GHz (Fig. 5.12f) indicate that the deviations due to the cell-to-cell interactions are inversely proportional to the frequency. Thus, the maximum value at 5 MHz, computed in the central cell, amounts to 0.6 V/m while the minimum value to 0.18 V/m. Likewise, the mean DC component is 0.41 V/m, determined by the maximum and minimum values in cells 3-4 that amount to 0.54 V/m and 0.28 V/m, respectively. For a frequency of 5 GHz and above, the cell-to-cell interactions modify the cell response so little that the cosinusoidal-like curves are deviated from that obtained for the single cell at this same frequency at most by 1 mV/m, except for the localized variations due to the cell-to-cell interactions that account for deviations up to 2 mV/m.

The transmembrane electric field is also computed for the forty-five three-layered arbitrarily-positioned cells used in previous simulations cases. Accordingly, the numerical results indicate that presence of cell-to-cell interactions cause deviations in the expected cosinusoidal-like pattern, which turns out to be inversely proportional to the frequency. These deviations reach maximum and minimum magnitudes at 3.48 V/m and 1.031 V/m respectively during the exposure to an incident field of 1 V/m with frequency 5 kHz (Fig. 5.12g). Such magnitudes are lower than those obtained in the
Figure 5.12: The transmembrane electric field in a-c) one, d-f) five, g-i) forty-five three-layered cells, and j-l) forty-five three-layered cells with frequency-dispersive materials exposed to an incident electric field of 1 V/m with frequency 5 kHz (left), 5 MHz (middle), and 5 GHz (right). In j-l), the frequencies are set to 5 GHz, 50 GHz, and 500 GHz, respectively.

five-cell case, which indicates that maximal values can only be obtained when two cells are touching each other. This situation, however, does not occur for the distribution of the forty-five arbitrarily-positioned cells since a minimum distance of 0.1 µ is reserved between two adjacent cells.

Nevertheless, due to the number of intersecting curves and despite the deviations intro-
duced by the presence of adjacent cells, which are confined to specific regions along the cell circumferences, the average response resembles the cosinusoidal-like shape obtained for the single-cell, with a maximum magnitude around 2.8 V/m, a minimum magnitude around 1.3 V/m, and a DC component of approximately 2 V/m. These values are significantly lowered with the increase in frequency, so that already at 5 MHz the maximum magnitude is within the interval 0.5-0.6 V/m and the minimum magnitude between the interval 0.2-0.3 V/m (Fig. 5.12h). Increasing the frequency up to 5 GHz reduces the cell-to-cell interactions to the extent that the average maximum and minimum values of the entire group of forty-five curves is comparable to the curve obtained for the single cell exposed the same frequency of 5 GHz (Fig. 5.12i), with differences of about 1-2 mV/m.

An additional case using the forty-five-cell arrangement and including frequency-dispersive materials indicates that at a frequency of 5 GHz the magnitudes of the transmembrane electric fields are approximately 69 % higher than those obtained with the non-dispersive materials at the same frequency, with a maximum magnitude at 76.5 mV/m and a minimum at 37.5 mV/m approximately. Despite the change in magnitude, the cosinusoidal-like general pattern is preserved, with deviations among the forty-five curves due to the cell-to-cell interactions that account for additional variations of $\pm 3-5$ mV/m in the maximum and minimum magnitudes (Fig. 5.12j).

Furthermore, increasing the frequency up to 50 GHz (Fig. 5.12k) increases approximately by 40 % the maximum and minimum magnitudes (39 mV/m and 19.5 mV/m respectively) as compared to those shown in Fig. 5.12i, with variations due to the cell-to-cell interactions among the forty-five curves that amount to additional differences in the magnitude of about $\pm 2-3$ mV/m.

A further increase in the frequency up to 500 GHz reduces the magnitudes of the cosinusoidal-like curves approximately 33 % with respect to the case with non-dispersive materials. Accordingly, the maximum transmembrane electric field is 15.6 mV/m, the minimum is 7.8 mV/m, and there are almost no variations due to the cell-to-cell interactions because all the forty-five individual curves are virtually overlapping each other, in a similar way to the result for the membrane electric potential shown in Fig. 5.11f.

5.6 Discussion

This chapter introduces a scattering-matrix-based modelling approach suitable to compute the electrical response of multi-layered spherical objects that represent a group of biological cells exposed to an incident electric field. Unlike the framework presented in Chapter 4, this approach relies on the derivation of an equivalent electrical network for the multi-layered cell that leads to a single expression suitable for computing the electric field at any point within the simulation domain.
Furthermore, by recurring to an adaptive cobweb-like mesh scheme so as to include the cell membrane, the two-layered cell structure from Chapter 4 is extended to a three-layered spherical cell that provides additional information about the electrical behaviour of the cell, especially in the cell membrane. In general, the inclusion of additional features to allow for material properties with frequency dispersions and different field orientations, provides numerical results indicating that the electrical response of a cell is modified by the presence of surrounding cells inducing cell-to-cell interactions. Moreover, the intensity and location of these interactions, especially at the cell membrane, is found to depend significantly on the number of cells, the intracellular distances, the relative location of adjacent cells, and the frequency of the exposure.

The three-layered model featuring the cell membrane allows to examine both the cell response to an incident electric field and the variations due to cell-to-cell interactions at a wider range of frequencies than the two-layered model presented in Chapter 4, which is limited to frequencies above the cut-off frequency of the cell membrane. Thus, numerical results indicate that the electrical response of both the membrane potential and the membrane electric field is maximal for all frequencies below the cut-off frequency up to 100-500 kHz, in agreement with the bioactive region below 1 kHz for which weak electric fields as low as several V/m are considered capable of inducing biological effects [108] and evidencing a frequency window for the interaction mechanisms due to weak electric fields to occur [1, 35].

Furthermore, and even though for frequencies below the cut-off frequency both the cytoplasm and the cell nucleus remain electrically shielded, the cell membrane in every cell engages in a frequency-dependent electrical activity that in addition to the number (density) and position (intracellular distances) of the surrounding cells (see Figs. 4.7 and 4.5), also depends on the location of the minimum distance to an adjacent cell measured from the cell circumference (Fig. 5.5). Therefore, the uncovered activity at the cell membrane might be a possible explanation for the experimentally observed modulation in the firing frequency of action potentials in excitable cells (neurons) exposed to a weak ELF (50 Hz) external fields, which ultimately might be related to modifications in the kinetics of calcium channels and the membrane surface charge [65, 66, 121]. It may also be related with molecular changes and transduction mechanisms presumably taking place during the exposure to fields with frequencies in the ELF range (up to 100-300 Hz) [4, 44, 60] that might have the ability to influence biochemical processes such as integrins signalling [60, 122], molecular transport and reaction rates [44].

In addition, the results for three-layered model for frequencies above the cut-off frequency are equivalent to those obtained with the two-layered model, as expected. Nevertheless, it must be highlighted that even for frequencies above the cut-off frequency, the three-layered model is a closer approximation to the cell inasmuch as it accounts for variations in both the membrane potential and the membrane electric field that, albeit
with magnitudes inversely proportional to the frequency (Figs. 5.5-5.12), might affect the cell homeostasis and possibly induce high-frequency (non-thermal) effects [4, 10, 11].

### 5.6.1 Validation of the implementation: one three-layered cell

In order to validate the scattering-matrix-based representation, the results of the frequency-dependent exposure of a one and two three-layered spherical cell are compared with the analytical solutions and the implementation presented in Chapter 4. Thus, adapting the material properties so as to simplify the three-layered spherical cells into single-layered cell results in a solution equivalent to both the numerical and the analytical solutions shown in Fig. 4.2. The same procedure to reduce two three-layered cells into two-layered cells, provides numerical results in agreement with those shown in Fig. 4.3.

Furthermore, numerical results shown in Fig. 5.3 for the cell membrane compare favourably with the analytical solution in (5.4). Thus, the maximum value of both the membrane potential and the membrane electric field displays a sigmoid-like frequency dependence that resembles the response from of a low-pass filter [120]. Noticing that indeed (5.4) corresponds to the expression of such a filter [61], the cut-off frequency can be expressed as \( \nu = 1/(2\pi \tau_m) \) [61, 120], which amounts to 959.8 kHz. By definition, the cut-off frequency of a low-pass filter, which in the case of the cells corresponds to the cut-off frequency of the cell membrane, is the frequency at which the magnitude of the output is equal to \( 1/\sqrt{2} \) the maximal output [120]. Furthermore, since the maximal output is 1.5 V (low-frequency), the magnitude at the cut-off frequency is 1.06 V. The numerical results are in agreement with both the value of the cut-off frequency and the magnitude of the membrane potential at this frequency, and follow the predictions given by (5.4). Therefore, the three-layered model results an accurate representation suitable to reproduce the cell membrane frequency-dependent behaviour characterized by a cut-off frequency of approximately 1 MHz, a value that compares favorably with previously reported approximations [61, 102].

Thus, while the intracellular electrical response is proportional to the frequency of the incident field (Fig. 4.6), the electrical response in the cell membrane is inversely proportional. Additional results obtained in five and forty-five cells also indicate that the cell-to-cell interactions induced by the surrounding cells follow the same frequency-dependent response. Accordingly, the variations in the intracellular compartments (cytoplasm and nucleus) are minimal for frequencies below 10-100 kHz, reaching maximum values at about 10 MHz. In contrast, the variations in the cell membrane are maximal for frequencies up to about one decade before the cut-off frequency, i.e., 100-500 kHz, and then decay towards minimum values which are reached approximately at 100 MHz. Therefore, possible interaction mechanisms between biological cells exposed to incident
electric fields with frequency below 10-100 kHz might be due to effects at the cell membrane level. In contrast, any possible cell effect due to the exposure to fields with frequencies above 10-100 MHz might be associated with the variations in the intracellular electric field, especially for frequencies in the range 10 MHz - 1 GHz for which the variations reach maximum values. Any possible effect caused by the exposure to an incident electric field with frequency ranging from 100 kHz to 10 MHz, might be the consequence of interaction mechanisms taking place due to variations in either the membrane potential, the intracellular electric field, or both.

5.6.2 Intercellular distances and circular arcs

Numerical simulations for the electric field distribution in five three-layered cells arranged in three different configurations with different intercellular distances (Fig. 5.4) indicate that these distances mediate in the strength of the cell-to-cell interactions, which are capable to modify the electrical response at each of the cell compartments. This observation is in agreement with the results presented in Chapter 4 involving two-layered cells. However, an examination of the additional variations in the cell membrane that are provided by the three-layered model points out that the angles between a line intersecting the centers of two adjacent cells and the equatorial lines therein are paramount to understand the irregularities observed in a cell due to its neighbors. In fact, it has been found that the deviations from the otherwise cosinusoidal shape of the membrane potential for one cell, which is analytically predicted by Schwam’s equation in (5.1) and (5.4), are most visible along the portion of the cell membrane (surface) closer to the adjacent cell.

In fact, using additional simulations involving two cells touching one another at different locations around the cell circumference, together with additional simulations for the five cells shown in Fig. 5.4a that touch at different locations around the cell circumference (results not shown), the portion (circular arc) of the cell membrane affected by the adjacent cell is centered at the contact point and spans a total length of $2\pi/3 \ (120^\circ)$. Beyond this length, the response of the cell membrane follows the expected cosinusoidal curve. Moreover, a closer look to the membrane potential in the central cell (cell 5) shown in Fig. 5.5 reveals deviations in the otherwise cosinusoidal curve that are virtually overlapping those displayed by the other four cells. Accordingly, the individual lengths of the deviations due to the cell-to-cell interactions are larger than those in the other cells. The explanation to this follows from the fact that for a cell with two (or more) adjacent cells, the contact points of which are separated an equivalent circular arc shorter than $2\pi/3$, the total circular arc enclosing the variations in the membrane potential is the summation of the circular arcs associated with each contact point.

Hence, the variations in the membrane potential of any cell are delimited by circular
arcs centered at the points of minimal intercellular distance with its adjacent cells, and are distributed along the arc distance between these same points of minimal intercellular distance. Thus, and in the light of the results shown in Fig. 5.5 from which the variations in the membrane potential depend on the position of the neighbor cells and the frequency of the exposure, the existence of the circular arcs bounding the influence of the cell-to-cell interactions serves to explain the numerous curvilinear variations present not only in the membrane potential (Figs. 5.9 - 5.11), but also in the membrane electric field (Fig. 5.12) of the forty-five cells.

These circular arcs open the possibility to a certain degree of fortuity in the occurrence of effects at the cell membrane level, since the location of adjacent cells in-vitro is essentially random as is the section of the membrane facing a neighbor cell. Hence, by virtue of the circular arcs, it could be a matter of chance that the aspecific portion of a cell (cell membrane) sensitive to the variations induced by the presence of an adjacent neighbor cell is in fact facing it and the interaction therein being able to trigger a biological effect.

Although experimental evidence is needed before reaching solid conclusions on this matter, this argument could be suitable to explain the lack of reproducibility of experimental results. Moreover, it would provide an additional perspective to cell manipulation by allowing the induction of localized variations in the cell membrane due to carefully positioned and aligned neighboring cells. Such a controlled cell arrangement would be suitable to (electro)-porate specific areas of the cell membrane [97], and might be especially relevant for the combination of two colliding cells during electrofusion [8].

5.6.3 Frequency-dispersive materials

The use of frequency-dependent material properties accounting for dielectric relaxations lead to changes in the values of the relative permittivity and conductivity that are most evident at frequencies above 1 GHz (Fig. 5.7). However, it must be highlighted that these dielectric relaxations are mostly based on experiments performed in aqueous solutions (physiological saline) and artificial in-vitro bi-lipid membranes [61]. Nevertheless, from Fig. 5.7 it is observed that the inclusion of (approximated) frequency dispersions provides material properties with values much different from the static values only for frequencies above the cell membrane cut-off frequency [39, 61]. Thus, using a material relaxation model with parameters as indicated in Table 5.1 results in an improved material model that modifies the electrical response at frequencies above 100 MHz (RF range) but that has no influence in the low-frequency (LF) range. For these frequencies, the numerical results indicate that the static material properties are enough to represent all the material regions.

However, additional relaxations such as the tangential flow of ions across the interfaces,
the surface effects due to the charge built-up at the material interfaces (Maxwell-Wagner and double layer effects [88, 92]), and changes in the material properties of the cell compartments due to changes in other variables such as the ionic (salt) concentrations and thermal variations [123] might induce specific frequency-dependent dispersions that deserve attention. In fact, experimental observations performed at frequencies lower than 50 Hz allow to conclude that the electrical properties of water exposed to an incident field change with time (aging effect). This behaviour, which could be another cause for the failure to reproduce experimental results, even at the same laboratory [33, 124], suggest the feasibility for the material properties to change at frequencies below the cell membrane cut-off frequency [124]. Nevertheless, using dielectric spectroscopy, more accurate values on the actual cell compartments have been identified [39, 125]. Interestingly, these values lead to dispersion curves that, in spite of differences in magnitude, are similar to those shown in Fig. 5.6, i.e., decreasing permittivity and increasing conductivity for frequencies in the RF range (above 100 kHz).

Even though a more accurate description of the dielectric relaxations at these frequencies might possibly change the magnitude of the interactions observed, the results for the forty-five three-layered cells with frequency-dispersive materials exposed to an incident electric field with frequency above 1 GHz indicate that reductions of 70-80 % in the static relative permittivities, together with increases of two-four orders of magnitude in conductivity with respect to the static values (Fig. 5.7), represent an increment in the electrical response at the cell membrane that at 5 GHz corresponds to magnitudes of the membrane potential and the membrane electric field 67.5 % and 69 % higher than in the non-dispersive case (Fig. 5.11f and 5.12j). This increment weakens for higher frequencies, so that at 500 GHz the values are 3 % and 33 % lower with respect to the non-dispersive case. In contrast, the intracellular electric fields at 5 GHz are only 1-3 % lower than in the non-dispersive case, whereas at 500 GHz they are 10.5-11.5 % higher. Thus, the use of material dispersions represents a significantly more accurate calculation of the variations in the intensity of the cell-to-cell interactions only for the exposure to incident electric field with frequencies above 1 GHz.

Specifically for exposures above 50 GHz, numerical results also indicate that the use of material dispersions reduce the strength of the cell-to-cell interactions. This is observed in the forty-five cells by the rapid convergence towards a single value of the intracellular electric field (Fig. 5.11c), the membrane potential (Fig. 5.11f), and the membrane electric field (Fig. 5.12l) at frequencies above 50 GHz. Therefore, the fact that the forty-five curves for the electrical response tend to overlap at 500 GHz suggests that no matter the number of simultaneously-exposed cells, the cell cluster could be reduced to an equivalent single cell of adjusted dimensions [107]. In fact, assuming the availability of material properties for each cell compartment including all possible relaxations, it is believed that unless the consequent frequency-dependent material properties were considerably different than those in Fig. 5.7, the magnitudes of the electrical response of
this equivalent cell for frequencies above 50 GHz will have magnitudes comparable to those obtained in the forty-five three-layered cells.

This convergence of the electrical response towards that of an equivalent cell might also have relevance in the further identification of possible interaction mechanisms activated by frequencies in the THz range, which are most likely not related to cell-to-cell interactions. However, a further exploration of the possible effects of THz exposure using the current approach is confronted with the fact that at these frequencies the quasi-static approximation supporting the derivation of (5.20) is insufficient to tackle possible changes in the cell electrical response [93]. Thus, attempting at solving the problem of multiple cells exposed to an incident field in the THz range requires a more general (full-wave) approximation, an alternative to which is presented in the following chapter.

### 5.6.4 Orientation of the incident electric field

Moreover, comparing the results for the intracellular electric field in multiple cells obtained for two orientations of the incident electric field [Figs. 5.9 and 5.10 (upper rows)] indicate that despite the symmetry of the spherical shape, cells exposed to different field orientations experience different intracellular electric fields along the same axis of reference. In other words, the exposure to different field orientations create different paths along which the intracellular electric field is maximized. The different location of these paths across the cell membrane might be associated with changes in local signal transduction patterns, ion (protein) transport and may even possibly affect the activity of enzymes at the cell membrane [41, 94, 107].

Additionally, it must be highlighted that due to the symmetry of the spherical shape, computing the intracellular electric field along the \( y \)-axis of a single cell exposed to an incident field oriented in the \( y \)-direction is equivalent to the results computed along the \( x \)-axis during the exposure to an incident field directed along the \( x \)-axis. Thus, the intracellular electric field in a single cell computed along the \( y \)-axis during the exposure to a \( x \)-oriented field is also equivalent to the field developed along the \( x \)-axis during the exposure to a field directed in the \( y \)-axis. Evidently, the presence of surrounding cells induce cell-to-cell interactions that change the equivalence of such fields due to the fixed location of the adjacent cells. However, the electrical distribution of the forty-five three-layered cells exposed to the \( y \)-directed field is such that the magnitude of the electric field across the domain has maximum and minimum values equal to those obtained for the \( x \)-directed field (Fig. 4.5c). Therefore, the cell-to-cell interactions induced during exposure to the \( y \)-oriented incident field appear to modify the intracellular electric field within the same range of variation as the \( x \)-oriented field does.

Furthermore, the results for the forty-five cells exposed to the 5 GHz reveal that the
steep variation in the magnitude of the intracellular electric field at the surface of the cell nucleus during exposure to an incident electric field oriented in the $x$-direction (Fig. 5.9c) is smoothed out by changing the orientation of the field to the $y$-direction (Fig. 5.10c). Therefore, since a single cell experiences simultaneously concave-like intracellular electric fields as shown in Fig. 5.9a-c along one axis and convex-like fields as shown in Fig. 5.10a-c along the other, the gradient of the electric field at the surface of the cell nucleus varies around the nucleus circumference. The variations might be associated with possible conformational changes in the embedded membrane proteins by modulating the nuclear membrane potential [112].

5.6.5 Importance of the intracellular electric fields

The multiple numerical results for the forty-five cells indicate that in order to compare the intracellular electric fields and draw conclusions based on the existence of gradients of electric field, it results convenient to compute the cytoplasmatic electric fields at the surface of the cell membrane instead that computing the maximal magnitude within the cytoplasm. In fact, as observed from the solutions obtained for the exposure to incident fields with frequencies of 5 kHz and 5 MHz (Figs. 5.9a-b), computing the maximal values in the cytoplasm limits the possibility to address the maximal range of variation among the forty-five cells, which in these cases is most prominent towards the surface of the cell membrane and might be related to (possible) interaction mechanisms involving the cell membrane, specifically, the mobilization of surface charge [65, 121].

Conversely, this behaviour is not present during the exposure to an incident electric field with frequency 5 GHz. In this case, the minimum values defining the range of variation within the cytoplasm are obtained at the nucleus surface (Fig. 5.9c), a condition that is repeated for frequencies in the range 5 MHz - 5 GHz. These variations are presumably more involved with effects on the cell nucleus than on the cell membrane, and thus, they are not considered in the definition of the range of variation within the cytoplasm. It must be noticed, however, that such a behaviour only applies to the exposure to an incident electric field oriented in the $x$-direction, since the change in the field orientation towards the $y$-axis leads to minimal values located at the surface of the cell membrane (Fig. 5.10c). Nevertheless, and even considering that for such a $y$-directed incident electric field the problem at hand comprises the maximum values which are located at the nucleus surface, the maximal range of variation within the cytoplasm is (unequivocally) defined by computing the maximum and minimum at the surface of the cell membrane and disregarding all other values near the cell nucleus that lie outside this range and that are assumed related to the behaviour of the cell nucleus instead.

Moreover, the existence of gradients of electric field at specific locations around the
nucleus surface, likely due to the difference in dielectric properties and the orientation of the incident field, might cause micro-thermal heating that eventually could derive in thermally-induced biological effects [10]. Although the micro-thermal heating is initially assumed to be negligible [10, 38], the variations in the magnitude of the intracellular electric fields induced by the cell-to-cell interactions might eventually be enough to rise the local temperature at the cell level 0.1-0.2 °C [10, 20, 59, 126]. However, the combination of the electrical behaviour presented in this chapter with a temperature analysis similar to that presented in Chapter 9 for the solution of the Bio-heat equation [80, 104], would be required prior to further concluding on this matter.

Assuming that the inner-most cell layer represents a mitochondria instead of the cell nucleus [93, 112], the variations in the gradient of electric field around the circumference of such mitochondria might open the permeability transition pore complex (MPTP) [93, 127]. This complex is a voltage-dependent channel implicated in cell apoptosis, which once opened, allows the transport of non-specific small molecules [127]. Since both a mitochondria and the cell nucleus are surrounded by a membrane of characteristics similar to those of the cell membrane [112, 115], it is feasible for these gradients of electric field developing on the organelle circumference to induce changes in the organelle membrane potential (electric field) that activate the MPTP complex. Nevertheless, further exploration thereof would require the introduction of an additional material layer to represent the nuclear membrane [93, 112], the quantification of the values needed for the activation of the MPTP complex, and a better identification of the exact material properties of the cell compartments, especially after considering the results shown in Fig. 4.9, for which a specific set of the static values of the material properties maximizes the intracellular electric fields specifically at the surface of the cell nucleus in two-layered cells.

Besides, experimental results demonstrate that there is a frequency window between 1 MHz and approximately 200 MHz in which electric fields can be used to manipulate and characterize the exposed cells [35, 81]. This window might be associated with the higher magnitude of the intracellular electric fields obtained for frequencies between 1 MHz and 500 MHz with respect to other frequencies, as observed in the five-cell case (Fig. 5.6) and in the forty-five-cell case (Fig. 5.9b,i). Furthermore, the difference in the intracellular electric field among simultaneously-exposed cells is higher in this range of frequencies, a behaviour that is maintained after changing the orientation of the incident electric field (Fig. 5.10i). Hence, the presence of surrounding cells introduces cell-to-cell electrical interactions that in some cells might be enough to increase the intracellular electric field above a threshold value needed to trigger an electrically-mediated response. These interactions are found to depend on both the position (intercellular distances) and relative location of the adjacent cells (circular arcs).

Considering an in-vitro cell culture in which the cell positions are essentially arbitrary,
Implications of the cell membrane in the cell-to-cell interactions

Numerical results suggest that during exposure to an incident electric field some cells will experience more intense intracellular electric fields than others, the difference being an additional factor that hampers the reproducibility of experimental results. Interestingly, changing the field orientation causes important variations in the cytoplasmatic electric field but no significant change in the electric field in the nucleus. Thus, any possible effects caused by alterations in the induced intracellular electric field due to the cell-to-cell interactions are most likely restricted to electrically-mediated process occurring in the cytoplasm, such as conformational alteration of dipole reorientation in biomolecules (proteins) [10], and variations in the Coulomb interactions that govern the intracellular movement of messenger proteins between the cell membrane and the nucleus [115].

5.6.6 Electric response of the cell membrane

The frequency response of the membrane potential indicates that the threshold value for the activation of electrically-sensitive membrane components such as ion channels increases with the frequency of the incident field, in agreement with previous models [10]. It is known that an incident electric field can open voltage-controlled channels by changing the membrane potential about 10-30 mV [108, 122]. Accordingly, provided the linearity of the current approximation with respect to the magnitude of the incident electric field, the opening of a voltage-controlled channel would require the exposure of a single cell to an incident electric field of at least 667 V/m at a frequency below the cut-off frequency of the cell membrane. This magnitude would increase with the frequency, so that the opening of a channel at frequencies above the cut-off value would require the exposure to fields of several kV/m.

Such electric fields have been already identified in experimental setups for frequencies higher than 1 MHz [10] and lead to the conclusion that prior to opening an ion channel, the exposure to such field magnitudes leads to harmful effects [9, 11]. Moreover, even though a magnitude of 667 V/m at low frequency might not lead to immediate cell (thermal) damage [14, 77], it seems very likely that prior to the opening of channels other thermal effects take place within the cell, especially if 200 V/m is assumed a maximal incident field magnitude for the (possible) effects to be considered as non-thermal [19, 114].

In addition, in the light of the linearity of the scattering-matrix model with respect to the magnitude of the incident field, and in accordance with the results shown in Fig. 5.9g, the exposure of forty-five three-layered cells to an incident field with magnitude of 200 V/m and frequency below the cut-off frequency would induce a maximum membrane potential of approximately 3.3 mV. Such value is above the presumed level of channel noise of 100 µV [4] and might be related with changes in membrane potential mediating the directional sensing mechanisms for cell electrotaxis [128].
Increasing the magnitude of the incident field to 600 V/m increases the membrane potential up to nearly 10 mV. Considering 10 mV as the initial variation in the membrane potential needed to activate voltage-controlled ion channels [108, 122], and since an incident electric field of 600 V/m is within a range of viability for the exposure of single cells [45, 64, 77], the numerical findings suggest that it is feasible for the exposure to an incident electric field with frequency below approximately 100-500 kHz to cause cell effects related to variations in the membrane potential which are the consequence of both the incident electric field and the cell-to-cell interactions.

However, even though the exposure of in-vitro cells to such magnitudes seems plausible, additional evidence suggests that the external electric field needed for the interior of an organ to experience an induced electric field of 1 mV/m is 560 V/m [107]. Assuming linearity with respect to the magnitude of the electric field, the numerical results suggest that in order to reach an induced (incident) electric field of 600 V/m, needed to activate voltage-controlled channels, the organ should be exposed to an external field of about 300 MV/m. This magnitude is similar to that obtained in calculations for the electric field just outside a cell in suspension due to an incident electric field originated in an external air compartment [19, 129].

Moreover, this magnitude is higher than that used in cell electroporation and thermotherapy [8, 80, 81, 82], and even higher than the value for the dielectric breakdown of air (about 1 MV/m), which makes it implausible if the organ (body) is assumed surrounded by air [19]. Therefore, since most of the exposure of an individual in a residential environment is usually to ELF electric fields with intensities below 100 V/m [23, 107], but in any case (e.g. outdoors near high-voltage power lines) to fields with magnitudes below the maximal of 5 kV/m allowed by internationally-standardized reference levels for general public exposure (see Table 2.2) [24], the exposure of an organ (body) to an ELF electric field strong enough to induce a (non-thermal) effect at the cell level seems very unlikely.

Additionally, in view of the results for the membrane potential at frequencies above the cut-off frequency of the cell membrane (Fig. 5.9d-f), the exposure of the forty-five cells to an incident electric field of 200 V/m would induce a membrane potential of about 3.4 mV, 0.6 mV and 26 µV if the frequency is 5 kHz, 5 MHz and 5 GHz, respectively. Accordingly, while frequencies ranging from 5 kHz and 5 MHz may still affect the cell membrane [10, 114], numerical results indicate that frequencies above 5 MHz induce variations in the membrane potential that weaken with the increase frequency and are probably not enough to affect the cell membrane, especially in the GHz-range, in which case the magnitude of the variations is lower than the 100-µV threshold overcoming channel noise [4]. Therefore, it is hypothesized that any possible interaction mechanism related to variations in the membrane potential is most likely due to the exposure of in-vitro cells cultures to incident electric fields with frequencies in the low-frequency
5.7 Perspectives

Despite the availability of numerical results providing insights into the variations in the intracellular electric fields, especially due to the presence of surrounding cells, it is difficult to determine the magnitude of the incident electric field capable of inducing a certain effect due to the contradictory findings reported in the literature.

Experimental evidence shows that ELF incident fields as low as $10 \mu V/m$ increase bone matrix formation and cell proliferation [18], while neutrophils metabolism is sensible to induced fields as low as $100 \mu V/m$ [57]. Furthermore, magnitudes of $480 \mu V/m$ reduce osteoclastogenesis in bone marrow cultures [2], and $9 \mathrm{mV/m}$ might be enough to alter existing transport and reaction rates [44]. In contrast, experimental evidence suggests that endogenous (ELF) electric fields of about 1-200 V/m are involved in physiological processes such as wound healing and embryo development [2, 58]. Thus, any incident electric field capable of producing a significant biological effect must also override the effect due to endogenous fields [4, 107]. Notwithstanding the lack of consensus, it is most likely that the possible biological effects are governed by a magnitude (frequency)-dependent window related to calcium influx [130].

A further exploration of the values of the incident electric field is related to induced variations in the membrane potential, which between 0.2 V and 1 V are enough to trigger the electroporation of the cell membrane [8, 83]. According to the numerical results shown in Fig. 5.9d-f, in order to induce a membrane potential of 500 mV in the forty-five simultaneously exposed cells, the incident electric field should have a magnitude of approximately 30 kV/m at low frequency (< 100-500 kHz), 167 kV/m at 5 MHz and almost 4 MV/m at 5 GHz. These results are in agreement with the values typically used in electroporation and electrofusion [8, 82]. Moreover, they provide numerical support to the (reported as surprisingly) high field intensity of 40 kV/m at 5 MHz to which fibroblastic cells can be exposed during several days [81]. Although numerical results also corroborate the experimental evidence according to which the magnitude of these incident electric fields depends on the position of the surrounding cells, the number of cells, and the orientation of the field [8], the evaluation of the additional influence of the cell-to-cell interactions should be accompanied by the quantification of the temperature increase that poses limits to the exposure [81].

In addition, the induced membrane potential along the cell circumferences following a cosinusoidal-like shape modified by the presence of the cell-to-cell interactions demonstrates that such an asymmetric distribution (positive and negative) causes hyperpolarization in one half of the cell, and depolarization in the other [4]. These two phenomena play an important role in the generation of action potentials and the transmis-
sion of electrical messages between cells, but also in the activation of voltage-controlled channels in the cell membrane, inducing several physiological effects such as affections in the concentration of ions, especially calcium [4, 14]. However, a proper quantification of the extent of both hyper- and depolarization requires including the resting membrane potential. This resting potential is paramount to accurately determinate variations in the membrane potential capable of activating ion channels, and to localize zones of cell membrane permeabilization [97], especially after considering the circular arcs in which the cell-to-cell interactions are most evident (Fig. 5.5), and the possible cases when the magnitude of the total induced membrane potential is close to the threshold value that triggers electroporation [8].

Furthermore, several types of cells have interconnections with other cells, thus creating cell networks as is the case for neurons and osteocytes [52, 131]. These interconnections are capable of modifying the electrical response of a single cell, improving the signal-to-noise ratio (higher intracellular electric fields) and equalling the membrane potential of a group of (interconnected) cells to that of a single cell [107]. However, the use of non-intersecting spheres to model a biological cell restricts the inclusion of such interconnections, especially if the minimal intercellular distance represents two cells sharing one point. Despite this limitation, the current approach is suitable to provide information on the interactions between multiple cells, in comparison with other approaches limited to one or two cells [41, 93, 96]. Moreover, the capability of the current approach to evaluate clusters of multi-layered arbitrarily-positioned spherical cells, with dispersive materials, represents an improvement over models of multiple cells recurring to transport lattices (equivalent circuits) [101] and symmetrical cell arrangements (fixed positions) [97].

A better approximation to the effect of a change in the direction of the electric field can be obtained in cells approximated using a geometry other than spherical. In general, human cells have an elongated (ellipsoidal) shape, with a major-to-minor axes ratio of about 7-10 [132]. Thus, changes in the orientation of the incident electric field are expected to cause variations in the electrical response of elongated cells with a greater degree of deviation along the two main axis parallel to the direction of field propagation than the values presented in Figs. 5.9 and 5.10 [41].

These variations, together with the analysis of cell-to-cell interactions, might lead to membrane potentials, associated with the hyper- and de-polarization of the cell membrane, that change both the opening probability of calcium channels and the distribution of surface charge, which is believed to be involved in the electrically-mediated mobilization of intracellular calcium [65, 121]. Similarly, the use of ellipsoidal shapes might lead to induced intracellular electric fields useful to elucidate the alterations of the electrical pathways (polarization effects) required to induce biochemical signalling and intracellular transport of biomolecules [94, 107, 115]. One way of performing this task is to
adapt the current approach to other systems of coordinates so as to deal with oblate and prolate spheroids [100], or recurring to parametrization, similar to that performed using a Casini oval to represent biconcave erythrocytes [95].

In any case, changing the geometry of the cell requires also the adaptation of the cobweb-meshing scheme. Although this approach enables the inclusion of the cell membrane, it limits the cell positioning so that the centers of the cells must be actual points of the mesh. This precludes the five cells shown in Fig. 5.4a to touch exactly at one point, or to be separated a distance equal to a quarter radius or half the radius. Although the error between the expected positions and those used are at most 12 % (quarter-radius case), such error can be significant in a more detailed exploration of possible cell-to-cell interactions due to careful cell positioning, such as that hypothesized for the circular arcs. This error can be also reduced to a minimum by increasing the number of divisions in the domain box, although doing so implies increasing the number of simulation points and, consequently, the computational cost.

5.8 Conclusion

This chapter has presented a simulation approach for simulating the electrical response of multiple biological cells exposed to an incident electric field. The cells are described as three-layered concentric spherical shells than can be arbitrarily positioned in a simulation domain so that the interactions between multiple non-intersecting cells can be accounted for. Although this approach is similar to that presented in Chapter 4, it reduces the formulation from Chapter 4 into one single expression suitable for calculating the electric field in every material region, but more importantly, by using an cobweb mesh-like approach, it improves the cell description by including of the cell membrane. Thus, the numerical results provide additional information about the membrane potential and membrane electric field which, together with the electric field at the interior of the cell, can be obtained in terms of the magnitude, frequency and orientation of the incident electric field. An additional feature is included to deal with frequency-dispersive materials for the different compartments.

All in all, numerical results provide insights into the electrical response of single and multiple cells exposed to an incident electric field, and reveal the existence of variations in the electrical response of the cell membrane due the cell-to-cell interactions caused by surrounding cells that depend on the position (intercellular distances) and the relative location (circular arcs) of adjacent cells. These interactions are believed to influence the cell response to specific magnitude and frequency windows of the exposure, and might be helpful in explaining the lack of reproducibility of experimental results.

Furthermore, the model provides numerical evidence that might direct the experimentalists as to where to look in the cell when it comes to possible interactions with an
incident electric field. More in detail, numerical findings suggest that the exposure to incident fields with frequencies below the cut-off frequency of the cell membrane (≈ 1 MHz) are most likely related to effects in the response of the cell membrane associated with changes in both the membrane electric potential and the membrane electric field, whereas the exposure to incident fields with frequencies above 1 MHz are most likely associated with changes in electrically-mediated biological processes occurring at the interior of the cell and governed by the intracellular electric fields.

The inclusion of frequency-dispersive materials confirms the calculations obtained with non-dispersive materials indicating that the exposure to incident fields with frequencies above 50 GHz are unlikely to cause biological effects related to variations in the intracellular electric field, especially because at this frequency the cell-to-cell interactions are virtually non-existent, a cell contingent can be reduced to an equivalent cell of adjusted size. However, at this frequency the quasi-static approximation that supports the mathematical formulation is at its limit of applicability. Thus, the exploration of possible effects at frequencies in the THz range can be more adequately conducted by resorting to a more general (full-wave) modelling approach, e.g., the one presented in the following chapter, which also allows analyzing the effect of different cell shapes (elongated cells) and non-concentric organelles (nucleus).
In recent years, the response of biological cells to the exposure to an incident electric field has captured much attention due to the beneficial effects of electrostimulation, but also has raised concerns about possible side-effects in detriment of health [3, 14, 59]. Although electric fields are used in physical therapy and clinic to increase tissue formation, reduce the wound healing time, induce pain relief and ablate undesirable tissue (tumors) [2, 67, 80], some physicians and scientist supported by the so-called group of electro-sensitive people defend the idea that the exposure to incident electric fields is related to sleep disorders, muscular and neuronal detriments, cancer and brain damage [3, 30, 133]. Unfortunately, there is no clear consensus on the matter, in general because the literature is rife with contradictory experimental conclusions with unreproducible results. Moreover, despite advances in molecular biology and improvements in experimental techniques for cell culture and cell visualization, very few details about the

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biological mechanism affected by the presence of the incident electric field are known [12, 14].

Thus, a general (multiscale) model of one or more biological cells exposed to an incident electric field may lead to the formulation of hypotheses on the interaction mechanisms that are suitable for guiding the experimental search of the underlying biological effects of the exposure [10]. However, obtaining a reliable model able to deal with the irregular shape of most biological cells is not trivial. Therefore, the cell shapes are simplified into regular shapes for which in most cases the analytical solution is attainable. As discussed in Chapters 4 and 5, layered spherical shells are a fair approximation to cells in suspension, and can provide insight to the electrical response of such geometries. However, this approach is based on the solution of the Laplace equation which, for simplicity, requires the use of concentric layers [93]. Therefore, the inclusion of cell organelles such as the cell nucleus is limited to the introduction of multiple layers, which can have different material properties and resemble inhomogeneities in the intracellular compartments [54, 93]. An alternative to deal with non-concentric organelles is a multiscale approach for the analysis of electroporation inside the cell in which both a spherical cell and a transport lattice (representing a system of electrolytes, membranes and electrodes) allow the quantification of the influence of an incident electric field in intracellular effects such as apoptosis induction [101, 134].

Additional evidence indicates that the morphology (shape) of the cells also affects their electrical response [41, 95, 96, 100]. In fact, spherical vesicles exposed to incident electric fields change into ellipsoids depending on, among other factors, the frequency of the exposure and the conductivity of the culture medium [135]. Since these vesicles are enclosures of a lipid bi-layer that resembles the cell membrane, the changes in shape observed are related to effects of the incident electric field in cell deformation, cell motility and membrane permeability [135]. Moreover, prolate (oblate) spheroids have been introduced as a more appropriate approximation to the shape of the cell. Accordingly, variations in both the membrane potential and the membrane electric field have been identified in terms of the elongation of the cell [100, 136], with consequences in the cell response especially during electroporation [8].

Other approaches to deal with non-canonical (irregular) shapes rely on the implementation of appropriate numerical methods (such as finite elements (FE), finite differences in time domain (FDTD), or integral equations (IE) [95, 96]) in which the shape of the cells poses no restrictions to the analysis. In particular, FE and FDTD methods are based on numerical schemes where the solution domain is divided into well-defined sets of points at which a local solution is generated and later used to assemble the general solution. Although these methods have been used to investigate possible interaction effects in both cells and tissues [96, 137], an inherent drawback is the computation of solutions at regions outside the objects of interest. Therefore, computing the electric
field distribution inside and outside a cell exposed to an incident electric field requires defining points inside the cell but also points outside the cell within a simulation domain. This results in large numbers of simulation points that increase the computational cost of the solution.

An alternative to avoid the requirement of points external to the cell is the use of implementations based on integral equations (IE), which can be either of volume (VIE) or surface (SIE) nature. Although both VIEs and SIEs representations have been employed to compute power absorption rates in the whole human body and sections thereof (limbs, head), and to investigate the interaction of the near-field of an antenna with biological tissues [37], VIEs are reliable in a wider range of frequencies than SIE in the sense that the size of the object (cell) can be much more smaller than the wavelength of the incident electric field. Therefore, this chapter is dedicated to presenting a simulation approach based on VIE to compute the electric field distribution in non-canonical shaped inhomogeneous cells exposed to an incident electric field. The approach is well suited to investigate the influence that the cell shape may have in possible interaction mechanisms between the incident electric field and a group of biological cells. The cells are allowed to have differences in terms of shape, location and size, resulting in ellipsoidal (elongated) arbitrarily-positioned cells with non-concentric ellipsoidal (elongated) nucleus, and different (arbitrary) sizes. These features increase the likeness in morphology of the simulated cells with respect to those in an in-vitro cell culture.

6.1 The Volume Integral Equation - VIE

The VIE model is a full-wave approach. Unlike the quasi-static approximation, this full-wave approach aims at finding the solution to the general set of Maxwell's equations given by (3.3a) - (3.3d). In principle, the electric field solution to a scattering problem (Fig. 6.1) can be expressed as the superposition of the incident $E_i$ and scattered $E_s$ fields, namely [138]

$$E = E_i + E_s.$$  \hfill (6.1)

The solution to the total field can be obtained by formulating the problem in terms of integral equations instead of differential equations, such as in (3.3a) - (3.3d). Consequently, the original scattering problem is transformed into an equivalent problem in which the inhomogeneities (caused by the presence of the scatterer) are substituted by equivalent induced polarization currents and charges [138]. Using (3.3a), (3.3b), and assuming sources at infinity, the total field is represented in terms of the equivalent
polarization \( J^P \) and magnetization \( M^P \) currents given by

\[
\nabla \times H = j\omega \varepsilon_0 \varepsilon_i E + j\omega (\varepsilon - \varepsilon_0 \varepsilon_i) \cdot E \quad \text{(6.2a)}
\]

\[
\nabla \times E = -j\omega \mu_0 \mu_i H - j\omega (\mu - \mu_0 \mu_i) \cdot H, \quad \text{(6.2b)}
\]

where \( \varepsilon, \mu \) are the constitutive properties of the scatterer with volume \( V \), as shown in (3.13a) and (3.13b), \( \varepsilon, \mu \) represent the material properties of the background medium in which the scatterer is immersed (Fig. 6.1b), \( \varepsilon_0 \) and \( \mu_0 \) are respectively the permittivity and permeability of free space, \( j \) is the imaginary unit so that \( j^2 = -1 \), \( \omega \) is the angular frequency, and \( \hat{I} \) is the identity dyadic.

The procedure of replacing the scatterer material with equivalent induced sources is known as the volumetric equivalence principle. The sources \( J^P \) and \( M^P \) radiate in the homogeneous background medium [138]. Recurring to the vector Laplacian given in (3.15), and in view of (6.1), (6.2) lead to [138]

\[
\nabla^2 E_i + k^2 E_i = 0 \quad \text{(6.3a)}
\]

\[
\nabla^2 E_s + k^2 E_s = j\omega \mu_0 J^P - \frac{\nabla \cdot J^P}{j\omega \varepsilon_0 \varepsilon_r}. \quad \text{(6.3b)}
\]

The expression in (6.3a) is the Helmholtz equation for the incident field. As mentioned in Chapter 2, its solution can be expressed in terms of a plane wave represented by [85, 89].

\[
E_i = E_0 e^{-jkr}, \quad \text{(6.4)}
\]
6.2 Solving the VIE

where \( \mathbf{k} \) is the wave vector.

