Simulations of tumor radius and surface charge density changes during the untreated solid tumor growth

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ABSTRACT
Understanding the untreated tumor growth kinetics and its intrinsic findings is interesting and intriguing. The aim of this study is to simulate changes of tumor radius and surface charge density changes during the untreated solid tumor growth. For this, the Gompertz and Poisson equations are used. Simulations reveal that the unperturbed solid tumor growth is closely related to changes in the surface charge density over time between the tumor and the surrounding healthy tissue. Furthermore, the unperturbed solid tumor growth is governed by temporal changes in this surface charge density. It is concluded that graphic strategies corroborate the correspondence between electrical and physiological parameters in cancer, which may have an essential role in its growth, progression, metastasis and protection against immune system attack and anti-cancer therapies. In addition, knowledge of surface charge density at tumor boundary may be relevant to understand the unperturbed tumor growth kinetics to propose taking into account the polarity of the substances in conventional therapies (e.g., chemotherapy and immunotherapy) or to design completely new ones.

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INTRODUCTION

The untreated solid tumor growth kinetics (TGK) exhibits a sigmoidal shape with three well-defined phases from the experimental point of view. The first phase that comprises the tumor cells is inoculated in the host until the tumor reaches its size $R_{T0}$. It grows slowly over time. The second is associated with the quick tumor growth. The third asymptotic phase is related to the balance between the production and loss of tumor cells\(^{1-3}\). Understanding the genesis and endogenous/mechanisms involved in TGK represents a challenge for researchers\(^{3,4}\). For this reason, different mathematical models have been proposed in the literature, being the Gompertz equation (GE) the most accepted one\(^5-7\). Recently, a new formulation of the Gompertz equation is reported in\(^8,9\). In addition, the modified Kolmogorov-Johnson-Mehl-Avrami equation is also suggested to describe TGK. Analysis of TGK involves only biological parameters of the solid tumor, but not those of the surrounding healthy tissue. Furthermore, the electrical properties of the tumor and surrounding healthy tissue are not included in the models used to describe TGK.

A close relation between physiological and electrical parameters in a biological system has been experimentally confirmed by means of a bioelectrical impedance analysis\(^{10,11}\), image technique of the electric current density\(^{12}\) together with other devices that quantify electrical properties\(^{13-17}\) and bioelectric potentials\(^{18-21}\) in different biological tissues. It has been documented in the literature different findings, such as: 1) differences between electrical conductivities ($\eta_k$, $k=1,2$) and electrical permittivities ($\varepsilon_k$, $k=1,2$) of the untreated malignant tumor ($k=1$) and the surrounding healthy tissue ($k=2$)\(^{13-15}\); 2) chemical and electrical (charged negatively) environments in untreated tumors\(^{20}\); 3) the breakdown of intercellular communication (gap junction) in the tumor due to low regulation in expression of the connexin\(^{17,22,23}\). Furthermore, electrical biopotentials in the tumor and in the surrounding healthy tissue are negative and positive, respectively\(^{17-19}\); cancer cells and some cells of the immune system are negatively charged\(^{20}\); and the electrical coupling among these cancer cells is weak\(^{1,19,20,22,23}\). The deregulation of intercellular communication has been associated with tumorigenicity and metastasis of a tumor\(^{20,22}\).

The aforementioned suggests that the bioelectrical activity is inherent in cancer, in all cell types and involved in many physiological mechanisms\(^{24,25}\). Although the correlation between physical and biological processes in unperturbed cancer are poorly understood in in vivo studies, the bioelectric properties and electrical states of cancer cells and microenvironment are closely related to their biological processes. These biological processes are involved in hypoxia, proliferation, growth, invasiveness, and metastasis of the unperturbed cancer (e.g., metabolism abnormalities -one of the emerging hallmarks of cancer-, as well as migration and shape changes of cancer cells)\(^{24-31}\).

As morphology, growth, regulation and metabolic activity of cancer cells differ from those of healthy cells, their electrical properties, ionic and faradic currents are also different\(^{24}\). This finding justifies why cancer and its surrounding healthy tissue are differentiated from the electrical point of view\(^{13-21}\). In both types of tissues, the bioelectric potential or bioelectricity is generated due to the uneven movement of ions (e.g., sodium ($\text{Na}^+$), potassium ($\text{K}^+$), calcium ($\text{Ca}^{2+}$), chloride ($\text{Cl}^-$)) and electrons across the plasma membrane via ion pumps\(^{24,32}\). Activity of $\text{Cl}^-$ channel is vital in migration and invasion of cancer cells as it regulates the cell volume. The concentrations of these ions and electrons are altered in cancer. This ionic imbalance contributes to alter the growth signaling, proliferation, angiogenesis, invasion and metastasis (cancer hallmarks)\(^{33}\). Leslie et al.\(^{33}\) report that deregulated $\text{Na}^+$ handling in tumors may lead to important physiological changes at the cellular level (e.g., altered $V_{\text{mem}}$ (membrane electrical potential difference), pH and metabolic activity). Furthermore, they
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conclude that deregulated Na\(^+\) balance in cancer may be also relevant for designing of new imaging biomarkers and cancer drugs.

Although bioelectrical pathways are still poorly understood in cancer cells\(^{24}\), active bioelectricity in cancer may be explained from the alterations in the imbalance of the charge between the intra- and extracellular compartments at the cancer cell membrane\(^{32}\), gene expression level and glutamate-dependent currents\(^{24,33-35}\), and both ionic and faradic). Ionic currents are due to the movement of charged ions, whereas faradic currents are produced electrons exchange from reduction and/or oxidation of biochemical molecules). Furthermore, electrically active cancer cells possess bioelectric circuitry that generates resting membrane potential and endogenous electric fields that influence cell functions and communication\(^{24}\).

Transport and distribution of charged carriers affect \(V_{\text{mem}}\) of the cancer cells and induce some polarizations at different levels (e.g., cellular, tissue, and organ) generating adversity of signaling responses. \(V_{\text{mem}}\) has an important role in high proliferation of cancer cells, the control of the critical cell functions, mitosis, deoxyribonucleic acid synthesis, and depletion of adenosine triphosphate.