The expression in (6.3b) is the Helmholtz equation for the scatterer field. The term associated with \( \mathbf{M}^P \) vanishes because in biological materials \( \mu_r \approx 1 \) and \( \mu \approx \mu_0 \) [4, 23].

The solution to this expression can be written in the form [138, 139]

\[
E_s = \omega^2 \mu_0 \int_V dV' G(\mathbf{r}, \mathbf{r}') (\mathbf{\alpha} \cdot \mathbf{D}) + \frac{1}{\varepsilon_0 \varepsilon_r} \nabla \int_V dV' G(\mathbf{r}, \mathbf{r}') \nabla' \cdot (\mathbf{\alpha} \cdot \mathbf{D}),
\]

(6.5)

where \( \mathbf{D} \) is the total electric flux density and \( \mathbf{\alpha} \) is given by

\[
\mathbf{\alpha} = \mathbf{I} - \varepsilon_0 \varepsilon_r \mathbf{\bar{\varepsilon}}^{-1},
\]

(6.6)

so that the polarization current \( \mathbf{J}^P \) reduces to

\[
\mathbf{J}^P = j \omega \mathbf{\bar{\alpha}} \cdot \mathbf{D}.
\]

(6.7)

In addition,

\[
G(\mathbf{r}, \mathbf{r}') = \frac{e^{-jk|\mathbf{r} - \mathbf{r}'|}}{4\pi |\mathbf{r} - \mathbf{r}'|},
\]

(6.8)

is the three-dimensional Green’s function that depends on both the position vector to the observation point \( \mathbf{r} \) and the position vector to the source point \( \mathbf{r}' \) [138]. Proceeding in accordance to (6.1), the total electric field reads

\[
E = E_i + \omega^2 \mu_0 \int_V dV' G(\mathbf{r}, \mathbf{r}') (\mathbf{\alpha} \cdot \mathbf{D}) + \frac{1}{\varepsilon_0 \varepsilon_r} \nabla \int_V dV' G(\mathbf{r}, \mathbf{r}') \nabla' \cdot (\mathbf{\alpha} \cdot \mathbf{D}).
\]

(6.9)

At the interface between two dielectric materials—such as those found in biological cells and tissues—the surface charge is zero and the normal component of the electric flux density is continuous. Hence, it is practical to express (6.9) solely in terms of the electric flux density \( \mathbf{D} \). Accordingly, the normalization with respect to the background permittivity \( \varepsilon_0 \varepsilon_r \) and the use of the constitutive relation given in (3.7a), yields

\[
\varepsilon_0 \varepsilon_r \mathbf{\bar{\varepsilon}}^{-1} \cdot \mathbf{D} = \mathbf{D}_i + k^2 \int_V dV' G(\mathbf{r}, \mathbf{r}') (\mathbf{\alpha} \cdot \mathbf{D}) + \nabla \int_V dV' G(\mathbf{r}, \mathbf{r}') \nabla' \cdot (\mathbf{\alpha} \cdot \mathbf{D}),
\]

(6.10)

which is the volume integral equation for the electric flux density [139, 140].

6.2 Solving the VIE

The solution of (6.10) is obtained by first discretizing the three-dimensional scatterer into a set of tetrahedral volume elements and then expressing the electric flux density as the linear combination of basis functions [139, 141]. Then, the integral equation is converted into a linear system [142], the numerical solution of which leads to the coefficients of the expansion of the electric flux density [139, 140, 141].
6.2.1 SWG basis functions

The SWG basis functions, which are named after Schaubert, Wilton and Glisson who first described them in 1984 [141], have properties adequate to solve electromagnetic scattering problems involving dielectric materials. In accordance with the concepts of discretization and interpolation, a continuous vector function such as the electric flux density $\mathbf{D}$ can be written as [139, 142]

$$
\mathbf{D} \approx \sum_{n=1}^{N} D_n \mathbf{f}_n(\mathbf{r}),
$$

where $D_n$ represents a set of $N$ coefficients, and $\mathbf{f}_n(\mathbf{r})$ are the basis functions. In the case of tetrahedral volume elements, $N$ corresponds to the number of facets and $\mathbf{f}_n(\mathbf{r})$ are the SWG basis functions associated with each facet.

Fig. 6.2a shows two tetrahedra $T_n^+$, $T_n^-$ that share a $n$-th facet, the area of which is denoted as $a_n$. The choice of either positive or negative index is related to the direction of the vector normal to this shared $n$-th facet [141]. Accordingly, the basis function therein is

$$
\mathbf{f}_n(\mathbf{r}) = \begin{cases} 
\frac{a_n}{3V_n} \mathbf{r}_n^+, & \mathbf{r}^+ \in T_n^+ \\
\frac{a_n}{3V_n} \mathbf{r}^-, & \mathbf{r}^- \in T_n^- \\
0, & \text{otherwise},
\end{cases}
$$

where $\mathbf{r}_n^+ = \mathbf{r}^+ - \mathbf{r}_f^+$, $\mathbf{r}_n^- = \mathbf{r}^- - \mathbf{r}_f^-$ are the position vectors of any point inside $T_n^+$, $T_n^-$, with respect to the free vertexes $v_f^+$, $v_f^-$. These free vertexes correspond to the vertexes not coplanar with the (shared) $n$-th facet (Fig. 6.2b). The vectors $\mathbf{r}^+$, $\mathbf{r}^-$ and $\mathbf{r}_f^+$, $\mathbf{r}_f^-$ are
given with respect to the origin of coordinates \( O \). \( V_n^+, V_n^- \) are the volumes of tetrahedra \( T_n^+, T_n^- \) respectively.

In addition, the normal component of \( f_n(r) \) is non-zero only across the \( n \)-th facet with which it is associated. In fact, when considering \( T_n^+ \), the normal component of \( \rho_n^+ \) is equal to the height \( h_n \). Since the volume of a tetrahedron is given by \( V = \frac{1}{3} a_n h_n \), it results in [141]

\[
\hat{n} \cdot f_n(r) = \begin{cases} +1, & r^+ \in T_n^+ \\ -1, & r^- \in T_n^- \end{cases},
\]

(6.13)

with the normal vector pointing outwards \( T_n^\pm \). Hence, the normal component of \( f_n(r) \) across the \( n \)-th facet is constant and continuous across the facet. It thus provides the necessary support to satisfy the boundary condition for dielectric materials given in (3.6c).

Furthermore, since the SWG functions are defined for pairs of tetrahedra and apply to a single facet, the continuity of the normal component guarantees that the field solution for these facets at the boundary of the scatterer can be obtained by defining the so-called half-SWG basis functions. These functions are SWG functions that instead of being associated with two tetrahedra are associated with one single tetrahedron, so that the terms referring to the second tetrahedra are set to zero [140, 141].

Besides, by using a representation of the gradient operator in spherical coordinates with respect to the position vector \( \rho_n^\pm \), the divergence of the SWG basis function is [141]

\[
\nabla \cdot f_n(r) = \begin{cases} \frac{a_n}{V_n}, & r^+ \in T_n^+ \\ \frac{a_n}{V_n}, & r^- \in T_n^- \end{cases}.
\]

(6.14)

This result simplifies the procedure of finding the solution to the electric flux density \( D \) from (6.10) using the Method of Moments.

### 6.2.2 The Method of Moments - MoM

The Method of Moments (MoM), also known as the method of weighted residuals, is a numerical technique used to transform the volume integral equation in (6.10) into a linear system [142]. Consider the general problem [142]

\[
\mathcal{L}\{D\} - D_i = 0,
\]

(6.15)

where \( \mathcal{L} \) is the integro-differential linear operator encompassing the terms of the electric field VIE in (6.10) that are related to the unknown total electric flux density \( D \), and \( D_i \) is the remaining known excitation term (incident field) in (6.10). Resorting to the
discretization of the electric flux density $D$ in terms of SWG functions given in (6.11),
the linearity of $\mathcal{L}$ allows to write

$$\mathcal{L}\{D\} \equiv \sum_{n=1}^{N} D_n \mathcal{L}(f_n). \quad (6.16)$$

Such an approximation induces an error or residual $R$ in the generalized problem represented by (6.15). In symbols,

$$\sum_{n=1}^{N} D_n \mathcal{L}(f_n) - D_i = R. \quad (6.17)$$

From here, the MoM aims at minimizing the residual function $R$ [142]. This is achieved by introducing the (symmetric) inner product between the basis function $f_n$ and the weighting function $f_m$, which reads [42, 142]

$$\langle f_m, f_n \rangle = \int_{T_m \cap T_n} f_m(r) \cdot f_n(r) \, dV. \quad (6.18)$$

Enforcing the projection of the residual function $R$ to be zero at every weighting function, i.e., $\langle f_m, R \rangle = 0$, yields

$$\sum_{n=1}^{N} D_n \langle f_m, \mathcal{L}(f_n) \rangle = \langle f_m, D_i \rangle, \quad (6.19)$$

which for $N$ weighting functions results in the $N \times N$ matrix equation $A \cdot x = b$ with matrix elements [140] given by

$$A_{m,n} = \langle f_m, \mathcal{L}(f_n) \rangle, \quad (6.20)$$

and the right-hand side vector elements given by

$$b_m = \langle f_m, D_i \rangle. \quad (6.21)$$

The vector $x$ corresponds to the unknown coefficients $D_n$ introduced for the discretization of $D$. 
6.3 Numerical implementation

Accordingly, and returning to (6.10), the expression in (6.19) now expands to

\[
\sum_{n=1}^{N} \left\{ \int_{V_m \cap V_n} dV \varepsilon_0 \varepsilon_r f_m(r) \cdot \bar{e}^{-1} \cdot f_n(r) - k^2 \int_{V_m} dV \int_{V_n} dV' G(r, r') f_m(r) \cdot \bar{\alpha} \cdot f_n(r') \right. \\
- \int_{V_m} dV f_m(r) \cdot \nabla \int_{V_n} dV' G(r, r') \nabla' \cdot (\bar{\alpha} \cdot f_n(r')) \bigg\} D_n = \int_{V_m} dV f_m(r) \cdot D_i,
\]

(6.22)

where the integrals are defined on a tetrahedral domain given by \( V_m = V_m^+ \cup V_m^- \) [140] and the weighting functions are the same SWG basis functions. Such an approach is also known as Galerkin’s form [42, 142].

By using the divergence theorem given by [43]

\[
\int_{V_m} dV f_m(r) \cdot \nabla \Psi = \oint_{\partial V_m} dS \hat{n} \cdot f_m(r) \Psi - \int_{V_m} dV \nabla \cdot f_m(r) \Psi,
\]

(6.23)

the equivalent MoM discretized form of (6.10) reads

\[
\sum_{n=1}^{N} \left\{ \int_{V_m \cap V_n} dV \varepsilon_0 \varepsilon_r f_m(r) \cdot \bar{e}^{-1} \cdot f_n(r) - k^2 \int_{V_m} dV \int_{V_n} dV' G(r, r') f_m(r) \cdot \bar{\alpha} \cdot f_n(r') \right. \\
- \int_{S_m} dS \hat{n} \cdot f_m(r) \int_{V_n} dV' G(r, r') \nabla' \cdot (\bar{\alpha} \cdot f_n(r')) \\
+ \int_{V_m} dV \nabla \cdot f_m(r) \int_{V_n} dV' G(r, r') \nabla' \cdot (\bar{\alpha} \cdot f_n(r')) \bigg\} D_n = \int_{V_m} dV f_m(r) \cdot D_i.
\]

(6.24)

6.3 Numerical implementation

The MoM equivalent form of VIE given in (6.24) was implemented in computer code written in Fortran (Intel Corporation, Santa Clara, CA, USA). At its core, the program consists of i) computing the entries of both matrix \( A \) and vector \( b \) given in (6.20) and (6.21) respectively, ii) assembling the algebraic system \( A \cdot x = b \), and iii) solving (inverting) it through LU factorization. The evaluation of the integrals in expression (6.24) was carried out by using normalized volume coordinates and Gaussian formulas.
Additional simplifications come from the application of the properties of the basis functions shown in (6.13) and (6.14) [140].

After solving (6.10), the corresponding electric field distribution inside the object can be computed using the constitutive relation shown in (3.7a) to rewrite the linear combination shown in (6.11) in terms of the electric field. Accordingly, the electric field at any point \( p \) within the object is given by

\[
\tilde{E}_p = \sum_{n=1}^{4} \bar{\varepsilon}_t^{-1} \cdot D_n f_n(r), \tag{6.25}
\]

where \( t \) denotes the tetrahedron containing the observation point \( p \), \( \bar{\varepsilon}_t^{-1} \) is the inverse permittivity tensor in tetrahedron \( t \), and \( D_n \) are the four coefficients associated with the four facets of tetrahedra \( t \), which are in turn associated with the basis functions \( f_n(r) \).

The electric field outside the object can be obtained by resorting to the source-field relationship for the electric field in an homogeneous space [138]. Accordingly, normalizing to the background medium, the solution to the Helmholtz equation shown in (6.3b) becomes

\[
\varepsilon_0 \varepsilon_r E_s = \frac{k^2}{V} \int dV' G(r, r') (\bar{\alpha} \cdot D) + \nabla \int dV' G(r, r') \nabla' \cdot (\bar{\alpha} \cdot D), \tag{6.26}
\]

which can be cast into

\[
E_s = \frac{k^2}{\varepsilon_0 \varepsilon_r} \int dV' \tilde{G}(r, r') \cdot \bar{\alpha} \cdot D. \tag{6.27}
\]

In (6.27), the term

\[
\tilde{G}(r, r') = \left( \bar{I} + \frac{\nabla \nabla}{k^2} \right) G(r, r'), \tag{6.28}
\]

corresponds to the dyadic Green’s functions [138], with \( G(r, r') \) given by (6.8).

According to (6.11), the electric flux density is a linear combination of basis functions. Hence, the expression for the scattered electric field \( E_s \) in (6.27) yields

\[
E_s = \frac{k^2}{\varepsilon_0 \varepsilon_r} \sum_{n=1}^{N} D_n \int dV' \tilde{G}(r, r') \cdot \bar{\alpha} \cdot f_n(r). \tag{6.29}
\]

For the results presented in the following section, the expression (6.29) was also used to compute the electric field inside the object. This was accomplished by dealing appropriately with the singularity of the Green’s function that occurs when the observation point is interior to the object [143].
Contrary to the implementations presented in Chapters 4 and 5, where the solutions corresponded to two-dimensional testing cases, the visualization of the current model is fully three-dimensional. Accordingly, the biological cells are represented by spheroid-like objects positioned in a three-dimensional domain. These objects are obtained by means of an automated meshing scheme devised so that the cell and nucleus position, together with the radii along any of the three dimensions, can take arbitrary values within a set of pre-defined ranges.

Hence, the objects can be arbitrarily positioned and take on a spheroidal shape that
corresponds to spheres elongated along any of the three principal axis. Furthermore, the nucleus can be non-concentric with respect to the cytoplasm, and also adopt an spheroidal shape. Thus, different shape combinations are attainable that range from the typically used cells with spherical cytoplasm and spherical nucleus (Fig. 6.3a), to cells with non-concentric nucleus (Fig. 6.3b), as well as with spheroidal cytoplasm and nucleus (Fig. 6.3c). Care is exercised to ensure that the cell nucleus is located in all cases within the cell cytoplasm, especially when the nucleus is non-concentric with respect to the cytoplasm and adopts a spheroidal shape. Additional meshing features include axis rotation so that the objects are not aligned to a given principal axis, and controlled positioning along the \(z\)-axis in order to simulate either a monolayer of confluent cells (cell centers with equal \(z\)-coordinate) or a group of cells in suspension (cell centers with arbitrary \(z\)-coordinate).

The automatically generated objects are then discretized into tetrahedra by splitting both the internal (nucleus) and the external (cytoplasm) spheroids into octets. The octets of each spheroid are defined by locating the intersections of their centered principal axes with their boundaries. Each octet is then meshed according to division rules over the principal axes and over the outer surface. Care is taken to ensure continuity of the mesh along the principal axes and to identify the set of tetrahedra corresponding to the cell nucleus, which are then associated with the corresponding material properties therein. The material properties of the remaining tetrahedra are those of the cytoplasm.

The electric field inside and outside the object is obtained by computing (6.29) in a set of points defined by a plane intersecting the center of the cells. These points are arranged in a structured two-dimensional mesh adapted to the contours of the intersected objects, which is based on the adaptive mesh presented in Chapter 5 (Fig. 5.2). Thus, each cell is defined within a cell box as shown in Fig. 6.3d and the contour of the domain is split into a number of intervals equal to the number of intervals used by tetrahedral mesh to divide the exterior of the cell. In this way, a point-coincidence criterion is defined, so that the contour of the cell (i.e., the border line created by the intersection of the plane and the cell) is divided exactly as dictated by the tetrahedral mesh, as shown in Fig. 6.3d. This same cobweb-like mesh is modified to deal with elongated cells and cells with non-concentric nucleus.

Finally, although all human cells have a cell membrane [51], the implementation of the expression in (6.24) works better when all the tetrahedra within the mesh have comparable sizes. Thus, considering that the thickness of the cell membrane (10 nm) is about 3 orders of magnitude smaller than the cell radius (10 \(\mu\)m), the inclusion of the cell membrane in the cell representation would result in a mesh with a significantly large number of tetrahedra, which increases dramatically the computational cost. However, since numerical results presented in Chapter 4 and 5 indicate that the cell membrane is negligible for the electrical response of (spherical) cells exposed to incident electric
fields with frequencies about one decade above the cut-off frequency of the cell membrane \((\approx 1 \text{ MHz})\), and since numerical evidence shows that this behaviour also holds true in ellipsoidal cells \([144]\), the current implementation is limited to investigating the electrical response of spheroidal (ellipsoidal) nucleated cells exposed to incident electric fields with frequencies above 50 MHz.

6.4 Results

6.4.1 One and two spherical cells

The numerical implementation of the VIE-based formulation presented in this chapter is validated by first considering a spherical cell of radius \(R_1 = 10 \mu\text{m}\), centered in a cubic domain of 50-\(\mu\text{m}\) side and exposed to an incident electric field (plane wave) of magnitude 1 V/m and zero frequency (static case). The wave propagates along the \(z\)-axis and is oriented along the \(x\)-axis. The relative permittivities of the extracellular medium and the cell amount to 80 and 60, respectively. The cell is discretized into 2048 tetrahedra that correspond to 4352 facets and an equal number of SWG functions. Note that the cubic domain does not have to be meshed since the solution is based on the cell (scatterer) only.

The numerical result for the electric field distribution in the \(xy\)-plane containing the center of the cell is shown in Fig. 6.4a. This distribution compares favourably with both the solution obtained using the dipole moment approach introduced in Chapter 4 and the analytical solution (Fig. 4.2a). Moreover, a plot of the magnitude of the electric field along a line running parallel to the \(x\)-axis and through the center of the cell (Fig. 6.4b) shows that the electric field inside the cell is essentially homogeneous with a magnitude of 1.09 V/m. This value compares favourably with the magnitude computed by using the equivalent dipole moment approach and the analytical solution (Fig. 4.2b). However, the curve is not completely smooth, and contains small fluctuations that deviate the intracellular electric field by a maximum of 5 mV/m (0.9\%) with respect to the constant reference value. Outside the cell the solution is smooth and continuous, as expected from Fig. 4.2.

A second validation case considers a two-layered spherical shell of external radius \(R_1 = 10 \mu\text{m}\) with a concentric nucleus represented by an sphere of radius \(R_2 = 2.5\mu\text{m}\). The cell is centered in a 50-\(\mu\text{m}\) side domain and exposed to a 1-V/m, 5-GHz incident electric field (plane wave) propagating along the \(z\)-axis and oriented along the \(x\)-axis. As shown in Chapters 4 (Fig. 4.6d) and 5 (Fig. 5.9i,j), the intracellular electric field at 5 GHz includes the electrical response of the nucleus and remains unchanged as the frequency is further increased. The material properties (relative permittivity and electrical conductivity) in the extracellular medium amount to 80 and 1.2 S/m, respectively. In the cytoplasm,
Figure 6.4: Magnitude [V/m] of the electric field on the \( xy \)-plane containing the cell center(s) (left column) and along a line running parallel to the \( x \)-axis and through the cell center(s) (right column) for a,b) a single spherical cell without a nucleus exposed to a 1-V/m static (zero frequency) electric field, c,d) a spherical cell with a concentric spherical nucleus exposed to a 1-V/m, 5-GHz incident electric field e,f) two spherical cells with a concentric nucleus exposed to a 1-V/m, 5-GHz incident electric field.
these values amount to 60 and 0.3 S/m, whereas in the nucleus these values amount to 50 and 0.3 S/m. This set of material properties is used in the remaining simulation cases presented in this chapter. The mesh for the nucleated cell contains 2366 tetrahedra (4844 facets), 469 of which are dedicated to mesh the nucleus.

The numerical result for the electric field distribution in the $xy$-plane intersecting the center of the cell is shown in Fig. 6.4c. This result compares favourably in magnitude and pattern distribution with the solution obtained when using the equivalent dipole moment approach presented in Chapter 4. The magnitude of the electric field along a line running parallel to the $x$-axis and through the center of the cell is shown in Fig. 6.4d. This plot reflects the variation in the intracellular electric field due to the presence of the cell nucleus. Inside the nucleus the curve displays fluctuations similar to those observed in the one-cell case (Fig. 6.4d). These variations amount to about $\pm 5$ mV/m the 1.15-V/m magnitude of the intranuclear electric field, a value that represents a difference of approximately 0.4 %.

A third validation case considers two cells with concentric nucleus, with the cytoplasms represented by 10-$\mu$m radius spheres and the nuclei represented by internal spheres of radius 2.5 $\mu$m. These two cells are positioned alongside the center of a cubic domain of 60-$\mu$m and separated a distance of 10 $\mu$m. The incident electric field is a 1-V/m, 5-GHz plane wave propagating along the $z$-axis and oriented along the $x$-axis. The cells are discretized into 1920 tetrahedra (960 per cell), with the nuclei accounting for 128 tetrahedra (64 per cell). The total number of facets is 3968.

The numerical result for the electric field distribution in the $xy$-plane intersecting the centers of the cells is shown in Fig. 6.4e. This result compares favourably in both the magnitude and the pattern distribution with the solution obtained using the dipole moment approach presented in Chapter 4 (Fig. 4.3e). The magnitude of the electric field along a line running parallel to the $x$-axis and through the center of the cells is shown in Fig. 6.4d. There, both the variation in the intracellular electric field due to the presence of the cell nucleus and the presence of a cell-to-cell interactions are displayed. The latter reduces the magnitude of the intracellular electric field in the section of the cytoplasm closer to the neighbor cell. This plot also compares favourably in both magnitude and pattern with that obtained using the equivalent dipole moment approximation (Fig. 4.3f), and displays fluctuations similar to those observed in the previous two cases. These fluctuations correspond to differences of about 20 mV/m (1.6 %) in the magnitude of the intranuclear electric field, which in both cells amounts in average to 1.13 V/m as indicated by the dotted lines. Note that this magnitude is 20 mV/m lower than the value registered in the single nucleated cell (Fig. 6.4d), a difference that corresponds to the variations caused by the presence of cell-to-cell interactions, as shown in Fig. 4.3f.
6.4.2 Spherical cell with non-concentric nucleus

One constraint of the approach based on the quasi-static approximation and the solution of Laplace’s equation (presented in Chapters 4 and 5) is that, no matter the number of layers the spherical shells representing the cells may have, they must be all concentric. Since biological cells do not necessarily have a concentric nucleus [51], the VIE approach turns out to be useful in obtaining the electric field distribution in cells with non-concentric nucleus.

In order to study such a type of cell, a two-layered spherical cell of 10-µm radius is centered in a 50-µm cubic domain and exposed to a 1-V/m, 5-GHz incident electric field (plane wave, z-propagating, x-directed). The nucleus therein is represented by a 2.5-µm sphere located inside the cell but not necessarily centered with it. The frequency is set to 5 GHz to obtain an intracellular electric field pattern clearly reflecting the presence of the nucleus, as concluded from the results shown in Fig. 4.6.

A cell with a concentric nucleus is first considered as a control case. This cell is discretized into 1629 tetrahedra that correspond to 3369 facets. Second, the effect of changing the position of the nucleus is evaluated by first displacing it 3.75 µm along the x-axis of the cell and towards the right-most point of the cytoplasm. Compared to the case of a cell with concentric nucleus, the center of the a 3.75-µm displaced nucleus is located midway between the surface of the cell and the surface of an otherwise concentric nucleus. The mesh therein consists of 1092 tetrahedra and 2295 facets. Third, the nucleus is displaced by an extra 3.75 µm after which the right-most point over the surface of the nucleus coincides with the surface of the cell. This displacement corresponds to the maximal possible displacement of the nucleus along the x-axis of the cell to avoid the nucleus to (partly) lie outside the cytoplasm. The mesh therein has 906 tetrahedra and 1926 facets. In the three cases, the mesh in the nucleus consists of 32 tetrahedra and 61 facets.

The numerical results are shown in Fig. 6.5 for both the electric field distribution in the xy-plane containing the center of the cell and a line running parallel to the x-axis and through the cell center. The solutions for the control case (Fig. 6.5a,d) are comparable with the results in the nucleated cell show in Fig. 6.4c-d. However, the fluctuations of the magnitude of the electric field inside the nucleus reach higher magnitudes, inducing variations up to 35 mV/m (3 %) in the average magnitude of the intranuclear electric field that in this case amounts to 1.14 V/m, as indicated by the dotted line. This magnitude is 10 mV/m lower than the magnitude registered in the nucleated cell shown in Fig. 6.4d, and is related to the differences in the number of tetrahedra used to mesh the cell in every case.

The displacement of the nucleus towards the right-most point of the cell along the x-axis has negligible effect on the electric field distribution outside the cell, except for the
6.4 Results

a) b) c) d) e) f)

Figure 6.5: Magnitude [V/m] of the electric field distribution in the $xy$-plane containing the center of a two-layered spherical cell exposed to an incident field of 1 V/m at 5 GHz and with a) concentric nucleus, b) nucleus displaced 3.75 µm along the $x$-axis of the cell and towards the right-most point of the cytoplasm, and c) nucleus displaced 7.5 µm along the $x$-axis of the cell so that the right-most point in the surface of the nucleus is also on the surface of the cell. The plots in d-f) display the magnitude of the electric field along a line that crosses the domain parallel to the $x$-axis and runs through the center of the cell. The dotted line inside the nucleus corresponds to the average value therein.

 maximal displacement case shown in Fig. 6.5f. In that case, the electric field at the surface of the cell is approximately 10 % lower than the other two cases. This value is compensated by a change in the curvature of the electric field response so that at $x = 45$ µm the magnitude is equal to that obtained at the same location in the other two cases (Figs. 6.5d-e).

Inside the cytoplasm, the displacement of the nucleus changes the distribution of the intracellular electric field. Thus, the 3.75-µm displacement of the nucleus shifts the dipole-like electric field pattern related to the presence of the nucleus. This shift causes the lobes of the curve for the electric field along the $x$-axis of the cell to change. Accordingly, the left lobe is smoothly extended whereas the right lobe is narrowed as compared to the those obtained in the concentric (control) case.

In turn, the 7.5-µm displacement induces a further shift of the dipole-like electric field pattern associated with the nucleus. This causes the right lobe of the intracellular electric field to be further extended as compared to the 3.75-µm displacement case. In contrast, the right lobe disappears. The magnitude of the electric field at the right-most
point over the nucleus surface (which is also over the cell surface) seen from outside the cell is 0.747 V/m. This value is approximately 10 % lower than that obtained in the left inflection (cytoplasm-medium change) point, which is in turn only 0.34 % higher as compared to the magnitude at the same point in the concentric (control) case. Inside the nucleus, the 7.5-µm displacement generates an (average) electric field of approximately 1.13 V/m. This magnitude is about 15 mV/m (1.3 %) lower than the average intranuclear electric field magnitude of approximately 1.14 V/m registered in the control case. In addition, the presence of distortions in the intranuclear electric field generate variations that amount to 35 mV/m (4.3 %) with respect to the average magnitude therein.

### 6.4.3 Two spherical cells with non-concentric nucleus

As shown in Fig. 6.4e,f and discussed in Chapters 4 and 5, the presence of surrounding cells induce cell-to-cell interactions that modify the electric field distribution inside and outside the cells. Thus far, these interactions have been found to be more related to the distance between adjacent cells than to the presence of the cell nucleus (Fig. 4.6a). However, the results obtained from the cell with non-concentric nucleus (Fig. 6.5c,f) indicates that the electric field inside the cell, at the cell surface, and in the surroundings of the cell surface depend on the location of the nucleus with respect to the center of the cell.

Therefore, in order to investigate how the position of the nucleus influences the cell-to-cell interactions, an arrangement of two nucleated spherical cells of 10-µm radius positioned next to each other along a line running parallel to the x-axis is analyzed. The cells are exposed to an incident (plane wave) electric field of 1 V/m at 5 GHz propagating along the z-axis and oriented along the x-axis. The nucleus of the left cell is displaced by 7.5 µm towards the right-most point over the cell surface (similar to the cell in Fig. 6.5c), while the nucleus of the right cell is displaced by 7.5 µm towards the left-most point over the cell surface. The cells are separated by 0.1 µm, a non-zero distance needed to generate the set of points for the visualization of the electric field using the cobweb-like mesh scheme. The arrangement is centered in a 50-µm side box acting as visualization domain. The tetrahedral mesh for the two cells contains a total of 1812 tetrahedra and 3852 facets, with 64 tetrahedra representing the nuclei (32 per cell).

The electric field distribution in the xy-plane containing the center of the cells is shown in Fig. 6.6a. The electric field in the region between the cells is lower than elsewhere, registering magnitudes of less than 0.8 V/m. The presence of an area registering low magnitudes of electric field, or cold region, extends into both the cytoplasms and the nuclei. In fact, the electric field for points in proximity of the neighboring cell reaches
Figure 6.6: Magnitude [V/m] of the electric field distribution in the \( xy \)-plane containing the center of two spherical cells with the nucleus sufficiently displaced from the cell center as to share one point with the cell surface and exposed to a 1-V/m, 5-GHz incident electric field oriented in the a) \( x \)-direction, b) \( y \)-direction. The magnitude of the electric field along a line running parallel to the \( x \)-axis and through the center of the cells when exposed to the a 1-V/m, 5-GHz incident electric field oriented along the c) \( x \)-direction, d) \( y \)-direction, and along the \( x \)-direction but changing the frequency to e) 500 MHz, and f) 50 MHz.
lower magnitudes than those for points distant from it. The intracellular electric field along a line running parallel to the $x$-axis and through the center of the cell is plotted in Fig. 6.6c. The magnitude of the electric field at the cold region between the cells has a minimum of 0.72 V/m. The cytoplasmatic electric field displays a pattern that in each cell is similar to that shown in Fig. 6.5c, regardless of the cell-to-cell interactions. Inside the nucleus, these interactions induce a significant variation in the electric field so that the use of a mesh with 32 tetrahedra discretizing the nucleus results in a smooth, distortion-free curve.

Changing the orientation of the incident electric field interchanges the location of those areas outside a single exposed cell with higher magnitudes of electric field (so-called hot regions) and those with low magnitudes. Therefore, the exposure of the two cells to a 1-V/m, 5-GHz incident electric field oriented along the $y$-direction results in the electric field distribution shown in Fig. 6.6b. The area between the cells now corresponds to a hot region, with magnitudes above 1.2 V/m. In addition, the electric field distribution outside the cells is such that, contrary to the exposure to a $x$-directed electric field, the maximum magnitudes are obtained towards the cell equator and the minimum towards the cell poles.

As a result, the electric field along a line running parallel to the $x$-axis and through the centers of the cells (Fig. 6.6d) has on average, a higher magnitude than that for the $x$-oriented incident field. In fact, the electric field between the two cells amounts to 1.33 V/m, a value that is maintained in the cell and decays exponentially across the nucleus until reaching 1.21 V/m at the opposite border of the nucleus. Inside the cytoplasm, the shape of the intracellular electric field curve displays an additional (exponential-like) decay, so that at the surface of the cell the magnitude lowers down to 1.12 V/m.

In Chapter 5, the intracellular electric field in spherical cells was found to be frequency-dependent (Fig. 5.9i,j). To review this behaviour with the two cells with a non-concentric nucleus, the frequency of the incident electric field is set to 500 MHz (Fig. 6.6e) and to 50 MHz (Fig. 6.6f). These two values are chosen as they provide intracellular electric fields that evidence the frequency-dependent electrical response of the nucleus (Fig. 4.6). For both these frequencies, the pattern or the intracellular electric field across the cytoplasm is similar to that obtained when the frequency is set to 5 GHz. Inside the nucleus, both the cell-to-cell interactions and the change in frequency account for a higher variation of the electric field, so that the maximum magnitude of the intranuclear electric field amounts to 1.53 V/m at 500 MHz and to 3.06 V/m at 50 MHz.

### 6.4.4 Ellipsoidal cell without nucleus

A second constraint of the quasi-static approximation approach and the solution of Laplace’s equation (Chapters 4 and 5) is that the shape of the cells is restricted to
spherical shells. Since the solution of the volume integral equation is independent of the shape of the scatterer (cell) [140, 142], the full-wave approach presented in this chapter is suitable for computing the electrical response of an ellipsoidal cell exposed to an incident electric field.

To test this, a 10-μm radius spherical representation used so far is elongated along the $x$-axis by a factor of two, such that the three radii of the resulting ellipsoid are given by $R_x = 20$ μm, $R_y = R_z = 10$ μm. This factor of two is estimated based on the shape of Chinese Hamster Ovary (CHO) plated cells [145], human bone marrow derived mesenchymal stem cells (hMSCs) undergoing osteogenic differentiation [47], and other numerical approaches to the ellipsoidal shape [41, 100, 136]. The resulting disc-like (prolate) ellipsoid is exposed to an incident electric field (plane wave) with magnitude 1 V/m and frequency 50 MHz that propagates along the $z$-axis and is oriented along the $x$-axis. The material properties (relative permittivity and electric conductivity) outside the cell are set to 80 and 1.2 S/m respectively, and to 60 and 0.3 S/m inside the cell. The cell is centered in a 50-μm side cubic domain and is discretized into 328 tetrahedra than account to 744 facets.

The result for the electric field distribution in the prolate cell, computed in the $x y$-plane containing the center of the cell, is shown in Fig. 6.7a. Accordingly, the electric field outside the cell resembles the dipole-like pattern obtained for the spherical cell with concentric nucleus (Fig. 6.4c), with the maximum electric field at the cell poles and the minimum at the cell equator. Inside the cell and around its center, the electric field is homogeneous and reaches a magnitude of 1.21 V/m. In the equatorial edges, that is, the tips of the cell, the magnitude of the intracellular electric field is 0.98 V/m and increases to 1.21 V/m when moving about 10-μm away from the cell surface along the $x$-axis of the cell and towards its center. In the polar edges, the electric field reaches a maximum magnitude of 1.28 V/m and decays to 1.21 V/m within the first 5 μm when moving along the $y$-axis of the cell and towards its center. The overall solution compares favorably with that obtained using CST, which indicates that the low number of tetrahedra used to mesh the prolate cell is enough to arrive at reliable results.

In order to identify the direction of the cell elongation that maximizes the intracellular electric field, the prolate cell is rotated an angle of $\frac{\pi}{4}$ radians counter-clockwise around the $z$-axis. The electric field distribution in the $x y$-plane containing the center of such a tilted cell is shown in Fig. 6.7b. Accordingly, the electric field pattern outside the cell has variations within a range of values equal to that obtained for the prolate cell. However, the dipole-like pattern reflects the rotation of the cell so that the zones in which these maximum and minimum magnitudes are concentrated do not correspond with the equator and the poles of the cell. Inside the cell and around its center, the magnitude of the electric field is approximately 1.46 V/m, with variations in the order of tenths of milli-volts per meter towards the regions closer to the zones of maximum
Influence of cell shape in the electrical response of biological cells

Figure 6.7: Magnitude [V/m] of the electric field distribution in the $xy$-plane containing the center of ellipsoidal cell exposed to a 1-V/m, 50-MHz incident electric field propagating along the $z$-axis and oriented along the $x$-axis. The angle (in radians) between the major axis of the ellipsoid and the electric field is a) zero (prolate cell), b) $\pi/4$ (tilted cell), and c) $\pi/2$ (oblate cell). d) The electric field distribution in a $yz$-plane containing the center of the oblate cell. The magnitude of the electric field along a line running parallel to the $x$-axis and through the center of the oblate cell exposed to a 1-V/m incident electric field with frequency e) 50 MHz, f) 500 MHz, and g) 5 GHz.
and minimum magnitudes in the extracellular media.

An additional rotation of $\frac{\pi}{4}$ radians in the counter-clockwise direction and around $z$-axis leads to an oblate cell, with the major axis parallel to the $y$-axis. The electric field distribution associated to this cell and computed across the $xy$-plane containing its center is shown in Fig. 6.7c. The electric field outside the cell recovers the dipole-like pattern obtained in the prolate cell, albeit adjusted to the particular elongation of the oblate cell (the tips corresponding with the poles). Inside the cell and around its center, the magnitude of the electric field is approximately 1.67 V/m. In the regions close to the cell surface, a maximum magnitude of 1.8 V/m at the cell poles and a minimum of 1.61 V/m at the cell equator are obtained. These magnitudes change to approximately 1.67 V/m within about 3- and 5-µm when moving towards the cell center and along the $x$- and the $y$-axis of the cell, respectively.

The intracellular electric field in the $yz$-plane containing the center of the oblate cell is shown in Fig. 6.7d. Accordingly, the electric field at every point over the cell surface has a magnitude of approximately 1.8 V/m and decays when moving radially away from the cell. Inside the cell and around its center, the magnitude of the electric field is approximately 1.67 V/m. From the cell surface and towards the cell center, a ring of about 3-µm wide is created by the decreasing magnitude that decays from 1.8 V/m down to 1.67 V/m.

These results indicate that the oblate cell maximizes the intracellular electric field as compared to the prolate and tilted cell. Thus, changing the frequency of the incident electric field to which the oblate cell is exposed, and computing the electric field along a line running parallel to the $x$-axis across the domain and through the center of the cell, results in the plots shown in Fig. 6.7e-g. These plots are similar in pattern to that obtained in the single spherical cell without nucleus (Fig. 6.4a), with the intracellular electric field constant and a magnitude inversely proportional to the frequency. Hence, at 50 MHz the intracellular electric field amounts to 1.67 V/m, at 500 MHz amounts to 1.19 V/m and at 5 GHz to 1.12 V/m. Outside the cell and at the border of the domain, the magnitude of the electric field amounts to 0.98 V/m and decays when moving towards the surface of the cell down to 0.23 V/m, 0.79 V/m and 0.83 V/m, respectively for each of the three frequencies.

### 6.4.5 Influence of the ratio of elongation

The elongation of an ellipsoidal cell can be expressed in terms of the ratio of elongation $r_e$, given by the ratio between the longest main axis and the shortest one. In the case of the oblate cell studied in the previous case, the fact that $R_x$ and $R_z$ are equal means that the ratio of elongation is determined by the relation $r_e = R_y/R_x$. This ratio corresponds to the factor of two used to obtain the initially prolate cell shown in
Fig. 6.7a. However, the value can be different from two in several types of elongated (oblate and prolate) cells, such as erythrocytes, muscle cells, retina photoreceptor cells, and even ellipsoidal-like micro-organisms like bacilli and yeasts [51, 145, 146].

Therefore, in order to address the variation in the ratio of elongation and the influence it can have in the magnitude of the intracellular electric field, the ratio of elongation of the oblate cell shown in Fig. 6.7c is set to the following values: 0.5 (prolate cell), 1 (spherical cell, control case), 1.5, 2, 5, 10, 25, 50 and 100 (extremely elongated cell). For each case, the resultant cell is exposed to a 1-V/m incident electric field (plane wave) propagating along the z-axis and oriented along the x-axis. The frequency of this field is first set to 50 MHz, then increased to 500 MHz and finally set to 5 GHz. The cell is centered in a rectangular box of 50-µm side along its x and z axis, and of $h_y$-µm side along its y axis, where

$$h_y = \begin{cases} 20r + 30, & r > 3 \\ 50, & \text{otherwise}. \end{cases}$$  \hspace{1cm} (6.30)

The mesh is adapted to the elongated shape of the cell, varying from 442 tetrahedra (997 facets) for the spherical case ($r_e = 1$) to 1232 tetrahedra (2736) for the extremely elongated case ($r_e = 100$).

The numerical results for the electric field computed at the center of the oblate cell as a function of the ratio of elongation are shown in Fig. 6.8a-c for the three frequencies. The points in the figures correspond to the values obtained at each value of $r_e$. The dashed lines correspond to the trend lines obtained by performing the regression of the values indicated by the dots. Accordingly, increasing the ratio of elongation increases the magnitude of the electric field at the center of the cell. This behaviour can be represented by a modified power-law function (see Discussion for details). This increment reaches a (virtually) unchanging state for ratios of elongation greater than 50. In fact, the values obtained for $r_e = 100$ differ from those obtained for $r_e = 50$ in less than 0.25 %, regardless of the frequency. Moreover, a change in frequency causes no effect to the pattern of the trend line other than an increase in the range of values of the magnitude of the electric field in the center of the cell. Hence, for a 50-MHz frequency, the electric field increases from 1.49 V/m when $r_e = 1$ up to 1.97 V/m when $r_e = 50$. Similarly, the electric field increases from 1.15 V/m to 1.24 V/m when the frequency is set to 500 MHz, and from 1.09 V/m to 1.14 V/m when the frequency is 5 GHz.

This frequency-dependent increment can be better observed by considering the electric field distribution in the $xy$-plane containing the center of a oblate cell with a ratio of elongation $r_e = 5$ exposed to a 1-V/m incident electric field with frequency of 50 MHz (Fig. 6.8d) and 5 GHz (Fig. 6.8e). Accordingly, the intracellular electric field has variations in the regions close to the cell surface, which are higher near the poles (tips). Around the center of the cell, however, the electric field is practically homogeneous. Outside the cell, the electric field is consistent with the dipole-like distribution,
6.4 Results

Figure 6.8: Magnitude [V/m] of the electric field at the center of an oblate cell as a function of the ratio of elongation when the frequency of the incident electric field is a) 50 MHz, b) 500 MHz, and c) 5 GHz. The electric field distribution in an oblate cell with \( r_e = 5 \) and frequency of d) 50 MHz, and e) 5 GHz.

exhibiting minimal magnitudes towards the cell equator and maximal towards the poles.

6.4.6 Ellipsoidal cell with nucleus

The numerical results shown in Fig. 6.7 suggest that the oblate shape maximizes the intracellular electric field. However, the question remains as to what shape would be best for the cell nucleus in an oblate cell to maximize the resultant intracellular electric field. To answer this question, the oblate cell shown in Fig. 6.7c \((r_e = 2)\) is endowed with a concentric nucleus that can have either a prolate, a spherical or an oblate shape. The initial radius of the nucleus is 2.5 \(\mu\)m (spherical case), and the ratio of elongation (along the \(y\)-axis) is also set to two, so that \(R_x = R_z = 2.5\mu\)m and \(R_y = 5\ \mu\)m for the prolate case. The oblate case is obtained by rotating the prolate nucleus an angle of \(\frac{\pi}{2}\) around the \(z\)-axis. The nucleated cell is exposed to a 1-V/m, 5-GHz incident electric field (plane wave, \(z\)-propagating, \(x\)-oriented) and is centered in a 50-\(\mu\)m side square.
Figure 6.9: Magnitude [V/m] of the electric field along a line running parallel to the \( x \)-axis (upper row) and through the center of an oblate cell exposed to a 1-V/m, 5-GHz incident electric field propagating along the \( z \)-axis and oriented along the \( x \)-axis and with a concentric a,d) prolate, b,e) spherical, c,f) oblate nucleus.

domain. Although the conditions for the mesh (number of divisions per cell octet and in the nucleus) are equal in the three cases, the mesh of the (oblate) cell with prolate nucleus has 1216 (2520 facets), whereas in the cell with spherical nucleus the number of tetrahedra amounts to 1336 tetrahedra (2760 facets), and to 1032 tetrahedra (2152 facets) in the case of the oblate nucleus. In the three cases, the nucleus is meshed with 32 tetrahedra.