Additionally, \(V_{\text{mem}}\) influences in the fail of ionic pumps at the cellular membrane and mechanism of contact inhibition, modulation of local concentrations of signaling molecules and ions; among other biophysical alterations in cancer\(^{13-16,18-21,24,28,30,37-39}\). High proliferation of cancer cells is due to the depolarization of their membranes by high intracellular concentration of sodium ions. The control of the critical cell functions is related to cell proliferation, migration, cell-volume control, regulation of the proliferative state, differentiation and pigmentation.

On the other hand, simulations evidence that \(V_{\text{mem}}\) is also involved in the spatiotemporal regulation of morphogenesis, which is an essential aspect for understanding of body plan control during development, regeneration and disease\(^{37}\). \(V_{\text{mem}}\) is also found in the interaction with heterogeneous networks that combines conventional gene regulatory network including bioelectrical signals, also known as bioelectricity-integrated gene and reaction network\(^{40}\). In turn, it and cell behavior are controlled by spatiotemporal bioelectrical patterns based on electric potentials and currents from steady and oscillatory multicellular states. These multicellular electric potentials influence on the spatiotemporal distributions of signaling ions and molecules that modulate biochemical pathways in cancer cells, and therefore in growth and regeneration\(^{41}\).

A depolarized membrane is considered a driving force for the production of Ca\(^{2+}\)\(^{(42)}\) and bioelectronic cancer regulator\(^{24}\). Both cases lead to affect proliferation (ion and ion channel non-specific), migration (due to its effect on Ca\(^{2+}\) concentrations), invasion and metastasis of cancer cells. Bioelectronic cancer regulator associates with initiate mitosis and deoxyribonucleic acid synthesis.

\(V_{\text{mem}}\) may be regulated in different ways, such as: the ion channel expression, the ionic composition of the extracellular environment, and the presence of bioelectronic gradients within cancer\(^{24,29}\). Payne et al.\(^{29}\) suggest that \(V_{\text{mem}}\) should be analyzed in two directions: \(V_{\text{mem}}\) effect on the cellular function (that contributes to the cancer phenotype) and how \(V_{\text{mem}}\) is affected (by the expression of voltage-gated ion channels and cell metabolism). Dhar et al.\(^{43}\) report that shift in metabolism is driven by the tumor microenvironment. This modifies \(V_{\text{mem}}\) from the production of lactate anions that contributes to that cancer cell surface is negatively charged\(^{31}\). Net negative charges on cell surfaces is a consequence of much higher rate of glycolysis of cancer cells, suggesting that the cell surface charge and cancer cell metabolism are correlated\(^{44}\).

Alterations in the metabolism of cancer cells involve higher rates of intracellular glucose import and glycolysis related to reduced pyruvate oxidation and increased lactic acid production (Warburg effect). As a result, cancer cells reprogram their metabolism during tumor growth\(^{24}\). There are evidence that the mitochondrial metabolic theory is better in describing the origin and management of cancer than the somatic mutation theory. The mitochondrial metabolic theory suggests that cancer arises from a slow disturbance of adenosine triphosphate synthesis through oxidative phosphorylation, its disruption causes genomic instability and somatic mutations in cancer, and not
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the opposite. These genomic changes are due to the acidification of the microenvironment and the excessive production/accumulation of reactive oxygen species (mutagenic and carcinogenic), which cause important damage to lipids, proteins, and nucleic acids in both the mitochondria and in the nucleus of cancer cells.

The results of Seyfried et al. agree with the possible existence of more than one bioelectrical pathway associated with a metabolic phenomenon. Robinson et al. affirm that these bioelectrical pathways affect ionic electrical-based communication among cancer cells, like: reactive oxygen species (ROS) and aberrant trans plasma membrane electron transport systems. Cancer cells use ROS to induce the reverse Warburg effect. Higher levels of ROS in these cells may be caused by malfunction in the redox balance, altered biological electron transfer reactions (higher electron transfer), a high energetic demand, and increased concentration of reduced bioelectrochemical mediators. These two later reasons are due to increased glycolysis rates that lead to oxidation of these mediators, among others. This cellular metabolic deregulations lead to changes in the blood bioelectricity (bioelectric potentials) and physiochemical properties. Furthermore, the trans plasma membrane electron transport systems participate in this oxidation and redox centers existing in cell membranes transport electrons across these membranes in the form of faradic currents.

Burgos-Panadero et al. give special attention to the tumor microenvironment (key factor in the genesis, growth, progression, metastasis and treatment of cancer) and how its understanding may suggest the proposal of new biomarkers and treatments for cancer patients. Furthermore, they suggest the normalization of the malignant tumor by remodeling healthy tissue morphogenesis in a non-malignant bioelectric/biophysical field and metabolism. Thus, the carcinogenesis may be a reversible process not necessarily related to mutation.

Wang et al. document that electrical conductivity and electrical permittivity significantly correlations with cell proliferation biomarkers in cell suspensions at different stages of breast cancer growth, being marked for the migration rate. These two physical magnitudes increase as the degree of breast cancer malignancy increases. In addition, they suggest that the impact of microenvironment on these two electrical properties of these cell suspensions should not be ignored. Therefore, these authors suggest that both physical properties may be used as a potential diagnostic method.

Levin and Martyniuk report that 1) cellular networks implement pattern regulation and plasticity from bioelectric mechanisms, 2) biological processes form bioelectric circuits from individual cell behaviors, 3) anatomical information encode in bioelectrical states, and 4) the bioelectric code allows a better control over spatiotemporal biological patterns.

Fan and Huang establish that plasticity and heterogeneity of cancer, and tumorigenesis depend on functions of ion channels, whose expressions and activities are deregulated. Ion channel functions may elucidate how electrical signals spread through a network of cancer and stromal cells and how ion channels perceive mechanical cues to control tumor malignancy to propose novel therapies addressed to target ion channels in cancer.

Electric potential gradients are established across multiple cells due to gap junctions and other cell-to-cell connections on a tissue level. In cancer, these gradients have an important role in altered migration and invasiveness of cancer cells, in which junction protein genes are strangely regulated. It has been reported that most cell types may migrate under small endogenous electric fields (phenomenon named galvanotaxis/electrotaxis), which activate different cellular signaling pathways depending on cell subtypes. These results agree with migration and shape changes of cell are driven by endogenous electric fields and changes in $V_{\text{mem}}$.

Measurement of bioelectricity may be carried out by microelectrodes and neutralized input capacity amplifiers, high-impedance micropipettes, potentiometry, fluorescence, electrical double layer in field-effect transistors, and electrical impedance spectroscopy. Additionally, vibrating probes, glass microelectrodes, microfluidic-based tissue/organ-on-a-chip devices, and endoscopes with inserted electronics to detect bioelectricity changes in real-time are used. Nanotechnology-based
bioelectronics with nano-sized devices is used to detect cancer at a stage earlier\cite{24,52-54}. Furthermore, Cervera et al.\cite{37} have been suggested to BioElectrical Tissue Simulation Engine modeling environment to simulate bioelectric states from ion concentrations and fluxes. Bălăț et al.\cite{46} propose a hopeful device/analyzer for highlighting the bioelectric potential of blood. Wang et al.\cite{44} recommend using bioelectricity-driven nanoparticle binding (as surface charges-mediated cell targeting) instead of static electrical potential via electrophoresis. Robinson et al.\cite{24} report that cancer bioelectricity is involved in its phenotypes; abnormally expressed ion channels; and growth, progression and metastasis processes. Cancer phenotypes include both cellular ionic and faradic currents. The growth is due to malfunctions in bioelectrical circuitry of their cells. The progression is a consequence of alterations of trans-plasma membrane electron transport. The metastasis comprises degradation of basement membranes, cancer cell invasion, migration, extravasation, and colonization.

Wang et al.\cite{44} establish that the bioelectricity is applied to capture electrostatically and magnetically circulating cancer cells from the entire blood, in addition to investigate the metabolic state of them. Ioro et al.\cite{27} consider the deregulation of ionic activity involving ion channels and transporters, transmembrane proteins, as a novel hallmark of cancer cells.

It has been documented in Electrodynamics of media that a surface charge density arises at the interface between two materials in contact with different electrical properties\cite{55}. Consequently, the existence of a surface charge density (\(\sigma_1\)) is expected at the tumor-surrounding healthy tissue interface, named \(\Sigma\). This happens for the following reasons: first, solid tumors have chemical and electrical environments\cite{21}. Second, the cancer and the surrounding healthy tissue are in contact and inhomogeneous\cite{8,9,56,57}. Third, both tissue types differ significantly in their electrical properties and thermal\cite{13-16,20,21,58,59}, and physiological parameters\cite{21,56,60}. Fourth, \(\sigma_1\) is due to synergism between an external volumetric current density (the source of electricity) and the Maxwell-Wagner-Sillars interfacial polarization (or Maxwell-Wagner interfacial polarization). The Maxwell-Wagner effect is an interfacial relaxation process that occurs for all two-phase multi-systems, in which the electric current must pass an interface between two different loss dielectrics\cite{57,61-64}. Fourth, the electrophysiological activity of cancer cells is higher in tumor regions near \(\Sigma\)\cite{1,8,9,11,65}.

The surface charge density has been measured in many biological and non-biological heterogeneous materials by means of the surface photovoltage effect, the vibrating probe technique, electrostatic force microscopy, among others\cite{66-68}. These experimental techniques may be used to measure \(\sigma_1\) at \(\Sigma\). Nevertheless, we are not aware that \(\sigma_1\) at \(\Sigma\) has been experimentally measured nor calculated theoretically. Estimation of \(\sigma_1\) at \(\Sigma\) presupposes the experimental knowledge of normal components of the flux density vector on both sides of \(\Sigma\), a procedure that is cumbersome and expensive (in time and resources) in preclinical and clinical studies. Furthermore, an analysis of TGK in terms of \(\eta_k, \varepsilon_k\) \((k = 1,2)\) and \(\sigma_1\) has not been reported in the literature. That is why, physic-mathematical models are suggested in this study. Therefore, the aim of this study is to simulate \(\sigma_1\) at \(\Sigma\) during the untreated tumor growth, in terms of two tumor kinetic parameters, tumor radius and electrical properties of the tumor and the surrounding healthy tissue. For this purpose, an approximate analytical expression for \(\sigma_1\) is proposed in terms of \(\eta_k, \varepsilon_k\) \((k = 1,2)\), the tumor radius and its kinetic parameters.

METHODS

Many biological processes require bridging biological scales (molecules to cells, to tissues, and to organisms). It has been suggested that cellular metabolism may be a key insight for how these scales are connected. For instance, metabolism can affect morphogen signaling via the plasma membrane potential at the cellular level\cite{69}. There is a consensus among researchers that biological and physical processes are closely related in biological systems\cite{1-65,70-74}. Nevertheless, a question arises, biological
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Changes (e.g., metabolism abnormalities) lead to physical changes (e.g., changes in $V_{\text{mem}}$ and electrical properties) or vice versa. We believe that physical changes (primary mechanisms) are the first alterations that occur in any biological system that lead to chemical changes, to biological modifications, and to clinical manifestations/alterations. This is why, we focus mainly in physical magnitudes.