The electric field along a line running parallel to the \( x \)-axis and through the center of the cell is show in Fig. 6.9 (upper row), for the three different shapes of nucleus. Accordingly, the pattern of the electric field in the three cases is similar to that obtained in a spherical cell with concentric (spherical) nucleus (Fig. 6.5a). Moreover, regardless of the shape of the nucleus, the magnitude of the electric field at the boundaries of the domain amounts to 0.98 V/m, whereas at the points corresponding to the surface of the cell, the magnitude amounts to 0.83 V/m. Inside the cell, the electric field at the points of inflection corresponding to the surface of the nucleus reaches magnitudes that can be fairly rounded to 1 V/m for the three types of nucleus. However, inside the nucleus, the (average) magnitude in the case of a prolate nucleus is approximately 1.14 V/m, whereas in the case of a spherical nucleus, this magnitude amounts to approximately 1.17 V/m, and to approximately 1.19 V/m for the case of the oblate nucleus.
Additional plots for the electric field along a line running parallel to the y-axis and through the center of the cell are shown in Fig. 6.9 (lower row), for the three possible shapes the nucleus can take on. The electric field at the boundary of the domain is equal for the three cases and amounts to 1.02 V/m. From this point and towards the cell, the electric field increases to an average value of 1.12 V/m at the surface of the cell, and then to approximately the same values obtained for the electric field inside the nucleus when computed along the x-axis of the cell (upper row in Fig. 6.9), despite the fluctuations. These fluctuations can be reduced by increasing the number of tetrahedra in the mesh. Although such an increase is irrelevant to draw conclusions from the results, it does represent an unnecessary increment of the computational time.

6.4.7 Two ellipsoidal cells

Although analyzing the response of a single cell is suitable for i) identifying the effect of the shape of the cell, ii) the orientation of the incident field, and iii) both the shape and location of the nucleus on the intracellular electric field, it does not allow studying the influence of the cell-to-cell interactions. To this purpose, an analysis is obtained by exposing two oblate cells to a 1-V/m, 5-GHz incident electric field (plane wave, z-propagating, x-oriented). The cells are positioned in a square box of 50-µm side in such a way that they are facing each other’s equator, with the centers aligned with respect to the x-axis and separated by a distance of 0.1 µm. An illustration of this arrangement is shown in the inset of Fig. 6.10a.

Three different conditions are analyzed: 1) cells without nucleus, 2) cells with concentric (oblate) nucleus, and 3) cells with non-concentric (oblate) nucleus. In the latter case, the nucleus is displaced by 7.5 µm along the x-axis of the cell and towards the neighboring cell, so that the nucleus and the cytoplasm share one overlapping point. The sizes, the ratio of elongation of both cell and nucleus, and the material properties are the same as in the previous simulation case. In the cells without a nucleus, the mesh is composed by a total of 656 tetrahedra and 1488 facets. In the cells with concentric nuclei, the mesh has a total of 2432 tetrahedra and 5040 facets, whereas in the cells with non-concentric nuclei the mesh has 2415 tetrahedra and 5006 facets. In the latter two cases, the nuclei account for 64 tetrahedra (32 per cell).

The electric field computed along a line running parallel to the x-axis and through the center of the cells is plotted in Fig. 6.10 (upper row). As expected from previous results, the patterns obtained are symmetrical with respect to a line running parallel to the y-axis and through the center of the domain. Regardless of the cell nucleus, the electric field at the boundary of the domain box amounts to 0.93 V/m, decaying down to 0.84 V/m when still outside the cell and increasing to 1.1 V/m at the cell surface. Across the cytoplasm, the cell-to-cell interactions cause the intracellular electric field in
both the cells without nuclei (Fig. 6.10a) and the cells with concentric (oblate) nuclei (Fig. 6.10b) to decay from a magnitude of 1.10 V/m down to 0.83 V/m. In the latter case, the presence of the nuclei increases the electric field in the cytoplasm to a maximal magnitude of about 1.17 V/m registered at the point over the surface of the nucleus distant to the neighbor cell. Inside the nucleus, the cell-to-cell interactions cause an approximately linear reduction in the magnitude of the electric field, so that at the point over the nucleus surface proximal to the neighboring cell it amounts to approximately 1.15 V/m.

For the cells with non-concentric nucleus (Fig. 6.10c), the section obtained when the cells are intersected by the $xy$-plane containing the center of the cells is equivalent to that of the two spherical cells with non-concentric spherical nucleus (Fig. 6.6a). Thus, the electric field along the $x$-axis of the cells is the same as that shown in Fig. 6.6c, with the cell-to-cell interactions causing a visible effect especially inside the nucleus. In this case, the minimum magnitude is registered in between the cells and amounts to 0.72 V/m, whereas the maximum intracellular electric field, registered at the surface of the nucleus distant to the neighboring cell, amounts to 1.11 V/m.
In addition, Fig. 6.6b shows a change in the orientation of the incident electric field that increases the intracellular electric field in two spherical cells positioned very close to one another. Such an increase is related to the fact that the change in the orientation of the field swaps the position of the hot- and cold-region of the electric field distribution outside the exposed cell. In an oblate cell without nucleus this distribution indicates that the hot region in the electric field external to the cell is located toward the poles (tips) of the cell (Fig. 6.7c). Therefore, a second configuration of two cells is proposed to take advantage of these extracellular hot-regions and maximize the intracellular electric field. These cells are positioned facing each other’s pole, with their centers aligned with respect to the x-axis, and with an intercellular distance of 0.1 µm. An illustration of this arrangement is show in the inset of Fig. 6.10d. In this case, the cells have a prolate shape, with elongation along the x-axis and ratio of elongation \( r_e = 2 \). The incident electric field corresponds to a 1-V/m, 5-GHz plane wave propagating along the z-axis.

To compensate the change in cell shape and register the maximum intracellular electric field, the field is oriented along the y-axis. This orientation guarantees that the hot regions in the extracellular electric field are concentrated towards the poles of the cells. The cells are centered in a box of 90 µm along the x-axis, and of 50 µm along the other two dimensions. Three different conditions for the nucleus are analyzed: 1) cells without nucleus, 2) cells with concentric (prolate) nucleus, and 3) cells with non-concentric (prolate) nucleus. In the latter case, the nucleus is displaced by 7.5 µm along the x-axis of the cell and towards the adjacent cell, so that the nucleus and the cytoplasm share one point. The sizes and ratio of elongation of the cell (nucleus) are the same as in the previous simulation case, only difference being the direction of the elongation. For the cells without nuclei, the mesh has a total of 656 tetrahedra and 1488 facets. For the cells with concentric nuclei, the mesh has a total of 2432 tetrahedra and 5040 facets, whereas for the cells with non-concentric the mesh has 1980 tetrahedra and 4136 facets. In all cases, the nuclei account for 64 tetrahedra (32 per cell).

The electric field computed along a line running parallel to the x-axis and through the center of the cells is plotted in Fig. 6.10 (lower row). The patterns obtained are symmetrical with respect to a line running parallel to the y-axis and through the center of the domain. Regardless of the cell nucleus, the electric field at the boundary of the domain box amounts to 1.02 V/m, increasing to approximately 1.12 V/m at the cell surface. Across the cytoplasm of the cells without nucleus and the cells with concentric nucleus, the presence of the cell-to-cell interactions induce variations in the electric field described by an increase up to a magnitude of approximately 1.18 V/m at the point over the cell surface proximal to the adjacent cell (Fig. 6.10d,e).

Similarly, the presence of the concentric (prolate) nucleus induces a maximum intracellular electric field of approximately 1.2 V/m, registered inside the nucleus (Fig. 6.10e). Inside the cells with non-concentric (prolate) nucleus, the electric field reaches a max-
imum of 1.33 V/m at the point over the surface of the cell (nucleus) closer to the adjacent cell, whereas the magnitude of the electric field at the opposite edge of the nucleus amounts to 1.21 V/m. These values are equal to those found in Fig. 6.6d, as the section obtained when intersecting the cells with a $xy$-plane containing their centers is equivalent to that of the two spherical cells with non-concentric spherical nucleus.

### 6.4.8 Five ellipsoidal cells with nucleus

A first analysis of the influence of the cell-to-cell interactions generated by multiple, simultaneously-exposed ellipsoidal cells is performed by assuming five cells arranged in a distribution that minimizes the intracellular distances. These cells correspond to prolate ellipsoids (elongated along the $x$-axis) positioned in a square box domain of 100-$\mu$m side and exposed to a 1-V/m, 5 GHz incident electric field (plane wave, $z$-propagating, $x$-oriented). The five cells are arranged in a symmetric configuration in which one cell is situated at the center of the domain, whilst the other four are centered in the corners of a square that shares its center with the domain. The center of the cells are all coplanar with respect to a $xy$-plane, thus creating a monolayer of cells.

The cells are positioned so that the minimal intercellular distance is 1.6 $\mu$m. The four external cells are provided with a non-concentric (prolate) nucleus positioned such that its surface has a point in common with the surface of its parent cell and the distance to the surface of the central cell is minimal. In contrast, the central cell has a concentric (prolate) nucleus. The sizes, the ratio of elongation of the cells (nuclei) and the material properties are the same as in the previous simulation case. The total mesh contains 4933 tetrahedra (10306 facets), 160 of which are dedicated to discretize the nuclei (32 tetrahedra per cell).

The numerical result for the magnitude of the electric field in a $xy$-plane containing the centers of cells is shown in Fig. 6.11a. The indexes therein serve to identify the cells. The magnitude of the electric field outside the cells registers higher magnitudes at the poles of the cells, and minimum magnitudes at the equator. In the region between two adjacent cells positioned with the same value in the ordinates (facing each other’s equator), the magnitude of the electric field is about 20 % lower than the magnitude of the incident electric field, whereas in the small regions between the four external cells and the central cell the magnitude of the electric field is at most 10 % higher than that of the incident electric field. Inside the cells, the cell-to-cell interactions, aided by the displacement of the nucleus in the four external cells, cause more intense variations in the intracellular electric field of the central cell (cell 5) as compared to the other cells.

As a consequence, the magnitude of the electric field observed towards the cell equator (or towards the tips in this case) is higher than that registered in the region close to the poles, generating inside this cell an electric field distribution similar to that generated
6.4 Results

Figure 6.11: Magnitude [V/m] of the electric field distribution in five ellipsoidal (prolate) nucleated cells arranged in a symmetric distribution that minimizes the intracellular distances. These cells are exposed to a 1-V/m, 5-GHz incident electric field oriented along the a) $x$-axis, and b) $y$-axis. The magnitude of the electric field along a line running parallel to the $x$-axis and through the center of each cell (normalized to the cell diameter), when the cells are exposed to a 1-V/m, 5-GHz incident electric field oriented along the c) $x$-axis, and d) $y$-axis.

in the central cell of the same type of cell arrangement using five spherical cells with spherical (concentric) nucleus (Fig. 5.4). In the four external cells, the intracellular electric field has variations with lower intensities in comparison to the central cell. In addition, the higher electric field is concentrated inside the nucleus and its close vicinity. The overall variations in the electric field are symmetrical with respect to a plane parallel to the $y$-axis and crossing through the center of the central cell.

The electric field along the $x$-axis of the five cells (normalized to the cell diameter) is plotted in Fig. 6.11c. The symmetry of the solution is evident, such that the curve for cell 3 (4) overlaps the curve for cell 1 (2). In addition, the more intense variations in the intracellular (cytoplasmatic) electric field of cell 5 generate a maximum electric field of about 1.07 V/m at a point with same $x$-coordinate as the point most proximal to the adjacent cells. Since the central cell has a concentric nucleus, the curve for its
intracellular electric field shapes accordingly, with an average internuclear electric field of approximately 1.07 V/m. The intracellular electric field in cells 1 (3) and 2 (4) displays an average magnitude of 1.04 V/m, with variations of few µV/m across the cytoplasm.

However, based on the results shown in Figs. 6.7 and 6.10 (lower row), the intracellular electric field of a configuration of ellipsoidal cells is maximized when the incident electric field is perpendicular to the axis along which the cells are elongated. Therefore, the same arrangement of five ellipsoidal cells is exposed to an incident electric field oriented along the $y$-axis. The numerical result for the magnitude of the electric field in a $xy$-plane containing the centers of cells is shown in Fig. 6.11b. Accordingly, the intracellular electric fields are higher than those obtained when the incident electric field is oriented along the $x$-direction. Furthermore, the intracellular electric field of the central cell displays a pattern with higher magnitudes at the poles and lower at the equator (tips).

Outside the cells, the electric field distribution creates regions of higher magnitude at the equator (tips) of the cells, and regions of lower magnitude at the poles. In between the external cells and the central cell, the electric field registers magnitudes at least 20 % lower than the incident electric field, whereas in the regions in between adjacent cells and with nucleus positioned at the same $y$-coordinate, the magnitude of the electric field can be 20 % higher as compared to the magnitude of the incident electric field.

Moreover, the electric field along the $x$-axis of the five cells (normalized to the cell diameter, plotted in Fig. 6.11d) reveals more intense variations in the intracellular electric field due to the presence of the cell-to-cell interactions than those obtained when the incident electric field is oriented along the $x$-axis. Accordingly, the intracellular electric field in the four external cells reaches a maximum magnitude of approximately 1.2 V/m (near the tips) and a minimum of 1.07 V/m (in the vicinity of the nucleus). In the central cell, the pattern of the intracellular electric field is similar to that of the ellipsoidal cell with concentric (ellipsoidal) nucleus shown in Fig. 6.9 (lower row), with the maximal magnitude registered inside the nucleus amounting (in average) to 1.16 V/m.

6.4.9 Five cells with nucleus and arbitrary shape, location and size

So far, the different shape-related characteristics of the VIE-based approach presented in this chapter have been used separately to identify conditions that maximize the intracellular electric field. However, a more realistic representation of cells exposed to an incident electric field requires the location, the shape and the size of both cytoplasm and nucleus to vary altogether from cell to cell. Thus, a final simulation including five cells is proposed, with the cells (nuclei) allowed to have different sizes. The cells are
arbitrarily-positioned within a square box of 100-µm and exposed to a 1-V/m, 5-GHz incident electric field (plane wave, z-propagating, x-oriented).

Both the cytoplasm and the nucleus of each cell can have an ellipsoidal shape, and the elongation (which is initially allowed along the three main axis) is set arbitrarily. The radii of the cytoplasms are set arbitrarily from the range 10-20 µm. In a first case, the nuclei are restricted to be concentric with respect to the cytoplasm. Hence, the radii of the nuclei vary arbitrarily from a minimum of 1 µm to a maximum of 10 µm.

In a second case, the nuclei are located anywhere within the cytoplasms. Therefore, to ensure that every nucleus is completely internal to its parental cell, the maximal nuclear radii is reduced to 5 µm. In both cases, the first cell is centered with the domain. The centers of all the cells are restricted to be coplanar with respect to the xy-plane, creating a monolayer of cells. For simplicity in the visualization of the results, the radius \( R_z \) of the ellipsoids representing the cytoplasms is fixed to 10 µm (cells having same height), and the displacement of the non-concentric nuclei is restricted to the x- and y-directions. The mesh for the cells with concentric nucleus has 4884 tetrahedra (10240 facets), with 232 tetrahedra dedicated to the five nuclei. In the case of cells with non-concentric nucleus, the mesh contains 4827 tetrahedra (10090 facets), with 192 tetrahedra discretizing the nuclei.

The magnitude of the electric field distribution in the xy-plane containing the centers of the cells is shown in Fig. 6.12a for the cells with concentric nucleus, and in Fig. 6.12b for the cells with non-concentric nucleus. The indexes therein serve to identify the cells. Due to the (arbitrary) positions of the cells and despite the differences in shape and size, the electric field outside the cells displays variations due to the presence of the cell-to-cell interactions that are similar to those obtained using spherical cells (Fig. 4.5c). In fact, the overall magnitude of the electric field ranges approximately between \( \pm 20 \% \) the magnitude of the incident electric field, with the low values (cold regions) concentrated at the equator of the cells and high values (hot regions) concentrated at the poles.

Inside the cells, both the nucleus and the cell-to-cell interactions account for variations of the intracellular electric field that, regardless of the position of the nucleus, are most evident in the regions where two adjacent cells are closer to one another, and hence, are especially noticeable in the central cell. The higher intracellular electric fields are observed in those cells featuring an oblate shape (cells 1 and 5 in Fig. 6.12a, and cells 4 and 5 in Fig. 6.12b), with magnitudes at most 18 % higher than the magnitude of the incident electric field. These values are confirmed by the electric field computed along a line running parallel to the x-axis and through the center of the cells, as shown in Fig. 6.12c,d. In fact, the higher intracellular electric field magnitudes are mostly confined to the nucleus, especially in those cells with an oblate shape.

In general, these intracellular electric fields display two types of patterns. In the first type (cell 2 (4) in Fig. 6.12a), the presence of the nucleus is evident and the shape of the
Figure 6.12: a, b) Magnitude [V/m] of the electric field distribution in five arbitrarily-shaped, arbitrarily-positioned cells. c,d) The magnitude of the electric field along a line running parallel to the x-axis and through the center of each cell (normalized to the cell diameter). e,f) Magnitude [V/m] of the electric field at the surface of the cells. Left column: Cells with concentric nucleus. Right column: Cells with non-concentric nucleus. The cells are exposed to a 1-V/m, 5-GHz incident electric field oriented along x-axis. The cells from each column are different in terms of shape, location and size.
curve is similar to that of a single spherical cell with (non-)concentric nucleus exposed to the incident electric field oriented along the $x$-direction (Fig. 6.5). In the other type (cell 1 (5) in Fig. 6.12a), the nucleus is barely visible (or not visible it all) so that all the distortions in the curve are caused by the presence of the cell-to-cell interactions.

The differences in the intracellular electric field in the five simultaneously-exposed cells motivated the calculation of the electric field at the surface of the cells, which is obtained by computing the expression (6.25) at the centroid of those tetrahedra with facets associated with half-SWG basis functions. The results are shown in Fig. 6.12e,f. Accordingly, the electric field at the surface of the cells displays variations that in some cases correspond with either the electrical polarization (equivalent electrical dipole moment) of the cells (cell 5, Fig. 6.12a), the cell-to-cell interactions (central cells), the internuclear distances (cell 5, Fig. 6.12b), or the oblate shape of the cytoplasm (cell 4, Fig. 6.12b), if not the combination of these factors. Regardless of their source, the magnitude of these variations ranges between 0.98 V/m and 1.12 V/m. The general distribution is symmetrical with respect to the $xy$-plane containing the centers of the cells, so that the values obtained when observing the cells from above (as in the figures) are the same as when the cells are observed from below.

6.5 Discussion

This chapter presents a mathematical approach suitable for computing the electric field distribution inside and outside biological cells when exposed to an incident electric field. Unlike the framework presented in Chapters 4 and 5, which is based on a quasi-static approximation, this approach is based on the volume integral equation for the electric flux density in an inhomogeneous scatterer (representing a cell). This full-wave approach results in a more sophisticated model able to deal with the spherical representation of a cell, but also with more realistic cell shapes including non-spherical (ellipsoidal) cytoplasms with non-spherical (ellipsoidal) non-concentric nuclei.

The model also enables the simulation of groups of cells with different attributes, such as shape, location and size (Fig. 6.12). The latter induce variations in the electric response that may be associated with biological effects. In addition, changes in the conditions of exposure such as frequency and orientation are also found to cause variations in the electrical response (Figs. 6.6 and 6.7). Therefore, the electrical response of biological cells exposed to an incident electric field depends on the adequate combination of both the attributes of the cells and the conditions of exposure. Since biological cells are living organisms that change shape, location and size, the electrical response is likely to vary from one set of cells to the other. Such variation in the attributes of the cells may provide clues to explain the lack of reproducibility between experimental results carried out in different laboratories using the same conditions of exposure.
6.5.1 Validation of the implementation: one and two spherical cells

The numerical results for one and two spherical cells (Fig. 6.4) compare favourably with both the analytical solution and the results obtained using the quasi-static approximation (Figs. 4.2 and 4.3). Specifically for the two-cell case, the results reflect the effect of the cell-to-cell interactions in the intracellular electric field. Therefore, the VIE, full-wave approach is a tool suitable for computing the electrical response of biological (spherical) cells. Since this approach is independent of the geometry of the cell, it is suitable for representing cells with more realistic non-spherical geometries.

An attribute of the solution obtained using the VIE approach is the presence of spurious fluctuations in the intracellular electric fields. In the case of a single cell without nucleus (Fig. 6.4a,b), these fluctuations cause variations of approximately 0.9 % in the (average) intracellular electric field. In the case of a single cell with nucleus (Fig. 6.4c,d), the fluctuations represent variations of approximately 0.4 %, and in the two nucleated cells the variations amount to 1.6 %. Ultimately, these percentages indicate that the presence of fluctuations do not significantly deviate the solutions from the those shown in Figs. 4.2 and 4.3.

Furthermore, in the results shown in Figs. 6.4 and 6.5, the same spherical cell with concentric nucleus is discretized using three different meshes. Namely, the cell of Fig. 6.4c,d has 2366 tetrahedra (469 in the nucleus), each of the cells of Fig. 6.4e,f has 960 tetrahedra (64 in the nucleus), and the cell of Fig. 6.5a,c has 1629 tetrahedra (32 in the nucleus). Comparing the results from these three cases, the maximal variation in the (average) intranuclear electric field is 20 mV/m (about 1.7 %) when the mesh has 960 tetrahedra. Therefore, the reduction of the number of tetrahedra in the mesh (cytoplasm and/or nucleus) yields intracellular electric fields that are still reliable and comparable with the values expected. Since such a reduction also decreases the computational cost, it has been decided to discretize the majority of the nucleated cells presented in this chapter with approximately 1000 tetrahedra (2000 facets), 32 of which are dedicated to the nucleus. Such a mesh results in an reasonable computational cost especially in the five-cell cases (Figs. 6.11 and 6.12), in which case the simulation time is 5-6 hours when using an i7-2600 CPU running at 3.40 GHz with 8 GB of memory (RAM).

Another characteristic of the implementation is that it uses a modified cobweb-like meshing scheme to create the set of coplanar points required to visualize the electric fields outside the cells (Fig. 6.3d). This meshing scheme makes use of the divisions set by the tetrahedral mesh (which are reflected onto the plane of interest) to mesh the interior of the cells (Fig. 6.3d). However, it relies in the point-coincidence criterion that preserves the continuity of the tetrahedral mesh and provides a smooth visualization of the electric field. When dealing with more than one cell, the cell-to-cell interactions
cause greater variations in the electric field in the regions between the cells (Fig. 6.11), which can be asymmetrical if the cells are arbitrarily positioned (Fig. 6.12).

An adequate visualization of these variations requires a refinement of the cobweb-like mesh to have more points along the contour of the cells (nuclei) than those inherited from the tetrahedral mesh. Although such refinement poses no difficulty, including additional points in the contours may cause the occurrence of localized regions with low electric field magnitude at the surface of the cell (nucleus). These distortions have to do with the tetrahedral model and the singular character of the Green’s function, and are similar to those obtained when the cobweb-like mesh is replaced by an automatic (unstructured) mesh (Fig. 6.12) and do not significantly change the overall electrical response. Nevertheless, an improvement of the cobweb-like mesh approach is required to find a configuration that extends the point-coincidence criterion to the refinement of the contour lines of both cells and nuclei.

6.5.2 Non-concentric nucleus

The implementation of a spherical cell with a non-concentric nucleus reveals that the electric field outside the cell is nearly unaffected by a change in the position of the nucleus with respect to the center of the cell. In fact, the displacement of the nucleus causes a variation in the intracellular electric field that corresponds to centering the dipole-like electric field pattern associated with the presence of the nucleus (Fig. 6.4d) with the new center of the nucleus. If this new center is positioned so that the nucleus touches the surface of the cell (maximum allowed nucleus displacement), the electric field at this point is reduced by 10 % with respect to a cell with concentric nucleus. This reduction results in a higher electric field gradient at the surface of the cell that may be related to electrical processes occurring at the cell membrane such as the activation of ion channels [10, 121] and the accumulation of surface charge [59, 108].

Furthermore, the reduction of 15 mV/m in the (average) electric field inside the nucleus obtained in this case indicates that changing the center of the nucleus with respect to the center of the cell is enough to trigger variations in the intranuclear electric field values. Although the variations are small (1.3 % with respect to the concentric case), they may be responsible under certain conditions of exposure for effects in the nucleus such as DNA fragmentation [33, 58] and DNA damage. The latter only if a strong causal relation to the exposure to an incident electric (magnetic) field can be successfully established [58].

The numerical results also demonstrate that a displacement of the nucleus with respect to the cell center modifies the distribution of the intracellular electric field, which may affect cellular pathways for charged molecules and ions such as the intracellular calcium [121]. Additional numerical tests are needed to further consolidate these hypotheses.
and rule out the possible interference that a refinement of the mesh may have in both the magnitude of the electric field at the inflection points over the cell surface and the fluctuations inside the nucleus.

In this regard, it is observed that even though in the three cases shown in Fig. 6.5 the meshing conditions—that is, the number of divisions in the octets of both the cytoplasm and the nucleus—are the same, the displacement of the nucleus allows reducing the total number of tetrahedra. This is because the meshing is performed first for the nucleus and then for the cytoplasm, which is discretized and adjusted to the edges and facets of the already meshed nucleus. Repeating the simulation case of the non-concentric spherical cell using a mesh of 1212 tetrahedra (2537 facets) with 264 tetrahedra for the nucleus (results not shown), confirms that a change in the mesh has no significant effect in the overall solution other than reducing the intensity of the distortions in the intranuclear electric field. In that case, the distortions are 64% lower than when the mesh has a total of 906 tetrahedra (1926 facets), 32 dedicated to mesh the nucleus. The trade-off for this reduction, however, is an increase in about 76% in the simulation time (from 25 to 44 minutes).

6.5.3 Non-concentric nucleus and cell-to-cell interactions

The arrangement of the two cells shown in Fig. 6.6a,b was devised based on the results obtained in Chapters 4 and 5 regarding the influence of the intracellular distance in the intensity of the cell-to-cell interactions. Accordingly, these interactions reach a maximum value when the intercellular distance is minimal (Fig. 5.4a). Therefore, and based on the observations obtained from the displacement of the nucleus (as shown in Fig. 6.5c), the arrangement of two cells maximizes the cell-to-cell interactions due to the cytoplasms, but also maximizes the influence of the cell nucleus in these cytoplasmatic interactions (Fig. 6.6).

The influence of the nucleus in the magnitude of the intracellular electric field depends on the orientation of the incident electric field. In fact, the intracellular electric field is maximized when the incident electric field is oriented along the \( y \)-axis. In that case, the intracellular electric field reaches a maximum magnitude of 1.33 V/m in between the cells and a minimum of 1.12 V/m in the cytoplasm. In contrast, the exposure to an \( x \)-oriented electric field yields a maximum intracellular electric field of 1.10 V/m inside the nucleus and a minimum of 0.72 V/m in the region between the cells. The reason for the variation is that changing the orientation of the incident electric field also changes the orientation of the dipole-like electric field distribution outside the cell. Hence, the electric field outside the cell is maximal at the poles of the cell when the electric field is oriented in the \( x \)-direction, and when is oriented in the \( y \)-direction, the maximal value is located at the cell equator. This behaviour, together with the effect of displacing the
nucleus, is responsible for the different patterns of the intracellular electric fields shown in Fig. 6.6c-d.

As concluded in Section 4.9.3, the highest variations in the magnitude of the intracellular electric field of spherical cells with concentric nucleus are obtained by increasing the number of exposed cells. However, comparing the magnitudes obtained for the two spherical cells with non-concentric nucleus (Fig. 6.6) with those in five (Figs. 5.4) and forty-five cells with concentric nucleus (Figs. 5.9c and 5.10c) shows that more intense variations the intracellular electric field can be obtained by adjusting the position of the nucleus and the orientation of the electric field.

In fact, the difference in the intracellular electric field across any of the two spherical cells with non-concentric nucleus is of 34.5% when the incident electric field is oriented along the $x$-axis and of 18.8% when oriented along the $y$-axis. In turn, the magnitudes of the intracellular electric field across any of the forty-five spherical cells with concentric nucleus exposed to an incident field oriented along the $x$-direction (Fig. 5.9c) show a maximal difference of 16.5% if the nucleus is considered, and of 13.5% if the nucleus is disregarded. Changing the orientation of the incident field to the $y$-direction restricts these variations to a maximal difference of 2.6% if the nucleus is considered, and of approximately 12% if the nucleus is disregarded (Fig. 5.10c). Hence, the cell-to-cell interactions due to the exposure of only two spherical cells with non-concentric nucleus are capable of modifying the intracellular electric field to a greater extent than the interactions due to forty-five cells with (and without) concentric nucleus.

These percentages of variation change when more than one neighbor cell is considered (Fig. 6.11 and 6.12). Nevertheless, the induced gradients of electric field may be associated with the movement of intracellular molecules [94] and the electrically-mediated drift of integrins controlling cell adhesion [14]. In fact, the intensity of the variations, especially at the part of the cell closer to an adjacent (neighbor) cell, may be related to changes in the surface charge, which is considered to play an important role during cell electrotaxis [63] and the activation of voltage-controlled (calcium) channels [65, 121].

Besides, as discussed in Chapter 5, the intracellular electric field in the two cells varies with respect to the frequency of the incident electric field, as shown in Figs. 6.6d-f. In fact, the intensity of the cell-to-cell interactions is inversely proportional to the frequency, similarly to the results shown in Fig. 5.9 for the intracellular electric field in multiple simultaneously-exposed cells with concentric nucleus. Therefore, the results shown in Figs. 6.6d-f correspond to the maximization of the intracellular electric field with respect to i) the position of the cell with respect to its proximal neighbor (minimal intercellular distance), ii) the position of the nucleus and a minimal internuclear distance (measured between the center of the nucleus of two adjacent cells), iii) the orientation of the incident electric field, and iv) the frequency of the incident electric field.

In addition, since the inclusion of frequency-dispersive materials only modifies the re-
response when the frequency of the incident electric field is over 1-5 GHz (Section 5.6.3), a further maximization due to changes in the material properties may be attainable only if other sources of dispersion (such as temperature, ionic concentration or aging [123, 124]) are considered, provided they affect the response at frequencies in the MHz range.

Another option is to change the set of material properties to those that maximize the region of high electric field magnitude in a single spherical cell with concentric nucleus as listed in Table 4.2. However, the maximum intracellular electric fields shown in Figs. 6.6d-f obtained at 50 MHz, 500 MHz and 5 GHz are already 9 %, 7 % and 3 %, respectively, higher than those obtained using that set of properties (Fig. 4.9b-d). Thus, a review of the values in Table 4.2 considering cells with non-concentric nucleus would be required to obtain a possible combination of parameters that further maximizes the intracellular electric field.

In any case, the numerical results suggest that it may be possible for cells exposed to an incident electric field to reach maximum values in their intracellular electric field as high as three times the magnitude of the incident electric field (Fig. 6.6f) as a consequence of their own attributes (shape, location, size) rather than due to the material properties (Fig. 4.9b-d). Such maximum values, under given circumstances of exposure (magnitude of the incident field, number of cells, position of the cells) might be capable of inducing an electrically-mediated biological response.

### 6.5.4 Ellipsoidal cells without nucleus

In some cells, such as erythrocytes [145], neurons (dendrites) [52], muscle cells [51], and confluent (plated) in-vitro cell in general [145], the cell shape is better approximated by means of (prolate, oblate) ellipsoids. Although the effect of exposing ellipsoidal (elongated) cells to an incident electric field has been studied by adapting the solution of Laplace’s equation to different systems of coordinates [100, 145] and by recurring to shape-independent methods such as finite elements [41, 95, 97, 136], the interest has been concentrated in the influence of the cell shape in the membrane potential (membrane permeabilization) rather than on the intracellular electric field.

Since the solution of the volume integral equation is not restricted to the spherical shape, the current approach can be used to compute the electric field inside and outside an ellipsoidal cell, as shown in Fig. 6.7. There, instead of changing the orientation of the incident electric field, the cell is rotated from an initial disk-like (oblate) shape into a cigar-like (prolate) shape in order to find the shape that maximizes the intracellular electric field. Accordingly, the numerical results indicate that the intracellular electric field is maximized when the major axis of the ellipsoid is perpendicular to the orientation of the incident electric field (oblate cell).
Note that this relation can also be obtained when considering a cell elongated along the $z$-axis. In fact, elongating a cell a factor of two along the $z$-axis and exposing it to a 1-V/m, 50-MHz incident field oriented along the $x$-direction, yields an electric field distribution in the $xz$-plane containing the center of the cell equivalent to that shown in Fig. 6.7c. Likewise, the electric field distribution in the $yz$-plane containing the center of the cell is equivalent to that shown in Fig. 6.7d, albeit rotated by an angle of $\frac{\pi}{2}$ radians. Across the $xy$-plane containing the center of the cell, the electric field distribution in the $z$-elongated cell has a pattern similar to that obtained for the single spherical cell case without nucleus (shown in Fig. 6.4a). The electric field is nearly constant inside the cell at a magnitude of 1.67 V/m and resembles the dipole-like pattern outside the cell. The maximum values, located at the cell poles, are lower than the magnitude inside the cell. The same result is obtained in the $xz$-plane of the oblate cell shown in Fig. 6.7c.

Furthermore, the variations in the magnitude of the intracellular electric field observed at the regions closer to the cell surface are at most 7.8 % higher than the magnitude of the electric field around the center of the cell. Although this difference is not significant to identify the direction of cell elongation that maximizes the intracellular electric field, it can be reduced by increasing the number of tetrahedra in the mesh. In fact, using a mesh composed of 2048 tetrahedra (4352 facets) results in virtually constant intracellular electric fields. Nevertheless, increasing the mesh requires a longer computational time.

As the oblate shape generates the highest intracellular electric field, the change in the frequency of the incident electric field at 50 MHz to 500 MHz and 5 GHz is found to reduce the electric field at the center of the (oblate) cell. This behaviour is also observed in the cytoplasm of the spherical cells with concentric nucleus discussed in Chapters 4 and 5. Specifically in a scenario of forty-five spherical cells exposed to the same frequencies (Fig. 4.6), the maximal magnitudes of the cytoplasmatic electric field are for the three frequencies 1.42 V/m, 1.17 V/m and 1.12 V/m, respectively. Even though these values account for the presence of the nucleus and the cell-to-cell interactions, they are 13 %, 2 % and 1 % lower than the values obtained using a single elongated (oblate) cell without a nucleus (Fig. 6.7c). Therefore, it is concluded that such a cell exposed to an incident electric field experiences an intracellular electric field that can be higher or similar in magnitude to that experienced by any of forty-five spherical nucleated cells simultaneously exposed.

Furthermore, increasing the ratio of elongation $r_e = R_y/R_x$ results in magnitudes of the intracellular electric field that are even higher than those experienced by the forty-five cells. This trend is observed for the three frequencies of interest(Fig. 6.8a-c so that the magnitude of the intracellular electric field $E_c$ (computed at the center of the cell) can be approximated by the function

$$E_c = E_i(E_{ecc} - \alpha \cdot r^{-\beta}),$$

(6.31)
where $E_i$ is the magnitude of the incident electric field, $E_{ee}$ is the magnitude of the intracellular electric field (normalized to 1 V/m) in an extremely elongated cell ($r_e = 100$), $\alpha$ is a factor that depends on the frequency, and $\beta$ is a factor that depends on the elongation of the cell. The expression in (6.31) is obtained from the regression of the results shown in Fig. 6.8 to determine the trendlines that best adjusts to the data from each of the three different frequencies. The fit of the data is given by a minimum r-square value (coefficient of determination) of 0.93. Using these trendlines, the factor $\alpha$ is approximated to $\alpha = 3.4615 f^{-0.49}$ with $f$ the frequency of the exposure, and $\beta$ to 1.15. Replacing into (6.31) returns values with a difference of less than 2% with respect to those shown in Fig. 6.8, and hence, is an expression suitable for calculating the magnitude of the intracellular electric field in a elongated cell.

The numerical results for the elongated cell exposed to a 50-MHz incident electric field indicates that a value of $r_e > 50$ induces an intracellular electric field magnitude (at the center of the cell) that is approximately 38% higher than the maximal magnitude observed in forty-five spherical cells (Fig. 4.6). This difference demonstrates that both the shape and orientation of the cell affect the magnitude and distribution of the electric field inside the cell [136]. Furthermore, the magnitude of the intracellular electric field in these very elongated cells can be nearly twice as intense as the magnitude of the incident electric field (Fig. 6.8a). This response may have significant relevance in cells with similar elongations (such as long neurons and muscle cells), and under certain circumstances of exposure (magnitude of the incident electric field), it may be enough to induce a (non-thermal) electrically-mediated biological effect. Nevertheless, this hypothesis is proposed considering the magnitudes registered at the center of the oblate cell (Fig. 5.7a-c). Additional work is needed to evaluate whether the variations in the magnitude of the intracellular electric field in the regions close to the cell surface, especially at the poles (Fig. 5.7d), are associated with the elongation of the cell or disappear when higher numbers of tetrahedra are used to mesh the cell.

In addition, the results shown in Fig. 6.7 pose additional questions. First, whether the presence of the cell nucleus further increases the intracellular electric field in an oblate cell. Second, which shape the nucleus must possess for that phenomenon to occur. Third, how the cell-to-cell interactions modify the electric field in a given (elongated) cell. In this regard, the presence of a concentric nucleus within an elongated (oblate) cell is evaluated to determine the shape that further maximizes the intracellular electric field when exposed to a 5-GHz incident electric field. Accordingly, the magnitude of the intracellular electric field reaches a maximum value of approximately 1.19 V/m at the center of an oblate cell with concentric oblate nucleus (Fig. 6.9c,f). This value is only 1.7% (4.2%) lower than the maximum value obtained in a oblate cell with spherical (prolate) nucleus, and only 20 $\mu$m higher (1.7% difference) than the maximum value reached by any of the forty-five spherical cells shown in Fig. 4.6d. Such a small difference suggests that, at least for the case of a single cell, the shape of the nucleus plays a not so
important role in the maximization of the intracellular electric field as both the shape of the cytoplasm and the frequency of the exposure. In these cases, a value of $r_e = 2$ and the exposure to an incident electric field at 50 MHz are enough to induce a 38% higher intracellular electric field as compared to the spherical cells.

Note that the latter value was obtained during the exposure to a 50-MHz incident electric field, while the value for the oblate cell with nucleus was obtained at 5-GHz. Lowering the frequency from 5 GHz down to 50 MHz reduces the magnitude of the electric field inside the nucleus of spherical cells (Fig. 4.8). Therefore, the probability to obtain an intranuclear electric field with magnitudes that differ more than 38% with respect to the spherical cells is rather low.

### 6.5.5 Two ellipsoidal cells with and without nucleus

The shape of both cell and nucleus that maximizes the intracellular electric field is used to evaluate the influence of the cell-to-cell interactions in two elongated cells. As discussed previously, the strength of the cell-to-cell interactions depends on the intercellular distance (Fig. 5.4). Since a shorter distance increases the interactions, the intercellular distance between the two ellipsoidal cells is set to a minimum of 0.1 µm.

For the case of two ellipsoidal cells without nucleus (Fig. 6.10a) and two ellipsoidal cells with a concentric nucleus (Fig. 6.10b), the cell-to-cell interactions cause a difference in the magnitude of the intracellular electric field across the cell of 24.5%. In the case of two ellipsoidal cells with a non-concentric nucleus (Fig. 6.10c), the effect of displacing the nucleus towards the cell surface increases the difference up to 34.5%. These percentages are higher than those found in forty-five spherical cells (Fig. 5.9c) and indicate that the gradients of intracellular electric field caused by both the displacement of the nucleus and the cell-to-cell interactions may be associated with changes in intracellular electrical pathways and with alterations in the surface charge [14, 94, 121].

The presence of a non-concentric (ellipsoidal) nucleus induces higher variations in the intracellular electric field than the concentric nucleus. These variations depend on the orientation of the incident electric field, so that the same configuration nucleus-shape leads to magnitudes (in between the cells) that are approximately 30% lower than the magnitude of the incident electric field when it is oriented along the $x$-axis, or 30% higher when it is oriented along the $y$-axis (Fig. 6.10c.f).

Furthermore, the maximization of the intracellular electric field observed in a oblate cell (elongated along the $y$-axis, Fig. 6.7) is due to the combination of both the cell shape and the orientation of the incident electric field. Therefore, elongating the shape of the cell along the $x$-axis (prolate cell) can be compensated by a change in the orientation of the incident electric field. Accordingly, the exposure of a prolate cell to an incident electric field oriented along the $y$-axis yields the same intracellular electric field as obtained
in the oblate cell (Fig. 6.7c). Changes in the orientation of a cell due to biological (chemical) processes—such as cell growth and cell migration (chemotaxis)—may cause variations in the intracellular electric field during its exposure to an incident electric field.

If the change in the cell orientation results in a proper alignment with respect to the incident electric field, the intracellular electric field can be maximized and reach magnitudes that, under certain circumstances of exposure, may be able to trigger (modify) an electrically-driven biological response. This hypothesis may result useful to explain the lack of reproducibility of some experimental results [1, 10, 33]. Additional work is required to better conclude on this, especially when considering the presence multiple cell-to-cell interactions.

The intracellular electric field obtained in two ellipsoidal cells with non-concentric ellipsoidal nucleus exposed to an incident electric field oriented either along the $x$-direction (Fig. 6.10c) or the $y$-direction (Fig. 6.10f) is equivalent to that obtained in two spherical cells with spherical non-concentric nucleus exposed to the same incident fields (Figs. 6.6c and 6.6d). In consequence, the minimization of the internuclear distance is a more effective way to induce variations in the intracellular electric field than changing the shape cell (nucleus).

For the two ellipsoidal cells without a nucleus exposed to an incident electric field oriented along the $x$-axis, the magnitude of the intracellular electric field at the two points over the cell surface shows a difference of 12.7%, with the point closer to the neighbor cell having the lowest magnitude (Fig. 6.10a). In the case of two ellipsoidal cells with a non-concentric nucleus, the effect of displacing the nucleus towards the cell surface increases the difference up to 18.8% (Fig. 6.10c). Changing the orientation to the $y$-axis yields differences of 34.5% and 24.5% for the cells with (Fig. 6.10f) and without nucleus (Fig. 6.10e), respectively. In these cases, the highest magnitudes are found at the point closer to the neighboring cell. These percentages indicate that the variations in the intracellular electric field most likely correspond to the sum of, on the one hand, the contributions associated with the minimization of the intercellular distance (12.7% and 24.5% for the $x$-oriented field and the $y$-oriented field, respectively) and, on the other hand, the contributions generated by the minimization of the internuclear distance (6.1% and 10%, respectively).

An additional analysis would be required to identify the minimal value of the internuclear distance at which the effect of the non-concentric nucleus overrides the effect of the cell shape, maximizing the intracellular electric field. This is especially relevant after considering the results shown in Fig. 6.10, where both the concentric nucleus and the lack of nucleus yield the same magnitude of electric field in between the cells. However, it must be stated that a minimal internuclear distance such that used in Fig. 6.10c,f is a special condition for two adjacent cells. It may be more common to observe pairs of
cells with large internuclear distances so that the variations in the intracellular electric field are due to the effect of the cell-to-cell (cytoplasmatic) interactions, rather to the proximity of the nuclei, as obtained in the arrangements of five cells shown in Figs. 6.11 and 6.12. Nonetheless, since a specific case of minimal intercellular and internuclear distances can take place right after cytokinesis, a more careful analysis of their combined influence may provide an insight into the effect of the incident electric field on cell division [147, 148].

6.5.6 Five ellipsoidal cells with nucleus

The analysis of five ellipsoidal cells provides conclusions on the exposure of multiple ellipsoidal cells to the incident electric field and the effect of the corresponding cell-to-cell interactions. Accordingly, maximal intracellular electric fields are again obtained when the incident electric field is oriented perpendicular to the direction of cell elongation (Fig. 6.11). Furthermore, the numerical results indicate that the influence of the electric field in ellipsoidal cells is not limited to a response at each cell, but to the presence of the surrounding cells. This result is as expected from the two ellipsoidal cells (Fig. 6.10) and the spherical cells (Fig. 4.4). In fact, the variations in the intracellular electric field of the five ellipsoidal cells, especially at the central cell, indicate that the presence of cell-to-cell interactions also in elongated cells induce gradients of electric field that might be associated with changes in electrically-mediated (intra-)cellular processes [11, 59, 121].

Moreover, the cell-to-cell interactions in the five ellipsoidal cells exposed to an incident electric field oriented along the $y$-axis (Fig. 6.11b) yield average magnitudes of the intracellular electric field 6-7% lower than the (constant) magnitude found for a single ellipsoidal cell (Fig. 6.7g). Additionally, and regardless of the orientation of the incident electric field, the range of variation of the intracellular electric fields in these cells is at most 10% with respect to the intracellular electric field in a ellipsoidal cell with concentric nucleus (Fig. 6.9c,f). These differences are most likely masked by the (thermal) noise generated by regular cellular mechanisms [19, 114]. Nevertheless, it may be that further deviations caused by the presence of additional (arbitrarily positioned) surrounding cells increase the difference to more 20%. Such difference is considered sufficient to cause changes in protein electroconformation, ion (molecules) movement in both the intra- and extra-cellular compartments [1, 44], and activate field-sensitive enzymes [101].

The fact that in the central cell the nucleus is concentric constrains the intracellular distances to a value that is not enough to induce variations in the intracellular electric field as intense as those obtained in two cells with (almost touching) non-concentric nucleus (Fig. 6.10c,f). Therefore, the extent of the variations in the intracellular electric field are determined by the combination of a series of conditions. In the real world, some
of these conditions (orientation, elongation, position of the nucleus) are also determined by the type of cell, the cell cycle, and the (bio-chemical) environment [15, 50, 149], to mention a few. Hence, unless they are all adequately controlled, the probability to have simultaneously all the conditions necessary to ensure a (maximizing) response is very low (if not due to fortuity). The low repeatability of such a set of conditions may be also related to the lack of reproducibility of experimental results.

The maximizing effect of an incident electric field oriented perpendicular to the direction of cell elongation is valid as long as the cells are elongated along only one direction. Although the uni-directional elongation simplifies the analysis of ellipsoidal cells, it is important to identify the combination of incident electric field orientation and cell elongation that maximizes the intracellular electric field when the elongation occurs along two or three directions. An initial approach to this problem is presented in Fig. 6.12, in which case both cells and nuclei are allowed to have elongations along the $x$ and $y$ directions.

Accordingly, the cell (oblate) shape is the predominant factor for the maximization of the intracellular electric field. This result is supported by the rather similar electric field distribution registered in the (oblate) cell, as shown in cells 1 and 5 in Fig. 6.12a,c and cells 4 and 5 in Fig. 6.12b,d. In the latter case, the displacement of the nuclei appears to play a less significant role in the maximization of the intracellular electric field. This finding is in agreement with the results obtained in the symmetrical arrangement of five cells shown in Fig. 6.11. In addition, it indicates that displacement of the nucleus seems to take part in the maximization of the intracellular electric field when both the intercellular and the internuclear distances are minimal (Fig. 6.10c,f).

The higher intracellular electric field (especially inside the nucleus) in cell 3, Fig. 6.12c, and to a lower extent in cell 3, Fig. 6.12e, suggests that the ratio of elongation of the nucleus may be an additional attribute to be considered for the maximization of the intracellular electric field, especially for some types of cells with elongated nucleus such as epithelial (endothelial) cells and muscle cells [49, 51]. This evidence is a generalization of the idea obtained from the results shown in Fig. 6.9 using one ellipsoidal cell (nucleus) where the shape of the nucleus is less important than the shape of the cell. Instead, it may play a role in inducing variations in the intracellular electric field when acting in combination with other characteristics of the cells such as size and ratio of elongation of both cytoplasm and nucleus.

Furthermore, in those cells in which the cell nucleus is very elongated along the direction parallel to the orientation of the incident electric field (cells 1 and 5, Fig. 6.12a) the intracellular electric field is rather homogeneous and display a pattern in which the cell nucleus is not clearly identifiable. The fact that under the same conditions of exposure the nucleus of only some cells is (electrically) visible may be related to the variability in the electrical response among simultaneously-exposed cells. Moreover, both an oblate
and prolate nucleus can yield the highest magnitude of the intranuclear electric field, as seen in cells 3 and 5, Fig. 6.11b. This result emphasizes the importance of the orientation of both cytoplasm and nucleus in the possible changes that the natural movement (rotation) of a cell can induce to its electrical response. In fact, biological cells are living organisms that change in response to their intrinsic machinery and their environment.

Finally, the electric field over the surface of the cell is helpful to further quantify the variation in the electric field response of a given cell caused by the changes in the attributes of the surrounding cells. Accordingly, and regardless of the position of the nucleus within the cells, the highest electric fields are observed over the surface of the cells elongated perpendicular to the direction of the incident electric field. The changes in the magnitude of the surface electric field can be related to the polarization of the cell, the presence of the cell nucleus (internuclear distances) and the surrounding cells inducing cell-to-cell interactions. Altogether, these changes correspond to variations of 10-12 % with respect to the magnitude of the incident electric field. Although such variations may not be enough to trigger a biological response, the gradients associated could be related to electrically-driven changes at the cell membrane level (permeability) and in the density of surface charge. These changes mediate in the activation of ion (calcium) channels, the flow of ions through the cell membrane, and the integrity of the cell membrane prior to electroporation and dielectric breakdown [10, 54, 55, 121].

6.6 Perspectives

Although some particular aspects for future work have been already indicated in the Discussion, there are general aspects that deserve special elaboration.

For a start, it must be noted that a careful analysis of the influence of the cell (nucleus) size in the intracellular electric field has not been conducted. The reason is that the results obtained for the electric field inside five cells with different sizes (Fig. 6.12a,b) indicate that the size is not so relevant for the variation (maximization) of the intracellular electric field as the shape of the cytoplasm (oblate cell) and the location of the nucleus (minimal internuclear distance). Nevertheless, a thorough, future evaluation of the incidence of changing the size of both cytoplasm and nucleus is required to attain curves similar to those show in Fig. 6.8 but regarding cell (nucleus) size and to assess whether the electric fields generated in multiple simultaneously-exposed cells have any dependence on the size.

Another aspect is the technical difficulty of including the cell membrane in the full-wave VIE-based approach. The reason is that the thickness of the cell membrane (about 10-nm [100]) would require a refinement of the mesh that results in a considerably large number of tetrahedra. More tetrahedra in the mesh translates into more SWG
basis functions and consequently a higher computational time. An strategy to obtain a solution for a cell with its cell membrane within an affordable computational time can be achieved by reducing the number of basis functions needed to represent a scatterer (cell) and implementing macro-basis functions [150]. Another strategy is to represent the cell membrane in terms of a suitable boundary condition similar to that used in [96] when implementing a solution for the exposure of two irregularly-shaped cells using finite elements analysis.

The inclusion of the cell membrane is needed i) to further study the influence of the shape of both the cell and nucleus in the membrane potential, ii) to analyze the possible changes that the shape, location and size of the cells (nucleus) may have in the conformation of the circular arcs introduced in Chapter 5 (Fig. 5.5), and iii) to consider the numerical evidence given in [144, 151] according to which the cell membrane in elongated cells such as long nerve cells and muscle cells do not completely shields the cell to low-frequency incident fields as it does in a spherical cell. Despite these limitations, the current VIE approach is suitable for computing the electric field distribution in cells exposed to incident electric fields with frequencies above the cut-off frequency of the cell membrane, but ultimately, above 50 MHz as concluded in Chapter 4.

It must be highlighted that the VIE in (6.10) has no limitation to conduct simulations in scenarios when biological cells are exposed to incident electric fields with frequencies in the terahertz range. In Section 4.9.6, the quasi-static approximation is recommended to study the exposure to an incident electric field with frequency up to 50 GHz. This value was obtained based on the ratio between the wavelength associated with the frequency and the size of most biological cells. However, exposing the cell shown in Fig. 6.4c,d to an incident electric field with frequency varying from 10 GHz to 10 THz indicates that the threshold of the quasi-static approximation, given by the prevalence of significant changes in the electric field distribution using VIE as compared to those obtained in Chapters 4 and 5, is around 30 GHz (results not shown).

In fact, the shape of the curve for the intracellular electric field at 20 GHz start to change from the pattern displayed in Fig. 6.4d into a pattern similar to obtained when the incident electric field is oriented along the $y$-axis (Fig. 5.10d). These changes result in an intracellular electric field at 10 THz that is nearly constant at 0.5 V/m (with the nucleus is barely identifiable), and a surface electric field reaching magnitudes 2.5 times the magnitude of the incident electric field. Although these results seem promising in evaluating the (possible) biological effects of the exposure to incident electric fields in the terahertz regime, additional numerical testing and analysis is needed before drawing conclusions.

Even though dealing with ellipsoidal cells with non-concentric ellipsoidal nucleus is an improvement compared to the quasi-static approximations presented in Chapters 4 and 5, the VIE approach is suitable for studying the effect of more realistic (irregular) cell
shapes. Such shapes can be extracted from a collection of images obtained from plated cells using imaging technology [96]. The results from cells with these irregular shapes may provide additional information to identify specific possible points of interaction between an incident electric field and biological cells with cytoplasmatic prolongations (neurons, fibroblasts), and cavities (erythrocytes) [14, 51]. Besides, the current meshing approach can be modified to obtained geometries suitable for studying cells undergoing cell mitosis [152], multinucleated cells, and nucleated cells with additional organelles such as mitochondria [134].

Finally, the simulations presented in this chapter are limited to five simultaneously exposed cells due to the computational cost. Therefore, the implementation of cases with higher number of cells such as the forty-five cells used in Chapters 4 and 5 is in line with the implementation of a strategy to reduce the number of basis functions needed to represent a cell (macro-basis functions), thus reducing the computational cost. In these scenarios with multiple cells, additional factors such as the influence of the cell elongation along more than one (preferential direction), the incidence of the cell (nucleus) size, and the effect of changing the material properties, may require additional attention.

6.7 Conclusion

This chapter presents a numerical approach to simulate multiple simultaneously exposed cells that have different shape, location and size. Based on the solution of the volume integral equation for the electric flux density, this full-wave approach is suited to study the possible effects of exposing biological cells to incident electric fields with frequencies above 50 MHz.

The numerical results show that the presence of a non-concentric nucleus modifies the intracellular electric field in both spherical and ellipsoidal cells. Furthermore, when the internuclear distance between two nucleated cells is minimized, the intracellular electric field can reach magnitudes 30 % higher or lower than the magnitude of the incident electric field, depending on the orientation of the incident electric field. In addition, changing the shape of the cells from spheres to ellipsoids indicates that higher intracellular electric fields are obtained in ellipsoidal cells when the orientation of the field is perpendicular to the major axis of the cell. In that case, the intracellular electric field at 50 MHz can have a magnitude 38 % higher than the magnitude of the incident electric field observed in a spherical cell exposed to the same field conditions.

An evaluation of the variation of the intracellular electric field (at the center of the cell) with respect to the ratio of elongation yields magnitudes of electric field inversely proportional to the frequency of the exposure that can be as high as two times the magnitude of the incident electric field. Moreover, setting ellipsoidal cells in combination
with ellipsoidal nucleus initially indicates that the shape of the nucleus is less important
to cause variations in the intracellular electric field than changing both the shape of the
cell and the location of the nucleus within the cytoplasm. In fact, it has been identified
that the cell-to-cell interactions in such cells are the combination of minimizing both
the intercellular and the internuclear distances.

Based on the different simulation configurations, it is concluded that the intracellular
electric field depends on i) the shape of the cell, ii) the elongation of the cell, iii) the
position of the nucleus, and iv) the shape of the nucleus. These attributes of the
cells are found to determine the cell-to-cell interactions, which are the result of adding
the contribution due to the presence of neighbor cells (intracellular distances) with
the contribution due to non-concentricity of the cell nuclei (intranuclear distances). In
addition to these attributes of the cells, the electrical response is found to be mediated by
the conditions of the exposure, such as the frequency and the orientation of the incident
electric field. As a consequence, the possible interaction between an incident electric field
and biological cells may not be limited to bio-chemical electrically-mediated mechanisms
but also to alterations in the electrical response that are created by differences in the
attributes of the cells (shape, location and size of cells and nuclei) and the conditions
of exposure (magnitude, frequency and orientation of the incident electric field).
Part III

Getting advantage of the electric field
Mathematical modelling of biological systems*

In previous chapters, the numerical identification of possible causal relations between biological cells and an incident electric field has been focused on the representation of individual cells. In general, this type of representation provides information about the cell-to-cell interactions generated between multiple cells simultaneously exposed to the incident electric field, and their dependance on the attributes of the cells (shape, location, size) and the conditions of the exposure (magnitude, frequency, orientation). However, another approach to model multiple cells is to consider them as a conglomerate that can be described in terms of a concentration or density [153, 154]. The solutions of these type of models provide information about the evolution of so-called spatial-temporal patterns that can be associated with biological processes [154, 155].

In developmental biology, the use of these models has derived in two main approaches: the gradient and the reaction-diffusion models. Gradient models are mathematical formulations especially devised for chemical substances that have differences in concentration and that after some time tend to reach a homogeneous steady-state [156]. In contrast, reaction models deal with chemical interactions between substances. These interactions generate complex patterns in space and time due to the presence of terms representing transport, synthesis and degradation [154, 156]. Some of these reaction-diffusion models are suitable for describing cell behaviour with solutions showing the formation of patterns due to changes in cell density as a consequence of aggregation or repulsion between the cells, or in response to specific chemicals substances [154].

In general, the reaction-diffusion models present two types of solution: 1) spatial-temporal patterns, and 2) traveling waves [154, 156]. In 1952, the British mathematician Alan Turing showed that a reaction-diffusion model defined in a closed spatial domain, due to small perturbations in the initial conditions and the appropriate set of parameters, evolves towards heterogeneous spatial patterns nowadays called Turing instabilities [154, 156, 157]. Pattern formation is typically observed during morphogenesis (shape development) in tissues, organs and limbs, but also is characteristic of the skin patterns in animals, like the spots and stripes in tigers and zebras [154, 156, 158]. The second type of solution is the traveling wave-like pattern. This solution is suitable for describing processes involving a transition (travelling wave) from a point of equilibrium to another point where an external stimulus is applied [154, 159].

Mathematical models of the reaction-diffusion type have been used to study numerically the influence of biological and mechanical stimulus during tissue formation, wound healing, and growth [156, 160, 161]. The have also proven useful to describe the influence of electric signalling mechanisms such as the electrically-guided cell migration or electro-taxis [158]. In order to perform an initial numerical exploration to models representing a biological system—such as a population of cells—that later can react to the presence of an incident electric field, this chapter introduces two different mathematical models used in developmental biology together with a numerical approach to implement them [154, 155, 156, 162, 163]. In what follows, the details of the reaction-diffusion models are introduced together with the two mathematical models. Details about the biological content of the terms used in every formulation are given to correlate them to the biological system [153, 154, 156]. Then, a implementation strategy based on the finite elements method is summarized and details about the discretization of the models are given. Next, the numerical results are shown, providing material to discuss the possibilities for the two models presented to be used in the study of the interaction between an electric field and a group of cells.

### 7.1 The reaction-diffusion equation

The movement of a given substance (chemical, protein) or population (bacteria, cells) is denoted by \( u(\mathbf{x}, t) \) and satisfies Fick’s Law, namely [155, 156, 158]

\[
\mathbf{J}(\mathbf{x}, t) = -D \nabla u(\mathbf{x}, t),
\]

(7.1)

where \( \mathbf{J} \) is the diffusive flux vector, \( D \) is the diffusion coefficient, and \( \mathbf{x} \) is the position vector. The negative sign indicates that the movement occurs from points of higher concentration towards points of lower concentration, i.e., opposite to the direction of the gradient vector [162, 163, 164]. In addition to diffusion movement, the interplay between
two or more species denoted by \( u(\mathbf{x}, t) \) induces a reaction mechanism. The latter can be expressed by a term \( f(u, \mathbf{x}, t) \) representing the change in the concentration of \( u(\mathbf{x}, t) \) due to interaction effects such as production, proliferation, consumption, degradation, among others [156, 158]. According to the principle of conservation, the rate of change of the amount of mass contained in a volume \( V \) must equal the sum of both the total flow of mass through the volume surface \( S \) (diffusion) and the amount of mass transformed within the volume (reaction). In symbols, this reads

\[
\frac{\partial}{\partial t} \int_V u(\mathbf{x}, t) \, dV = -\int_S (\mathbf{J} \cdot \hat{n}) \, dS + \int_V f(u, \mathbf{x}, t) \, dV, \tag{7.2}
\]

where \( \hat{n} \) is the unit vector normal to the surface \( S \). Applying the divergence theorem and combining (7.1) with (7.2) yields

\[
\frac{\partial}{\partial t} \int_V u(\mathbf{x}, t) \, dV = \int_V \nabla \cdot (D \nabla u(\mathbf{x}, t)) \, dV + \int_V f(u, \mathbf{x}, t) \, dV, \tag{7.3}
\]

which can be written in differential form as [156]

\[
\frac{\partial u(\mathbf{x}, t)}{\partial t} = \nabla \cdot (D \nabla u(\mathbf{x}, t)) + f(u, \mathbf{x}, t), \tag{7.4}
\]

an expression known as the reaction-diffusion equation. This equation is useful to represent the formation of spatial-temporal patterns describing the behaviour of the species denoted by \( u(\mathbf{x}, t) \) when interacting within the volume \( V \) [156, 157, 162].

### 7.2 Biological models

There exist different models formulated in terms of reaction-diffusion equations that are suitable to describe numerically complex biological phenomena occurring in nature [153, 154, 155, 162, 163]. Good examples are the glycolysis model, used to explain both the synthesis of glucose into cellular energy and the formation of morphogenetic patterns [155, 156, 162, 163], and the chemotaxis model, the solution of which depicts a traveling wave-like pattern used to describe cell migration in response to a given chemical stimulus within the surrounding environment [158, 162]. These two models yield spatial-temporal patterns suitable for the exploration of the interaction between electric fields, cells and tissues, and they meet the stability criteria defined by Turing [165].
7.2.1 Glycolysis model

In cell biology, the process by which the glucose molecule is synthesized to provide energy to the cell is called glycolysis. Roughly speaking, the process consists of a sequence of specific chemical reactions preceding the conversion of glucose into pyruvate and ATP, the latter being a metabolic exchange unit for the living organism [162]. This conversion process is mathematically described by a set of two non-dimensional reaction-diffusion equations for the glucose concentration \( u(x,t) \) and the pyruvate concentration \( v(x,t) \) given by [156, 162]

\[
\frac{\partial u}{\partial t} = D_u \nabla^2 u + \delta - ku - u^2 v \\
\frac{\partial v}{\partial t} = D_v \nabla^2 v + ku + uv^2 - v,
\]

where \( D_u, D_v \) are the diffusion coefficients for glucose and pyruvate respectively, and \( k, \delta \) are constants. The biological interpretation corresponds to the motion of the substances caused by both the diffusion mechanism regulated by the diffusion coefficients and the interactions between the substances described by the reaction terms. These interactions are given for the glucose in terms of a constant production with coefficient \( \delta \), a linear consumption with coefficient \( k \), and a non-linear consumption. Similarly, the reaction term for the pyruvate is expressed in terms of a linear production with coefficient \( k \), a non-linear activation, and a linear consumption [158, 162]

7.2.2 Chemotaxis model

Chemotaxis refers to the cell migration directed by the gradient of concentration of a certain external chemical stimulus existing in the extracellular environment, known as chemoattractant, which causes a pulling effect in the cells typically towards the site of higher chemical concentration [158, 162]. Thus, the mathematical model of chemotaxis describes the chemically-driven movement of cells as a travelling wave, the speed of which is controlled by the chemoattractant concentration [162]. Since this kind of cell movement appears concomitantly with other cellular processes such as cell division (mitosis) and cell death (apoptosis), a complete model for the cell dynamic including chemotaxis is given by the non-dimensional equations [162]

\[
\frac{\partial C}{\partial t} = \nabla \cdot [D_C \nabla C - H_C C \nabla Q] + r_C \left( 1 - \frac{C}{C_h} \right) f(Q, W_Q) - \delta_C C \\
\frac{\partial Q}{\partial t} = D_Q \nabla^2 Q + r_Q C g(C, W_C) - (\delta_Q + \delta_{QC} C) Q.
\]

where \( C(x,t) \) is the cell density and \( Q(x,t) \) is the chemoattractant concentration.
In this model, cell migration is described by the transport caused by gradients of concentration. First a diffusion term with diffusion coefficient $D_C$ represents the transport caused by the gradient of cell density. Then, an additional transport term controlled by the cell density and the chemoattractant sensitivity $H_C$ represents the pulling action of the chemoattractant. The negative sign indicates a pulling effect against the chemoattractant concentration gradient, i.e., towards the site of higher concentration.

The cell density reaction term is given by a production term controlled by the coefficient $r_C$. This term includes the growth of the cell population due to cell proliferation, represented by the logistic function $(1 - C/C_h)$ where $C_h$ is the equivalent to the carrying capacity [158]. This function behaves like a sigmoid curve, resulting in an initial low proliferation rate that increases with time up to the carry capacity. The activation function $f(Q,W_Q)$ represents a minimum concentration of chemical needed to initiate cell mitosis, and the additional linear term with coefficient $\delta_C$ represents the cell death.

Likewise, the chemoattractant dynamics are given in terms of a diffusion term with diffusion coefficient $D_Q$ and a reaction term comprised of a production term and a degradation term. The production term is controlled by the coefficient $r_Q$ and includes the activation function $g(C,W_C)$ representing the minimum chemical concentration needed for the cells to initiate chemical synthesis [162]. The degradation term is given by both the natural chemical degradation with coefficient $\delta_Q$ and the consumption of chemical required by the cells to migrate and proliferate, controlled by the coefficient $\delta_{QC}$ [162].

The activation functions $f(Q,W_Q)$, $g(C,W_C)$ in equations (7.6) refer to a type of logistic functions used to activate (or inhibit) specific terms of the formulation with respect to the threshold value of the control variable, in this case identified by $W_C$ and $W_Q$ [158, 162]. In its general form, these functions are expressed as

$$f(K,W_K) = \frac{\alpha^K}{K^p + W_K^p},$$

(7.7)

where $W$ is the control variable, $W_K$ is the control threshold value, $p$ is a parameter related to the slope of the function at the threshold value $W_K$, and $\alpha$ determines whether the function behaves as activator or inhibitor. Hence, if $\alpha = K$ then $f(K,W_K)$ is an activation function for values of $K$ greater than $W_K$. Otherwise, if $\alpha = W_K$ then $f(K,W_K)$ is an inhibition function for values of $K$ greater than $W_K$. A graphical representation of these behaviors can be seen in Fig. 7.1 for three different values of the slope parameter $p$. 
Figure 7.1: Graphical representation of the behavior of a) the activation function and b) the inhibition function. Threshold value \( W_K \) is 0.5. Dashed line, \( n = 5 \). Dotted line, \( n = 10 \). Continuous line, \( n = 20 \).

7.3 The Finite Elements Method - FEM

The finite elements method (FEM) is a numerical approach to the solution of partial differential equations. The basic assumption behind the method is that any object defining a solution domain can be divided into a set of constitutive parts or *elements* each one of them defining a subdomain. By means of a systematic discretization process that transforms the partial differential equations into a system of linear algebraic equations [156, 166, 167], the FEM can be easily implemented to find the solution of problems involving complex geometries and in a wide range of applications, which also include bioelectromagnetics [80, 154, 168, 169].

The linearization of any system of equations can be achieved by using the so-called *weighted residual method* [168, 169, 170, 171]. To elucidate, consider a linear differential
operator $L$ applied to a function $u(x)$ which yields the function $F(x)$, i.e.,

$$ L\{u(x)\} = F(x). \quad (7.8) $$

Assume that the function $u(x)$ can be approximated in terms of a linear combination of a set of basis functions $N^i(x)$ with a set of $n$ unknown coefficients $u^i$, viz.,

$$ u(x) \cong \tilde{u}(x) = \sum_{i=1}^{n} N^i(x) u^i. \quad (7.9) $$

Therefore, plugging $\tilde{u}(x)$ into (7.8) yields an approximation to $F(x)$. The approximation is then characterized by an error or residual given by

$$ R(x) = L\{\tilde{u}(x)\} - F(x) \neq 0. \quad (7.10) $$

Moreover, consider $\Omega$ the solution domain where $u(x)$ is defined, and subdivide it into a $N_e$ number of grid elements or subdomains $\Omega_e$. Therefore, by virtue of (7.10) and the assumed linearity of the operator $L$, a local residual $R_e(x)$ exists at each subdomain $\Omega_e$. Minimizing the residual at every subdomain $\Omega_e$ by introducing a set of $n$ weighting functions $W^i$, it holds true that

$$ \int_{\Omega_e} W^i(x) R_e(x) d\Omega_e = 0 \quad i = 1, 2, 3, \ldots n. \quad (7.11) $$

In the case of the reaction-diffusion equations used in biologically-inspired mathematical models such as the glycolysis and chemotaxis formulations in (7.5) and (7.6), the implementation of the FEM allows the calculation of the involved variables at every point inside the domain $\Omega$ and at each time step. Using residuals and weighting functions, the glycolysis model in (7.5)) can be rewritten at a local subdomain $\Omega_e$ as

$$ \int_{\Omega_e} W^i \frac{\partial u}{\partial t} d\Omega_e = \int_{\Omega_e} W^i \nabla \cdot (D_u \nabla u) d\Omega_e + \int_{\Omega_e} W^i (\delta - ku - u^2 v) d\Omega_e \quad (7.12a) $$

$$ \int_{\Omega_e} W^i \frac{\partial v}{\partial t} d\Omega_e = \int_{\Omega_e} W^i \nabla \cdot (D_v \nabla v) d\Omega_e + \int_{\Omega_e} W^i (ku + uv^2 - v) d\Omega_e. \quad (7.12b) $$

Applying Gauss’s theorem to the second order derivative term and writing in the form of residuals yields

$$ \int_{\Omega_e} W^i \frac{\partial u}{\partial t} d\Omega_e + \int_{\Omega_e} D_u \left( \nabla W^i \cdot \nabla u \right) d\Omega_e - \int_{\Omega_e} W^i (\delta - ku - u^2 v) d\Omega_e = 0 \quad (7.13a) $$

$$ \int_{\Omega_e} W^i \frac{\partial v}{\partial t} d\Omega_e + \int_{\Omega_e} D_v \left( \nabla W^i \cdot \nabla v \right) d\Omega_e - \int_{\Omega_e} W^i (ku + uv^2 - v) d\Omega_e = 0. \quad (7.13b) $$
where the diffusion coefficients are assumed to be constant and \( \hat{n} \cdot \nabla u = \hat{n} \cdot \nabla v = 0 \) on \( \partial \Omega_e \). The integral forms shown in (7.13) are referred to as the *weak form* of the partial differential reaction-diffusion equations of the glycolysis model [169]. A similar approach can be followed to obtained the weak form of the partial differential equations of the chemotaxis model.

Using proper summation (assembling) of the particular solutions at each subdomain \( \Omega_e \), a global representation for the domain \( \Omega \) can be obtained. By means of a suitable set of weighting functions, this global representation turns the original partial differential equations into a set of \( n \) algebraic equations with \( n \) unknown values \( u^i \) [169], which is solved iteratively as detailed in Appendix A.

### 7.4 Numerical implementation and results

The elementary matrix \( AMATRIX^{(e)} \) and the elementary \( RHS^{(e)} \) vector (see Appendix A), associated with the equivalent elementary forms of the glycolysis model (A.6) and the chemotaxis model (A.17), are implemented in a subroutine written in Fortran. This subroutine is used by the commercial FEM software Abaqus 6.11 [172] to first assemble both the global matrix \( AMATRIX^{(g)} \) and the global vector \( RHS^{(g)} \), and then solve the resulting global system (A.21) [169, 172]. Additional information such as the position and connectivity of the grid points, numerical parameters, initial and boundary conditions, and time step are defined externally and read by Abaqus during execution. The solution is finally written to a plain-text output file used for further postprocessing.

#### 7.4.1 Glycolysis model

Three test cases were analyzed using the glycolysis model in (7.5). In the first test case, the model was implemented for a bi-dimensional domain \([0, \pi] \times [0, \pi]\). Results of this implementation are shown in Figs. 7.2 and 7.3. The numerical parameters used were: \( D_u = 1.0, D_v = 0.0518, \delta = 1.75, \text{ and } k = 0.05 \) [155, 162]. The solution was obtained after 25000 iterations using a time step \( \Delta t = 0.1 \). The bi-dimensional grid consisted of 2601 nodes distributed among 2500 quadrilateral patches defined by Lagrangian bi-linear elements. Initial conditions are given by a random perturbation of 5% around the steady-state \( u_s = 0.5622, v_s = 1.75 \) [165], as shown in Figs. 7.2a and 7.3a. The flux of both substance is zero at the boundaries, i.e., \( \hat{n} \cdot \nabla u = \hat{n} \cdot \nabla v = 0 \) on \( \partial \Omega \).

Numerical results show the formation of a spot-like spatial-temporal pattern defined by zones of high and lower concentration. The fact that higher concentrations of a substance are found in zones where the other substance acquires lower concentrations and vice versa, is an indication of the production-consumption relationship between the
two substances. This relationship is denoted in the model equations (7.5) by means of the non-linear reaction terms, and more specifically, by the difference in sign. In addition, the maximum and minimum values that both substances reach after 25000 iterations are such that the average concentrations are the same initial conditions. In consequence, the reaction mechanism is such that both synthesis and consumption of a substance occur at the same rate but in an oscillatory way. This means that an increment of glucose (pyruvate) results in a consumption of pyruvate (glucose) [156].

For the second test case, a $[0,5\pi] \times [0,5\pi]$ bi-dimensional domain was used. Numerical parameters, grid, initial conditions, boundary conditions, and simulation time are left unchanged. Changing the domain size results in the spot-like spatial-temporal patterns shown in Fig. 7.4. The solution for pyruvate follows the same pattern but with opposite phase, as observed in the previous simulation case. Moreover, the average concentration

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{glycolysis_model.png}
\caption{Glycolysis model in two dimensions. Glucose concentration. a) Initial condition, b) t = 750, c) t = 1500, d) t = 2500.}
\end{figure}
of glucose after 25000 iterations is about 3.4 % higher than the initial condition of $u_s = 0.5622$ shown in Fig. 7.4a. This suggests that the reaction kinetics needed for the formation of the spot-like pattern is affected by a change in the length of the square domain. The third test case was also conducted in the bi-dimensional domain $[0, 5\pi] \times [0, 5\pi]$ but using the following set of numerical parameters: $D_u = 1.0$, $D_v = 0.08$, $\delta = 1.2$, and $k = 0.06$ [162]. The initial conditions were given as a perturbation of 5 % of the steady-state $u_s = 0.8$, $v_s = 1.2$. Grid, boundary conditions are same as in the previous two cases. The simulation time corresponds to 5000 iterations using a time step $\Delta t = 0.1$. Results shown in Fig. 7.5 correspond to the formation of stripe-like patterns in the concentration of glucose. An almost two-fold increment in the magnitude of the glucose concentration suggests that a significantly higher kinetic reaction between the substances is needed to obtain the stripe-like pattern formation. The higher values reached by the peaks of glucose concentration are the consequence of changing the
parameters $\delta$ and $k$, both controlling the kinetic reaction and the oscillatory behaviour between the production and consumption rates of glucose and pyruvate. This change leads also to a faster pattern formation compared to the two previous simulation cases. More specifically, a simulation time five times shorter is needed for the stripe-like pattern to appear. This reduction is also associated with the increased kinetic reaction between the two substances.

Finally, due to the consequent increase in concentration, the initial condition shown in Fig. 7.5a resembles an homogeneous distribution. However, zooming into the values reveals a randomly-distributed pattern similar to those in Figs. Fig. 7.2a and 7.4a.
7.4.2 Chemotaxis model

The chemotaxis model was solved for the bi-dimensional domain $[0,1] \times [0,1]$. The numerical parameters used were: $D_C = 0.001$, $D_Q = 0.001$, $H_C = 2.0$, $r_C = 3$, $r_Q = 0.001$, $C_h = 1$, $W_C = 0.1$, $W_Q = 0.0195$, $\delta_C = 0.07$, $\delta_Q = 0.05$, $\delta_{QC} = 0.05$, and $n = 30$ [162]. The grid consisted of 2500 quadrilateral patches defined by Lagrangian bilinear elements and 2601 nodes, as detailed in Appendix A [169]. The solution was obtained after 200 iterations with a time step of $\Delta t = 0.05$, for a total simulation time of 10.
The initial conditions are given by

\[ C(x, y, 0) = \begin{cases} C_0, & \text{if } x \leq x_i, y \leq y_i \\ 0, & \text{if } x > x_i, y > y_i \end{cases} \]

(7.14)

\[ Q(x, y, 0) = Q_0, \]

where \( C_0 = 1.0, Q_i = 0.2, x_i = 0.1, \) and \( y_i = 0.1 \) [162]. The flux of both the cell density and the chemotactic concentration is zero at the boundaries, i.e., \( \hat{n} \cdot \nabla C = \hat{n} \cdot \nabla Q = 0 \) on \( \partial \Omega \).

The numerical results are shown in Figs. 7.6 and 7.7. Due to diffusion, the cell density that initially is confined to a 0.1-side square as shown in Fig. 7.6a starts to migrate. This migration is represented by a spatial-temporal pattern that resembles a travelling wave. Following from the behaviour of the logistic function, after \( t = 2.5 \) the rate of cell proliferation is still low [158]. At the same time and in response to diffusion, cells are induced to move in the direction of the cell density gradient. Thus, these two effects combined explain the reduction in the cell density at the location of the initial condition, as shown in Fig. 7.6b, a reduction that is reinforced by the natural cell death term.

Migrating cells consume part of the chemoattractant available. Since this consumption occurs at a rate faster than the rate of chemoattractant production, the result is an increased reduction in the chemoattractant concentration at the region where the cells are present, as shown in Fig. 7.7b. This reduction gives rise to a gradient of chemoattractant concentration which activates the mechanism of chemotaxis, contributing to the cell migration pattern shown in Figs. 7.6c and 7.6d. The action of the natural degradation throughout the domain explains the diminution in the chemoattractant concentration at locations where no cells are present, as shown in Figs. 7.7b, 7.7c and 7.7c.

Finally, the activation of the logistic function allows a higher rate of cell proliferation that increases the cell density behind the migration front up to the value of the carrying capacity, as shown in Figs. 7.6c and 7.7d. The interplay between proliferation and cell death maintains the cell density unchanged as the cells continue to migrate.

7.4.3 Combined approach: tissue formation model

In this test case a combined approach is implemented. Accordingly, both the glycolysis model and the chemotaxis model are coupled and solved as a single set of partial differential equations. The purpose of this approach is to obtain the formation of spot-like spatial-temporal patterns in combination with the travelling wave-like cell migration front. Such a formation resembles the appearance of spots alongside a cell migration front line, an spatial-temporal pattern useful to represent tissue formation
This is accomplished by means of the non-dimensional coupling mechanism given by

$$\frac{\partial G}{\partial t} = \gamma Cv - \beta CG,$$

where $G$ is the tissue formation, $u$ is the concentration of the chemical activator, taken from the glycolysis model, $C$ is the cell density taken from the chemotaxis model, and $\gamma = 0.05$ and $\beta = 0.05$. According to (7.15), the tissue formation is described by the synthesis carried out by cells in the presence of a chemical signal, which is controlled by the coefficient $\gamma$, and a degradation due to cell shear during cell migration [174], which is controlled by the coefficient $\beta$. This coupled model is solved for the bi-dimensional domain $[0, 1] \times [0, 1]$, using a grid with 2500 quadrilateral patches defined by Lagrangian bilinear elements and 2601 nodes. Initial conditions for the equations of the glycolysis model are the same as mentioned above for the spot-like pattern implementation. For the equations of the chemotaxis model, the initial cell density is $C_0 = 1$ for all the nodes with the $x$-coordinate equal to one, i.e., the nodes belonging to the right border of the grid. $Q_0$ remains at 0.2. The flux at the boundaries is zero, i.e., $\hat{n} \cdot \nabla G = 0$ on $\partial \Omega$.

The numerical solution is obtained after 25000 iterations with a time step $\Delta t = 0.1$. 

Figure 7.6: Chemotaxis model in two dimensions. Cell density. a) Initial condition, b) $t = 2.5$, c) $t = 5$, d) $t = 7.5$. 

[154, 158, 173].
like in the glycolysis model, plus 200 iterations with a time step $\Delta t = 0.05$, like in the chemotaxis model. This definition is required, since the time needed for the appearance of the spot-like spatial-temporal pattern of the glycolysis model is larger than the simulation time needed for the travelling wave-like pattern of the chemotaxis model to cover the entire simulation domain. In order to overcome this mismatch, the first 25000 iterations are used to obtained the spot-like patterns, while both the chemotaxis model and the coupling scheme are delayed by dividing the time-scale by a factor of 1000. At time 2500, the time scaled is re-adjusted to the time parameters of the chemotaxis model, allowing the travelling wave-like pattern to start moving from right to left. The adjustment of the simulation time allows for the solution of the set of equations at two different timescales. This is practical to implement a model of biological process involving tissue formation occurring at a timescale of days or weeks that depends on the outcomes of previous events occurring at a timescale of minutes or hours [175, 176]. A more detailed description of how tissue formation depends on the time scale is presented in Chapter 10.

Results of the simulation are shown in Fig. 7.8. The tissue formation, considered as the appearance of the spot-like pattern, is concomitant with the advance of the cell migration wave-like front, in this case, travelling from right to left and along the $x$-axis. The magnitude of the spot-like formation is the same as that obtained for the
Figure 7.8: A tissue formation preliminary model using a glycolysis-chemotaxis coupled approach. Results correspond to the movement of the cells initiated at $t_p = 2500$ a) $t = t_p$, b) $t = 2.5 + t_p$, c) $t = 5 + t_p$ d) $t = 7.5 + t_p$.

solution of the glycolysis model shown in Fig. 7.4. This result suggests that coupling the spot-like formation with a travelling cell migration keeps the spot-like formation unaltered but introduces an additional control to its appearance throughout the domain, which is mediated by the cell migration front. The resultant travelling spot-like pattern formation might be suitable to describe the formation of canaliculi and osteons during woven bone formation in a fracture healing scenario [177, 178].

7.5 Discussion

In the case of the glycolysis model, the formation of the expected spatial-temporal patterns depends on the small perturbations of the steady-state, which are used as initial conditions. These perturbations lead to the formation of patterns in response to the Turing’s instabilities [166]. As shown in Figs. 7.2 and 7.3, the solution presents lo-
cal shifts between the maximum and minimum concentration values of the two coupled substances, due to the nature of the kinetic reaction that describes an oscillatory conversion [162]. Similar shifted mechanisms have been observed in several pairs of biological agents, such as predator-prey populations, growth-feeding mechanism, epidemiological distributions within an environment, among many others [157].

Besides, the selection of the numerical parameters must be consistent with the expected response. It has been observed that variations in parameter values lead to variations in the solution, as shown in Figs. 7.4 and 7.5. Moreover, numerical results show that modifications in the solution domain also affect the spatial-temporal patterns in a way that is independent on changes in the numerical parameters [155]. Interestingly, changes in the appropriate numerical parameters also might speed up the formation of specific (desirable) spatial-temporal patterns, as is the case of the stripe-like patterns shown in Fig. 7.5. Therefore, there is a trade-off between stability, spatial-temporal patterns and simulation time that depends on both the numerical parameters used and the size of the computation domain. This dependence has been investigated elsewhere to explore the formation of spatial-temporal patterns in growing domains [166, 167].

Furthermore, the formation of different spatial-temporal patterns according to changes in both the numerical parameters and the domain is a well known fact of the reaction-diffusion models [155, 166]. However, the lack of knowledge for choosing the adequate set of parameters might represent a problem for the validation of a mathematical model against the real biological problem. As a consequence, mathematical models should be understood as tools providing approximate solutions whose accuracy depends on the availability of experimental (real) data to validate the numerical findings.

Regarding the model of chemotaxis, the mathematical description is suitable to represent cellular movement transport phenomena. The travelling wave-like spatial-temporal pattern obtained is similar in behaviour to the cell migration mechanism observed towards a chemical stimulus, in this case, the chemoattractant signal [158, 162]. According to the numerical results, there is a balance between the production term for the cells (proliferation due to mitosis) and the cell death in (7.6)a. This balance leads to the appearance of a constant density of cells in the migration front. This also implies a constant chemical consumption as observed in Fig. 7.7, which can be maintained because of a secondary balance between both the chemical production and the degradation terms in (7.6)b [179]. Thus, the presence of these numerical balance provides an homogeneous pattern formation throughout the domain that preserves the wave-like nature shown in Fig. 7.6. Once again, this balance depends on the set of numerical parameters, which also need to be adjusted according to the dimensions (units) of the variables involved [160, 180].
7.6 Conclusion

The numerical results obtained after the implementation of the models presented in this chapter show that the reaction-diffusion equations are suitable to describe features from biological systems such as the formation of spot and stripe patterns that might be useful to mathematically describe some of the spatial-temporal patterns observed during tissue formation such as the spot-like osteons during bone fracture healing [177], the fibrin fibers (stripes) during blood clotting [174] and the spotted-striped vascular network formation during wound healing [181]. Furthermore, the implementation using the FEM approach allows to obtain solutions similar to those reported elsewhere and obtained with different numerical techniques [155, 156, 162, 163]. Accordingly, the numerical approach is suitable to be used to obtain the solution of other complex mathematical models with accurate results provided that the numerical parameters, the domain size and the time restrictions are well described.

Besides, the travelling wave-like cell migration front obtained after solving the chemotaxis model is not only suitable to describe the cell migration process [154, 162], but also to describe in a coupled scheme complex biological processes such as tissue formation [161, 180]. Therefore, applications of this type of coupled mechanisms have been devised. Details about the biological processes inspiring these applications, the mathematical descriptions obtained and the results of the implementation will be the matter of the following chapters.
Electrotherapy techniques are widely used for treating delayed bone unions and non-unions that appear during fracture healing [2, 182]. These techniques exploit the ability of an electric field to control bone deposition and resorption [67], and they have been proven useful to stimulate the formation of other tissues such as cartilage, ligament, muscle and skin [45, 76]. However, the dosimetry of the applied signal is still a matter of discussion and both the adequate frequency and magnitude of the electric field required to induce a given effect are not clearly defined [4, 12]. Nevertheless, it is accepted from experimental evidence that the biological effects of electrostimulation are more a consequence of the magnitude (intensity) of the stimulus rather than of the frequency [12, 67]. Moreover, since the effects of a static electric field may last for hours, days and even weeks, they may influence long-lasting biological processes such as growth development and wound healing [12]. Therefore, static electric fields are commonly used in the search of interaction pathways between the cells and the electric field [14].

Electrotaxis is the cell migration in the presence of a static or extremely low frequency (ELF) electric field [4, 45, 122]. This migration occurs along a direction parallel to the electric field vector and overrides chemical migration cues [45]. This chapter is dedicated to introduce a mathematical model for the electrotaxis in osteoprogenitor cells. The formulation is based on the chemotaxis model introduced in Chapter 7. The reliability of the model is evaluated using different electric field intensities and different configurations of both electrical and chemical stimuli, mimicking experimental setups [183, 184, 185]. Accordingly, we have found that the cell migration speed is affected by
the combination of an electrical and a chemical term. In addition, the numerical results allow to conclude that cell migration is faster when both stimuli orient cell migration coherently. In contrast, a reduced speed is obtained when the electric field vector is opposed to the direction of the chemical stimulus.

These conclusions are supported by numerical relations obtained from the results allow for the quantification of the cell migration speed under application of both a chemical and an electrical stimulus. Additional calculations for the cell colonization of a substrate also show mediation of the electric field magnitude. Therefore, the term electro-osseoconduction is introduced to account for the electrically induced cell colonization. Since numerical results compare favourably with experimental evidence, the model can be extended to other types of cells, and can be employed to explore the influence of the electric field during wound healing (see Chapter 9).