Assumptions

1. The second and third phases of TGK are only considered in this study because the tumor radius/volume can be experimentally measured.
2. There is a three-dimensional, conductive, anisotropic and heterogeneous region consisting of two linear, anisotropic and heterogeneous media (tumor and the surrounding healthy tissue) separated by an interface $\Sigma$ (Fig. 1). Solid tumor (medium inside $\Sigma$, named medium 1) is considered as a heterogeneous conducting sphere of radius $R_T$ (in m) of constant mean conductivity ($\eta_1$, in S/m) and mean permittivity ($\varepsilon_1$, in F/m). The surrounding healthy tissue (medium outside $\Sigma$, named medium 2) is supposed to be a heterogeneous infinite medium of constant mean conductivity ($\eta_2$, in S/m) and mean permittivity ($\varepsilon_2$, in F/m).
3. Tumor tissues have larger conductivity and permittivity values than homologous normal tissues.
4. The source of electricity is neglected because the tumor is unperturbed.
5. Maxwell-Wagner effect occurs physiologically between the tumor and the surrounding healthy tissue.
6. Changes in $\sigma_{12}$ at $\Sigma$ govern the unperturbed TGK, taking into account that the tumor contour has higher weight in growth, metastasis and aggressiveness of the untreated solid tumor than tumor mass$^{1,8,9}$.
7. Intrinsic electrical activity at the cellular level in an unperturbed solid tumor, represented by the electromotive force field ($\vec{E}_f$), is due to endogenous electrical biopotentials ($\phi$) in it.
8. In a first approximation, $\vec{E}_f$ depends only on the distance to the tumor center.
9. Cancer cells that are at $\Sigma$ do not significantly contribute to $\vec{E}_f$.

As solid tumor and surrounding healthy tissue are anisotropic and heterogeneous media (formed by cells, water, ions, molecules, macromolecules, among others)$^{13,14}$, their electrical conductivities and electrical permittivities are real symmetric second-order tensors$^{15,58}$. Let be $\vec{\eta}$ and $\vec{\varepsilon}$ real symmetric second-order tensors of electrical conductivity and electrical permittivity, respectively$^{59}$. Consequently, there is an orthonormal base (which defines the so-called principal axes of the medium) in which $\vec{\eta}$ is represented by the diagonal matrix $\text{diag}(\eta_1, \eta_2, \eta_3)$, where $\eta_1$, $\eta_2$ and $\eta_3$ are electrical conductivities according to these main axes. If these diagonal elements are replaced by their mean value, named $\eta (\eta = (\eta_1 + \eta_2 + \eta_3)/3)$ in this approximation, the tensor $\vec{\eta}$ corresponds to the scalar matrix $\eta I$, where $I$ is the identity matrix of order 3, as in$^{59}$. The tensor $\vec{\varepsilon}$ is treated in the same manner and its mean value is $\varepsilon$. Average values of these two electrical properties are reported in the majority of theoretical$^{75-78}$ and experimental$^{10,11,13-15,17,79-83}$ studies. That is why, mean values of $\eta_1$ and $\varepsilon_1$ (for the tumor), and $\eta_2$ and $\varepsilon_2$ (for the surrounding healthy tissue) are considered in the Assumption 2.

A diversity and complexity of non-spherical geometries$^{1-3,5,6,8,19}$ and irregular borders$^{12,13,15,56,84}$ are observed in tumors during their growths in preclinical and clinical studies. This makes very difficult to establish a single spatiotemporal pattern of these two aspects for simulations. This tumor spherical geometry is observed in in vitro$^{85-88}$ in vivo$^{89,90}$ and clinical$^{85}$ studies. Furthermore, in preclinical studies, the tumor spherical shape is observed at beginning of TGK and after changes to ellipsoidal
shape\textsuperscript{1-3,8}. Castañeda et al.\textsuperscript{8} suggest the preponderant role of the tumor border in TGK. Effects of the irregular border and changes in $\sigma_{12}$ at $\Sigma$ in TGK are poorly understood. These reasons are used to argue the tumor spherical shape considered in Assumption 2.

Larger conductivity and permittivity values of tumor tissues (Assumption 3) are experimentally corroborated in several studies\textsuperscript{10,11,13-18,20,21,57,79,80,83}. These values may be explained from malignant tumors have a significantly higher water content\textsuperscript{13,14}, higher concentrations of ions and electrons, and altered cellular metabolism\textsuperscript{24-54,56,70-74} with respect to those in the surrounding healthy tissue.

$\vec{E}_f$ is due to constant and/or time-varying endogenous electrical currents produced by diffusion and concentration of different positive and negative charge carriers (electrons, ions and molecules) that occur in the heterogeneous biological tissue\textsuperscript{13,14} due to altered physical and biological processes in cancer as previously mentioned\textsuperscript{1-65,70-74}. In turn, these endogenous electrical currents have electric and/or magnetic fields associated. Both fields may be static or variable in time, as reported in\textsuperscript{13-15}. These electrical currents, electric fields and/or magnetic fields are weak due to the breakdown of intercellular communication in the tumor\textsuperscript{17,22,23}. If $\vec{E}_f = 0$, the biological tissue die. Therefore, we believe that $\phi$ and $\vec{E}_f$ have essential roles in the integral function of the untreated biological tissue.

**Theory**

The **Assumptions** section and the close relationship between physical and biological aspects in cancer\textsuperscript{1-65,70-74} allow us to consider that $\phi$ and $\vec{E}_f$ are related, in a first approximation, by means of the equation

$$\nabla \cdot \eta \vec{E} - \nabla \phi + \vec{E}_f = 0,$$

where $\eta$ is the symmetric second-order tensor of the electrical conductivity of any linear, anisotropic and non-homogeneous medium (for example, a biological tissue). This tensor is used in previous studies\textsuperscript{15,58,59}.

Eq. (1) is obtained by combining the continuity equation ($\nabla \cdot \vec{J} + \partial \rho / \partial t = 0$) for the static case ($\partial \rho / \partial t = 0$) and law of Ohm ($\vec{J} = \eta \cdot (\vec{E} + \vec{E}_f)$), valid for media of linear conduction. In this case, $\vec{J} = \vec{J}(\vec{r})$ is the electric current density $\vec{J}(\vec{r}) = \rho(\vec{r}) \vec{v}(\vec{r})$, where $\rho(\vec{r})$ is the electric charge density and $\vec{v}(\vec{r})$ the velocity field of electric current carriers.