8.1 Cell migration: electrotaxis

Cells in a static electric field tend to align themselves perpendicularly to the electric field lines [45, 186]. This cell alignment is related to cell adhesion and tissue contraction events guided by polarization effects [4]. Cell movement and speed are also biased by a static electric field. Exposed cells migrate parallel to the electric field lines in response to an effect called galvanotaxis or electrotaxis. This phenomenon depends on the electric field magnitude [45, 186] and on the presence of molecular activators [185, 187]. Since a static electric field induces reorientation of cell surface and signalling molecules [186, 187], the application of such stimulus appears to be more important for cell migration than chemical and haptotactic signals [4, 186]. Indeed, the electric field overrides other cell migration cues [45] and therefore, it has been employed in cell recruitment applications such as wound healing [12].

Several cell types change their initial migration direction when an electric field is applied and even change their migration direction when the polarity of the electric field is reversed [4, 185]. Furthermore, cells derived from the same tissue can migrate in opposite directions, and even cells from the same genotype may show different behaviors. Experiments conducted with rat calvaria and human osteoprogenitor cells showed that, although cell migration in both types of cells is increased in the presence of an external electric field, the cell migration direction is opposite. Osteoprogenitor cells from rat calvaria were found to migrate towards the cathode, whereas the human osteoprogenitor cells migrated towards the anode [77]. This same difference in the cell migration direction has been observed in other types of cells such as fibroblasts, keratinocytes and endothelial cells [4]. Although it is clear that cell migration by electrotaxis is oriented towards a preferential electrode [4], the different cell migration directions, resulting when an external electric field is applied, could represent a complication in the clinical
8.1 Cell migration: electrotaxis

treatment of a specific cell or tissue type. Therefore, cell migration and electrotaxis should be measured and quantified for specific tissues and cell types in order to define the electric performance that may be expected during clinical applications.

In addition, the amount of cells recruited for cell migration depends on the magnitude of the electric field [188]. Therefore, cell electrotaxis is useful to direct cell migration but also to control the number of cells that reach a surface. In oral implantology, the term osteoconduction is used to refer the cell colonization of the non-corporeal bioactive surface of a dental implant [149, 189]. This process depends on the biocompatibility of the implant material and on the surface irregularities. Moreover, cells are able to colonize the implant surface only under certain biological conditions [190]. The presence of an electric field alters the electrical configuration of the surface by redistributing ionic heads in signalling proteins such as integrins [21]. This redistribution creates electrical forces that mediate the cell attachment to the bioactive surface, thus improving the cell colonization [4, 191]. Therefore, the number of cells arriving to a substrate might be increased by the increment of both cell migration speed and cell colonization induced by the application of a static electric field.

Although a great amount of knowledge regarding cell and tissue interactions with the electric field comes from experiments [14, 67, 77, 192], different computational approaches have been also used to obtain specific information on the interactions between cells and electric fields. A physics-oriented model for cell motion was found to be useful in controlling and planning cell motion during the exposure to an electric field [193]. A complementary approach that used modelling techniques of automatic control described cell migration by electrotaxis as an input-output controller [194]. However, although the results of these models are in agreement with experimental observations, neither the cell density nor the dynamics of the biochemical mechanisms involved during the biological response were considered.

A description for the cell density is included in a model of cell receptors used to investigate the electro-chemical sensing [195]. Nevertheless, this model focuses on cell orientation rather than cell migration and the influence of the chemical environment. Although these features have been tackled in several numerical frameworks describing cell behaviour, tissue formation and wound healing [196, 197], the influence of an electric field has not been considered. In order to close the gap, this chapter introduces a mathematical model to study the behaviour of a contingent of osteoprogenitor cells due to the action of both an electric field and the chemical environment. The mathematical description aims at reproducing features of the electric field effects on cell behaviour and numerically quantifying cell migration by electrotaxis. Numerical results provide mathematical relations for cell recruitment and cell colonization of a surface in the presence of the electric field. The results shed light on the relation between the electric field, cell migration and surface colonization for osteoprogenitor cells.
8.2 Mathematical model

A setup for the exposure of an in-vitro cell culture of osteoprogenitor cells to an electric field [185] is mathematically described by means of three model variables: the osteoprogenitor cell density \( C_o(x,t) \), the osteoprogenitor chemical concentration \( Q_o(x,t) \), and the external electric potential \( \Phi(x) \). Cell differentiation is not considered in order to maintain the same type of cells throughout the experiments and to reduce the model complexity to only one type of cell. For the same reason, the osteoprogenitor chemical concentration brings together in one single variable the chemical signalling present during bone healing. This signalling is provided by the activation of platelets (releasing platelet-derived growth factor (PDGF)) [198], and the activation of both the transforming growth factors beta (TGF-\( \beta \)) and the fibroblast growth factor (FGF) [199]. Other types of external stimulus, such as mechanical forces and fluid shear forces that are known to influence cell behaviour and cell response [200], are not considered.

8.2.1 Osteoprogenitor cells

The mathematical description of the migration of the osteoprogenitor cells is based on the random dispersion mechanism present in the chemotaxis model given by (7.6) [158, 162]. Accordingly, the following equation holds

\[
\frac{\partial C_o}{\partial t} = \nabla \cdot \left[ D_C o \nabla C_o - H_C o C_o \nabla Q_o - E_C o C_o \nabla \Phi \right] \tag{8.1}
\]

where \( C_o \) is the density of osteoprogenitor cells. The cell migration is caused by a linear diffusion term with coefficient \( D_C o \) together with a linear chemotactic term with coefficient \( H_C o \). Electrotaxis is modeled as the action of the electric potential gradient on an anisotropic electrical sensitivity coefficient \( E_C o \), the value of which is different from zero only in the direction of the electric field vector. The negative sign indicates that osteoprogenitor cells migrate opposite to the electric field vector [77]. The cell dynamics is complemented in the reaction term by the cell proliferation, which is modelled with a logistic linear term [162] with coefficient \( r_C o \) and carrying capacity \( C_i \). In addition, the minimum density of osteoprogenitor chemical required to initiate cell migration [158, 162] is represented by the threshold value \( W_{Q_o} \) of an activation function similar
8.2 Mathematical model

to that shown in Fig. 7.1a. The parameter $p$ refers to the slope of this cell proliferation activation function. The reaction term in (8.1) is complemented by the cell death given as a linear term with coefficient $\delta_{C_o}$.

### 8.2.2 Osteoprogenitor chemical

The evolution of the concentration of the osteoprogenitor chemical $Q_0$ is mathematically described by the equation

$$
\frac{\partial Q_0}{\partial t} = D_{Q_o} \nabla^2 Q_0 + (r_{Q_o} + r_\Phi \Phi) C_o - (\delta_{Q_o} + \delta_{Q_C} C_o) Q_o, \quad (8.2)
$$

The random dispersion is given by a linear diffusion term with coefficient $D_{Q_o}$. The production of the osteoprogenitor chemical is modelled as a linear term with coefficient $r_{Q_o}$, which is related to the amount of chemical produced during the cell migration and proliferation [201]. An additional term with coefficient $r_\Phi$ represents the increased chemical production in the presence of the electric field [76]. The chemical consumption is modeled by a natural linear decay term with coefficient $\delta_{Q_o}$ and a nonlinear term with coefficient $\delta_{Q_C}$, representing the amount of chemical consumed by the osteoprogenitor cells.

### 8.2.3 Electric stimulus

In order to analyze the mechanism of cell electrotaxis, the density of osteoprogenitor cells is assumed to be exposed to an incident electric field. Since human cells can be reasonably modelled as spheres with a radius of about 5-20 $\mu$m [40], and electrotaxis has been mostly observed when cells are exposed to static electric fields or electric fields in the ELF-range (less than 300 Hz) [122, 184, 185], the electrical response of the cells can be fairly approximated by using a quasi-static approach [40, 93]. Since cells and tissues of the human body have both dielectric properties [14, 105], the net charge inside a Gaussian surface enclosing the osteoprogenitor cell-like culture is assumed to be zero. Therefore, Poisson’s equation in (4.3) becomes Laplace’s equation as in (4.4) and reads

$$
\nabla^2 \Phi = 0, \quad (8.3)
$$

where $\Phi$ is the electric potential, related to the electric field $\mathbf{E}$ through the expression in (4.2), i.e., $\mathbf{E} = - \nabla \Phi$. 
8.3 Description of the simulation

The mathematical model given by (8.1) - (8.3) has been solved numerically by following the discretization technique described in Appendix A. The computational domain is a 1 mm-side square representing part of the bottom of a cell culture dish. The bi-dimensional grid consisted of 2601 nodes distributed among 2500 quadrilateral patches associated with Lagrangian bi-linear elements. The osteoprogenitor cells were numerically seeded in a 0.2-mm-side square-shaped area in the center of the domain. The initial condition for the osteoprogenitor chemical was assumed to be $5 \times 10^4$ mg/ml [160, 196].

The electric field is assumed generated by a voltage generator (battery) as shown in Fig. 8.1. This power source is assumed connected to the sides of the domain, with the positive electrode indicating the direction for cell electrotaxis. Thus, the nodes at the negative electrode are set to a boundary condition equal to zero (ground), whereas the nodes at the positive electrode are set to $\Phi = EL$, where $E$ is the magnitude of the electric field and $L$ the width of the domain. The intensities range from 0 V/cm to 10 V/cm [45, 64, 77], and the electric field direction depends on how both electrotaxis and chemotaxis are expected to influence cell migration. To this end, in some cases an osteoprogenitor chemical boundary condition was applied to the right side of the domain as shown in Fig. 8.1b-c [160, 196]. The simulation consisted of 1500 time steps each one equivalent to one minute of real time. The numerical parameters were obtained from the available literature and from other numerical results. A more detailed explanation of these values is presented in Appendix B.

Four simulation cases, depicted in Fig. 8.1, have been considered based on information extracted from the available literature. These cases correspond with different ways of stimulating the osteoprogenitor cells migration by using either or both the electrical stimulus and the chemical stimulus, as follows.

### 8.3.1 Electrotaxis only

The first simulation case, sketched in Fig. 8.1a, reproduces an experimental condition where cells are exposed to a static electric field only in order to assess their directional migration. The magnitude of the applied electric field varies in the range 0-10 V/cm. Cell position has been observed at three different time steps, namely after 700, 1200 and 1500 minutes of exposure. As a control test, the electric field has been replaced with a chemical stimulus of $(1-3) \times 10^{-2}$ mg/ml. Numerical information from this control condition has been also obtained from the time steps corresponding to 700, 1200 and 1500 minutes of exposure.
8.3 Description of the simulation

8.3.2 Electrotaxis overrides chemotaxis

The second simulation case is similar to the one shown in Fig. 8.1a, but includes a chemical stimulus in the right side of the domain, as sketched in Fig. 8.1b. The purpose of this additional chemical stimulus is to assess the overriding effect that the electric field has over the chemical stimulus [45]. The magnitude of the electric field varies in the range 0-10 V/cm and the chemical stimulus varied in the range (1-15)\times10^{-2} \text{ mg/ml}. Cell position has been observed after 700, 1200 and 1500 minutes of exposure.

Figure 8.1: Grid, initial osteoprogenitor cells density and boundary conditions. a) Electrotaxis only. b) Electrotaxis overriding chemotaxis. c) Electrotaxis aided by chemotaxis. d) Electrotaxis and chemical flux.
8.3.3 Electrotaxis aided by chemotaxis

The third simulation case, depicted in Fig. 8.1c, aims at evaluating possible changes in the cell migration profile when both the electric field and the chemical stimulus direct cell migration towards the same direction [45]. As in the previous cases, the electric field magnitude varies in the range 0-10 V/cm and cell position has been observed after 700, 1200 and 1500 minutes of exposure.

8.3.4 Electrotaxis and a perpendicular chemical flux

The fourth simulation case provides additional support to the overriding effect of the electric field on the chemical stimulus by excluding any possible chemotactic effects in the electrically-directed cell migration. Accordingly, a continuous cross flow of osteoprogenitor chemical perpendicular to the electric field vector was used as indicated in Fig. 8.1d [12, 45]. This flow was modelled by including an additional convective term in (8.2), which then reads

\[
\frac{\partial Q_o}{\partial t} + \vec{v} \cdot (\nabla Q_o) = \text{Diffusion} + \text{Production} - \text{Consumption},
\]

where \(\vec{v}\) is the velocity of the osteoprogenitor chemical cross flow, and the diffusion, production and consumption terms are the same as in (8.2). In this simulation case, the electric field is fixed to 5 V/cm and no osteoprogenitor chemical boundary condition is applied. Cell position has been observed after 700, 1200 and 1500 minutes of exposure.

Cell position is calculated in all cases by measuring the distance between the middle point of the cell contingent and its lateral borders. The measurements are taken for points located along an horizontal line crossing the center of the domain. Due to the balance between cell migration, cell proliferation and cell death, the movement of the cells is such that the cell density at the central area where the initial condition is defined is always the maximal density, i.e., \(C_0 = 1\). Therefore, the observation of the cell position is conducted using as reference the middle point of the cell contingent, and the lateral positions for which the cell density is reduced 5 % the maximal value.

Furthermore, cell migration speed \(v\) is calculated from the relation \(v = d/t\) as the net migration distance \(d\) divided by the duration of the migration \(t\) [185, 186]. Additional information for the number of osteoprogenitor cells colonizing the anode site is obtained by measuring the osteoprogenitor cell density in the middle point of the corresponding boundary.
8.4 Numerical results

8.4.1 Cell migration depends on the magnitude and direction of the electric field

Numerical results show that cell migration depends on both the electric field magnitude and the chemical stimulus, as shown in Fig. 8.2. The spatial distribution of the rounded pattern shows that cell migration is also a consequence of the cell diffusion mechanism.

Results of the simulations for the case in which only the electrotaxis is present show that the cell migration is directed by the electric field and towards the anode, as shown in Fig. 8.2a-c. Furthermore, the distance covered by the cells depends on the electric
field magnitude in such a way that for electric fields lower than 1 V/cm the cells appear to remain at their initial position, as observed in Fig. 8.2c.

Results for the case in which electrotaxis overrides chemotaxis (Fig. 8.2d-f), show that the overriding effect holds true for electric fields higher than a threshold value below which cell migration is reduced and directed by the chemical stimulus. This fact can be observed as the slight deformation of the rounded pattern in the side facing the chemical stimulus as seen in Fig. 8.2f.

Results for the case in which electrotaxis is aided by chemotaxis (Fig. 8.2g-i) show that cell migration can be oriented in a single direction when both electrical and chemical stimulus are applied. Furthermore, results of this case indicate that the cell migration also depends on the direction of the electric field vector. Fig. 8.1c shows that particularly for this case the electric field vector points towards the right side of the domain. As a result of this, cell migration is reversed as shown in Fig. 8.2g-i. Hence, a change in the direction of the electric field vector switches the cell migration direction.

### 8.4.2 Cell migration speed

Cell positions were observed after 1200 minutes of exposure to the electric field (Fig. 8.2) to calculate the migration speed at each case. Results for the case in which only electrotaxis is applied lead to a linear relation between the migration speed and the electric field magnitude, as shown in Fig. 8.3a. Accordingly, osteoprogenitor cells in the presence of only an electric field migrate towards the anode at a rate of $7.32 \times 10^{-9} \text{ mm}^2/(\text{mV s})$, yielding a cell migration speed due to electrotaxis of $3.66 \times 10^{-6} \text{ mm/s}$ when a 5-V/cm electric field is applied. As observed in Fig. 8.3a, a small value for cell migration speed remains when no electric field is applied. This value is calculated as $4.39 \times 10^{-7} \text{ mm/s}$, and corresponds to the cell migration due to diffusive effects. In consequence, the total cell migration speed, described as the summation of the contributions due to both electrotaxis and diffusive effects, accounts to $4.01 \times 10^{-6} \text{ mm/s}$.

Additional calculations using a control test for cell migration under the effect of the chemical stimulus only lead to a logarithmic relation between the applied chemical stimulus and the migration speed. According to this relation, cells under a chemical stimulus of $2 \times 10^{-2} \text{ mg/ml}$ migrate at a speed of $9.64 \times 10^{-7} \text{ mm/s}$, as shown in Fig. 8.3b. The chemical stimulus of $2 \times 10^{-2} \text{ mg/ml}$ is defined as the average chemical concentration produced by cells under a numerical control test with no chemical and electrical stimuli [179, 196]. The speed of $9.64 \times 10^{-7} \text{ mm/s}$ is 23.54 % the speed obtained when an external electric field of 5 V/cm is applied, a value that is comparable with experimental observations [64, 77].

Results for the case in which electrotaxis overrides chemotaxis also lead to a linear relation between the cell migration speed and the electric field magnitude, as shown in
8.4 Numerical results

Figure 8.3: Numerical relations for cell migration speed. a) Electrotaxis only. b) Chemotaxis only (control). c) Electrotaxis overrides chemotaxis. d) Electrotaxis aided by chemotaxis.

Fig. 8.3c. This relation shows that for very low electric field intensities, the cell migration is negative. On the one hand, this relation means that under the given conditions, cell migration is mainly induced by chemotaxis and directed towards the chemical stimulus source. On the other hand, the relation indicates that, as the electric field magnitude increases, the cell migration is positive thus mainly controlled by the electrotaxis and directed towards the anode.

By calculating the intersection of this linear relation with the x-axis, it is found that the electric field magnitude counteracting the chemical stimulus is 0.35 V/cm. Interestingly, the test for this value showed that there is a deviation of the cell contingent from the initial condition towards the chemical stimulus. This deviation is attributed to a remaining action of chemotaxis as shown in Fig. 8.2f. However, using the obtained electric field threshold value and the simulation environment shown in Fig. 8.1b, cells movement towards the chemical stimulus is calculated as 2 µm. Additional calculations when no electric field is applied lead to a cell movement towards the chemical stimulus equal to 46 µm. Therefore, an electric field of 0.35 V/cm might be considered as the electric field needed to counteract the effect of an opposed chemical stimulus of 2x10^{-2} mg/ml. In consequence, electric field intensities above this threshold value
show that electrotaxis overrides chemotaxis, a result that correlates with experimental observations in which the threshold electric field magnitude is found to be 0.125 V/cm [45].

Using the linear relation shown in Fig. 8.3c, it is possible to calculate the migration speed of the osteoprogenitor cells under the conditions given in Fig. 8.1b. Accordingly, osteoprogenitor cells migrate at a rate of 8.63x10^{-9} \text{ mm}^2/(\text{mV s}), corresponding to a cell speed of 4.31x10^{-6} \text{ mm/s} when an electric field of 5 V/cm is applied. However, Fig. 8.3c shows a negative value for the cell migration speed when no electric field is applied. The negative sign indicates that cell migration is directed towards the chemical stimulus, i.e., opposite to electrotaxis. By subtracting the diffusive effect, it has been found that cells migrate by chemotaxis at -7.40x10^{-7} \text{ mm/s}. Therefore, the total cell migration speed is given as the summation of the contributions given by the electrotaxis, chemotaxis and diffusion effects, which amounts to 4.01x10^{-6} \text{ mm/s}. This value is close to the speed obtained in the electrotaxis only case (Fig. 8.3a), and can be correlated with the experimental results in which a chemical stimulus opposed to the electric field vector reduces the cell migration speed [45].

Similarly to the two cases mentioned previously, a linear relation shown (Fig. 8.3d) between the migration speed and the electric field magnitude was obtained for the case in which electrotaxis is aided by chemotaxis. This relation consists of two contributions. The first contribution is related to the electric field, which leads to a cell migration speed by electrotaxis of 7.84x10^{-9} \text{ mm}^2/(\text{mV s}). Assuming an electric field of 5 V/cm, the cell migration speed amounts to 3.4x10^{-6} \text{ mm/s}. The second contribution accounts for the chemotaxis and diffusive effects. Therefore, subtracting the diffusion term found in Fig. 8.3a, the cell migration speed by chemotaxis is found to be 7.41x10^{-7} \text{ mm/s}. The total cell migration speed is obtained as the summation of the electrotaxis, chemotaxis and diffusion contributions and amounts to 5.0976x10^{-6} \text{ mm/s}. This total cell migration speed is higher than the speed obtained in the electrotaxis only case. In fact, there is an increase of 19.65 % when compared to the result shown in Fig. 8.3a and an increase of 18.65 % when the value is compared to the electrotaxis-overrides-chemotaxis case, shown in Fig. 8.3c.

8.4.3 Cell colonization can be electrically controlled

Cell colonization is determined for all the cases by evaluating the cell density at the anode site for different electric field intensities after 1500 minutes of exposure. Results show that when the cells are exposed to the electric field only, the cell density and the cell redistribution at the anode site increases with the electric field. In fact, an electric field of 2 V/cm allows cells to barely reach the anode, whereas an electric field of 3 V/cm leads to an initial colonization and cell redistribution that increases for electric
8.4 Numerical results

Figure 8.4: Cell density (in mg/ml) during colonization of the anode site for the electrotaxis only case. $t = 1500$ min. The magnitude of the incident electric field is a) 5 V/cm, b) 3 V/cm, c) 2 V/cm.

Fields up to 5 V/cm, as seen in Fig. 8.4.

Besides, after plotting the data obtained for cell position, it has been found that the magnitude of the external electric field and the cell density colonizing the anode site are related in a logarithmic way, as shown in Fig. 8.5. Using this relation, the cells exposed to the electric field only arrive at the anode (cell density equal to zero) when the magnitude of the incident electric field is 1.73 V/cm. It has also been found that the cell density at the anode increases to reach the maximum normalized density value (cell density equal to one) when an incident electric field with magnitude 2.42 V/cm is applied. This means that for magnitudes lower than 1.73 V/cm the cells are not able to reach the anode (negative cell densities). In contrast, for magnitudes higher than 2.42 V/cm, the cells colonize the anode and also begin to proliferate over it (cell densities greater than one).

Results for cell colonization during the case in which electrotaxis overrides chemotaxis show that after 1500 minutes the cell density and the cell redistribution exhibit a behaviour similar to that of observed when only the electric field is applied (Fig. 8.4). Moreover, the results indicate that there is also a logarithmic relation between the electric field magnitude and the cell colonization of the anode as shown in Fig. 8.5b. Using this relation it was found that cells arrive at the anode when an external electric field of 1.63 V/cm is applied. It was also found that the cell density at the anode increases to reach the normalized concentration value when an external electric field of 2.36 V/cm is applied. These values represent a difference of 5.85 % and 2.63 % with respect to the values obtained for the electrotaxis only case, namely, 1.73 V/cm and 2.42 V/cm respectively.

Finally, results for the case in which electrotaxis aids chemotaxis show that the cell density at the surface of the anode is higher and largely redistributed compared to case in which only the electric field is applied. Accordingly, it is found that cell colonization
at the anode is achieved using lower electric field intensities. The logarithmic relation between the electric field magnitude and the cell density colonization of the anode, shown in Fig. 8.5c, indicates that for the this exposure case the cells arrive at the anode when an external electric field of 1.02 V/cm is applied. Similarly, it is calculated that the cell density at the anode increases to reach a normalized concentration value when an external electric field of 1.67 V/cm is applied. These values represent a difference of 41.14 % and 31.28 % with respect to the values obtained for the case in which only the electric field is applied. These results suggest that when the chemical stimulus directs migration in the direction of the electric field vector, lower electric field intensities are needed for the cells to reach and colonize the anode up to a the maximum normalized density value, i.e., $C_0 = 1$.

### 8.4.4 Electrotaxis is independent of chemotaxis

An electric field of a given magnitude may override the chemical stimulus, as shown in Fig. 8.2d-f. A more detailed observation of the independence of the electrotaxis from any chemical cue can be observed from the results shown in Fig. 8.6, which correspond to the electrotaxis and chemical flux case. Accordingly, cells migrate towards the anode in response to electrotaxis even during exposure to a constant chemical flux perpendicular
8.5 Discussion

Figure 8.6: Cell density (in mg/ml) for the electrotaxis and chemical flux case.  
a) t = 700 min. b) t = 1200 min. c) t = 1500 min.

to the electric field vector.

Evaluation of the cell position after 700 minutes shows that the cell migration speed under the influence of the perpendicular chemical flux equals that obtained in the case in which only the electric field is applied. This result is observed after comparing the position (especially that of the center point) of the cell contingent shown in Figs. 8.2a and 8.6a, and despite the fact that the scale of the figures is not the same. Moreover, the cell colonization and the cell redistribution over the anode after 1500 minutes (Fig. 8.6c) is also similar to that obtained for the case in which only the electric field is applied (Fig. 8.4). However, a slight deviation of the cell contingent in the direction of the chemical flux is observed, caused by the drift force of the perpendicular osteoprogenitor chemical flux [45, 77, 185].

8.5 Discussion

Numerical simulations reproduce the cell migration directed by an external electric field as experimentally observed [45, 77]. Results show that an increase in the electric field magnitude increases the rate of cell migration (Fig. 8.2). The values calculated for the migration speed of the osteoprogenitor cells indicate that the exposure to a 5-V/cm electric field increases the migration speed 23.54 % as compared to the case in which no electric field is applied. This value is comparable with experimental results measured for similar types of cells, where the presence of the electrical stimulus increases cell migration in about 10-20 % [77]. Furthermore, reversing the direction of the electric field vector changes the direction of the cell migration in agreement with experimental reports and the electrotaxis concept (Fig. 8.2g-i) [12, 45]. Therefore, being able to reproduce cell migration also towards the cathode site, the model may be suitable to analyze other types of cells, seeded in different configurations, considering that the direction of cell migration due to electrotaxis also depends on the cell genotype [4].
Despite the agreement between the simulation results and previously reported experimental findings, still the model relies on two simplifications of the complex series of biological and electrical mechanisms involved during cell migration due to diffusion, chemotaxis and electrotaxis.

The first simplification deals with an idealization of the osteoprogenitor cell culture. Cell dynamics is described as a reaction-diffusion process including cell migration and proliferation. However, cell differentiation is not considered in order to deal with the same cell lineage throughout the simulations. Additionally, it is assumed that both the electrical and chemical stimulus affect cell dynamics in such a way that no interaction between them is present. Such interactions however have been observed experimentally [183] and may mediate the response of the cell to the electric field. In this respect, the mathematical description is simplified to account only for linear interactions between the osteoprogenitor cell density, the osteoprogenitor chemical concentration and the electric field. Higher order interactions are not considered, since the current numerical approach lead to numerical results that compare favourably with experimental observations.

The second simplification concerns with the electrical behaviour of the osteoprogenitor cells. These cells respond to an external electric field through electrotaxis [4, 45]. Cellular excitation due to the presence of the electric field leads to increased synthesis rates of chemical signals [76]. Both behaviors have been mathematically described as part of the cell dynamics. However, the electrical properties of the osteoprogenitor cells and of the chemical signal are not considered in the model. Polarization effects in the cells and the chemical signal are not considered either. Hence, the movement of proteins and other charged particles in the presence of the electric field are not included in the model. Although the model establishes a numerical approach for the electric field effects on cell migration, a phenomenon well known from experimental reports [45, 64, 77], in its current form the model is not intended to explore the underlying electrochemical mechanisms of electrotaxis, but to provide additional insight into the suitability of use electric field for inducing cell migration and promoting wound healing [158, 196].

Additionally, it must be considered that the control state in the numerical approach is related to a condition where cell migration is directed by chemotaxis [162]. A control state without chemical and electrical stimuli neglects cell migration, as described in equation (8.1). However, a control condition including a chemical stimulus is in agreement with the biological problem since cell migration is triggered by the presence of a physical stimulus inducing changes in the cellular signalling mechanism [14, 149].

By applying an electric field directing cell migration towards the anode and a chemical stimulus directing cell migration towards the opposite direction (Fig. 8.2d-f), simulation results provide a numerical insight into the overriding effect of the electric field. Such an effect turns out to be independent on further chemical gradients as indicated by the complementary numerical case that reproduces experimental assays in which a constant
8.5 Discussion

Chemical flux perpendicular to the electric field vector has no influence on electrotaxis (Fig. 8.6) [185].

Nevertheless, it has been found that the overriding effect of the electric field is only present when the electric field magnitude is higher than a certain threshold value, a behaviour shown in experimental reports [45, 185]. This threshold value has been calculated from the numerical results (Fig. 8.3c) as 0.35 V/cm, value comparable in order of magnitude to the 0.125 V/cm reported for corneal epithelial cells [45] and lower than 2 V/cm, which is the minimal electrical stimulation value for sarcoma osteoprogenitor cells (SaOs-2) [77]. Despite these differences, which may be explained as differences in the physiology and the genotype between corneal epithelial cells, SaOs-2 cells and the osteoprogenitor cells, numerical results can be correlated to the range of the threshold electric field magnitude obtained through experimental means.

Furthermore, it has been found that the chemical stimulus influences the total cell migration speed. Numerical results show that for an electric field of 5 V/cm there is a reduction of 2% in the cell migration if the chemical stimulus works against the electric field. In contrast, an increase of 19.65% has been found for chemical stimulus directing cell migration in the same direction than the electric field. These observations are in concordance with the behaviour of SaOs-2 cells [77] and provide quantification values for the change in cell migration due to the directness of the chemical stimulus, values that are not available in the reviewed literature.

Scientific curiosity prompted the use the numerical relations shown in Fig. 8.3a-b to calculate the value of the chemical stimulus needed to induce a migration speed equal to that obtained when an electric field of 5 V/cm is applied. Accordingly, the chemical stimulus needed turns out to be five orders of magnitude greater than the average chemical concentration observed throughout the rest of the numerical simulations. Since such a high chemical concentration might saturate the cell culture, from a chemical standpoint, numerical results indicate that there is no suitable way to move the cells at the same speed as that impressed by an electric field. Hence, the electrical behaviour of cells considered in terms of cell migration should be exploited as a possible mechanism to reduce healing time and improve new tissue formation in ways that are not achievable by means of chemical stimulation [36, 200].

A similar behaviour reviewing the function of the chemical stimulus in the presence of an electric field has been addressed experimentally [45, 185], although results are given for the total cell migration speed. Since the numerical approach allows splitting the total cell migration speed into three terms or effects, namely, electrical, chemical and diffusive, the quantification of the interaction between the electric field and the chemical stimulus can be done more effectively. Numerical results show increases of the electrical component of the total migration speed for increased electric field intensities. This facts provides numerical evidence to the enhanced cell motility derived from the exposure to
electric fields as experimentally observed [14, 202]. Additionally, results show that the chemical component of the total migration speed is reduced when an external electric field is applied. This behaviour might be explained by considering that the exposure to the electric field increases the synthesis of signalling molecules in the cell, but it also reduces its ability to sense the chemotaxis stimuli [187]. Further research, however, of both numerical and experimental nature should be performed before conclusive remarks can be made.

Moreover, numerical results for cell colonization and cell redistribution indicate that, compared to the case where only the electric field is applied, lower electric field intensities are needed for the cells to colonize the anode when both the electric field and the chemical stimulus are applied. Accordingly, a 5.85 % less intense electric field is needed for the osteoprogenitor cells to reach the anode when electrotaxis overrides chemotaxis, while a 41.14 % less intense electric field is needed when electrotaxis is aided by chemotaxis. These differences can be explained in the light of the magnitude and direction of the electrical and chemical terms in the total cell migration speed. Observing the electrical terms only, when the chemical stimulus is opposed to the electric field, the term acquires a higher value as compared to the electrotaxis only case. This means that there is a stronger electrical sensitivity on the osteoprogenitor cells and in consequence, the electric field needed to reach the anode might be lower. However, the additional load imposed by the term due to the opposed chemical stimulus reduces the net electrical effect and in consequence the required electric field is 6.22 % lower than when no chemical is applied. Hence, the 5.85 % lower electric field magnitude needed for the cells to reach the anode in the presence of a chemical concentration directing cell migration opposite to the electric field is due to a combination of both a reduced electrical term given by a higher cellular sensitivity to the electric field stimulus, and the opposition generated by the chemical load itself. In contrast, an even higher electrical sensitivity of the cells due to both electrotaxis and chemotaxis directing cell migration towards the same direction, causes a higher electrical term in the total cell migration speed and a chemical effect also increasing the total speed. In consequence, the electric field needed to colonize the anode is 41.14 % lower than in the electrotaxis only case.

Numerical results show that the density of viable cells that colonize the anode during the case in which electrotaxis aids chemotaxis is lower than the values found for the cases in which only the electric field is applied and in which electrotaxis overrides chemotaxis (Fig. 8.5). This is possibly due to an increase of the electro-chemical sensitivity of the cells [200]. Such sensitivity is related to the electrical component of the total cell migration speed. Numerical results for the case in which electrotaxis aids chemotaxis show that this component is increased mainly because the chemical stimulus directs cell migration in the same direction as the electrical stimulus. Therefore, the numerical approach is able to provide insight into the experimentally observed electrochemical response [45, 200]. This response is caused by the application of both an electric field
that increases their cell migration speed and a the chemical stimulus that acts as a control mechanism. In consequence, since an electric field of 10 V/cm is near the maximum electric field ensuring the viability of osteoprogenitor cells [64], the reduction in the density of cells colonizing the anode during the electrotaxis aided by chemotaxis case might be considered as a first indication of cell damage due to exposure to the electric field. Such damage can be associated with an increase in temperature beyond the maximum temperature bearable by the cells. A more detailed discussion on the possibilities of thermal damage is presented in Chapter 9.

Despite the differences caused by the presence and behaviour of the chemical effect, an electric field increases cell colonization and cell redistribution at the anode in the same way that cells colonize and redistribute on certain substrates due to their surface topography and biocompatibility [189, 190]. This effect, called osteoconduction, allows tissue formation in direct contact with a bioactive surface made of non-biological material, and it is the base for wound healing and anchorage of implants [189]. Since exposure to the electric field increases the cell density over the anode and this cell density can be mediated by the magnitude of the electric field as shown in Fig. 8.5, it is reasonable to conclude that a so-called electro-osteoconduction phenomenon may increase the rate of tissue formation over a given substrate. Such effect might be the cause for reduced healing times and improved tissue formation around implants when an electric field is applied, as experimentally reported elsewhere [192, 203]. A deeper exploration of this effect is detailed in Chapter 10.

8.6 Perspectives

The numerical framework presented in this chapter allows the reproduction of biological features observed in osteoblastic cells when exposed to an electric field. Nevertheless, since the model relies on few assumptions about the cell dynamics and the interaction with the electric field, further work should be done to overcome inaccuracies of the numerical results.

As a first step, a better description of the electric field should be used in order to account polarization effects, especially the drift of ions and charge particles inside the cells that may influence the cell migration profile [14].

Secondly, osteoblastic cells should be considered as individual cells rather than a contingent of cells initially seeded in the center of the simulation domain. A discrete cell implementation may be suitable to determine the particular behaviour of single cells when exposed to both electrical and chemical stimuli. Furthermore, cell differentiation should be included in the cell dynamics representation in order to extent the model to other biological scenarios.
In third place, a better indication for the chemical stimulus should be used. For the implementation of the cases shown in Fig. 8.1b-8.1c this chemical stimulus was assumed to be $2 \times 10^{-2}$ mg/ml. Although this value is consistent with experimental and numerical literature [64, 77, 196], changes in this value lead to changes in both the response of cells and the cell migration profile [162]. As a consequence, the current numerical implementation creates a simulated scenario that may change if the chemical stimulus is modified.

A mechanism for the chemical stimulus to vary might be electrically-dependent. It has been reported that an electric field may influence the chemical gradients in-vitro and cell migration not only by the electrotaxis effect but also by an electrically-mediated chemotaxis [183], which might be considered as an electrically-driven increase of the chemical stimulus at the boundary. Such scenario may lead to variations in the cell migration speed that better correlate to the experimental reports considering that the cells preferential response to the electrical stimulus could be affected by the presence of an electrically-activated chemical stimulus.

In addition, numerical parameters should be better validated since the exact value of many of them is unknown and adjustment was needed in order to obtain spatial-temporal patterns as expected. Therefore, predictions obtained from mathematical models justify lines of experimental work aimed to obtain complementary data for biological processes described by the mathematical formulations. Furthermore, a specific experimental protocol should be used to validate the quantitative numerical results on cell migration speed and cell colonization. Such experimental approach is needed in order to further validate the numerical observations allowing the numerical model to be used as a tool to reduce time, effort and resources in other experimental setups. Nevertheless, it should be highlighted that the parameter values and numerical results can be correlated with experimental reports, making the model feasible from a biological standpoint.

Finally, the logarithmic nature of the relations found for cell colonization (Fig. 8.5) show a tendency for a saturation value. This saturation might be related to an electric field magnitude at which no more cells are recruited and are able to proliferate. Such effect refers to cell apoptosis by electroporation of the cell membrane [83]. In consequence, a similar saturation profile could be associated with the electrically-dependent cell migration schemes (Fig. 8.3) when electric field magnitudes higher than 10 V/cm are applied. A proper numerical validation of this saturation hypothesis should deal with the thermal accumulation due to the presence of the electric field (Chapter 9), and with a refinement of the mathematical description to address the cell behaviour near or during electroporation. Such scenario may be useful to analyze the electric field dosimetry for cell migration and electro-osteoconduction as well as the influence of the electric field on cell damage [36, 200].
8.7 Conclusion

The current mathematical description is suitable to be used for the prediction of the cell migration speed when osteoblastic cells are exposed to both electrical and chemical stimuli. Results for cell colonization at the preferential electrode allowed to introduce the term electro-conduction as a way for the cells to colonize and proliferate over a given surface in the presence of an incident electric field. These features make the model suitable to be used in applications regarding different types of cells, wound healing and tissue formation. Among the future applications that are being considered for this model are the extension to time-harmonic electric fields and a better characterization of the electrical behaviour of cells at the individual level in order to provide further insight into the interaction between electric fields and biological cells.
Electric fields are known to influence cell and tissue activity. This influence can be due to thermal or non-thermal effects. While non-thermal effects are still matter of discussion, it is a fact that thermal effects might be detrimental for cell and tissue viability due to thermal damage, the phenomenon being exploited in applications like hyperthermia and tissue ablation. This chapter is dedicated to presenting a model aimed at investigating the influence of thermal damage in the consolidation of bone formation during electrostimulation. The model is an extension of the chemotaxis model presented in Chapter 8, which is here improved to analyze thermal variation, thermal damage accumulation, and the formation of new bone matrix in an injury (fracture) site. This new model is suitable to conduct dosimetry studies in support of other different electrostimulation techniques aimed at improving bone and soft tissues repair.

9.1 Bone electrostimulation and thermal damage

Electrostimulation refers to the application of an electric field in order to boost tissue recovery [67, 182]. It has been used to stimulate molecular and cellular responses in a wide range of tissues, among which muscle, ligament, bone, cartilage, and nervous and cardiovascular systems are the most studied [2, 76]. Especially in bone, application of electrostimulation is known to stimulate osteoprogenitor cells proliferation [76, 182]

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and increase the rate of new bone formation or *osteogenesis* [204]. Electrostimulation is thus suitable to improve the treatment of non-unions, fractures, and bone diseases such as osteoarthritis [2, 76].

Electrostimulation elicits biological responses in bone that are similar to those generated by mechanical stimulation [4, 67]. The compressive load induces an electronegative potential within the bone matrix that stimulates bone deposition. In contrast, the application of a tensile load induces an electropositive potential that causes bone matrix resorption [67]. Similarly, electrostimulation is known to control bone synthesis by stimulating specific signalling mechanisms in osteoprogenitor cells [67, 182] thus inducing cell proliferation and differentiation, and also affecting the cell migration response [45].

Furthermore, exposure to an electric field induces heating at the wound site [104, 105, 205] and excessive heat causes cell death and tissue damage [46, 205]. Detrimental as it is for cell and tissue viability [46], yet excessive heat is also used in hyperthermia for cancer treatment [105], and in tissue ablation to induce electroporation and improve drug delivery [5, 80]. In both techniques, the temperature increase activates a cascade of biological events that in normal conditions are not beneficial for the viability of both cells and tissues. Therefore, improving bone formation during electrostimulation requires careful control of the temperature to avoid an increment that may affect the viability of the new tissue formation [2, 46, 78]. The temperature is mediated by the dielectric properties of biological tissues which transform part of the energy of the electric field into heat [5, 80].

Despite the available experimental data, the underlying biological mechanisms influenced by the electric field during electrostimulation which are beneficial for bone formation are still unclear [2]. Furthermore, the thermal distribution associated with wound healing and tissue formation with and without exposure to an electric field are a current matter of research [10, 11]. Fortunately, numerical simulations provide additional insights about the behaviour of cells and tissues during electrostimulation, especially when the temperature mediates the biological response. Accordingly, numerical relations have been formulated to establish a relation between cell viability and temperature increase [46, 205]. Moreover, the temperature-dependent reversible and irreversible electroporation during exposure to electric fields has been evaluated [80], and a quantification of the temperature distribution during tissue ablation has been obtained [5].

Based on ideas derived from these numerical works and on the mathematical model presented in Chapter 8, this chapter is dedicated to introducing a numerical model to investigate the influence of thermal damage in the formation of new bone during electrostimulation. The approach allows for the analysis of the influence of both electric fields and heating during the migration of osteoprogenitor cells into the wound site and the subsequent synthesis of a new bone matrix. The numerical results are in agreement with experimental observations on both bone formation [204] and cell (tissue) damage.
by heat accumulation [46, 77, 80], and can provide additional insights into the incidence of the thermal effect during electrostimulation.

9.2 Bone healing and electrostimulation

9.2.1 Biological overview of bone healing

The bone healing process is a complex biological sequence of events that involve a vast number of cells, proteins and other molecules [178]. In short, the healing of any trauma caused to an intact tissue starts with blood pouring out of the damaged blood vessels. This spilled blood activates the inflammatory response which provides the signalling environment necessary for the migration of osteoprogenitor cells [175]. Concomitantly, the blood coagulation process transforms the spilled blood into a network of fibers. This network creates a temporary plug into the damaged vessels and paves the way for the osteoprogenitor cells to start the wound healing process [175, 178, 198, 206].

The bone repair process initiated by the osteoprogenitor cells follows two different mechanisms depending on the nature of the affected bone [178]: intramembranous bone formation and endochondral bone formation. During intramembranous formation the osteoprogenitor cells start migrating into the wound site using blood clot and the vascular network as a scaffold [201]. Due to the biochemical signalling environment, these cells differentiate directly into osteoblasts, which produce new primary (woven) bone. This primary bone, however, undergoes a remodelling process until it acquires biomechanical properties similar to those of the surrounding intact bone [177, 207]. The intramembranous bone formation is typical of flat bones, for instance, the skull, the jaw and the facial bones.

In contrast, during endochondral bone formation, the inflammatory response is followed by the formation of a cartilaginous callus [178]. This callus is formed by osteoprogenitor cells recruited during the inflammatory process and undergoes a continuous replacement into primary (woven) bone as the cells differentiate into osteoblasts. Further remodelling is needed to replace the woven bone into a rigid bone matrix with the adequate biomechanical properties [178, 207]. The endochondral bone formation is typical of long bones, for instance, the humerus in the arms and the femur in the legs.

9.2.2 Effects of electrostimulation in bone

In general, there are four modalities for bone electrostimulation [2, 67]. The most basic way of electrostimulation is the connection at the injury site of a pair of electrodes which create a static electric field between them [45, 204]. Such method has been used to study bone formation around implantable devices [204] and to evaluate cell migration
Predicting thermal damage during bone electrostimulation and alignment in-vitro [45, 188]. Several treatments in physical therapy are based on this technique, although more complicated stimuli are preferred, such as sinusoidal, saw-tooth, biphasic, square and pulse waveforms [2, 35].

A more sophisticated way of applying electrostimulation in bone is by means of a pair of electrodes, one of them inserted into the injury site while the other is applied on the skin [67, 182]. The electrodes are then connected to a power source that delivers an electrical current that starts to flow through the less-resistive path across the tissue, thus closing the electrical circuit. This method improves both fracture healing and osteogenesis by increasing the osteoblastic activity and angiogenesis directly at the injury site [182].

There are two additional modalities of electrostimulation in which the field delivered is time-harmonic, namely, the capacitive coupling and the inductive coupling. In the capacitive coupling [2], two external plate-like electrodes are connected to an electromagnetic source that generates a uniform electric field at the injury site. This modality increases the proliferation of both osteoblasts and chondrocytes [67] and upregulates the expression of growth factors such as BMPs and TGF-β, which act as chemical inductors for osteogenesis [2, 182]. In contrast, the inductive coupling consists of an external coil, used to generate a magnetic field across the injury site. This magnetic field is made uniform by means of a second coil as in Helmholtz configuration [2, 67]. In view of Faraday’s Law, a time-harmonic magnetic field has an associated electric field. However, although the associated electric field governs the polarization (ion drift) effects, the predominant stimulus is the magnetic field. This magnetic field is capable of improving bone healing when applied to treat fracture non-unions, osteoarthritis and other bone-related issues, modulates the formation of bony and cartilagenous matrices, and inhibites osteoclastic (bone resorption) activity [67].

In addition, the biological response of the bone to the electrostimulation depends on the magnitude and frequency of the applied stimulus [2]. An electric field as low as 0.48 $\mu$V/cm affect the response of osteoclasts, while fields up to 6-10 V/cm affect osteoblastic proliferation and migration [2, 45]. Static electric fields and field with frequency up to 100-300 Hz are widely used as they mimic bio-electrical signals. Significant results during stimulation of bone formation and osteoblastic proliferation are obtained when the frequency ranges between 10-30 Hz. Frequencies between 20-200 KHz are used during capacitive coupling, while the application of burst in the millisecond range (1-10 KHz) for a lapse of several hours is an accepted practice when using the inductive coupling [2, 67].

Despite the differences in frequency, it is believed that the biological effects due to electrostimulation are more a consequence of the magnitude of the stimulus rather than of the frequency itself [4, 67]. This observation is in accordance with the fact that at the frequencies typically employed in electrostimulation (lower than 200 KHz), the wavelength of the electric field is several orders of magnitude larger than the size of
both the electrodes, the injury site and even the cells. Therefore, the electric field can be fairly described using a quasi-static approximation. Furthermore, since the effects of static fields last hours, days and even weeks [12, 14], they can intervene in long-lasting biological processes such as growth development and wound healing [4, 21]. An example of such an effect is the cell electrotaxis [45, 77] addressed in detail in Chapter 8. This type of cell migration is determinant to reduce the wound healing time and increase the rate of new bone formation [4, 45].

9.3 Mathematical model

The complex biological process of bone formation during electrostimulation is modelled as the migration into the wound site of osteoprogenitor cells which synthesize a new bone matrix. We assume that, as a consequence of the Joule effect, the exposure to the electric field increases the tissue temperature and may eventually cause tissue damage [5]. Therefore, the synthesis of new bone is on the one hand stimulated by the electric field, but on the other hand negatively affected by thermal damage. A mathematical formulation based on a set of partial differential equations is used to describe the biological process and quantify the possible thermal damage during electrostimulation.

9.3.1 Osteoprogenitor cells

Similarly to the model presented in Chapter 8, the synthesis of new bone at the wound site is carried out by the density of osteoprogenitor cells \( C_o(x,t) \) which satisfies the following equation

\[
\frac{\partial C_o}{\partial t} = \nabla \cdot \left[ \sum_{\text{Migration}} D_{C_o} \nabla C_o - H_{C_o} C_o \nabla Q_o - E_{C_o} C_o \nabla \Phi \right] + r_{C_o} C_o \left( 1 - \frac{C_o}{C_i} \right) g(Q_o, W_{Q_o})
\]

\[
- \delta_{CT}(T) g(T, W_T) C_o - \delta_{CK} g(K, W_K) C_o - \delta_{C_o} C_o,
\]

where the cell transport is induced by three mechanisms described in (8.1), i.e., the gradient of the cell concentration, the gradient of the so-called osteoprogenitor chemical concentration \( Q_o(x,t) \), and the electric potential gradient that induces cell electrotaxis. Similarly to (8.1), cell proliferation follows a logistic function with carrying capacity \( C_i \) and controlled by the coefficient \( r_C \). The function \( g(Q_o, W_{Q_o}) \) follows (7.7) and corresponds to an activation function as shown in Fig. 7.1, where \( W_Q \) is the threshold value given in terms of the chemical concentration \( Q_o(x,t) \).
Cell death is described by three different terms representing cell necrosis, cell apoptosis and cell natural death. Cell necrosis refers to the cell death caused by an acute cellular injury, which can be due to extreme temperature. In contrasts, cell apoptosis corresponds to the activation of a cascade of biochemical events due to the presence of the thermal stimulus that ultimately lead to changes in cell morphology and induce programmed cell death. Natural cell death corresponds with death due to longevity [47, 208].

Based on experimental data [205], cell necrosis due to thermal action is assumed to be linearly dependent on temperature $T$, with the necrosis coefficient described as the temperature-dependent function $\delta_{CT}(T)$. The activation function $g(T,W_T)$ refers to the minimal temperature needed to trigger cell necrosis [46, 205], where $W_T$ is the threshold temperature value. The cell apoptosis is assumed to be linearly dependent on the cell density and activated by the thermal damage accumulation $K(T,t)$ [46], with $\delta_{CK}$ the cell apoptosis coefficient adapted from experimental data [205]. The function $g(K,W_K)$ represents an activation function where $W_K$ is the maximum thermal damage at which blood perfusion at the injury site ceases and thermally-induced coagulation is started [5]. Finally, cell death due to longevity is assumed linear with respect to the cell density and controlled by the coefficient $\delta_{Co}$.

### 9.3.2 Osteoprogenitor chemical

The cell migration in (9.1) is mediated by the chemical environment expressed in terms of an osteoprogenitor chemical concentration $Q_o(x,t)$, which is described in the same way as in (8.2), viz.

$$\frac{\partial Q_o}{\partial t} = D_{Q_o} \nabla^2 Q_o + (r_{Q_o} + r_{\Phi} \Phi)C_o - (\delta_{Q_o} + \delta_{QC} C_o)Q_o .$$

This chemical concentration brings together the vast amount of signalling molecules controlling the osteoprogenitor cell behaviour, especially growth factors as TGF-$\beta$, BMPs and FGF [175, 178, 207].

### 9.3.3 Bio-heat equation

The temperature variation due to electric field exposure is obtained by solving the well-known bio-heat equation, given by [5, 80, 104]

$$\rho_i \alpha_i \frac{\partial T}{\partial t} = \beta_i \nabla^2 T + \omega_i \alpha_i (T_b - T) + M_{HG} + \sigma_i |\nabla \Phi|^2 ,$$  

(9.3)
where the temporal variation relative to the material density $\rho_i$ and the heat capacity $\alpha_i$ is equal to the contributions due to the thermal diffusion, proportional to the thermal conductivity $\beta_i$, and a reaction term. This reaction term comprises a production term relative to blood perfusion, which refers to the amount of heat added or removed by the flow of blood. Accordingly, if the local temperature $T$ is below the arterial temperature $T_b$ the term becomes positive, in which case the flow of blood works as a source of heat. On the contrary, when the local temperature $T$ is higher than $T_b$ the term becomes negative, and the blood flow is useful to remove the excess of temperature [5]. The perfusion term is controlled by blood perfusion term $\omega_b$ and the blood heat capacity $\alpha_b$.

In addition to the perfusion term, the reaction term includes the metabolic heat generation $M_{HG}$ and the rate of electromagnetic power deposition. The former is the amount of heat naturally produced by the cells to maintain their biological functions and those of tissues. The latter refers to the power density that generates heating through the Joule effect. This power density $P$ is given in terms of the current density $J$ and the electric field $E$ as $P = J \cdot E$. Since $J = \sigma_i E$ and $E = -\nabla \Phi$, then $P = \sigma_i |\nabla \Phi|^2$, where $\Phi$ is the electric potential. The sub-index $i$ is an indicator of the type of tissue, which is assumed to mainly be either blood vessels (initial phase of tissue recovery) or bone matrix (later phase of recovery) [178].

### 9.3.4 Thermal damage

The thermal damage accumulation $K(T, t)$ refers to the amount of damage that cells and tissues might experience due to an excessive increase of temperature caused by the exposure to an electric field. This exposure can be of either high magnitude but short duration, or low magnitude but long duration. Therefore, the thermal damage is due to a combination of both magnitude (temperature) and time [80], and is modelled using an Arrhenius-like equation given by [5, 80]

$$
\frac{\partial K}{\partial t} = A \exp \left( -\frac{E_a}{RT} \right) g(C_o, W_{C_o}),
$$

where $A$ is the so-called damage frequency factor, $E_a$ is the activation energy, and $R$ is the universal gas constant [80, 205]. The activation function $g(C_o, W_{C_o})$ ensures that the thermal damage mediates bone formation. The threshold coefficient $W_{C_o}$ corresponds to a minimum cell density needed to start the replacement of the blood clot into new bone tissues [175, 178, 201].

### 9.3.5 Bone formation

The new bone formation $B(x, t)$ is described by means of a transformation mechanism that represents the conversion of the blood clot into primary bone [175, 178]. This
conversion is given by

$$B = 1 - \exp \left( -r_{BC} C_o - r_{BQ} \Phi Q_o \right), \quad (9.5)$$

where $r_{BC} \Phi$ and $r_{BQ} \Phi$ are control parameters related to cell adhesion and proliferation [160], and $\Phi$ is the electric potential. Since the electric field influences both cell adhesion and cell proliferation, the transformation mechanism is found to be also dependent on the electric field magnitude. Both processes are increased when the cells are exposed to the electric field [4, 67]. Accordingly, and assuming this electrical dependence to be linear, $r_{BC} \Phi = r_{BC}(1 + c_1 \Phi)$, and $r_{BQ} \Phi = r_{BQ}(1 + c_2 \Phi)$, where $r_{BC}$ and $r_{BQ}$ are control coefficients for cell adhesion and cell proliferation [160, 209], and $(1 + c_1 \Phi), (1 + c_2 \Phi)$ are electrical mediation terms with $c_1$ and $c_2$ the coefficients for both the electrically-mediated cell adhesion and cell proliferation.

9.4 Description of the simulation

The mathematical model described in (9.1) - (9.5) is completed with (8.3), which is used to obtain the electric potential $\Phi$. The set of partial differential equations is solved in a bi-dimensional 1-mm-side square domain, as shown in Fig. 9.1. This domain represents a section of a bone fracture wound site that is repaired via intramembranous bone formation, i.e., no callus formation is involved [178]. Such wound site is typical during orthodontic treatments, for instance, during distraction osteogenesis [210] or after the insertion of a dental implant in the jaw bone [201, 211]. As shown in Fig. 9.1, the host bone surface is assumed to be at the left side and the receptor surface at the right side, with cell migration occurring from left to right.

The initial conditions are $5 \times 10^{-4}$ mg/ml for the osteoprogenitor chemical concentration $Q_0$ [160, 196], $37 \, ^\circ \text{C}$ for the tissue temperature [5], and zero for the remaining variables. A constant cell density of $10^6$ cells/ml is assumed at the host bone surface. However, in the simulations this condition is normalized in order to obtain a cell density within the range [0-1] (normalized but non-dimensionless). This normalization avoids dealing with the differences in the initial cell seeding number used during experimental protocols [64, 77, 196, 212]. In accordance with the same normalization, the cell saturation value $C_i$ is set to one [162]. Additionally, the wound healing process at the fracture site is simplified as the formation of a new bone matrix by the migrating osteoprogenitor cells. Therefore, the initial inflammatory response and the blood coagulation process following the injury are not considered.

The electrostimulation is described as an ideal DC voltage source that delivers a constant electric field across the wound site. This source is assumed connected to the sides of the domain, with the positive electrode at the receptor surface. Therefore, the nodes at the negative electrode are set to a boundary condition equal to zero (ground). In contrast,
9.4 Description of the simulation

Figure 9.1: Sketch of the simulation domain. The electric field resulting from connecting an ideal voltage source directs cell migration towards the receptor surface [4, 45].

the nodes at the positive electrode are set to $V_B = EL$, where $V_B$ is the potential, $E$ is the desirable electric field magnitude and $L$ the width of the domain. Five simulation scenarios were considered, i.e., when $E$ is equal to 0 V/cm, 2.5 V/cm, 5 V/cm, 7.5 V/cm and 10 V/cm, respectively.

Furthermore, the variation in temperature depends on the thermal properties of the material at the wound healing site. This material changes in time from the initial fibrin matrix (blood clot) into the new bone matrix [178]. Therefore, assuming that a (normalized) cell density of $C_t(x, t) = 0.7$ cells/ml is needed to start the transformation of blood clot into the new bone tissue, whenever the cell density is lower than $C_t$ the material properties are equal to those of blood vessels. For locations where the cell density is equal or greater than $C_t$ the material properties are those of cancellous bone [87, 104].

Numerical parameters used in (9.1) and (9.2) are similar to those used in the model presented in Chapter 8. The parameter values used for the implementation of the thermally-inspired equations in (9.3)-(9.4) are taken from both experimental and numerical data [80, 87, 104]. The complete set of parameters is listed in Appendix C.
The numerical solution is carried out by means of the finite elements method, following the numerical discretization technique described in Appendix A. The bi-dimensional grid consisted of 2500 quadrilateral patches and 2601 nodes. The simulated time was equivalent to 6000 minutes, or 4.17 days. This time was obtained from the results shown in Chapter 8, and it represents the time needed for the cell density to travel across the injury site to colonize the receptor surface. This time is in agreement with treatment protocols used in electrostimulation according to which the duration ranges from weeks to months with exposures between 10 and 24 hours a day [45, 67]. Results are observed along a line parallel to the \( x \)-axis which intersects the \( y \)-axis at 500 \( \mu m \).

9.5 Numerical results and discussion

The simulation results for the cell density after 3000 minutes of exposure to the electric field are shown in Fig. 9.2. Using the position of the cell front at different times, it is possible to calculate the cell migration speed. Accordingly, in the presence of electric field, cell migration speed is 12.25 \( \mu m/h \) and 12.90 \( \mu m/h \) when exposed to electric fields of 2.5 V/cm and 5.0 V/cm, respectively. When no electric field is applied, the rate of cell migration towards the receptor surface amounts to 9.92 \( \mu m/h \) (Fig. 9.2a). This value is comparable with experimental observations on cell migration speed when no electric field is applied [4, 64, 77].

The electrically-mediated increment in cell migration speed is similar to that experimentally observed [45] and explains the displacement towards the receptor surface of the cell density front line for the 2.5-, 5.0- and 7.5-V/cm electric fields compared to the case in which no electric field is applied. This response is due to cell electrotaxis, which provides a direct relation between the magnitude of the applied electric field and the increase in cell migration speed as discussed in Chapter 8. However, the electrically-mediated increase in cell migration is obtained for electric fields with intensities lower than 7.5 V/cm. Already at this magnitude, the cell density shows a reduction 100 \( \mu m \) away from the host bone surface of 18 % the density obtained in the case when no electric field is applied (Fig. 9.2a). A similar reduction pattern is also present during exposure to 2.5- and 5.0-V/cm electric fields, although the difference in cell density with respect to the unexposed case is 0.86 % and 4.22 %, respectively. In contrast, after 3000 minutes of exposure to a 10-V/cm electric field a notably diminution in the cell density is observed. Accordingly, the cell density 100 \( \mu m \), 300 \( \mu m \) and 500 \( \mu m \) away from the host bone is 60.72 %, 51.92 % and 59.86 % the density obtained when no electric field is applied, and the cell density front line is slower as compared to the case in which no electric field is applied.

Such reduction in the cell density is related to the increase in temperature during electrostimulation, i.e., the higher is the electric field magnitude, the more intense is
the temperature induction due to the Joule effect [46, 80]. The thermal induction implies an elevation in the temperature that for the case of the osteoprogenitor cells should be maintained below a viability threshold of 40-42 °C in order to prevent cell apoptosis and eventual cell necrosis [46, 78, 205]. As shown in Fig. 9.3, the use of a 10-V/cm electric field increases the temperature above this viability range. This result leads to conclude that the reduced cell density shown in Fig. 9.2a is a consequence of a thermally-induced cell death.

Both the increment in cell migration for electric fields up to 7.5 V/cm and the reduction in cell density due to thermal damage, especially at 10 V/cm, are preserved after 6000 minutes of exposure, as shown in Fig. 9.2b. However, 200 μm away from the host bone surface, there is a reduction in the average cell density compared to the unexposed case even for electric fields of 2.5 V/cm and 5.0 V/cm. This reduction amounts to 12.97 % and 22.97 % the density of the unexposed case, respectively. As shown in Fig. 9.3, the 7.5-V/cm electric field causes a temperature increase which is enough to activate cell apoptosis and necrosis. However, Fig. 9.2b shows that after 6000 minutes of exposure, the 7.5-V/cm electric field induces a reduction in the cell density similar to that caused by the 5.0-V/cm electric field. The reason for this is that both the electrically-induced cell proliferation and migration balance out the thermally-induced cell death and maintain the cell density close to the threshold value of 70 % required to initiate (primary) bone formation.

Conversely, after 6000 minutes of exposure to a 10-V/cm electric field, there is a marked reduction in the cell density as compared to the unexposed case, as shown in Fig. 9.2b. This considerable reduction is due to the associated temperature increase as observed in Fig. 9.3. Since the temperature is higher than the viability threshold for the osteoprogenitor cells, the cell migration is hampered [46, 78]. Therefore, electric field intensities above 10 V/cm are found to reduce the viability of cells under migration. This 10-V/cm electric field threshold compares favourably with experimental results in which and absence of cell electrotaxis possibly due to cell death in osteoprogenitor cells exposed to electric field intensities of about 12 V/cm is observed [64]. Furthermore, Fig. 9.2 shows an increased accumulation of osteoprogenitor cells near the positive electrode for electric field intensities of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm. This accumulation is related to cell electrotaxis, and to the activation of a so-called electro-osteococonduction property at the receptor surface, as explored in more detail in Chapter 8. Accordingly, the presence of the electric field increases the cell migration speed to the point that cells start to accumulate at the receptor surface. This accumulation, together with the electrically-mediated and biochemical activation of signalling molecules –especially BMPs and TGF-β [2, 67]– increases the formation of new bone directly over the receptor surface [160]. This formation is mediated by the proper anchorage of the osteoprogenitor cells onto the receptor surface. This anchorage can be
electrically controlled by altering the adhesion of integrins to the receptor surface due to differences in surface charge [191] and also by increasing the mechanical retraction of the tissues under formation with electric forces created between the cells and the surface [60, 188]. Therefore, the presence of the electric field might increase the capability of a given surface (material) to act as a scaffold for tissue regeneration [2, 188].
As already mentioned, an external electric field induces thermal induction at the injury site, with the average temperature shown in Fig. 9.3. Accordingly, after 30 minutes of exposure, the application of a 5.0-V/cm electric field increases the temperature from the initial 37 °C to 40.3 °C, whereas a 7.5-V/cm electric field is responsible for an average
temperature of 44.4 °C. Considering that the maximal temperature for the viability of osteoblasts is 40-42 °C [46, 78], the application of a 7.5-V/cm electric field is detrimental to the cell viability and induces cell death by necrosis.

However, the exposure to a 7.5-V/cm electric field increases cell migration speed as shown in Fig. 9.2. Therefore, and because (primary) bone formation is initiated in areas where the cell migration front has already occurred, the more and faster the cells redistribute across the injury site, the larger is the area occupied by the new bone. Despite the thermally-induced cell death, the results in Fig. 9.2b reveal that the minimal (normalized) cell density needed for bone formation, namely $C_t = 0.7$ cells/ml, is achieved along most of the domain but especially at the sides. This result indicates that, despite the increment in temperature caused by the exposure to the 7.5-V/cm electric field, there must be a simultaneous reduction in the temperature allowing for the bone formation to be feasible. Interestingly, such a reduction in temperature can be explained with the aid of the material properties used to solve the bio-heat equation in (9.3). Accordingly, if the cell density at a given location is higher than $C_t$, the material properties of that location correspond with those of cancellous bone. In other cases, the material properties are those of blood vessels. Since the values of the thermal parameters for cancellous bone are approximately 2-3 times lower than those of blood vessels (see Appendix C), locations indicating presence of cancellous bone lead to a reduction in the temperature, which is concomitant with the movement of the cell front line.

Such a reduction in temperature is shown in Fig. 9.3b, in which the temperature during
9.5 Numerical results and discussion

Exposure to an electric field of 7.5 V/cm starts to drop from 44 °C after 1500 minutes of exposure to 39.3 °C after 4000 minutes, a value which is maintained during the rest of the simulation time. Similar cooling down responses are observed for the 2.5- and 5.0-V/cm electric fields. When observed from a biological perspective, the wound healing conversion mechanism transforming the granulation tissue into a new bone matrix might be responsible for maintaining a viable thermal environment for the proliferation and migration of osteoprogenitor cells when an electric field is applied [5, 76, 178]. Such a transformation is represented in the model by the change in the material properties needed to solve (9.3).

Furthermore, results for the average temperature shown in Fig. 9.3 also show that the application of an electric field of 10 V/cm increases the temperature to 50 °C, which is maintained during the whole exposure time. Since such a temperature is above the osteoblastic viability range of 40-42 °C, it follows that the excessive cell death especially due to necrosis reduces significantly the cell density at the injury site after 6000 minutes of exposure. This leads to bone formation 400 µm away from the host bone surface and towards the receptor surface that is lower than 50 % the maximal amount. In fact, the amount of bone formation at the receptor surface is reduced by 67.9 % as compared to the unexposed case. It must be emphasized, however, that although the average temperature in this specific case is over 49 °C as shown in Fig. 9.3, the density of osteoprogenitor cells surviving the thermal stress and still migrating is approximately 60 % of the maximal amount. This density is localized near the host bone surface after 6000 minutes of exposure, since at this boundary a density of cells is assumed to be entering the injury site. Moreover, it can be noticed from Fig. 9.2b that despite the high temperature, a part of the localized cell density still redistributes itself along the injury site, in response to electrically-mediated cell proliferation and migration.

Such surviving cell density might be explained as a balance between the cell proliferation rate and the thermally-induced cell death. Experimental observations performed on erythrocytes reveal that the survival rate at temperatures between 48 °C and 52 °C is 60 % between 50 minutes and 100 minutes of exposure, and about 20 % after 200 minutes [205]. Moreover, the osteoprogenitor cells proliferation rate \( r_C = 3.57 \times 10^{-3} \text{ min}^{-1} \) used in (9.3) (see Appendix C) corresponds to the value when no electric field is applied. Accordingly, and assuming no cell death, the cell density would have increased by 35.7 % and 71.4 % after 100 minutes and 200 minutes, respectively. These increments alone would be enough to ensure that after 100-200 minutes of exposure there is a remnant of cells withstanding the thermal damage. In the long term, however, it is observed that the cell density is influenced by the electric field, as seen in Fig. 9.2b. Therefore, it is the simultaneous activity of cell proliferation and cell death, together with the presence of the electric field and the adequate temperature, what accounts for the survival of the cell density especially when the electric field magnitude is 7.5 V/cm.
In addition to features regarding cell migration and accumulation at the receptor surface, the model is suitable to evaluate the effect of the electrically-induced osteoprogenitor cell death due to both apoptosis and necrosis during bone formation. Cell apoptosis is the programmed cell death due to accumulation of stress, in this case, thermal stress [46, 205]. Its activation depends on the level stress and is an intentional (expected) mechanism that can even be reversed [205]. The evaluation of the activation of cell apoptosis due to the presence of the electric field is performed by means of the thermal damage. Fig. 9.4 shows the results for the thermal damage due to different electric field intensities.

A thermal damage factor of 1, obtained after solving the integral in (9.4), represents a 63 % probability of cell (tissue) death (damage), and correlates to the point at which tissue coagulation first occurs [5]. A thermal damage factor of 4 is associated with a 98 % probability of cell death. Accordingly, 6000 minutes of exposure to electric field magnitudes below 5.0 V/cm induce a maximal thermal damage factor of about 2, which corresponds with a probability of 86 % cell death. Although this probability is high, results in Fig. 9.2b show that even though exposure to the electric fields up to 5.0 V/cm causes cell death by apoptosis, the electrically mediated cell proliferation balances the cell density variation out in order to maintain a cell density around 70 % of the normalized value. As shown in Fig. 9.5, such cell density is favorable for a higher bone formation as compared to the unexposed case.

Furthermore, for a 7.5-V/cm electric field, a thermal damage of near 4.5 at the host bone surface, as shown in Fig. 9.4 further explains the reduction in cell density observed in Fig. 9.2a 100 µm away from the host bone surface. However, despite the thermal damage, Fig. 9.2b shows that the cell proliferation mechanism dominates and counteracts the cell death along the injury site, an expected result since electrostimulation is known to increase proliferation of osteoprogenitor cells [67, 182]. In contrast, the application of a 10-V/cm electric field induces extreme thermal damage (5-fold higher 100 µm away from the host bone surface) which cannot be counterbalanced by the cell proliferation, and leads to the low cell densities shown in Fig. 9.2 as compared to the unexposed case.

Moreover, since the thermal damage is defined for the locations where the cell density is above 70 % of the maximal value, and since from Fig. 9.2 for an electric field of 10 V/cm the cell density is on average lower than this threshold, the thermal damage drops to zero at about 400 µm away from the host bone. From this point onward, after 6000 minutes of exposure the injury site is considered to have a predominant density of blood vessels and granular tissue rather than osteoprogenitor cells. This assumption is further supported by the results in Fig. 9.5, in which the bone formation is lower than in the unexposed case, reinforcing the idea that an electric field magnitude of 10 V/cm is detrimental for both the viability of osteoprogenitor cells and bone formation.
Figure 9.5: Simulated percentage of new bone matrix formation after 6000 minutes of exposure to different electric field magnitudes. EF = electric field.

In addition, the presence of a contingent of cells that survives the thermal stress caused by the exposure to an electric field of 10 V/cm as observed in Fig. 9.2b, is responsible for the decelerated formation of a new bone matrix within the first 400 μm of the injury site, as shown in Fig. 9.5. However, since this bone matrix formation falls below the threshold value of 70 % established for the tissue replacement to be carried out, it can be considered as a bone matrix with an inappropriate biomechanical structure that might be similar to the fibrous connective tissue that appears around implants that undergo undesirable in-site motion [213, 214].

Despite this formation, it is notorious that after 6000 minutes of exposure the application of a 10-V/cm electric field is detrimental for the (primary) bone formation process as compared to the unexposed case, expected when such an electric field magnitude increases the temperature as shown in Fig. 9.3. Conversely, the amount of new bone near the receptor surface increases with the application of electric field intensities up to 7.5 V/cm, as shown in Fig. 9.5, similarly to the bone formation observed during application of electrostimulation in-vivo [204, 215, 216]. Accordingly, the amount of new bone at the receptor surface after 6000 minutes of exposure to an electric field of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm is 39.5 %, 46.0 % and 48.9 % higher as compared to the unexposed case, respectively.

The numerical results for the exposure to an electric field with magnitude 10 V/cm show a deceleration in the cell migration which after 6000 minutes leads to a cell density equal to about 50 % the density obtained when no electric field is applied (Fig. 9.2a).
reason for this reduction is the excessive heating caused by the 10-V/cm electric field (Fig. 9.3) and the accumulation of thermal damage especially near the host bone surface (Fig. 9.4). In consequence, the contingent of osteoprogenitor cells near the host bone is reduced due to thermal-induced necrosis and apoptosis [46], which leads to a lesser density of cells able to migrate across the wound site (Fig. 9.2b). Such a lower density of migrating cells is translated into a lower bone formation, as noted in Fig. 9.5 in which about 200-μm away from the host bone surface a 10-V/cm electric field is detrimental for the bone formation as compared with the unexposed case.

9.6 Perspectives

The ability of electrostimulation to increase bone formation is affected by both an increase in the number of osteoprogenitor cells (due to electrotaxis) and a bearable thermal stress [45, 46]. Accordingly, a long-term bone electrostimulation might be successful to increase the rate of bone formation and reduce the healing time when an electric field up to 7.5 V/cm is applied. Further applications of the model may deal with dosimetry analysis for other clinical modalities of electrostimulation used to improve bone and soft tissue repair such as time-harmonic fields and pulsed electromagnetic fields (PEMF), more specifically, by means of a setup in which electrostimulation is activated until the temperature reaches the viability threshold of 40-42 °C and deactivated to allow cooling down. The analysis of the effect the a repeated application of an electric field might help to devise an efficient electrostimulation protocol for bone formation.

Furthermore, it is hypothesized that an increased electrically-mediated bone formation over the receptor surface may be beneficial in applications such as anchorage of implants [216]. Since implants are mostly made of biomaterials and have bioactive surfaces that strive for improving the interaction with the physiological environment [217, 218], the formation of new bone (tissues) in direct contact with the biomaterial of an implant during the first stage of recovery after implantation is paramount for the success of the treatment [201, 219, 220]. A detailed discussion about the formation of new tissues over the surface of an implant is presented in Chapter 10, where the proper anchorage or osseointegration of a dental implant is assessed numerically from the standpoint of both the biological process of wound healing and the influence of electrostimulation.

In addition, the mathematical formulation for the temperature and the thermal damage during the exposure to an incident electric field may be used in combination with the individual cell models presented in Chapters 4-6 to better evaluate the feasibility of changes in the shape, location and size of the cells to induce non-thermal effects. Nevertheless, the first limitation to carry out this analysis is the limited availability of numerical parameters for the thermal properties of cells and cell compartments. Hence, a combination of both numerical and experimental research is further needed to draw
sound conclusions regarding any possible non-thermal effect.

9.7 Conclusion

This chapter is dedicated to present a numerical approach for quantifying the thermal effect of electrostimulation during bone formation across a fracture site. The numerical results show that the use of electrostimulation associated to electric fields with magnitude as high as 7.5 V/cm is beneficial for both the migration of osteoprogenitor cells into the wound site (Fig. 9.2) and the synthesis of new bone matrix (Fig. 9.5). Moreover, an electric field with magnitude up to 7.5 V/cm is capable to improve new bone formation directly at the receptor surface. Although the results from the simulations compare favourably with experimental data on electrically-guided cell migration [45, 77], temperature withstanding capability of (bone) cells [46, 47], and bone formation in the presence of electric fields [2, 68], additional experimental research to better identify the implications of using electric fields during bone formation at the cell and molecular level is required to better evaluate the numerical findings.
Cell behaviour and tissue formation are influenced by an electric field. There are several protocols for the exposure to electric fields that aim at increasing the rate of tissue recovery and reducing the healing times in wounds [2, 36, 76, 77]. However, the underlying mechanisms of interaction between the electric field and both cells and tissues are still a matter of research. Although most of the knowledge on cell behaviour and tissue formation during exposure to an electric field comes from experimental protocols [4, 45, 204], mathematical models and numerical simulations have been used to provide information about the interaction between proteins, cells, tissues and the electric field during the healing process [160, 196, 221]. Reaction-diffusion models have been proven suitable to describe cell migration and proliferation in the presence of chemical stimuli, as shown in Chapter 7 [160, 164, 196, 212]. Still, as discussed in Chapter 8 and Chapter 9, these type of models are also suitable to analyze the effect of electrical stimulation (electrostimulation).

In this chapter a mathematical model is introduced for the influence of electrostimulation during wound healing and new bone formation or osteogenesis. The clinical scenario corresponds to the wound healing process and osteogenesis at the interface between a dental implant and the host bone. Due to the reaction-diffusion nature of the model, numerical results represent spatial-temporal patterns that account for the

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influence of the electric field during blood clotting, osteoprogenitor cell migration, granulation tissue formation, and deposition of bone formation. In addition, the model describes the electrically-mediated cell behavior and tissue formation that lead to increased osteogenesis over rough implant surfaces. Since the numerical results compare favorably with experimental evidence, the model can be used to predict the outcome of using electrostimulation in other types of wounds and tissues.

10.1 The bone-dental implant interface

Teeth anchorage may be at risk due to diseases and infections of the oral cavity, which may have as consequence the need for tooth (root) removal [211]. Another source of damage comes from hard impacts at both face and mouth level during an accident, which can cause tooth fracture and even cause a tooth to be knocked out [210, 222]. Current dental techniques allow replacement of missing teeth using different types of prosthesis. In some of these techniques, the anchorage of the prostheses to the jaw bone is achieved by the insertion of a dental implant. The dental implant is a biomaterial screw-like device that replaces the root of the missing teeth, as shown in Fig. 10.1 [189, 190]. Once in place, the dental implant supports the artificial crown or dental prosthesis that replaces the missing tooth.

The insertion of the dental implant initiates a sequence of wound healing events that create a firm, stable and long-lasting connection between the bone and the implant, a process called \textit{osseointegration} [189]. An adequate osseointegration is conditioned to both the acceptance of the implant by the living tissues and the formation of viable bone over the implant surface [189, 190]. Healing at the connection zone or \textit{bone-dental implant interface} depends on biological and patient-related factors [189], the implant design and surface [217, 219], the load distribution between the bone and the implant [177, 190, 207], the surgical procedure used during the insertion of the implant [190, 224], and more recently studied, the electrical properties of the biological tissues [14, 76].

During wound healing and bone formation, several biological and biochemical events take place at the bone-dental implant interface. These processes can be summarized as a sequence of events started with the insertion of the implant. The insertion causes bleeding, which activates the cascade of biochemical events leading to blood clot and the formation of a fiber-like matrix [198]. Using the scaffold created by these fibers, osteoprogenitor cells start migrating towards the implant surface in order to repair the damaged tissues [175, 178]. Accordingly, new vascular structures are formed and a new bone matrix is deposited in direct contact with the implant surface [175]. This bone matrix is constantly remodeled until it reaches a biomechanical stability similar to that of the original bone, a process that leads to the adequate osseointegration of the implant [175, 177, 178].
Furthermore, when a foreign device is inserted in the jaw bone, the surface interacts with the living tissues in such a way that the biological events that lead to bone formation and osseointegration are modified [201, 225]. In consequence, the success of the bone-implant interface depends on two additional phenomena related to the implant surface: osteoinduction and osteoconduction [189, 201]. Osteoinduction is the recruitment of stem cells that are somehow stimulated to develop into bone-forming cells [189]. Osteoconduction is the migration of these cells towards the implant surface. As mentioned in Chapter 8, these cells colonize the surface and start to deposit new bone directly over it [189, 201]. Both phenomena depend on the biocompatibility of the implant material and the surface characteristics [217]. More specifically, implants with a bioactive surface and irregular surface topographies have better chances of being osseointegrated than those without special coatings or with smooth surfaces [201, 219].
10.2 Bone formation around a dental implant

Bone formation at the bone-dental implant interface is the result of complex biological and biochemical events involving different types of cells and molecules \[175, 176, 177, 178\]. These events can be simplified into four successive stages \[175, 178\]: 1) bleeding and blood clotting, 2) fibrinolysis, 3) fibroplasia, and 4) osteogenesis.

The surgical procedure for implant insertion implies making an injury to intact jaw bone tissues causing bleeding \[201\]. During the bleeding stage, the damaged blood vessels constrict and the platelets from the bloodstream are activated to form a plug which temporarily stops the blood loss \[198, 226\]. The temporary plug is then replaced by a haemostatic plug formed by the reaction kinetics between two blood proteins, specifically, thrombin and fibrinogen\[198, 227\]. Thrombin converts fibrinogen into fibrin fibers \[198, 227\]. These fibers accumulate to form the fibrin clot, which completely detains the blood pouring about 10 minutes after the injury \[228\]. This fibrin clot also protects the tissue left exposed after implantation \[175, 226\]. Then, a cleansing activity also known as fibrinolysis and carried out mainly by macrophages and neutrophils, starts the degradation of some of the fibrin fibers to create paths for the migration of the osteoprogenitor cells \[201, 229\].

About four days after the injury the fibroplasia stage begins. During this stage, the fibrin clot is replaced by a new extracellular matrix known as granulation tissue. This new matrix is mainly composed of collagen and new capillaries \[175\] and provides mechanical and nutritional support for the migration of the osteoprogenitor cells \[229\]. Cell migration is stimulated by several molecules released during blood clotting and platelet activation, such as the platelet-derived growth factor (PDGF), the transforming growth factor beta (TGF-\(\beta\)) \[201\] and the fibroblast growth factor (FGF) \[199\].

The osteogenesis stage initiates around fourteen days after injury. During this stage, new bone starts to appear along the vascular structures \[229\]. Concomitantly, the granulation tissue is replaced by new collagen fibers which are slowly mineralized to create the new bone matrix \[207\]. From oral implantology it is known that the bone formation (osteogenesis) aimed at repairing the injury caused in the jaw bone after the insertion of a dental implant is a two-way process: direct osteogenesis or contact osteogenesis \[201\]. In direct osteogenesis, the new bone is deposited from the host bone surface and towards the implant surface. In contact osteogenesis bone deposition is started at the implant surface and evolves towards the host bone. Although contact osteogenesis increases the rate of bone formation at the interface \[201\], a clinically accepted osseointegration rate of 80 % is usually reached within 3-4 months after implant placement \[190, 201\]. During this time lapse, bone modelling and remodelling processes work together to fully restore the biomechanical integrity of the new bone and to consolidate the anchorage of the dental implant \[178, 207\].
10.3 Effects of electrostimulation in wound healing

The four stages leading to bone formation at the dental-implant interface are affected by an electric field. It has been found that platelets and fibrinogen, among many other molecules present during blood clotting, tend to migrate towards the positive electrode of the applicator when a potential difference is applied to a blood sample [230]. This behavior is the principle of electrothrombosis and of a platelet measurement technique called electrical aggregometry. During electrothrombosis, platelets are activated to form a thrombus or blood clot after the application of a static electric field [231]. In electrical aggregometry, two platinum electrodes are immersed in a blood plasma sample. Platelets respond to the electrical stimulus by accumulating at the polarized electrodes, making it possible to calculate the number of available cells [231].

As detailed in Chapter 8, cell migration direction and speed are directed by a static electric field [45, 64, 77]. Since a static electric field induces reorientation of cell surface and signaling molecules such as integrins [14, 191], the application of this kind of stimulus appears to be more important for cell migration than chemical and hapotactic signals [4, 186]. In fact, as shown in Chapter 8, an electric field is able to override a chemical migration cue [45]. Accordingly, and since electrotaxis has been observed in many different types of cells [4, 45], the use of an electric field might play significant role during cell recruitment for wound healing and new tissue formation [12].

Based on initial researches conducted in the 1800's, it is recognized that biological tissues and especially bone have a characteristic electrical behavior [12, 67]. In the presence of a compression load, bone responds with an electronegative potential that stimulates new bone deposition. In contrast, when tension is applied, an electronegative potential inducing bone resorption is obtained [67]. This electrical bone response to mechanical loading is considered the underlying mechanism for the bone remodelling process in the presence of mechanical stress, a mechanisms also known as Wolff’s Law [67].

Therefore, bone electrostimulation is based upon the electrical control that can be exerted in both bone deposition and resorption [182]. Accordingly, electrostimulation techniques using electric fields are used to increase bone formation and bone healing in delayed union and nonunions in long-bone fractures [182]. Since an electric field seems to replace the metabolic activity induced by the mechanical action of gravity and one’s body weight, electrostimulation has useful applications for the prevention of bone loss during immobilization procedures, prolonged stays in bed, and space missions [186, 207].

Furthermore, the electric field stimulates bone formation around implants and reduces the healing time post-surgery [204]. Experimental studies conducted on animal protocols and dental implants have shown that electrostimulation increases the rate of wound healing at the bone-dental implant interface [192, 204]. Although in general this increase leads to higher osseointegration rates, important details regarding the dosimetry of the
stimulus such as magnitude, exposure time, and duration of the treatment need to be fully validated against the effect on osseointegration [192, 204].

10.4 Mathematical model

Most of the biological mechanisms of the bone-dental implant healing process are influenced by an electric field. However, the mathematical description considered in this chapter accounts only for the most significant elements. More specifically, the model first describes blood clot formation as the consequence of a kinetic reaction between thrombin \( (T_h(x,t)) \) and fibrinogen \( (F(x,t)) \) to produce fibrin \( (f(x,t)) \) [198]. After formation of the fibrin network, the osteoprogenitor cells \( (C_o(x,t)) \) start to migrate from the host bone surface towards the implant surface. The assumption of a single cell contingent encompasses the different cell types present during wound healing, especially macrophages, fibroblasts and osteoblasts [175, 201]. Cell migration is assumed to occur in response of an osteoprogenitor chemical \( (Q_o(x,t)) \), representing the complex set of growth factors, proteins and other molecules present during cell migration and proliferation [199]. As a consequence of cell migration, the fibrillar network is replaced by granulation tissue \( (G(x,t)) \). The bone-dental implant interface is assumed to be placed between two plates connected to a DC power source. Therefore, the electrical stimulus is given as the electric potential \( (\Phi(x)) \) across the interface, similarly to the models presented in Chapters 8 and 9.

10.4.1 Thrombin

The equations for thrombin and fibrinogen are a modified form of the glycolysis model in (7.5) used in Chapter 7 to describe stripe-like spatial-temporal patterns as shown in Fig. 7.5. Accordingly, the thrombin concentration is modeled via a reaction-diffusion equation, namely

\[
\frac{\partial T_h}{\partial t} = \nabla \cdot (D_{T_h} \nabla T_h - T_h E_{T_h} \nabla \Phi) + k_1 P - k_2(\Phi) T_h F^2 - k_3 T_h, \tag{10.1}
\]

where \( D_{T_h} \) is the diffusion coefficient and \( E_{T_h} \) is the electrical dispersion. The negative sign indicates migration towards the negative electrode, since thrombin carries positive electrical charge [232]. Thrombin production is modeled as the conversion of a given concentration of prothrombin \( P \) into thrombin [233]. A coupling coefficient \( k_1 \) controls this conversion. The non-linear term represents the transformation mechanism between thrombin and fibrinogen, with \( k_2(\Phi) = k_2(1 - c_1\Phi) \) where \( k_2 \) is the transformation parameter [221]. The term \( (1 - c_1\Phi) \) is the electrical mediation, where \( c_1 \) is the anisotropic electrical control coefficient for thrombin. The negative sign thereof accounts for lower
thrombin concentration near the positive electrode, which can also be understood as a higher amount of thrombin used in the transformation mechanism [232, 234]. Finally, thrombin decay is modeled by a linear term with coefficient $k_3$.

### 10.4.2 Fibrinogen

Similarly to thrombin and in accordance with (7.5)b, the fibrinogen concentration is modeled via a reaction-diffusion equation that reads

$$\frac{\partial F}{\partial t} = \nabla \cdot (D_F \nabla F + F E_F \nabla \Phi) + k_2(\Phi)T_h F^2 + k_3 T_h + k_4(\Phi)G_p - k_5 F, \quad (10.2)$$

where $D_F$ is the diffusion coefficient and $E_F$ is the electrical dispersion. The positive sign represents the attraction towards the positive electrode in view of the negative electric charge of fibrinogen [230]. The non-linear term $k_2(\Phi) = k_2(1 + c_2 \Phi)$ represents the transformation mechanism with $k_2$ the transformation parameter [221], $(1 + c_2 \Phi)$ the electrical mediation term, and $c_2$ the electrical control coefficient for fibrinogen. The positive sign thereof accounts for higher amounts of fibrinogen near the positive electrode [230, 234]. The production of fibrinogen in the presence of thrombin is described through the coupling coefficient $k_3$. Additional production is related to the fibrinogen contained in the granules $G_r$ released by the platelets once they become activated [226]. More precisely, $k_4(\Phi) = k_4(1 + c_3 \Phi)$, where $k_4$ is related to the fibrinogen released by the activated platelets [221, 233], $(1 + c_3 \Phi)$ is the electrical mediation, and $c_3$ is the electrical control coefficient for platelet activation [231]. Finally, fibrinogen decay is modeled by a linear term with coefficient $k_5$. Additional details on how (10.1) and (10.2) correspond with a modified form of the glycolysis model in (7.5) are given in Appendix D.1.