**Isotropic media**

Assumptions 2-5 allow considering that $\vec{D} = \varepsilon \vec{E}$ and the medium is considered isotropic in this approach, where $\vec{D}$ is the induction field (flux density vector). Taking this into account, and assuming that the medium is electrically homogeneous, Eq. (1) has the form

$$\nabla \cdot \eta \cdot (-\nabla \phi + \vec{E}_f) = 0,$$

Therefore,

$$\nabla^2 \phi = \nabla \cdot \vec{E}_f.$$

**Boundary conditions**

The region of interest is assumed as a heterogeneous biological tissue formed by the solid tumor (with average values $\eta_1$ and $\varepsilon_1$) surrounded by the surrounding healthy tissue (with average values $\eta_2$ and $\varepsilon_2$), as shown in Fig. 1. It is reported in the literature that $\eta_1 > \eta_2$ and $\varepsilon_1 > \varepsilon_2$\textsuperscript{10,11,13-18,20,21,24-54,56,57,70-74,79,80,83}.
According to the continuity equation for the static case, the current density normal components of the tumor \( J_{1n} \) and the surrounding healthy tissue \( J_{2n} \) are continuous at \( \Sigma \):
\[
J_{1n} = J_{2n}.
\]
Therefore,
\[
\eta_1 E_{1n} = \eta_2 E_{2n} \Rightarrow \eta_1 \frac{\partial \phi_1}{\partial n} = \eta_2 \frac{\partial \phi_2}{\partial n},
\]
where \( E_{1n} \) is the normal component of the electrical field of the tumor. \( E_{2n} \) is the normal component of the electrical field of the surrounding healthy tissue. \( \phi_1 \) is the electrical potential in the tumor and \( \phi_2 \) the electrical potential in the surrounding healthy tissue. The normal derivatives of \( \phi_1 \) and \( \phi_2 \) are \( \frac{\partial \phi_1}{\partial n} \) and \( \frac{\partial \phi_2}{\partial n} \), respectively.

Eq. (4) is valid if \( \mathbf{E}_f = 0 \) at \( \Sigma \) (see Assumption 9). The positive normal to the tumor surface is indicated as a unit vector \( \mathbf{n} \) (represented schematically in Fig. 1 by \( \mathbf{a} \)) draw from the surrounding healthy tissue (medium 2) into the tumor (medium 1). According to this convention, medium 2 lay on the negative side \( (\mathbf{n}_2 = -\mathbf{n}) \), and medium 1 on the positive side \( (\mathbf{n}_1 = \mathbf{n}) \). Taking this into account as well as the matching boundary condition for \( \mathbf{D} \), \( D_{1n} - D_{2n} = \sigma_{12} \), Eq. (5) and \( \mathbf{D} = \varepsilon \mathbf{E} \) result for \( \sigma_{12} \) the expression
\[
\sigma_{12} = \varepsilon_2 \left[ \frac{\eta_1}{\eta_2} - \frac{\varepsilon_1}{\varepsilon_2} \right] \frac{\partial \phi_1}{\partial n} = -\varepsilon_2 \left[ \frac{\eta_1}{\eta_2} - \frac{\varepsilon_1}{\varepsilon_2} \right] E_{1n},
\]
where \( D_{1n} \) and \( D_{2n} \) are the normal components of the flux density vector \( \mathbf{D} \) in the tumor and the surrounding healthy tissue, respectively.

**Fig. 1 Schematic representation of a spherical tumor surrounded of its healthy tissue.** Variables \( \phi_0 \) and \( \phi_s \) denote the electrical potentials in the center and the periphery of the tumor, respectively. \( R_{T0} \) is the initial tumor radius. \( \eta_i \) and \( \varepsilon_i \) represent the electrical conductivities and electrical permittivities of the tumor \( (i = 1) \) and the surrounding healthy tissue \( (i = 2) \). \( \mathbf{n} \) denotes the inward unit normal vector to the boundary \( \Sigma \) (interface that delimits both tissues).

**Calculation of the free electric charge surface density \( \sigma_{12} \)**

Strictly speaking, the problem to be solved for the calculation of the electric potential is Eq. (3) subject to the matching boundary conditions for \( \phi \) and \( \frac{\partial \phi}{\partial n} \).
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\[
\begin{aligned}
\left\{ \begin{array}{l}
\nabla^2 \phi = \nabla \cdot \vec{E}_f(r) \\
\phi_1 = \phi_2 \\
\eta_1 \frac{\partial \phi_1}{\partial n} = \eta_2 \frac{\partial \phi_2}{\partial n}
\end{array} \right.
\end{aligned}
\]  
\tag{7}

where \( \vec{r} \) \in \Sigma.

The spherical model of the tumor has been used in the literature\(^7,85-93\). As \( \phi \) at \( \Sigma \) may be experimentally measured, conditions that may be replaced by a condition of Dirichlet and the work region is only inside the spherical tumor, of radius \( R \), the solution of the problem of Poisson into the tumor in spherical coordinates \((r, \theta, \varphi)\) \( 0 \leq r < R \) \( 0 \leq \theta \leq \pi \) and \( 0 \leq \varphi \leq 2\pi \) is given by

\[
\phi_1(r, \theta, \varphi) = \phi_{1h} + \phi_{1p} = \sum_{n=0}^{\infty} \sum_{m=0}^{n} r^n p_n^m (\cos \theta) (A_{nm} \cos m\varphi + B_{nm} \sin m\varphi) + \phi_{1p},
\]
\tag{8}

where \( p_n^m (\cos \theta) \) are the generalized polynomials of Legendre and \( \phi_{1p} \) is a particular solution any of Eq. (3) in the tumor.

Assumption 7 supposes that \( \nabla \cdot \vec{E}_f = 2/r \). In this case, the solution (Eq. (8)) is bounded and it does not depend on the coordinates \( \theta \) and \( \varphi \), given by

\[
\phi_1 = A \frac{r}{R} + A_{00}.
\]
\tag{9}

Constants \( A \) and \( A_{00} \) in Eq. (9) are calculated from \( \phi_0 = \phi_1(r = 0) \) and \( \phi_k = \phi_1(R, 0, 0) \), being \( A = \varphi_s - \varphi_0 \) and \( A_{00} = \varphi_0 \). As a result, \( \phi_1(r) \) is given by

\[
\phi_1(r) = \frac{\varphi_s - \varphi_0}{R} r + \varphi_0, \quad 0 \leq r \leq R
\]
\tag{10}

In Eq. (10), the difference between \( \varphi_0 \) and \( \varphi_s \) represents the tumor heterogeneity from the electrical point of view. The term \( (\varphi_s - \varphi_0)/R \) is interpreted as the linear radial gradient of \( \phi_i(r) \).