### 10.4.3 Fibrin

The conversion mechanisms between thrombin and fibrinogen create a fibrin fibrillar network [198, 227]. The formation of this network is modeled by the exponential mechanism [221]

$$\frac{\partial f}{\partial t} = \left[ \frac{\alpha f_{\text{max}} (1 - \exp(-\alpha t - \beta f - \beta_\Phi \Phi f))}{1 - (\beta + \beta_\Phi \Phi) f_{\text{max}} \exp(-\alpha t - \beta f - \beta_\Phi \Phi f)} \right] \left( \frac{W_F p}{f + W_F p} \right), \quad (10.3)$$

where $\alpha$ is the fibrin activation rate [221, 227], $\beta$ is the blood quality factor related to both the number of activated platelets and the concentration of fibrinogen released by them [226], and $\beta_\Phi$ is the electrical blood factor related to the activation of the negatively charged platelets that accumulate near the positive electrode [235]. The parameter $f_{\text{max}}$ corresponds to the maximum fibrin concentration [174]. Parameters $W_F$ and $p$ are the
threshold and the slope of an activation function similar to that shown in Fig. 7.1a that transmits the fibrinogen transformation pattern to the fibrillar formation.

10.4.4 Osteoprogenitor cells and chemical

The migration of osteoprogenitor cells in response to both chemical and electrical stimuli is modeled based on a modified form of the chemotaxis model in (7.6) [162, 236] and likewise presented in (8.1) and (8.2), namely,

$$\frac{\partial C_o}{\partial t} = \nabla \cdot \left[ D_{C_o} \nabla C_o - H_{C_o} C_o \nabla Q_o - E_{C_o} C_o \nabla \Phi \right] + r_{C_o} C_o \left[ 1 - \frac{C_o}{C_i} \right] \left[ \frac{Q_o^D}{Q_o^P + W_{Q_o}^P} \right] - \delta_{C_o} C_o,$$

(10.4)

$$\frac{\partial Q_o}{\partial t} = D_{Q_o} \nabla^2 Q_o + (r_{Q_o} + r_{\Phi} \Phi) C_o + r_{QD} \left( \frac{f^P}{f^P + W_f^P} \right) - (\delta_{Q_o} + \delta_{Q_C} C_o) Q_o,$$

(10.5)

where $D_{C_o}$ and $Q_{C_o}$ are the diffusion coefficients for both the cells and the chemical, $H_{C_o}$ is the chemotactic sensitivity of the cells, and $E_{C_o}$ is the electrical sensitivity coefficient. Its negative sign indicates cell migration towards the positive electrode [77]. Cell proliferation is modeled assuming a logistic growth with coefficient $r_{C_o}$ and carrying capacity $C_i$ [162]. Parameters $W_{Q_o}$ and $p$ referred to the threshold and slope of an activation function for cell migration in the presence of a minimum chemical stimulus [162]. Parameters $r_{Q_o}$ and $r_{\Phi}$ refer to the chemical production, the former being the chemical released by the cells during migration and proliferation [201], and the latter the increased chemical production in the presence of the electric field [68]. Finally, $r_{QD}$ refers to the chemical released by the activated platelets [198], which accumulate at higher fibrin formation sites [226], as indicated by the activation function with threshold $W_f$.

10.4.5 Granulation tissue

The replacement of the fibrin network by granulation tissue is modeled using a modified form of the tissue formation equation in (7.15) that couples the model of glycolysis with the model of chemotaxis, as shown in Fig. 7.8. Accordingly,

$$\frac{\partial G}{\partial t} = [r_{GF} F + r_{G\Phi} G \Phi - \delta_{G} G] \left[ \frac{C_o^P}{C_o^P + W_{C_o}^P} \right].$$

(10.6)

Since the fibrin fibrillar network is transmitted from fibrinogen, and new tissue is formed along the fibrin fibers [175, 176], the granulation tissue formation is initiated with the
presence of fibrinogen, coupled with the coefficient $r_G$. An increased tissue formation observed during exposure to the electric field [186, 237] is modeled as a linear term with coefficient $r_B$. Tissue degradation is coupled via the coefficient $\delta_G$. The parameters $W_C$ and $p$ are the threshold and the slope of an activation function associated with the minimum cell density needed to start the conversion of the fibrin network into granulation tissue.

### 10.4.6 Osteogenesis

The percentage of new bone formation due to osteogenesis is modeled using the transformation mechanism in (9.5), viz.

$$B = 1 - \exp\left(-r_{BC}(\Phi)C_o - r_{BQ}(\Phi)Q_o\right), \quad (10.7)$$

where $r_{BC}(\Phi)$ and $r_{BQ}(\Phi)$ are control parameters related to the influence of the electric field in both cell adhesion and cell proliferation as mentioned in Chapter 9 [4, 237]. Hence, $r_{BC}(\Phi) = r_{BC}(1 + c_4\Phi)$, and $r_{BQ}(\Phi) = r_{BQ}(1 + c_5\Phi)$, with $r_{BC}$ and $r_{BQ}$ the control coefficients for cell adhesion and cell proliferation [160], and $(1 + c_4\Phi)$, $(1 + c_5\Phi)$ the electrical mediation. The parameters $c_4$ and $c_5$ are the electric coefficients for both cell adhesion and cell proliferation.

### 10.5 Description of the simulation

The mathematical model given by (10.1)-(10.7) is supplemented with (8.3), which is used to obtain the electric potential $\Phi$. The set of partial differential equations is solved on a bi-dimensional domain that represents a section of the bone-dental implant interface, as shown in Fig. 10.2 [160, 221]. The width of the interface was obtained from experimental data [217, 238] for both the thickness of the layer of proteins attached to the implant surface and the volume of necrotic tissue left behind by the insertion procedure.

The initial conditions are zero for the equations representing fibrin, osteoprogenitor cells, granulation tissue, and new bone formation. The initial values for thrombin and fibrinogen correspond to small perturbations of the steady-state of the modified form of the glycolysis model as mentioned in Chapter 7. The initial condition for the osteoprogenitor chemical concentration is $5\times10^4$ mg/ml [196]. For the osteoprogenitor cell density $C_o(x, t)$, we assume a density of $1\times10^6$ cells/ml [196] at the host bone side, which is normalized as mentioned in Chapter 8.

Moreover, an injury area 5-µm thick located at the proximity of the implant surface as shown in Fig. 10.2c (corresponding to the contact zone between the tissue in formation
and the implant surface [238]) is used to define a boundary condition for the osteoprogenitor chemical $Q_o(x,t)$. Accordingly, (10.4) is rewritten for the subdomains within this area as

$$
\frac{\partial Q_o}{\partial t} = D_{Q_o} \nabla^2 Q_o + r_{QI}R_S + r_{Qo}C_o + (r_{qI}C_o + r_{qI}f)\Phi - (\delta_{Qo} + \delta_{Qo}C_o)Q_o.
$$

(10.8)

Upon activation, platelets release cytoplasmatic granules which contain fibrinogen and signalling molecules for the migration of the osteoprogenitor cells [226]. The implant surface is assumed to have surface irregularities able to activate platelets [218]. Therefore, the parameter $R_S$ in (10.8) controls the presence of this irregularities, namely,
$R_S = 0$ indicates a smooth surface and $R_S = 1$ corresponds to a rough one. The increment of the chemotactic signal near the implant surface due to platelet activation at the injury area is represented in (10.8) by the coefficient $r_{Qf}$.

An increased chemical signalling over a rough surface is responsible for an enhanced bone formation near the implant surface, allowing for contact osteogenesis [201, 218]. In contrast, the smooth surface lacks additional platelet activity at the injury area, and this lowers the osteoprogenitor chemical concentration near the implant surface [201, 218] and the chances of contact osteogenesis. Moreover, since platelets accumulate near the positive electrode but over the fibrin fibers [174, 235], an additional production term with coefficient $r_{\Phi I}$ is introduced to account for an electrically-mediated increase in platelet activation and consequent chemical release [231].

As it was done in Chapters 8 and 9, the electrostimulation is described as an ideal DC voltage source that delivers a constant electric field across the wound site. This source is assumed connected to the sides of the domain, as shown in Fig. 10.2d. The positive electrode is placed on the implant side to exploit the electrically-induced drift of thrombin and fibrinogen due to polarization that leads to higher blood clot density near the implant surface [230]. This choice also ensures that the osteoprogenitor cells migrate due to electrotaxis towards the implant surface [77]. The nodes at the positive electrode are set to $\Phi = E L$, where $E$ is the magnitude of the desired electric field and $L$ the largest width of the domain, i.e., 0.55 mm. The nodes at the negative electrode are set to zero (ground).

The electrostimulation influences bone deposition at the bone-dental implant interface [192], and this influence is believed to depend on the magnitude of the electrical stimulus [45, 192, 239]. Accordingly, four simulation cases were considered, i.e., when $E$ is equal to 0 V/cm, 2.5 V/cm, 5.0 V/cm and 7.5 V/cm, respectively. Higher electric field intensities are not considered, in view of the thermal damage that an electric field above 7.5 V/cm can cause, as explored in detail in Chapter 9. The endogenous electric fields and currents present after an injury and during wound healing are not considered [12]. Moreover, the influence of the electric field in long-lasting biological processes, such as wound healing and bone deposition, has been observed for fields in the ELF range (0-300 Hz) [12, 64, 77]. Therefore, given that at these frequencies a quasi-static approximation is a fair approximation to the problem, an electrostimulation scenario involving time-harmonic fields is not considered.

The numerical solution is carried out by means of the finite elements method, following the discretization technique described in Appendix A. The bi-dimensional grid consists of 9132 quadrilateral patches and 9350 nodes with as many linear elements associated. The values of the numerical parameters are obtained from literature and are detailed in Appendix D.2. The simulation corresponds with the first 21 days of healing at the bone-dental implant interface [199, 229]. The simulation time is adjusted in such a way
that the first 10 minutes refer to the fibrin network formation [228]. Then, using the fibrin network as support, the osteoprogenitor cell migration begins 3 days after injury [199, 229], and takes 5 days to cover the bone-dental implant interface. The formation of the granulation tissue begins 4 days after injury [175, 176], and lasts 3 days. The final 14 days are dedicated to the new bone formation [199, 229]. The exposure to the electric field is maintained throughout the simulated 21 days to reproduce experimental protocols [67, 215].

10.6 Numerical results

10.6.1 Formation of the fibrin network

The fibrin density in the presence of an electric field is higher near a preferential electrode [231]. Numerical results relevant to the fibrillar formation 10 minutes after the implant insertion are shown in Fig. 10.3. The stripe-like pattern resembles a blood clot fibrillar network that covers the entire interface [174, 175]. The dimension of the fibers is comparable with the width and length of fibrin fibers formed under experimental conditions [174, 227]. The fibrillar formation is uniform throughout the interface when no electric field is applied, as shown in Fig. 10.3a. In this case, the maximum fibrin density is below the maximal value of 2.5 mg/ml found experimentally [174].

The fiber density increases near the positive electrode when the electric field is applied [234, 235]. Therefore, the fibrin formation is attracted and increased in the vicinity of the positive electrode. In contrast, formation near the negative electrode remains unchanged, since no fibrin is attracted to it (Fig. 10.3b-d) [234]. Averaging the fibrin formation at the injury area demonstrated that the fibrin attraction depends on the magnitude of the electric field [234, 240]. Accordingly, no electric field exposure yields a maximal fibrin density at the injury area of 1.75 mg/ml, whereas exposure to an electric field of 7.50 V/cm yields a maximal density of 2.25 mg/ml. Values computed for electric fields of 2.5 V/cm and 5.0 V/cm are 1.98 mg/ml and 2.14 mg/ml, respectively.

10.6.2 Osteoprogenitor cells migration

Figure 10.4 shows the results for the cell migration pattern 5 days after injury. In all cases, the migration pattern is directed from the host bone surface (right) toward the implant surface (left). The fibrin fibrillar network supports cell migration, a process depending on both the surface roughness and the electric field [45, 219]. The surface roughness mediates in the chemotactic response, whereas the electric field induces electrotaxis, as described in Chapter 8. Therefore, when no electric field is applied and a smooth surface is assumed (Fig. 10.4a), the cell migration towards the implant surface...
Figure 10.3: Fibrin formation after 10 minutes of exposure to an incident electric field with magnitude a) zero, b) 2.5 V/cm, c) 5.0 V/cm, and d) 7.5 V/cm.

is mediated by the cellular chemical stimulus only in such a way that 5 days after injury the lower-left side of the interface is still not reached by the migration front. In contrast, when no electric field is applied and a rough surface is assumed, cells are also instructed to migrate towards the implant surface in response to the chemical release originated from the platelet activation at the injury area [226], as shown in Fig. 10.4b. This chemotactic stimulus is strong enough to initiate cell accumulation over the entire implant surface 5 days after injury.

The presence of the electric field induces cell migration by electrotaxis, increasing the cell accumulation over the implant surface depending on the electric field magnitude, as shown in Figs. 10.4c-h. In fact, even for the implant with smooth surface exposed
to a 2.5-V/cm electric field (Fig. 10.4c) there is more cell redistribution along the interface and a higher cell accumulation over the implant surface as compared to both the unexposed smooth and rough implants. Furthermore, the presence of surface irregularities (which add to electrotaxis the chemical release at the injury area) improves cell migration and increases cell colonization of the implant surface in agreement with experimental observations [188]. These results also confirm that a higher electrically-mediated cell migration speed and an increased cell accumulation over the implant surface can be obtained when both electrotaxis and chemotaxis induce cells to migrate towards the same direction, as shown in Figs. 8.3 and 8.5 for a 5-V/cm electric field.

An observation of the electrically-mediated cell redistribution and cell accumulation over the implant surface 7 days after injury is obtained by evaluating the cell density along a straight line running parallel to the $x$-axis and crossing the $y$-axis at 0.1 mm. The results plotted in Fig. 10.5a show that the application of an electric field of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm increases by 25 %, 65 % and 110 %, respectively, the cell accumulation over a smooth surface as compared to that obtained without electric field (Fig. 10.5a). Similarly, increases of 27 %, 61 % and 92 % in cell accumulation over the implant surface are obtained during exposure to 2.5-, 5- and 7.5-V/cm electric fields compared to the unexposed case (Fig. 10.5b).

Cell migration is activated by the presence of both electrical and chemical signaling [45]. While the electrotactic stimulus is uniform for the entire interface and depends on the electric field magnitude only, the chemical signal varies due to the presence of surface irregularities. Since both the surface irregularities and the electric field induce higher platelet activation at the injury area with consequent higher release of chemical signaling, exposure to the electric field induces higher chemical concentrations near the implant surface that decay exponentially along the interface due to diffusion, as showed in Fig. 10.5c-d for points along a straight line running parallel to the $x$-axis and crossing the $y$-axis at 0.1 mm.

Numerical results indicate that in the absence of the electric field, the presence of surface irregularities increases by 1.4 times the osteoprogenitor chemical concentration at the injury area as compared to the case of a smooth surface. This result is in agreement with experimental observations in surfaces with topographical irregularities of 1 $\mu$m [241]. Furthermore, a 1.9-fold increase in the chemical concentration near the implant surface is observed for a smooth surface exposed to a 2.5-V/cm electric field. This reveals that even at low electric field intensities induces a chemical activation higher (but comparable) to that observed in an unexposed rough surface [219, 241], an indication that the electric field provides a surface with a better osteoinductive capacity. A smooth surface exposed to electric fields of 5.0 V/cm and 7.5 V/cm leads to 2.7-fold and 3.5-fold increases respectively, as compared to the unexposed case. Likewise, a rough surface exposed to electric fields of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm shows
Figure 10.4: Osteoprogenitor cell density (in $10^6$ c/ml) 5 days after injury during exposure to an incident electric field with magnitude a, b) zero, c, d) 2.5 V/cm, e, f) 5.0 V/cm, and g, h) 7.5 V/cm. Left column: Smooth surface ($R_s = 0$). Right column: Rough surface ($R_s = 1$).
10.6.3 Granulation tissue formation

The replacement of the fibrin network with granulation tissue begins with the migration of the osteoprogenitor cells [175]. Numerical results in Fig. 10.6 show the dependence of this replacement on both the fibrin and osteoprogenitor cells spatial-temporal patterns,
following the pattern inheritance initially described in (7.15). Specifically, in Fig.10.6a-c the granulation tissue inherits the fibrillar appearance of fibrin shown in Fig. 10.3 and travelling wave-like pattern of the osteoprogenitor cells shown in Fig. 10.4. Since fibrin formation is unaffected by the surface roughness, and in (10.6) tissue formation is controlled by a threshold in cell density that can be reached with or without the presence of surface irregularities, the formation of granulation tissue is the same for both smooth and rough implant surfaces.

Furthermore, the electric field increases the granulation tissue density near the implant surface. Accordingly, the exposure to electric field intensities of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm 5 days after injury accelerates the accumulation of new tissue in the injury area due to the electrically-mediated cell migration and accumulation over the implant surface as shown in Fig. 10.4d-f. Furthermore, when an electric field of 7.5 V/cm is applied, the granulation tissue appears over the lower part of the implant surface before the arrival of the inherited cell migration front. This is possible because both the higher fibrin availability and stronger chemotactic stimulus accelerate in the few cells available the production of new tissue even before the arrival of the migration front [201]. Insight into this increment can be obtained with the aid of Fig. 10.6g, where the average granulation tissue density is evaluated 6 days after injury along a line crossing the interface at \( y = 0.1 \text{ mm} \).

### 10.6.4 New bone formation

Bone formation is a consequence of the interplay between the migration of the osteoprogenitor cells density and the chemotactic stimulation, both of which are mediated by the presence of the electric field and the surface irregularities. Numerical results plotted in Fig. 10.7 for the bone formation along a line crossing the interface at \( y = 0.1 \text{ mm} \) indicate that when no electric field is applied, the bone formation 14 days after injury over a smooth surface is especially low near the implant, with a formation of about 60 % at the injury area (Fig. 10.7a). With the rough surface, the bone formation is 74 % about 0.1 mm away from the implant surface, about 80 % half-way the interface, and about 85 % at the host bone surface (Fig. 10.7b).

In addition, numerical results show that 21 days after injury the bone formation along the entire interface is about 85 % for the case of a smooth surface, and above 86 % for the rough surface case, with a peak almost reaching 90 % at the injury area. This peak of bone formation indicates that the presence of surface irregularities increases the osteoconduction of the implant, as its known from experimental and clinical evidence [189, 218, 219]. In fact, as observed from the cell accumulation over the implant surface shown in Fig. 8.4, the smooth surface allows for direct osteogenesis only, while the rough surface enables both direct and contact osteogenesis.
When the electric field is applied, the bone formation over both a smooth and a rough surface increases with time in response to both direct and contact osteogenesis. Hence, the electric field facilitates osteoinduction and osteoconduction even over smooth sur-
Figure 10.7: Bone formation (in %) 14 days (a-b) and 21 days (c-d) after injury and during exposure to different electric field magnitudes. Left column: Smooth surface ($R_s = 0$). Right column: Rough surface ($R_s = 1$). EF = electric field.

Numerical results show that the 2.5-, 5- and 7.5-V/cm electric fields lead to average bone formation percentages 14 days after injury above 85 % along the interface (Figs. 10.7a - 10.7b), a value that increases to 90-95 % 21 days after injury (Fig. 10.7c - 10.7d). During exposure to the electric field, the bone formation directly over the implant surface is higher than it is near the host bone, being maximal for the case in which the 7.5-V/cm electric field is applied.
10.7 Discussion

All in all, the numerical results show that the biological events activated during wound healing at the bone-dental interface can be electrically controlled. Accordingly, numerical evidence shows that exposure to an electric field increases the fibrin density at the positive electrode in agreement with experimental reports [234, 240]. Since an electrical stimulus induces formation of a thicker network of fibrin and blood components directly over a metallic surface [231, 242], the stripe-like fibrin pattern near the implant surface plotted in Figs. 10.3b-10.3d reproduces fibers thicker than those obtained without the electric field (Fig. 10.3a). This in turn corresponds to larger [242] and denser [234] fibrin fibers. As a consequence, the distances between individual fibers may be smaller than the grid size and hence, individual fibers are not clearly identifiable. This explains the apparent homogeneous fibrin concentration near the implant surface observed in Figs. 10.3b-10.3d.

Regarding osteoprogenitor cell migration, numerical results represent the idea of electrotaxis or cell migration guided by an electric field [45]. Therefore, the cell migration speed depends on the electric field magnitude [236], in accordance with the results detailed in Chapter 8. Furthermore, the cell density over the implant surface increases with the electric field, as shown in Fig. 10.4, with the surface roughness being a mediator for an even larger increase. Comparing the cell accumulation over both a smooth and a rough implant surface at the three different electric field intensities analyzed shows that the average maximal increase in cell accumulation achievable with the rough surface is 25 %, a value close to the one observed experimentally [243]. Therefore, according to the results shown in Fig. 10.5, higher cell accumulation over the rough implant surface can be obtained by using a static electric field thanks to both increased platelet activation and electrically-mediated cell adhesion effects [14, 191, 219, 226].

However, the electrically-mediated increment in cell accumulation over the surface leads to reduced cell density spots located about 0.1 mm away from the implant surface for both the smooth and the implant surface, as shown in Figs. 10.4. These spots are created by the strong electrotaxis effect, which combined with the irregular surface, induce (by both electrical and chemical stimulation) cell accumulation over the implant surface that even cells that are not part of the migration front line are forced to migrate further away from their position and towards the implant. Nevertheless, the time evolution for electric fields of 5 and 7.5 V/cm shows that these spots are totally filled if cell migration is allowed to last 10 % longer than what was initially conceived. This extra time represents the additional time required by the cells to proliferate and replace the removed cells, and has no consequences on the subsequent modeling stages.

As for the osteoprogenitor chemical concentration, the increments observed when using both a rough surface and the electric field are mainly due to platelet activation [218].
Therefore, platelets aggregating over the positive electrode release their cytoplasmatic granules and create an electrically-mediated gradient of chemotactic signaling for cell migration [231, 242], in a similar way as due to the presence of surface irregularities [218, 219]. As a consequence, the migration of osteoprogenitor cells at the host bone is mediated by a gradient of chemical originated at the implant surface [226]. Moreover, the increment observed in the smooth surface case with different electric field intensities is consistent with the electric field having a stronger effect on cell migration than the surface irregularities [188]. This effect has been found to depend on the electric field intensities, which leads to higher cell migration rates [188].

Further interest in the numerical results for both cell density and chemical concentration motivates the following calculations. From Fig. 10.5, the values obtained at the implant surface in the case of an implant with a rough surface without electric field were subtracted from those obtained for a smooth surface without electric field. This yields the net increments due to the surface roughness only. These values are then added to the values obtained at the implant surface when both a smooth surface and the three different electric fields were used, results that can be considered as the net increment due to the electric field only. Hence, the addition of the separated surface and electric field effects leads to a difference of less than 1% in the cell migration at the implant surface as compared to that obtained when the two effects are combined. For the osteoprogenitor chemical concentration, the calculated difference is about 4%. Consequently, the effect that both surface roughness and the electric field have on the osteoprogenitor cell density and the chemical concentration appears to be additive, as experimentally found during corneal cells alignment in the presence of the electric field and surface grooves [188].

The numerical results also show increments in the granulation tissue formation alike to those reported in literature for soft tissue repair with and without the electric field [36, 186]. Accordingly, the tissue density when no electric field is applied is almost constant around 3 µg/ml, as shown in Fig. 10.6g. In contrast, the application of electric fields of 2.5 V/cm, 5.0 V/cm and 7.5 V/cm causes increases of 23 %, 40 % and 57 % respectively, which are comparable with increments of about 20-50% in the production of collagen-like matrices stimulated by an electric field [36, 186].

The effect of both the electric field and surface roughness in the osteoprogenitor cell density and the chemical concentration causes changes in the bone formation profile, as shown in Fig. 10.7. The numerical results indicate that the bone formation near a smooth implant surface is lower as compared to the formation near a rough surface, as clinically demonstrated [201, 244]. Furthermore, the similarities in curves obtained for both smooth and rough surfaces indicate that the presence of electric field induces contact osteogenesis in both types of surface. This is accomplished by the electrically-mediated osteoconduction (and osteoinduction) of the surface, as detailed in Chapter 8.
In addition, the ability of an implant surface to retain the fibrillar network during the contraction exerted by the migrating cells is critical for the cell colonization of the surface [201]. Surface irregularities play a major role in this retention, enhancing the bonding capacity of the surface [217, 247]. During wound healing, the fibrin fibers of the fibrillar network created during blood clotting adhere directly to the implant surface. A surface endowed with a bioactive coating and surface irregularities of micrometer size withstands better the fiber detachment due to the contractile forces generated during cell migration than a smooth surface lacking of any surface treatment [217, 219, 225]. Similarly, fiber retention can be reinforced by an electric field by influencing both the integrins and the focal contacts intervening in cell adhesion to the implant surface [14].

Accordingly, although bone formation 21 days after injury in both the smooth and rough surfaces is above the clinical accepted osseointegration rate of 80% (Fig. 10.7c-d) [189, 190, 201], it is reliable to assume that the contractile forces generated during cell migration might lead to fiber detachment and the reduction of the bone formation at the injury area, especially for the smooth surface. In fact, displacements (contractions) of the fibrillar network up to 500 µm are related to the formation of a gap between the new bone and the implant surface [213] that inevitably leads to the formation of soft tissue instead of bone [189]. A similar condition is achieved due to implant movements up to 150 µm which might be related to loading of the implant with forces generated during chewing [248].

Interestingly, the electric field influences both the integrins and the focal contacts controlling cell adhesion and increasing the bonding capacity of the implant surface [14, 21]. This leads to a a denser fibrin network over the implant surface as shown in Fig. 10.3. Therefore, the contraction of the matrix produced during cell migration might be overcome by the electric field in such a way that the number of fibers detached from the implant surface is not large enough to completely prevent cell colonization at the injury area, especially when the presence of the electric field increases the number of available fibers attached to the surface. In addition, the electrically-induced polarization on both the smooth and rough surfaces creates a surface electric charge that mediates the absorption of biomolecules needed for cell adhesion directly over the implant surface [191]. Since the presence of the electric field induces adhesion forces similar to those found at molecular level during cell adhesion and tissue contraction [60], it is plausible to assume that the contractile forces over the fibrillar matrix can be reduced with the presence of the electric field due to a consequent counteracting electro-mechanical force [14, 60, 122].
10.8 Perspectives

While the numerical results are in good agreement with data found in the literature, the model relies on a simplified sequence of the biological events that lead to bone formation at the bone-dental implant interface. Thereby, future work should be done to obtain even more accurate predictions. A first modification should include the bone remodeling stage started after the formation of the primary bone, around day 21 after injury [175, 207]. During this stage, the new bone matrix undergoes constant replacement due to hormonal and physical stimuli to acquire the same biomechanical integrity as the host bone [207]. Therefore, in order ensure bone formation rates above 80 % and a long-time electrically-induced osseointegration, a biological description for the tug-of-war between bone deposition and bone resorption, observed during bone remodelling, should be implemented together with a convenient description of the physical environment.

A second modification should include time-harmonic incident electric fields [36, 67, 182]. As discussed in Chapters 4 and 5, and because a human cell can be fairly approximated by an sphere with radius in the range 10-100 µm, the quasi-static approximation is valid for incident electric fields with frequency within the range typically used in electrostimulation, namely, 0-60 kHz [2, 67]. Nonetheless, an adaptation of the equations of the current model may be required to include frequency-dependent phenomena. In addition, the presence of frequency dispersions may lead to a different set of numerical parameters than those listed in Appendix D.2.

Additional work to investigate the influence of the electric field in the implant surface at the micro-(nano)scale level of interaction can be done to implement a model that combines the electrical representation of individual cells such as in Chapter 5 with an adequate cell dynamic. This cell dynamic should include the cellular processes described by the chemotaxis model shown in Chapter 7, applied to individual cells instead of cell population. This can be accomplished using numerical techniques such as the Pott's cellular model, cellular automata and integro-differential equations used model individual organisms (cells) and their interactions within a swarm (population) [249, 250]. Such an approach might be useful to assess the electrically-induced cell adhesion, the retention of electrically-guided molecules, and the electric surface charge activity, all processes affecting the osteoinduction and osteoconduction of the implant surface.

Moreover, it must be highlighted that in this work the stimulation of bone formation using a static electric field is similar to the application of direct electric current [67, 182], if we consider that the tissues at the interface have an electrical conductivity and thus the presence of a static electric field induces a direct electric current. Conversely to direct current electrostimulation, we have assumed that the bone around the implant is influenced by the electric field. Although this effect has been experimentally observed [204], a more detailed approximation may also deal with the effect of the direct electrical
current.

Furthermore, a dedicated experimental protocol to assess the electrically-mediated osseointegration of a dental implant is needed to fully validate the numerical results. Such a protocol may also provide additional information for the selection of numerical parameters. Nevertheless, and despite the lack of clear information, the set of values described in Appendix A has biological relevance in the light of the good agreement between numerical results and experimental data. Consequently, the mathematical model appears suitable to investigate the effect of an incident electric field in other types of wounds and tissues.

10.9 Conclusion

The numerical results suggest that a rough surface allows a higher bone formation at the injury area compared to the smooth surface, at the same time that it reduces the time needed to reach a bone formation of 80 % needed for osseointegration (Fig. 10.7). Moreover, since bone formation along the interface exposed to the electric field is higher than 80 % 14 days after injury, the numerical results show that the accepted osseointegration rate of 80 % [189] is reached in even less time with the exposure to the electric field than with the use of a rough surface. This result is in agreement with experimental observations on bone formation and osseointegration of dental implants, indicating higher bone deposition rates in shorter times when the electric field is applied [192, 204, 245]. Additionally, the increase in the bone percentage found numerically in the presence of an electric field is comparable with values reported for electrically-induced osteogenesis in rabbit mandible. In this case, the bone mineral density (BMD) measured after 10 and 20 days of the exposure to the electric field, is 90 % and 94 %, respectively [215]. Therefore, the presence of the electric field during the wound healing process of the bone-dental implant interface is suitable to improve the primary stability of the implant [213] and obtain higher osseointegration rates in a shortened healing time [215, 245].
This thesis presents the numerical results obtained from various numerical approaches used to investigate the occurrence of causal relations between biological cells and an incident electric field. These approaches are divided into two types: those dealing with an individual cell representation and those dealing with an aggregation of cells represented by a cell density (concentration).

11.1 Individual cells

In this type of representation, presented in Chapters 4-6, each cell is treated individually and described in terms of cell compartments (nucleus, cytoplasm and membrane). Each compartment is described by dielectric materials and a zero total charge, and is capable of influencing the electrical response of the cell. Therefore, the electric field outside the cell is the incident electric field modified in the close vicinity of the cell by an electric dipole-like electric field distribution. Inside the cell, both the nucleus and the cell membrane generate additional electric fields that modify the overall electric response.

Inside and outside a cell, the electric field is modified by the onset of cell-to-cell interaction created by the surrounding cells. These interactions depend on two groups of characteristics. In the first group, related to the attributes of the cells, the cell-to-cell interactions vary with i) the number of simultaneously exposed cells (cell density), ii) their position (intercellular distance), iii) the relative location of the adjacent cells (circular arcs), iv) the presence and location of the nucleus (internuclear distance), iv) the shape of both the cytoplasm and the nucleus (elongation), and v) the material properties (dispersions) at each cell compartment. In the second group, related to the conditions of the exposure, the interactions vary in terms of the frequency of the incident electric field, its magnitude and its orientation.
Any variation in the (intracellular) electric field lower than 10% of the magnitude of the incident electric field is most likely masked by both the thermal noise and other sources of noise generated by regular cellular mechanisms [19, 114]. In fact, variations from 10% to 20% are likely insufficient to trigger responses in the cells and can be overridden by the metabolic activity (homeostasis) of the cells [73, 114], the protective stress-response [72, 73], the endogenous (intracellular) electric fields [10, 114] and the changes in the electric (negative) charge of intracellular messenger proteins during phosphorylation [115]. Hence, it may well be that only those variations above 20% the magnitude of the incident electric field are enough to induce an electrically-mediated biological response.

Taking these considerations into account, the two groups of characteristics were evaluated in order to determine their ability to induce (localized) changes in the magnitude of the electric field distribution inside and outside the cells. The results allow concluding that if the right combination of characteristics is achieved, the electrical response of a cell (or group of individual cells) exposed to an incident electric field varies in a way that may be associated with possible biological effects. In other words, depending on the attributes of the cells and the conditions of exposure, the variations in the electric field distribution can be higher than 20% the magnitude of the incident electric field. For some specific cases, such as when the material properties (electrical permittivity and conductivity) are changed to maximize the intracellular electric field (Fig. 4.9b) or for extremely elongated cells (Fig. 6.8a), the variations reach twice the magnitude of the incident electric field. Said response may be initially associated with a change (to some extent) in protein electro-conformation mechanisms, ion (molecules) movement in both the intra- and extra-cellular compartments, and activated field-sensitive enzymes [101].

In addition to the variations in the electric field distribution, two types of effects in the electrical response of the cell have been identified. These effects strengthen the idea of the existence of a frequency window for the interaction mechanisms to occur [1, 35]. The first type of effects relies on the fact that the cell membrane provides an electric shield to the interior of the spherical cell during the exposure to incident fields with frequencies below approximately 1 MHz. Hence, the effects observed above 1 MHz are most likely related to effects occurring in the interior of the cell and may be governed by the intracellular electric field. These effects are possible associated with the conformational alteration of dipole reorientation in biomolecules (proteins) [10], variations in the Coulomb interactions (that govern the intracellular movement of messenger proteins between the cell membrane and the nucleus [115]), gradients of electric field around the circumference of organelles such as nucleus and mitochondria [93, 127], transport mechanisms associated with electro-osmotic flows [94, 115], molecular transport and reaction rates [44].

In the second type of effects, the shielding effect of the cell membrane reduces any
possible influence of the exposure to incident fields with frequency below 1 MHz to variations in both the transmembrane electric field and the transmembrane electric potential. These effects may be associated with modifications in the kinetics of calcium channels and the membrane surface charge [65, 66, 121], the activity of enzymes at the cell membrane [41, 94, 107], ion (protein) transport and the gating of voltage-controlled channels by changing the membrane potential [108, 122], the generation of action potentials, and how the transmission of electrical messages between cells is altered by the hyper(de)-polarization of the cell membrane [4, 14].

The numerical results using the individual cell representation provide information to better identify interaction mechanisms between cells and incident electric fields that may be capable of triggering a biological response. Although determining the nature and extent of such response is beyond the scope of this thesis, the numerical results provide target windows for future experimental work. These windows may help to explain the associations between the biological response and both the attributes of the cells and the conditions of the exposure. In fact, it can be argued that part of the lack of consensus about the interaction mechanisms corresponds to a mismatch between where in the cell (organelle, process, molecule) the electric field is causing an effect and where the observations are carried out. Hence, the identification of these target windows constitute a first step in resolving these discrepancies.

11.2 Group of cells

A group of cells, as used in Chapters 8 - 10, is defined by means of a cell density. The latter is expressed by a reaction-diffusion equation that contains terms associated with the cell migration, the cell proliferation (mitosis), the cell differentiation and the cell death (apoptosis). Since these cellular processes depend on chemical, mechanical and electrical stimuli, the spatial-temporal evolution of the cell density is obtained by solving a set of (partial differential) equations that, taken as a whole, describe the associations between the cells and their environment.

The numerical results indicate that the presence of an electric field modifies the behaviour of the cells and the chemical and mechanical conditions. This capability is the working principle of the different modalities of electrostimulation, a set of techniques used in clinical practice to enhance tissue formation, improve wound healing and relief pain. In some cases, especially those related to bone cells and tissue, the electrostimulation is generated by a static (zero frequency) electric fields, which induces an electrically-guided cell migration or electrotaxis.

Therefore, the description in terms of reaction-diffusion equations of the relation between the cells, the chemical stimulus and the incident electric field that takes place during electrotaxis (Chapter 8), leads to a mathematical framework suitable for study-
ing the risks and the benefits of using (static) electrostimulation. Then, an analysis of the temperature and the consequent thermal damage generated during electrostimulation (Chapter 9) defines a threshold for the magnitude of the incident electric field. Based on this limit, the use of electrostimulation is presented as a means to improve the bone healing process around a dental implant, increasing the rate of tissue formation and reducing the wound healing time (Chapter 10).

The numerical results show that the presence of an incident electric field with magnitude ranging from 250 V/m to 750 V/m increases the rate of cell migration. In contrast, magnitudes higher than 750 V/m are found to be detrimental to the viability of the cells. This is because the exposure to such magnitudes is capable for increasing the temperature in cells (tissues) above a safety threshold of 40-42 °C, which induces cell death. Moreover, the presence of an incident electric field proved more effective in inducing cell migration than the chemical stimulation (chemotaxis), which is present during the different stages of cell proliferation and differentiation. In fact, under certain conditions of exposure (magnitude and orientation), the electric field can override the chemical stimulus or add to it, inducing a purely electrical or an electro-chemical cell migration.

Either way, the cell migration is oriented along the direction of the electric field, which regulates the rate of cell accumulation over a surface. This mechanism, referred to as \textit{electro-osteoduction}, provides a surface with an increased bioactive capacity so that cells and tissues under formation can better adhere to it. Such an effect is combined with the presence of surface irregularities that also increase the adhesion of the cells. Therefore, both the rough surface and the presence of an incident electric field when applied to the case of a dental implant, promote a higher rate of new bone tissue formation (osseointegration).

These results demonstrate that the models based on reaction-diffusion equations are suitable for evaluating the effect of an incident electric field on biological cells and tissues. Furthermore, they enable the exploration of the biological consequences that changes in the (chemical, mechanical, electrical, thermal) environment of cells and tissues have in the biological response. However, the fact that these models provide new insights into how changes in the stimuli influence the biological response does not mean that they also provide additional information about what inside the cells (tissues) is affected by the change in the stimuli.

In other words, these models rely on a rather limited knowledge of the intricate processes inside of the cells, which in essence are considered as \textit{black boxes}. In fact, the formulations are based on a input/output approach where more attention is paid to the spatial-temporal response of the group of cells rather than to the response of each individual cell. Therefore, the numerical framework presented in this thesis is an effective way to improve electrostimulation-based therapies by controlling the exposure
11.3 Perspectives

The computational models presented in this thesis provide clues to improve current hypotheses about the possible non-thermal effects in biological cells due to the exposure to an incident electric field, proposing elements to explore the possible mechanisms of interaction. These clues are divided into those associated with how the attributes of the cells (shape, location, size) and the dynamic of a density of cells (migration, differentiation, apoptosis) change the cell response in the presence of different conditions of exposure (magnitude, frequency, orientation). This division resulted in the two types of models treated in Parts II and III.

Further work is required to implement a numerical framework that combines the effects on the individual cells that form a cluster (micro-scale) with the response of a cell density (macro-scale). On the one hand, such implementation will correspond to a generalization of the cell-to-cell interactions discussed in Chapters 4 - 6 and deal with a more realistic number of cells (about $10^6$ cells/ml). On the other hand, an approach to the changes in cell dynamics during electrostimulation that integrates the response of individual cells may help to explain the (bio-chemical) mechanisms affected by the presence of the electric field. This approach may represent a comprehensive multiscale model strategy suited to better understand the interaction mechanisms between the cells and the electric field. It may also provide plausible hypotheses for transduction processes (possibly occurring during electrostimulation such as the electrically-induced cell adhesion), the retention of electrically-guided molecules, and the activity of surface charge.

In addition to a combined approach, further exploration using the individual cell models should be carried out. In particular, the equivalent model for a spherical multi-layered cell presented in Chapter 5 can be used to study electroporation. The influence of the cell-to-cell interactions and especially the localized variations in the membrane potential that generate the so called circular arcs (Fig. 5.5) may have implications associated with localized changes in the permeability of the cell membrane. Such analysis may indicate that the electroporation of the cell membrane is not only determined by the conditions of the exposure (magnitude, frequency, exposure time) but also by the number and location of surrounding cells. A similar study can be performed to evaluate whether the higher cell-to-cell interactions occurring when the intercellular distance is minimized.
may have implications during cell electrofusion by modifying the electric field at the surface of the adjacent cells (Fig. 6.10).

Unlike the behaviour of spherical cells, numerical evidence given in [144, 151] indicates that in elongated cells, such as long nerve cells and muscle cells, the cell membrane does not completely shield the interior of the cell when exposed to low-frequency incident fields. To verify this phenomenon and to analyze the effect of the cell-to-cell interactions in the cell membrane of non-spherical cells, one option would be to adapt the mathematical framework presented in Chapter 5 to deal with non-spherical cells. Another option would be to include the cell membrane in the full-wave approach presented in Chapter 6. In this particular option, extra care must be exercised to reduce the computational cost derived from meshing a cell with its membrane. A possible alternative to this is the implementation of a reduction strategy based on macro-basis functions [150].

For a cell at rest, the unbalance in electrical charge (ionic concentration) between the intra- and the extracellular compartments is responsible for the membrane potential [15, 49]. Since most cell membranes contain charged molecules (lipids and proteins), both the inner and outer membrane surfaces bear a layer of net charge [92]. Thus, at the proximity of the cell membrane, the interaction between the bulk charge (ionic concentration) and the charged membrane surface sets up an ionic double layer of free charges, which can be assumed uniformly smeared over the surface [92, 98]. This surface charge plays a significant role during many physiological functions of the cell, such as cell adhesion, cell migration and binding of receptors to the membrane surface [63, 92].

Since this free charge spans a distance no larger than a couple of nanometers from the membrane, it can be treated as a double layer of charges [112]. Recurring to the principle of superposition, the equivalent electrical network of the multi-layered spherical cell introduced in Chapter 6 (Fig. 5.1b) can be extended to include these charge, with the difference between them generating the membrane potential [251]. This inclusion will be an additional feature to further investigate possible changes in the membrane potential of an individual cell due to changes in the attributes of the cells (shape, location, number) and the conditions of the exposure (magnitude, frequency, orientation).

A significant addition to the individual cell models will be the analysis in the time domain. Such analysis is especially important to investigate variations in the capacitive response (charging time) of the cell membrane and their implications in the membrane potential [82, 93]. Furthermore, an analysis in time may allow the consideration of variations in the conductivity of the cell membrane due to the time-dependent membrane potential [100]. These changes are related to the activation of voltage-controlled channels, especially relevant during (electro-)poration [82, 96]. Such variation in the electrical properties at the cell membrane may add to the relation between the intracellular electric fields, the cell-to-cell interactions, and the possible biological effects.

The plausibility of the induced intracellular electric field causing an effect in cell be-
haviour greatly depends on the magnitude of the incident electric field [23, 114]. Accordingly, the magnitude of 1 V/m used in the simulations shown in Chapters 4-6 is not intended to evaluate the plausibility of cellular effects due to this specific electric field. Instead, it is intended to elucidate the response of the intracellular electric field to both the presence of neighboring cells and to changes in the simulation parameters with results that are linearly proportional to the incident electric field.

Experimental data suggests that an increase in the cell temperature of 0.1-0.3 °C is enough to exclude non-thermal effects [10, 20, 59, 126]. In order to determine the electric field magnitude needed to cause this increase, the combination of (2.2) and (2.3) leads to the expression

$$|E| = \sqrt{\frac{2\rho c \Delta T}{\sigma \Delta t}}.$$  \hspace{1cm} (11.1)

Approximating $\rho$ to 1000 Kg m$^{-3}$, using the heat capacity $c$ of water [$c = 4181$ J (Kg °C)$^{-1}$)] [252], the conductivity equal to 0.3 S/m, and assuming that an increase of 0.3 °C is obtained after a short exposure time (< 1 s), (11.1) yields an electric field magnitude of approximately 2.9 KV/m. In-vitro experiments associated with (possible) non-thermal effects indicate exposure times of several (2-24) hours [10, 126]. Such an exposure time reduces the magnitude of the electric field obtained from (11.1) to about 10 V/m.

The range of variation from 10 V/m up to 2.9 KV/m indicates, on the one hand, that it may be possible to reach different magnitudes of electric field that are capable to induce a thermal effect in the cells, provided a sufficient time of exposure. On the other hand, based on the considerations on the thermal noise for a chloride ion described in Chapter 2, the highest magnitude of 2.9 KV/m will result in a kinetic energy $W_K$ of 9.6324x10$^{-15}$ eV. This value is at least eleven orders of magnitude lower than the 25.5 meV that correspond to thermal noise [18] and, hence, incapable of eliciting a biological response. This contradiction demonstrates the existence of the so-called mechanisms paradox commented in Section 1.4, and indicates the lack of correspondence between different efforts attempting at identifying possible non-thermal effects.