The electric field intensity in the tumor is calculated from \( \vec{E}_1 = -\nabla \phi_1 \), given by

\[
E_1(r) = \frac{\varphi_0 - \varphi_s}{R}, \quad 0 \leq r \leq R.
\]
\tag{11}

Eq. (11) shows that the electric field is uniformly distributed in the entire tumor volume. If Eq. (11) is substituted in Eq. (6), the following expression is found for \( \sigma_{12} \), given by

\[
\sigma_{12} = -\varepsilon_2 \left[ \frac{\eta_1}{\eta_2} - \varepsilon_1 \right] \left[ \frac{\varphi_0 - \varphi_s}{R} \right].
\]
\tag{12}

Eq. (12) gives the dependence of \( \sigma_{12} \) with \( \varphi_0 \), \( \varphi_s \), \( R \), \( \eta_k \) and \( \varepsilon_k \) \((k = 1, 2)\) for a fixed time after tumor cells are inoculated in the organism. \( R \) is any tumor radius higher and equal than \( R_m \), where \( R_m \) is the minimum tumor radius measured in preclinical studies or the first tumor radius detected in clinics \((1,2)\). The term \( (\eta_1/\eta_2 - \varepsilon_1/\varepsilon_2) \) represents the difference between the conductive and dielectric ratios of the tumor and the surrounding healthy tissue.

Several experimental studies report that \( R \) of untreated tumors changes in the time \( t^{1,3,5,6} \). As a result, \( \sigma_{12} \) is expected to depend on \( t \). For this, GE is used.

\textbf{Gompertz equation}

GE is given by

\[
V_T(t) = V_{T0} e^{\left(\frac{\alpha}{\beta}(1-e^{-\beta t})\right)},
\]
\tag{13}

where \( V_T(t) \) represents the tumor volume at a time \( t \) after tumor cells are inoculated into the host. The initial tumor volume \( (V_{T0}) \) is given by the initial condition \( V(t = 0) = V_{T0} \). The parameter \( \alpha \) \((\alpha > 0)\) is the intrinsic growth rate of the tumor. The parameter \( \beta \) \((\beta > 0)\) is the growth deceleration factor due to endogenous antiangiogenic process\(^{2,3,7,9}\).

As the tumor is assumed a spheroid, \( V_T(t) \) in GE corresponds to the volume of a sphere \( (V_T(t) = 4\pi R_T^3(t)/3) \), where \( R_T(t) \) is the spheroid tumor radius at a time \( t \). As \( R_T(t) \) and \( V_T(t) \) depend on \( t, R \) in
Eq. (12) is replaced by $R_T(t)$. As a result, $\sigma_{12}$ is a function of $t$, named $\sigma_{12}(t)$. Substituting $V_T(t)$ in Eq. (13) results

$$R_T(t) = R_T = R_{T0} \sqrt{\frac{e(\alpha)}{(\eta_1)}(1-e^{-\beta t})},$$

where $R_{T0}$ satisfies the initial condition $R_T(t = 0) = R_{T0}$ (Fig. 1).

The substitution of Eq. (14) in Eq. (12) allows to express approximately $\sigma_{12}$ in terms of $R_{T0}$, $\phi_0$, $\phi_i$, $\eta_1$, $\epsilon_i$, $\epsilon_2$, $i$, $i_0$, $\alpha$, $\beta$ and $t$, unprecedented in the literature. In this study, six graphic strategies for untreated tumors are analyzed: three for $R_T$ ($R_T$ versus $t$, $dR_T/dt$ versus $t$, and $dR_T/dt$ versus $R_T$) and three for $\sigma_{12}$ ($\sigma_{12}$ versus $R_T$, $d\sigma_{12}/dt$ versus $t$, and $d\sigma_{12}/dt$ versus $\sigma_{12}$), where $dR_T/dt$ is the first derivative of $R_T$ respect to $t$ whereas $d\sigma_{12}/dt$ is the first derivative of $\sigma_{12}$ with respect to $t$. From these six graphic strategies, four graphic strategies are only shown in this study: $R_T$ versus $t$, $dR_T/dt$ versus $R_T$, $\sigma_{12}$ versus $R_T$, and $d\sigma_{12}/dt$ versus $\sigma_{12}$.

The parameter $\epsilon_2$ in Eq. (12) is calculated by the expression $\epsilon_2 = \epsilon_2 \epsilon_0$, where $\epsilon_0$ (8.85x10^{-12} F/m) is the vacuum permittivity and $\epsilon_2$ (4x10^{10}) the relative permittivity of the muscle. Muscle is one of tissues where tumor cells are inoculated more frequently, by the subcutaneous way. This is why, the muscle and its electrical properties are chosen in this study to characterize the healthy tissue that surrounds the tumor.

**Simulations**

Values of $\alpha$ (0.6 days^{-1}) and $\beta$ (0.2 days^{-1})^{3}; $R_{T0}$ (5.6 mm)^{19}, and different values of $\phi_0$ - $\phi_i$ (between -145 and -25 mV) and $\eta_1$/$\eta_2$ - $\epsilon_i$/$\epsilon_2$ (between 1 and 5) are used for simulations. In this study, we only show results for $\phi_0$ - $\phi_i$ (-145 and -25 mV) and $\eta_1$/$\eta_2$ - $\epsilon_i$/$\epsilon_2$ (1, 3, and 5). Values of $\alpha$ and $\beta$ (corresponding to a fibrosarcoma Sa-37) and $R_{T0}$ (corresponding to a Sa-1 sarcoma) are chosen because the fibrosarcoma belongs to the group of sarcomas.