In fact, since electric field magnitudes as low as 100-500 $\mu$V/m have been associated with a remarkable metabolic sensitivity in neutrophils [57] and reduced cell (osteoclasts) proliferation in bone marrow [2], and a field of 1 mV/m can sensed by audioreceptor cells of the ear, electric fields with magnitudes smaller than 10 V/m seem to be still related to non-thermal effects [4]. Therefore, it may be the case that the thermal noise is an inadequate biophysical metric to irrefutably define the limit of the electric field magnitude necessary to induce a (beneficial or hazardous) non-thermal effect [1, 9, 59]. It could also be that the experimental data (measurement) is incorrect or incomplete. Therefore, only a continuous interplay of theoretical and experimental can create reliable knowledge on the matter [9].
The application cases reviewed in Chapters 8-10 correspond to the dual interest of studying the effects of electrostimulation as both a risk and a promoter for health. The findings indicate that such a dual study is necessary to improve our understanding of the conditions of exposure that might cause beneficial or hazardous biological responses. Therefore, the assessment of the influence of the electric field during tissue formation (as shown in Chapter 10), combined with the thermal accumulation and eventual cell (tissue) damage due to an excessive exposure (as shown in Chapter 9), will be useful in assisting the design of the next generation of prosthetic devices (hip implants, spine screws, artificial knees, among others) with built-in electrostimulation devices. A numerical platform to improve the design and testing of future (wearable) medical devices should then include a complete mathematical framework dealing with the biological, chemical, electrical and thermal dynamic of cells and tissues.

It is challenging to establish interactions between the cells and the incident fields based solely on a numerical standpoint. Therefore, the implementation of numerical models must be accompanied by an adequate and oriented experimental approach. The latter is not only needed to validate the numerical findings, but also to provide feedback for the modelling task. This feedback is required to endow the models with features aimed at improving the accuracy (expectancy) of the findings and to explore conditions that are technically or economically challenging from the experimental standpoint. Furthermore, a more complete understanding of the cell is paramount to improve the numerical approximations to the interaction mechanisms between the cells and the incident electric field. Such an understanding, aside of unraveling the mechanisms affected by the electric field, will also make possible new therapeutic alternatives and devices (electroceuticals) that take advantage of using electric fields in healthcare.
Appendices
To obtain the weak form of a biologically-inspired partial-differential-equations model such as the one given in (7.13), the unknown variables (in this case $u$ and $v$) can be approximated using the linear combination in (7.9) between an appropriate set of basis functions $N^i$ and the values of the unknowns $u^i$, $v^i$ which are defined at given points within the solution domain [170]. In a similar way, and using a number of discrete points equal to those used to discretize both $u$ and $v$, the geometrical variables $x$, $y$ and $z$ are expanded in terms of the basis functions, a method known as isoparametric formulation. Therefore, for a given subdomain $\Omega_e$ which defines a fixed number $m$ of unknown discrete points within a domain $\Omega$, both variables $u$ and $v$ and the geometrical variables $x$, $y$ and $z$ can be written as

\[
\text{Variables} = \left\{ \begin{array}{l}
    u = \sum_{i=1}^{m} N^i u^i = N \cdot u \\
    v = \sum_{i=1}^{m} N^i v^i = N \cdot v \\
\end{array} \right. \tag{A.1a}
\]

\[
\text{Geometry} = \left\{ \begin{array}{l}
    x = \sum_{i=1}^{m} N^i x^i = N \cdot x \\
    y = \sum_{i=1}^{m} N^i y^i = N \cdot y \\
    z = \sum_{i=1}^{m} N^i z^i = N \cdot z,
\end{array} \right. \tag{A.1b}
\]

where $N$ is a row vector containing the basis functions. Consider a bi-dimensional...
subdomain \( \Omega_e \) for which a transformation of coordinates is used to rewrite the spatial coordinates into normalized coordinates defined in the interval \([-1,1]\) as shown in Fig. A.1. By doing so, the four elementary basis functions, each one associated with one of the four points or nodes of the subdomain \( \Omega_e \), are given by

\[
N_1(\xi, \eta) = \frac{1}{4}(1 - \xi)(1 - \eta)
\]

\[
N_2(\xi, \eta) = \frac{1}{4}(1 + \xi)(1 - \eta)
\]

\[
N_3(\xi, \eta) = \frac{1}{4}(1 + \xi)(1 + \eta)
\]

\[
N_4(\xi, \eta) = \frac{1}{4}(1 - \xi)(1 + \eta),
\]

where \( \xi \) and \( \eta \) are the normalized coordinates of the bi-dimensional subdomain \( \Omega_e \) [168, 169, 170]. These basis functions are known as\textit{Lagrangian polynomials} written in normalized coordinates [169].

The introduction of normalized coordinates also implies the transformation of coordinates of the integrals present in the weak form of the partial differential equations (see (7.13)). Therefore, an additional variable transformation is needed to rewrite the domain differential \( d\Omega \) accordingly. This is accomplished by introducing the \textit{Jacobian} \( J \) of the transformation, which for the bi-dimensional subdomain \( \Omega_e \) is given by
\( \mathbf{J}(\xi, \eta) = \begin{pmatrix} \frac{\partial \xi}{\partial x} & \frac{\partial \xi}{\partial y} \\ \frac{\partial \eta}{\partial x} & \frac{\partial \eta}{\partial y} \end{pmatrix} \). \tag{A.3} 

The Jacobian is required to write an integral defined in spatial coordinates into normalized coordinates \[170\], viz.,
\[
\int_{\Omega^{(e)}} f(x, y) d\Omega_e = \int_{-1}^{1} \int_{-1}^{1} f(x(\xi, \eta), y(\xi, \eta)) |\mathbf{J}(\xi, \eta)| d\xi d\eta, \tag{A.4}
\]

where \(|\mathbf{J}|\) denotes the determinant of the Jacobian.

The spatial discretization achieved so far is complemented with an additional discretization for the time domain. This time discretization is accomplished by means of the \textit{backward Euler method} \[169, 253\]. Accordingly, the time derivative of the variable \(u\) is written in discrete form as
\[
\frac{\partial u}{\partial t} \approx \frac{\Delta u}{\Delta t} \approx \frac{u^k - u^{k-1}}{\Delta t}, \tag{A.5}
\]

with \(u^k\) the value of \(u\) at the current time step, \(u^{k-1}\) the value of \(u\) at the previous time step, and \(\Delta t\) the size of the time step. A similar approach is followed for the time discretization of variable \(v\).

In consequence, by transforming the integrals of the weak form following the equivalent Jacobian expression in (A.4), and considering the so-called \textit{Galerkin method} according to which the weighting functions \(W^i\) are the same basis functions \(N^i\) \[170, 171\], the equivalent FEM forms for the glycolysis model equations in (7.13), applicable at the subdomain \(\Omega_e\), become \[166, 167, 170\]
\[
\frac{M^{(e)} \cdot \Delta u}{\Delta t} + K^{(e)} \cdot u - (\delta R^{(e)} - k M^{(e)} \cdot u - S^{(e)}) = 0 \tag{A.6a} 
\]
\[
\frac{M^{(e)} \cdot \Delta v}{\Delta t} + K^{(e)} \cdot v - (k M^{(e)} \cdot u + T^{(e)} - M^{(e)} \cdot v) = 0, \tag{A.6b} 
\]
where,

\[
(K^{(e)})_{ij} = \int_{\Omega_e} \nabla N^T \nabla N \, d\Omega_e
\]  \hspace{1cm} (A.7a)

\[
(M^{(e)})_{ij} = \int_{\Omega_e} N^T N \, d\Omega_e
\]  \hspace{1cm} (A.7b)

\[
(R^{(e)})_{ij} = \int_{\Omega_e} N^T \, d\Omega_e
\]  \hspace{1cm} (A.7c)

\[
(S^{(e)})_i = \int_{\Omega_e} (u \cdot N)^2 (v \cdot N) N^T \, d\Omega_e
\]  \hspace{1cm} (A.7d)

\[
(T^{(e)})_i = \int_{\Omega_e} (v \cdot N)^2 (u \cdot N) N^T \, d\Omega_e
\]  \hspace{1cm} (A.7e)

In (A.7), the index \(i\) refers to the weighting functions while the index \(j\) here refers to the basis functions. Additionally, the integrals are expressed in normalized coordinates following (A.4).

Since the equivalent forms shown in (A.6) are defined in the normalized coordinate system bounded by the interval [-1, 1], the discretization of the set of partial differential equations corresponding to the glycolysis model is completed by resorting to the Gaussian quadrature method to solve the integrals numerically [170]. According to this method, the integration of a function in the range [-1,1] can be obtained by a weighted sum of function values calculated at the so-called Gaussian points. Therefore

\[
\int_{-1}^{1} \int_{-1}^{1} f(x(\xi, \eta), y(\xi, \eta)) |J(\xi, \eta)| \, d\xi d\eta \approx \sum_{r=1}^{GP_x} \sum_{s=1}^{GP_y} f(\xi_r, \eta_s) |J(\xi_r, \eta_s)| \, P_r \, P_s
\]  \hspace{1cm} (A.8)

where \(GP_x\) and \(GP_y\) are the number of Gaussian integration points, \(\xi_r\) and \(\eta_s\) are the evaluation points for the function \(f(x, y)\) in normalized coordinates, and \(P_r\) and \(P_s\) are the weight coefficients [170].

It must be observed that due to the non-linear nature of the partial differential equations in (7.5), the vectors \(S^{(e)}\) and \(T^{(e)}\) in (A.7) are calculated using the values of the variables \(u\) and \(v\) computed in the previous time step \(k - 1\). Furthermore, the solution of (A.6) amounts to finding its zeros, which can be achieved for the case of non-linear equations through the Newton-Raphson method [169]. Accordingly, a given set of non-linear
differential equations with variables \( u \) and \( v \) can be written at every current time step \( k \) in terms of the Taylor series as

\[
F(x + H) = F(x) + J_F(f_{u_h}, f_{v_h}) H + O(H^2), \tag{A.9}
\]

where \( x \) is a vector containing the variables \( u_h \) and \( v_h \) obtained in the current iteration \( h \), \( H \) is a vector containing the increments \( \Delta u_h \) and \( \Delta v_h \), \( O(H^2) \) is the quadratic error, and \( J_F \) is the Jacobian of the vector function \( F = (f_{u_h}, f_{v_h}) \) which is given by

\[
J_F(f_{u_h}, f_{v_h}) = \begin{pmatrix}
\frac{\partial f_{u_h}}{\partial u_h} & \frac{\partial f_{u_h}}{\partial v_h} \\
\frac{\partial f_{v_h}}{\partial u_h} & \frac{\partial f_{v_h}}{\partial v_h}
\end{pmatrix}. \tag{A.10}
\]

Neglecting the term \( O(H^2) \), and setting \( F(x + H) \) to be zero, (A.9) turns into

\[
J_F(f_{u_h}, f_{v_h}) H = -F(x). \tag{A.11}
\]

The solution of (A.11) is obtained by iterating as many times as required by the nonlinearities for the solution to converge towards a tolerance value close enough to zero, i.e., \( H \approx 0 \). The value of the vector function \( F \) at the new iteration \( h + 1 \) is computed by solving (A.11) for the vector \( H \) at the current iteration \( h \) and then solving for \( H = (u_{h+1} - u_h, v_{h+1} - v_h) \).

However, the solution of (A.9) can be simplified by solving for \( f_{u_h} \) and \( f_{v_h} \) separately and sharing the results for the variables \( u, v \) at the beginning of every new iteration [156, 166]. Therefore, (A.6) is rewritten using (A.9) in terms of

\[
\mathcal{F}(u_h + \Delta u_h) = \mathcal{F}(u_h) + \frac{\partial \mathcal{F}(u_h)}{\partial u} \Delta u_h \tag{A.12a}
\]

\[
\mathcal{G}(v_h + \Delta v_h) = \mathcal{G}(v_h) + \frac{\partial \mathcal{G}(v_h)}{\partial v} \Delta v_h. \tag{A.12b}
\]

where \( \mathcal{F} \) and \( \mathcal{G} \) denote the expressions at the left side of the equal in (A.6). Here, higher order terms of the expansion in Taylor series can be neglected considering that the basis functions \( N^1 \) in (A.2) are first-order differentiable functions.
Similarly to (A.11), it holds true then that

\[
\frac{\partial \mathcal{F}(\mathbf{u}_h)}{\partial \mathbf{u}_h} \Delta \mathbf{u}_h = -\mathcal{F}(\mathbf{u}_h), \quad (A.13a)
\]

\[
\frac{\partial \mathcal{G}(\mathbf{v}_h)}{\partial \mathbf{v}_h} \Delta \mathbf{v}_h = -\mathcal{G}(\mathbf{v}_h), \quad (A.13b)
\]

which can be rewritten as

\[
\mathbf{AMATRIX}_u^{(e)} \Delta \mathbf{u} = -\mathbf{RHS}_u^{(e)} \quad (A.14a)
\]

\[
\mathbf{AMATRIX}_v^{(e)} \Delta \mathbf{v} = -\mathbf{RHS}_v^{(e)}, \quad (A.14b)
\]

where \(\mathbf{AMATRIX}^{(e)}\) is generally known (from mechanics-related problems) as the elementary stiffness matrix and contains the derivatives of the terms of the equivalent FEM forms of the partial differential equations. In turn, \(\mathbf{RHS}^{(e)}\) is known as the elementary right-hand-side residual vector, and contains the equivalent FEM forms in (A.6).

Hence, for the particular case of the differential equations of the glycolysis model in (A.6), the matrix \(\mathbf{AMATRIX}^{(e)}\) is given by

\[
\mathbf{AMATRIX}_u^{(e)} = \mathbf{M}^{(e)} \frac{1}{\Delta t} + \mathbf{K}^{(e)} + k\mathbf{M}^{(e)} + \frac{\partial \mathbf{S}^{(e)}}{\partial \mathbf{u}}, \quad (A.15a)
\]

\[
\mathbf{AMATRIX}_v^{(e)} = \mathbf{M}^{(e)} \frac{1}{\Delta t} + \mathbf{K}^{(e)} + \mathbf{M}^{(e)} - \frac{\partial \mathbf{T}^{(e)}}{\partial \mathbf{v}} , \quad (A.15b)
\]

where, as a result of the chain rule, \(\frac{\partial \mathbf{S}^{(e)}}{\partial \mathbf{u}}, \frac{\partial \mathbf{T}^{(e)}}{\partial \mathbf{v}}\) are matrices equal to each other and with entries given by

\[
\left( \frac{\partial \mathbf{S}^{(e)}}{\partial \mathbf{u}} \right)_{ij} = \left( \frac{\partial \mathbf{T}^{(e)}}{\partial \mathbf{v}} \right)_{ij} = 2 \int_{\Omega_e} (\mathbf{u} \cdot \mathbf{N})(\mathbf{v} \cdot \mathbf{N}) \mathbf{N}^T \mathbf{N} \, d\Omega_e. \quad (A.16)
\]

Following a similar approach, the equivalent FEM forms of the partial differential equations of the chemotaxis model in (7.6) at a subdomain \(\Omega_e\) are
\[
\frac{M^{(e)} \Delta C}{\Delta t} + D_C K^{(e)} \cdot C - H_C L^{(e)} - r_C f(Q, W_Q) R^{(e)} + \left( \frac{r_C f(Q, W_Q)}{C_h} + \delta_C \right) M^{(e)} \cdot C = 0
\]

(A.17a)

\[
\frac{M^{(e)} \Delta Q}{\Delta t} + D_Q K^{(e)} \cdot Q - r_Q f(Q, W_Q) R^{(e)} + \left( \delta_Q + \delta_Q C \right) M^{(e)} \cdot Q = 0,
\]

(A.17b)

where the following definition, additional to those in (A.7), must be considered

\[
(L^{(e)})_i = \int_{\Omega_e} (\mathbf{C} \cdot \mathbf{N}) \nabla Q \nabla N^T d\Omega_e.
\]

(A.18)

By analogy with (A.12), (A.13) and (A.14)

\[
\begin{align*}
AMATRX^{(e)}_C &= M^{(e)} \frac{1}{\Delta t} + D_C K^{(e)} - H_C \frac{\partial L^{(e)}}{\partial \mathbf{C}} + \left[ \frac{r_C f(Q, W_Q)}{C_h} + \delta_C \right] M^{(e)} \quad \text{(A.19a)} \\
AMATRX^{(e)}_Q &= M^{(e)} \frac{1}{\Delta t} + D_Q K^{(e)} + \left[ \delta_Q + \delta_Q C \right] M^{(e)}, \quad \text{(A.19b)}
\end{align*}
\]

where \( \frac{\partial L^{(e)}}{\partial \mathbf{C}} \) is given by

\[
\frac{\partial L^{(e)}}{\partial \mathbf{C}} = \int_{\Omega_e} \nabla Q \nabla N^T \mathbf{N} d\Omega_e.
\]

(A.20)

The algebraic equations (A.6) and (A.17), which can be reduced to a system such as in (A.14), are defined at a given subdomain \( \Omega_e \). Furthermore, in (A.6) and (A.17) vectors \( \mathbf{u}, \mathbf{v}, \mathbf{C}, \) and \( \mathbf{Q} \) contain information related to the four nodes of the bi-dimensional subdomain \( \Omega_e \) as shown in Fig. A.1. Since the reduction to a system such as in (A.14) is valid for every subdomain \( \Omega_e \), the general reduction of the system of partial differential equations at the domain \( \Omega \) is given by the generic system

\[
AMATRX^{(g)}_\alpha \Delta \alpha = -RHS^{(g)}_\alpha,
\]

(A.21)
where $\alpha$ denotes any of the variables of the partial differential equations, $\text{AMATRX}^{(g)}$ is the global stiffness matrix and $\text{RHS}^{(g)}$ is the global right-hand-side vector.

Since in (A.21) the vector $\alpha$ contains information related to all the nodes of the spatial grid discretizing the domain $\Omega$, the elementary solutions $\text{AMATRX}^{(e)}$ and $\text{RHS}^{(e)}$ at every subdomain $\Omega_e$ are distributed or assembled within the global solutions $\text{AMATRX}^{(g)}$, $\text{RHS}^{(g)}$ in accordance with the specific nodes to which they are related to [169, 170]. Therefore, $\text{AMATRX}^{(g)}$ is a $n \times n$ matrix and $\text{RHS}^{(g)}$ is a vector of length $n$, with $n$ the total number of nodes in the mesh.

Furthermore, the solution at the nodes shared by more than one element are obtained by adding up the contributions coming from each shared element, which are properly distributed within $\text{AMATRX}^{(g)}$ during the assembling procedure. This assembling procedure and subsequent solution of the system in (A.21) is carried out by the commercial software Abaqus 6.11 (Dassault Systemes, Providence, RI, USA) after reading a subroutine written by the user in Fortran. This subroutine contains the discretization of both $\text{AMATRX}^{(e)}$ and $\text{RHS}^{(e)}$, as in (A.15), (A.6) and (A.19), (A.17) [172]. Additional input files should be provided to the Abaqus solver which contain relevant information for the execution, such as the mesh, the initial and boundary conditions, the initial conditions, the numerical parameters and the simulation time.
Appendix B

Numerical parameters for the model of cell electrotaxis

The numerical parameters used in (8.1) - (8.3) were mostly obtained from the available literature, especially numerical work dealing with tissue formation [160, 196, 212]. In cases where relevant data was not available, numerical experimentation was performed to choose values that would allow the formation of spatial-temporal patterns as expected.

- $D_{Co}$: The diffusion constant of the osteoblastic cells was set to $5.0 \times 10^{-4}$ mm$^2$/min [196, 212].

- $H_{Qo}$: The chemotactic sensitivity coefficient was initially estimated as $6.95 \times 10^{-4}$ (mm$^2$/min)(ml/mg) [196]. However, by numerical experimentation the value finally used was $5.0 \times 10^{-4}$ (mm$^2$/min)(ml/mg).

- $E_{Co}$: The electrical sensitivity coefficient was estimated as the ratio of the cell velocity without electric field to the maximum electric field intensity for osteoblastic cells to migrate. The cell velocity without exposure to the electric field was estimated from [45, 77] as $2.0 \times 10^{-4}$ mm/min. The maximum electric field intensity before electroporation and cell death is around 1.2 KV/cm [22, 77]. Accordingly, the parameter was set to $5.0 \times 10^{-4}$ mm$^2$/V min.

- $r_{Co}$: The cellular proliferation rate was taken directly from [160]. The value was $3.57 \times 10^{-3}$ min$^{-1}$.

- $\delta_{Co}$: The cell death coefficient was also taken directly from [160]. The value was $1.388 \times 10^{-5}$ min$^{-1}$.
• $C_i$: The cell saturation coefficient was assumed to be the unit (in cells/ml) based on the assumption that cell density is normalized [162].

• $W_{Q_o}$: The threshold value for the activation function was set to $1.0 \times 10^{-3}$ (mg/ml) [160].

• $p$: This parameter is related to the slope of the cell proliferation activation function. The value used in the simulations was 10.

• $D_{Q_o}$: The diffusion constant of the osteoblastic chemical is $2.88 \times 10^{-3}$ mm$^2$/min [160].

• $r_{Q_o}$: The osteoblastic chemical production rate was estimated through numerical experimentation using as starting point the value $5.0 \times 10^{-5}$ min$^{-1}$ [160, 212]. After numerical adjustments, the value used in the simulations was $1.0 \times 10^{-5}$ min$^{-1}$.

• $r_{\Phi}$: No information was found for the osteoblastic electro-chemical production rate. However, it is known that electrical stimulation induces synthesis of osteoblastic chemical [76]. Since chemical production depends on the electric field intensity, the parameter is related to the electric field magnitude. The parameter was set to $5.0 \times 10^{-5}$ (V min)$^{-1}$ through numerical experimentation.

• $\delta_{Q_o}$: The decay coefficient of the osteoblastic chemical was determined from the mid-life of some growth factors [254]. Accordingly, the parameter was adjusted to $2.0 \times 10^{-3}$ min$^{-1}$.

• $\delta_{Q_C}$: The coefficient of osteoblastic chemical consumption was calculated from the rate of cell proliferation $r_{C_0}$ and a concentration of reference for the osteoblastic chemical [212]. In consequence, the parameter was adjusted to $3.0 \times 10^{-5}$ min$^{-1}$ (ml/cells).

• $\bar{v}$: The magnitude of the convection velocity in (8.4), used in the forth analysis case, was calculated from [45, 255] when performing a similar experiment. The value finally used was $1.0 \times 10^{-3}$ mm/min.
The numerical parameters used in (9.1) - (9.5) were mostly obtained from the available literature. However, in some cases the values were modified based on the numerical parameters used in the model of electrotaxis presented in Chapter 8 and other numerical models [160, 196, 212].

- \( D_C, H_C, E_C, r_C, \delta_C \): The diffusion coefficient, the chemotactic sensitivity, the electrical sensitivity, the cell proliferation and the natural cell death coefficients are adapted from the model presented in Chapter 8 and from [160]. The values are \( 1.02 \times 10^{-5} \) mm\(^2\) min\(^{-1}\), \( 2.0 \times 10^{-3} \) mm\(^2\) ml (min mg)\(^{-1}\), \( 1.02 \times 10^{-5} \) mm\(^2\) (V min\(^{-1}\), \( 3.57 \times 10^{-3} \) min\(^{-1}\), and \( 1.388 \times 10^{-5} \) min\(^{-1}\) respectively.

- \( \delta_{CT}(T), \delta_{CK}, W_T, W_K \): The cell necrosis coefficient is adapted from experimental data reporting a survival rate of 20% of the erythrocytes cultivated in-vitro at various temperatures [205]. Accordingly, \( \delta_{CT}(T) = \delta_{CT} \exp(0.18T) \) min\(^{-1}\), with \( \delta_{CT} = 1.47 \times 10^{-7} \). The apoptosis coefficient is obtained by linearization of the survival rate for the same type of cells at temperatures between 44-60 °C [205]. After averaging, \( \delta_{CK} = 4 \times 10^{-3} \) min\(^{-1}\). The minimal temperature to initiate necrosis is assumed as \( W_T = 42 \) °C [46, 78, 205], and the maximum bearable thermal damage is set to \( W_K = 1 \) min\(^{-1}\) [5, 80].

- \( D_Q, r_Q, r_\Phi, \delta_Q, \delta_{QC} \): The parameters for the osteoprogenitor chemical concentration are \( 2.88 \times 10^{-3} \) mm\(^2\) min\(^{-1}\), \( 1.0 \times 10^{-5} \) min\(^{-1}\), \( 5.0 \times 10^{-5} \) min\(^{-1}\), \( 2.0 \times 10^{-3} \) min\(^{-1}\), and \( 3.0 \times 10^{-5} \) ml (mg min\(^{-1}\)) respectively [160, 196, 212].

- \( \rho_b, \alpha_b, \beta_b, \omega_b, T_b, \sigma_b \): These parameters correspond with the material properties of the blood vessels. Accordingly, the volume density is \( \rho_b = 1056 \) Kg m\(^{-3}\), the heat
capacity is $\alpha_b = 3456 \text{ J (Kg} ^\circ \text{C})^{-1}$, and the thermal conductivity is $\beta_b = 0.46 \text{ W (m} ^\circ \text{C})^{-1}$ [87]. The blood perfusion rate is $\omega_b = 1 \text{ Kg m}^{-3} \text{ s}^{-1}$ [80] and the arterial temperature is $37 ^\circ \text{C}$ [5, 80]. Finally, the electrical conductivity is $\sigma_b = 0.25 \text{ S m}^{-1}$ [87].

- $\rho_m$, $\alpha_m$, $\beta_m$, $\sigma_m$, $M_{HG}$: These parameters correspond with the material properties of the cancellous bone. Accordingly, the volume density is $\rho_m = 1920 \text{ Kg m}^{-3}$, the heat capacity is $\alpha_m = 1613 \text{ J (Kg} ^\circ \text{C})^{-1}$, the thermal conductivity is $\beta_m = 0.39 \text{ W (m} ^\circ \text{C})^{-1}$ [104], and the electrical conductivity is $\sigma_m = 0.0755 \text{ S m}^{-1}$ [87]. Finally, the metabolic heat generation $M_{HG}$ is equal to $576 \text{ W m}^{-3}$ [104].

- $A$, $E_a$, $R$, $W_C$: The frequency factor $A$ and the activation energy $E_a$ are taken from an experimentally-based approximation quantifying the thresholds for reversible heat response in erythrocytes [205]. Accordingly, the parameters are $4.1\times10^{38} \text{ min}^{-1}$ and $2.55\times10^5 \text{ J mole}^{-1}$, respectively. The universal gas constant $R$ is $8.3145 \text{ J (mole K)}^{-1}$. Finally, in order to maintain the agreement with the selection of the material properties for solving (9.3), the cell density threshold value $W_C$ is set to 0.7.

- $r_{BC}$, $r_{BQ}$, $c_1$, $c_2$, $p$: The control parameters for cell adhesion $r_{BC}$ and cell proliferation $r_{BQ}$ are based on experimental observations on osteoblastic-like cell cultures [209] and chosen as in [160] as 1 and 75, respectively. The coefficients for the electrically-mediated cell adhesion $c_1$ and cell proliferation $c_2$ are set to reproduce experimental observations according to which the increment of the bone matrix (when applying the electric field) is in the order of 10-20 % [204, 215]. Therefore, the values are 0.4 and 0.16, respectively. Finally, the slope of the activation functions described as in (7.7) corresponds to $p = 10$. 
Numerical parameters for the model of osteogenesis in dental implants

Dimensionless form of the kinetic reaction equations

Equations (10.1), (10.2) are cast into dimensionless form by using reference values for time, space, thrombin and fibrinogen concentrations, and the electric potential [221]. The normalized variables are defined through the following relations:

\[ t = \bar{t} \cdot t_{ref} \] (D.1)
\[ x = \bar{x} \cdot L_{ref} \] (D.2)
\[ T_h = u \cdot T_{h_{ref}} \] (D.3)
\[ F = v \cdot F_{ref} \] (D.4)
\[ \Phi = \phi \cdot V_{ref}. \] (D.5)

Plugging (D.1), (D.5) into (10.1) and (10.1), the dimensionless values yield the dimensionless form of the kinetic reaction equations for thrombin and fibrinogen including the electrical effect:

\[ \frac{\partial u}{\partial t} = \nabla \cdot (D_u \nabla u - uH_u \nabla \phi) + \delta - (1 - \gamma_1 \phi)uv^2 - ku \] (D.6a)
\[ \frac{\partial v}{\partial t} = \nabla \cdot (D_v \nabla v + vH_v \nabla \phi) + (1 + \gamma_2 \phi)uv^2 + ku + (1 - \gamma_3 \phi)\gamma - v. \] (D.6b)

Equations in (D.6) are a modified form of the glycolysis model used in morphogenesis applications [158]. Therefore, the values of the parameters observed in these equations...
correspond to the values needed to obtain spatial-temporal patterns resembling fibers permeating the simulation domain [162]. In some cases, the dimensionless values were determined upon information for the dimensional values taken from the available literature. In some other cases, numerical experimentation was employed to determine the dimensionless values involved in the new terms related to the electric field. The dimensional values correspond to

\[
D_u = D_{Th} \frac{t_{ref}}{L_{ref}^2} = 1.0 \quad (D.7)
\]

\[
D_v = D_F \frac{t_{ref}}{L_{ref}^2} = 0.08 \quad (D.8)
\]

\[
H_u = E_{Th} \frac{t_{ref} V_{ref}}{L_{ref}^2} = 2.0 \quad (D.9)
\]

\[
H_v = E_F \frac{t_{ref} V_{ref}}{L_{ref}^2} = 2.0 \quad (D.10)
\]

\[
\delta = k_1 \frac{t_{ref} P}{T_{href}} = 1.2 \quad (D.11)
\]

\[
k = k_3 t_{ref} = 0.06 \quad (D.12)
\]

\[
k = k_3 \frac{t_{ref} T_{href}}{F_{ref}} = 0.06 \quad (D.13)
\]

\[
\gamma = k_4 \frac{t_{ref} G_p}{F_{ref}} = 0.03 \quad (D.14)
\]

\[
\gamma_1 = c_1 V_{ref} = 0.2 \quad (D.15)
\]

\[
\gamma_2 = c_2 V_{ref} = 0.2 \quad (D.16)
\]

\[
\gamma_3 = c_3 V_{ref} = 0.2545 \quad (D.17)
\]

\[
1 = k_2 t_{ref} F_{ref}^2 \quad (D.18)
\]

\[
1 = k_2 t_{ref} T_{href} F_{ref} \quad (D.19)
\]

\[
1 = k_5 t_{ref}. \quad (D.20)
\]

To reconcile the double representation available for \(k_2\) and \(k_3\) (as given in (D.12), (D.13) and (D.18), (D.19)), is convenient to adopt the following convention:
Additionally, assuming a prothrombin concentration equal to the concentration of granules released by activated platelets [221], it is possible to reduce the concentrations of prothrombin and of the released granules to a single parameter as follows:

\[ T_{\text{ref}} = F_{\text{ref}} = C_{\text{ref}}. \]  \[ (D.21) \]

\[ P = G_p = C_{\text{ref}}. \]  \[ (D.22) \]

**Parameters of the model**

Most numerical parameters were obtained from previous simulations [160, 221]. Additional values were extracted from the available literature and in the cases where no related information was found, numerical experimentation was performed to arrive at plausible values.

**Thrombin, fibrinogen and fibrin**

\( t_{\text{ref}}, \ L_{\text{ref}}, \ C_{\text{ref}} \): The reference value for time \( t_{\text{ref}} \) is 10 minutes, as this is the time needed for the formation of the blood clot [228]. The reference value for length \( L_{\text{ref}} \) corresponds with the maximum distance between the host bone border and the implant surface. Considering the region of necrotic tissue after implant placement 0.2 mm wide [238], and a screw type dental implant with threads 0.35 mm deep, the value of the parameter is 0.55 mm. Finally the reference concentration \( C_{\text{ref}} \) is assumed as 1 mg/ml [221].

\( D_u, \ D_v, \ \delta, \ k, \ \gamma \): These parameters correspond to those needed for the glycolysis model to form stripe-like spatial-temporal patterns as shown in Fig. 7.5. Accordingly, \( D_u = 1.0, \ D_v = 0.08, \ \delta = 1.2, \) and \( k = 0.06 \) [162]. The parameter \( \gamma \) is a complement to the model described by (7.5) and has a value of 0.06 [221].

\( D_{Th}, \ D_F \): The thrombin diffusion constant is set to 5.0417x10\(^{-4}\) mm\(^2\)/s [221]. The fibrinogen diffusion constant is 5.647x10\(^{-5}\) mm\(^2\)/s as in [221].

\( k_1 - k_5 \): The set of parameters involved in the reaction terms are obtained from the dimensionless form (D.7) - (D.20). The values are 2.0x10\(^{-3}\) s\(^{-1}\), 1.7x10\(^{-3}\) (s (mg/ml)\(^2\))\(^{-1}\), 1.0x10\(^{-3}\) s\(^{-1}\), 50x10\(^{-6}\) s\(^{-1}\), and 1.7x10\(^{-3}\) s\(^{-1}\), respectively.

\( E_F, \ E_{Th} \): No information was found for the electrical dispersion of both thrombin and fibrinogen. Therefore, the values are calculated from numerical experiments so as to obtain spatial-temporal patterns which are biologically meaningful. Accordingly, the value 1.8x10\(^{-3}\) mm\(^2\) (V \cdot s)\(^{-1}\) is set for both parameters.
\(c_1, c_2, c_3\): The electrical control coefficients for thrombin and fibrinogen \(c_1, c_2\) are both set to 0.3636 V\(^{-1}\) using numerical experimentation. The electrical control for platelet activation \(c_3\) is calculated assuming that platelets (in the presence of a 750-mV/mm electric field) are activated in a similar way as in during thrombocytosis [221]. Accordingly, the numerical parameter was calculated as 0.4627 V\(^{-1}\).

\(\alpha, \beta, \text{and } \beta_\Phi\): The fibrin activation rate \(\alpha\) and the the blood quality factor \(\beta\) are 0.5 s\(^{-1}\) and 0.37 (mg/ml)\(^{-1}\) respectively [221]. The electrical blood factor \(\beta_e\) is calculated observing that the denominator of (10.3) is zero when \(\beta_\Phi = 0.072\) (V mg/ml)\(^{-1}\). Accordingly, the numerical parameter is set to 0.02 (V mg/ml)\(^{-1}\).

\(f_{\text{max}}\): The maximum concentration of fibrin \(f_{\text{max}}\) is obtained from the experimental analysis of the blood clot composition as 2.5 mg/ml [174].

\(W_F, p\): The threshold for the activation function controlled by fibrinogen \(W_F\) is 1.2 mg/ml [160]. The slope \(n\) of all the activation/inhibition functions is 10.

**Osteoprogenitor cells density and chemical concentration**

\(D_C, D_Q\): The diffusion constant for both the osteogenic cells and the osteogenic chemical are taken from [160] as 1.02x10\(^{-5}\) mm\(^2\)/min and 2.88x10\(^{-3}\) mm\(^2\)/min respectively.

\(H_Q, E_C\): The chemotactic sensitivity of the cells \(H_Q\) is set to 2.0x10\(^{-3}\) mm\(^2\)/min (ml/mg) as in [160]. The electrical sensitivity is derived as 1.0x10\(^{-5}\) from the cell speed [45, 77] and the maximal electric field intensity that cells can withstand before undergoing irreversible electroporation of the membrane [64].

\(r_C, r_Q, r_{CI}, r_{QI}\): The production coefficients are taken from [160] as 3.57x10\(^{-3}\) min\(^{-1}\), 5.76x10\(^{-5}\) min\(^{-1}\), 1.6x10\(^{-4}\) mg/(ml min), and 1.0x10\(^{-6}\) min\(^{-1}\), respectively. However, \(r_Q\) was numerical determined as 1.0x10\(^{-5}\) min\(^{-1}\) so as to obtain osteogenic chemical concentrations comparable with data on cell exposure to electric fields [36, 186].

\(r_\Phi, r_{\Phi I}\): Since no related experimental data was found, the electrically-mediated chemical production, \(r_\Phi\), is numerically adjusted to 5.0x10\(^{-5}\) min\(^{-1}\). The chemical released by the electrically activated platelets, \(r_{\Phi I}\), is also numerically adjusted to 5.0x10\(^{-4}\) (V min\(^{-1}\)).

\(\delta_C, \delta_Q, \delta_QC\): The death coefficients are 1.388x10\(^{-5}\) min\(^{-1}\), 2x10\(^{-3}\) min\(^{-1}\), and 3x10\(^{-5}\) min\(^{-1}\) (ml/mg), respectively, as in [160].

\(C_I, W_Q\): The cell saturation coefficient is set to 1x10\(^{-3}\) c/ml assuming a normalized cell density [162]. The threshold for the activation function controlled by the chemical \(W_Q\) is 1x10\(^{-3}\) mg/ml [160].
Granulation tissue

$r_G, r_\Phi$: The production rate $r_G$ is $8.33 \times 10^{-5} \text{ min}^{-1} (\text{ml/mg})$ [160]. The electrically-mediated production rate $r_\Phi$ is set to $2.0 \times 10^{-2} (\text{V min})^{-1}$, based on the fact that electrostimulation enhances the synthesis of collagen by 20-50\% [36, 186].

$\delta_G, W_{C_o}$: The degradation coefficient and the threshold for the activation function controlled by the osteogenic cells are $1.235 \times 10^{-2} \text{ min}^{-1}$ and 0.5 c/ml, respectively [160].

New bone formation

$r_{BC}, r_{BQ}$: The control parameters for cell adhesion and cell proliferation are based on experimental observations on osteoblastic-like cell cultures [209] and set as in [160] to the values 1 and 75, respectively.

$c_4, c_5$: The coefficients for electrical mediation in cell adhesion and cell proliferation are set to reproduce experimental observations according to which the increment of the bone matrix (when applying the electric field) is the order of 10-20\% [204, 215]. Therefore, the values are 0.4 and 0.16.

$W_U$: The threshold value for the activation function controlled by the displacements is set to the minimal displacement of the fibers required to obtain a successful osseointegration of the dental implant [213]. Accordingly, the value is set to 0.4 mm.
Bibliography


On a Tuesday morning, in early 2011, I entered the office shivering. I was wearing a pair of jeans, two sweaters, a jacket, a scarf, a beanie and gloves. The mercury measured only a couple of degrees above zero, an unreasonably low temperature for someone coming from a tropical country. I was welcomed by Prof. Peter Zwamborn and Prof. Anton Tijhuis, my two promoters. We held a fruitful meeting that helped us to set conditions to, eventually, work together. In the afternoon, and despite the cold, I went out and wandered around Eindhoven. I reflected over what we discussed in the meeting. But at the same time, I paid attention to the looks of the city. Once I returned to the hotel I was staying at, I had it clear in my mind: no matter the weather, the cultural differences, and the fact that I was far away from home, I could do it. I could make my life here.

Now that I have finished, I thank my two promoters for accepting me to work in the Electromagnetics group. Thank you for the numerous discussions that helped me to find the way towards the results presented in this book. I recognize the efforts you made to take me to the Netherlands, and the additional support you provided me during the difficult moments. I also must thank dr. Vito Lancellotti, my daily supervisor. Our communication was at first complicated, marked by our own cultural differences and the distance between our professional backgrounds. However, we managed to sort out these issues and we ended up in a cordial mutual understanding. Thank you for sharing with me your knowledge on Electromagnetics and numerical methods. I appreciate your dedication to have regular discussions, check up on my progress, and review my reports.

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My life while working at TU/e was marked by the disappearance of a brother. On a very unfortunate Thursday morning I realized, once more, that every day counts. For it can be the last. You told me I will make it. You told me not to succumb. I wish you were here to give you a big hug and thank you for that support. I had to forgive myself for what I should have said and I could not. For what I could have done and I did not. There is peace in my soul because I know, Juanito, that your spirit rests in the glory of the Lord. Your charming smile will remain in my memory. Forever. Because as you put it in your notes, *todos los días sale el sol*.

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After four years, I have learned to appreciate the life style of the Dutch people. I have also adapted to the differences the Netherlands has with respect to my motherland, Colombia. A great part of this adaptation comes from having invested time in learning the Dutch language. Dus, ik wil hier ook mijn docenten Nederlands op de TU/e bedanken voor hun overtuiging dat iedereen de Nederlandse taal kunt leren. Ik wil ook de vrijwilligers van Humanitas, een Stichting in Eindhoven die bijna een jaar me heeft geholpen om mijn Nederlands te verbeteren, bedanken. Het was een ongekende luxe om met Martijn van der Ven en Majid Abdí te praten. Met jullie heb ik een heleboel geleerd over de mensen die in deze vlakke land wonen. Het was ook gezellig om één keer per week Nederlands en Spaans op de TU/e met Nick Jaensson te praten. Femke, bedankt voor jouw steun, voor jouw help nadat ik mijn rug geblesseerd heb, en voor
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Then you, Liliana, my companion during this journey away from our motherland. Words are not enough to express how much fortunate I am to have you by my side. Words fall short whenever I want to tell you how much I love you. I know how much you have missed your parents and your sister, your relatives, your friends, our city, the local customs. I know how much you have longed for finding here what there is for granted. But you have persisted. Your endurance has given me strength to succeed. Your reliance has committed me not to give up. While writing this, I weep tears of joy. For you are by my side. What was inscribed in the ring is everlasting, no matter that now the ring is lost. Rings can be replaced. You cannot. Te amo, siempre lo haré. Tienes el sentido de aventura que yo tengo, y por eso quisiera que vayamos el uno al lado del otro hasta el final del camino, hasta el final del viaje. ¿Vamos?

To all others whose names escape from this note: I thank your contributions, your support, and your words of encouragement and confidence. I also thank the reader, if any, who patiently goes through this book in its entirety.

Last, but certainly not least, my gratitude to the source of my inspiration, the endower of my intellect, my ultimate companion, my savior, my God. Yours is the greatness and the power, the glory and the victory. I confessed to you my wishes, you listened to my pledges, and I have trusted unfaithfully your directions. Forgive my sins and transgressions, of which there are many. I put my future endeavors in your hands, this humble servant of yours, whose heart and soul will never stop exalting you. Thank you.
Curriculum Vitae

Juan Vanegas-Acosta was born on February 9th, 1984, in Bogota, Colombia. He enrolled in electronics engineering at the Universidad Nacional de Colombia (UNAL) in Bogota in 2001 and obtained his BSc. degree in 2006. His interest in the application of engineering in medicine motivated him to enroll in the MSc program Biomedical Engineering at his alma mater in 2007, obtaining the degree in 2009 with a thesis awarded Summa Cum Laude. During this period, he dedicated himself to the research in computational biology applied to bone healing, working in cooperation with the Israeli dental implants manufacturer MIS. He also completed an internship in Mexico City, Mexico, carrying out in-vitro cell cultures of osteoblastic cells. To continue with this research, he started a Ph.D. in Mechanical Engineering at UNAL in early 2010. In the same year he completed an internship at the University of Delaware, USA, working in simulations for tissue ablation. In mid 2011 he was appointed to a Ph.D. position at the Department of Electrical Engineering at the Technische Universiteit Eindhoven (TU/e), the Netherlands, to conduct research aimed at investigating the possible interactions mechanisms between electromagnetic fields and biological cells. While working at TU/e, he continued with his previous research at UNAL. In 2013 he obtained the Ph.D. degree from UNAL. He finished his research at TU/e in December 2015, the results of which are presented in this thesis.
The following list of products summarizes the results obtained during the course of the work presented in this thesis.

**Journal articles**


**Contributions in conferences**

List of publications


Awards

Vanegas Acosta, J.C. (2012). Funded by COST TD-1104 to attend the Electroporation based technologies and treatments international scientific workshop and postgraduate course. 18-24 November 2012, Ljubljana, Slovenia.