A computer program is implemented in the Matlab® software (version R2012b 64-bit, University Institute for Research in Mathematics and Applications, University of Zaragoza, Zaragoza, Spain) to calculate and simulate the tumor radius, free electric charge surface density and their first derivative in time. These calculations are performed on a PC with an Intel(R) core processor (TM) i7-3770 at 3.40 GHz with a Windows 10 operating system. All calculations take approximately 1 min.

**RESULTS**

Fig. 2 shows simulations of $R_T$ versus $t$ (Fig. 2a) and $dR_T/dt$ versus $R_T$ (Fig. 2b). Nevertheless, Fig. 3 reveals simulations of $\sigma_{12}$ versus $R_T$ (Fig. 3a, b) and $d\sigma_{12}/dt$ versus $\sigma_{12}$ (Fig. 3c, d). The last two graphic strategies are shown for three values of $\eta_1$/$\eta_2$ - $\epsilon_i$/$\epsilon_2$ above-mentioned and two values of $\phi_0$ - $\phi_i$ = -145 mV (Fig. 3a, c) and -25 mV (Fig. 3b, d).

The simulations of $R_T$ versus $t$ and $\sigma_{12}$ versus $t$ (figure are not shown) have similar behaviors. When time elapsed, $R_T$ and $\sigma_{12}$ grow up to their asymptotic values reached for $t = 40$ days, called $R_{T,f}$ and $\sigma_{12,f}$ respectively. The value of $\sigma_{12,f}$ (stationary condition for $\sigma_{12}$) is less negative than $\sigma_{12,0}$ and its value depends on $\phi_0$ - $\phi_i$ and $\eta_1$/$\eta_2$ - $\epsilon_i$/$\epsilon_2$, where $\sigma_{12,0}$ is the value of $\sigma_{12}$ at $t = 0$. Although the graphs of $dR_T/dt$ versus $t$ and $d\sigma_{12}/dt$ versus $t$ are not shown in this study, it can be proved that both graphs evidence similar behaviors. These graphics show that positive values of $dR_T/dt$ and $d\sigma_{12}/dt$ decrease asymptotically to zero when time increases. Fig. 2b reveals that $dR_T/dt$ decreases non-linearly to zero when $R_T$ increases, while $d\sigma_{12}/dt$ decreases when $\sigma_{12}$ is less negative (Fig. 3c, d). In addition, two stages are identified from the graphic strategies shown in Figs. 2 and 3: the first grows rapidly (positive slope) and the second stationary ($R_T$ and $\sigma_{12}$ are constant over time).
Surface charge density changes in tumor growth

**Fig. 2** Unperturbed tumor radius. Simulations of (a) $R_T$ against time $t$, (b) $dR_T/dt$ versus $R_T$.

**Fig. 3** Free electric charge surface density in unperturbed tumor. Simulations of (a) $\sigma_{12}$ versus $R_T$ for $\phi_0 - \phi_s = -145$ mV, (b) $\sigma_{12}$ versus $R_T$ for $\phi_0 - \phi_s = -25$ mV, (c) $d\sigma_{12}/dt$ versus $\sigma_{12}$ for $\phi_0 - \phi_s = -145$ mV, (d) $d\sigma_{12}/dt$ versus $\sigma_{12}$ for $\phi_0 - \phi_s = -25$ mV. Three values of $\eta_1/\eta_2 - \varepsilon_1/\varepsilon_2$ (1, 3 and 5) are shown in each sub-plot.

**DISCUSSION**

The lack of experimental data to corroborate theoretical results constitutes the main limitation of this study. Despite, $\sigma_{12}$ is a direct consequence of Eq. (12) if $(\eta_1\varepsilon_2 - \eta_2\varepsilon_1) \neq 0$, corroborating the existence of a multi-system with two different media (two different loss dielectrics in contact), in agreement with Martinsen et al.\textsuperscript{61} By contrast, the condition $\eta_1\varepsilon_2 - \eta_2\varepsilon_1 = 0$ supposes two identical loss
dielectrics (density of free charges is equal to zero at Σ)\textsuperscript{61}, in contrast with the experiment\textsuperscript{13-16,18-21,56-59}. It should be noted that loss dielectrics are dielectrics that have finite conductivities, as assumed in this study (η\textsubscript{1} for the tumor and η\textsubscript{2} for the surrounding healthy tissue), meaning that induced electrical charges in both biological tissues can move but not as freely as they would in a perfect conductor.

Due to Maxwell-Wagner effect, both free and bound surface charge densities contribute to σ\textsubscript{12} and the current densities \( J_1 \) (in the tumor) and \( J_2 \) (in the surrounding healthy tissue) on both sides of Σ. \( J_1 \) and \( J_2 \) are different because physical processes (\( \text{V}_{\text{mem}} \), electric potential gradients, endogenous electric fields, electrical properties, bioelectrical state) and biological processes (metabolism, regulation, among others) differ significantly in these two types of tissues\textsuperscript{10-54,57-65,70-74}. Consequently, average electrical density is larger at one place and smaller at another and therefore more electrical charges are moved into some region than away from it. This may explain why a volume density of charge arises in both biological tissues. It should be noted that \( J_1 \) and \( J_2 \) are due to ionic current (e.g., sodium, calcium, hydrogen ions, among others) and faradic (e.g., electrons), in agreement with Robinson et al.\textsuperscript{24}

The similarity between the graphs of \( R_T \) versus \( t \) and \( σ_{12} \) versus \( t \) confirms the close relation between the physiological and electrical parameters of an untreated tumor, in agreement with Robinson et al.\textsuperscript{24}

The change from the second to third phase of TGK corresponds to the variation from \( σ_{12-0} \) to \( σ_{12-f} \) at Σ, confirming that charge surface density at the interface between two loss dielectrics changes in time and depends also on \( η_k \) and \( ε_k \) (\( k = 1,2 \)) defined above, thicknesses of two loss dielectrics and the relaxation time of the interfacial polarization. In turn, this relaxation time depends on \( η_k \) and \( ε_k \) (\( k = 1,2 \)) and these two thicknesses, as reported in\textsuperscript{61-63}. As the tumor is unperturbed, the electric potential that appears in the expression of the charge surface density is an endogenous electric potential due to intrinsic electrical sources in the biological tissue, due to biophysical alterations in cancer, in agreement with\textsuperscript{24-54}. It should be noted that this endogenous electric potential should not be confused with the electric potential applied to a tissue by means of electrode. Furthermore, it is not discarded that \( η_k \) and \( ε_k \) (\( k = 1,2 \)) and this relaxation time of the interfacial polarization change in time too, as reported Vu et al.\textsuperscript{94} This may be explained because \( η_1 \) and \( η_2 \) exhibit non-linear behavior due to biological tissues are non-linear systems\textsuperscript{1,95}, being marked for \( η_1 \) due to the untreated tumor grows in time.

The motion of electrical charges in both biological tissues involved during the tumor growth happen in different time scales, named relaxation times (\( τ \)), being \( τ_1 \) for the tumor (\( τ_1 = ε_1/η_1 \) and it depends on the tumor histological variety) and \( τ_2 \) for the surrounding healthy tissue (\( τ_2 = ε_2/η_2 \) and it depends on the tissue type). These aspects may suggest that both tissues cannot be perfect conductors (\( τ_1 \) and \( τ_2 \) then to zero because \( η_1 \) and \( η_2 \) are infinite) or perfect dielectrics (induced volume charges cannot move).

The temporary change from \( σ_{12-0} \) to \( σ_{12-f} \) at Σ coincides with the relaxation time of the interfacial polarization. As a result, this change depends on the electrical properties and biological characteristics of the tumor and surrounding healthy tissue. This change is faster for the tumor histological variety, more aggressive (greater difference of \( α \) with respect to \( β \), the electrical potential gradient is more intense (greater permissible difference between \( φ_o \) and \( φ_s \)) and the greater difference is between ratios of electrical properties of the tumor and surrounding healthy tissue (maximum permissible value of \( η_1/η_2 - ε_1/ε_2 \)). This may suggest that the most undifferentiated tumors (most aggressive) are those that have the greatest differences between \( φ_o \) and \( φ_s \) and their biophysical processes (electric-biological states: \( \text{V}_{\text{mem}} \), electrical properties, deregulate metabolism) differ more from those of the surrounding healthy tissue, in agreement with\textsuperscript{24-39,41,45}. We hypothesize that the greatest differences between \( φ_o \) and \( φ_s \) may suppose the existence of strong electric potential
gradients in cancer and therefore higher mobility of ions, electrons, charged molecules and cancer cells that diffuse to Σ. This may explain spatiotemporal diffusion simulations in untreated tumors, being marked for more aggressive cancer7.

In the literature is often reported $V_{\text{mem}}$ values for cancer cells$^{20,21,24,40}$. We do not know studies that report $\phi_o$ and $\phi_s$ values from experimental or theoretical points of view. The electrical potentials have been measured inside the tumor$^{18,20}$. Although values of $\phi_o - \phi_s$ are not detailed in previous studies, we establish this difference to explain electric potential gradients$^{12,22,23,50}$, endogenous electric fields$^{24,27}$ in cancer, and taking into account distributions of electric potentials quantified in$^{18-20}$ and other studies not cited here (i.e., studies of Habal and Schauble that experimentally report values of electric potentials in the tumor and in the surrounding healthy tissue).

Although $\sigma_{12}$ at Σ has not been experimentally measured or theoretically calculated for most undifferentiated tumors, we cannot affirm that $\sigma_{12}$ values (Fig. 3) correspond to one of aggressive tumor histological varieties in preclinical or clinical study. Nevertheless, small variations of $\sigma_{12}$ (in the range of -0.01 to -0.001 C/m²) for these tumor types may facilitate metastasis process of their cancer cells. We believe that these small values of $\sigma_{12}$ may be relevant to protect cancer from the immune system and anti-cancer therapy attacks.

The change from $\sigma_{12,0}$ (more negative) to $\sigma_{12,f}$ (less negative) at Σ may mean that negativity of $\sigma_{12}$ changes dynamically over time during the untreated tumor growth. This may also lead to dynamic changes over time of both chemical$^{56,65,96}$ and electrical$^{51}$ environments of the tumor. These dynamical changes of $\sigma_{12}$ and their possible biophysical explanations given in this study may be related to dynamical changes in bioelectrical fields in cancer growth$^{73}$. Furthermore, our simulations confirm the close relationship between bioelectricity and biological processes during TGK, in agreement with Robinson et al.$^{24}$, Schwab and Stock$^{72}$ and Silver et al.$^{73}$ The cancer bioelectric handling has been suggested as a useful tool to understand bioelectrical fields that change dynamically during cancer growth. Additionally, cancer bioelectricity may be used as possible anti-cancer therapeutic targets. These two aspects remain unclear yet$^{26,72,73}$.

We believe that complex dynamic changes of $\sigma_{12}$ at Σ are self-regulated during entire TGK. This guarantees the maximum survival of the untreated tumor, counteracts the attack of cellular and humoral components of the immune system, and inhibits the antitumor effect of therapies (i.e., immunotherapy and chemotherapy). In addition, they may be related to endogenous electric biopotentials$^{3,14,18,20,21}$, mechanical properties$^{7,8,9,77-99}$, endogenous angiogenesis processes$^{1,8,9,56,91,97-100}$, dynamic structural transformations, contour deformation in time$^{1,8,9,101}$, heterogeneity and anisotropy$^{1,8,9,56,92,102}$, growth$^{1,8,9,56,91,99,102,103}$, and metastasis$^{56,101,104-106}$ of the untreated solid tumor. Furthermore, these dynamics changes of $\sigma_{12}$ at Σ may permit the tumor evasion/protection against the attack of the immune system$^{56,107,108}$.

Tumor dielectric properties$^{13-16,20,21,58,61}$ may be affected by its water content$^{13-15}$, blood vessel density$^{8,109}$, heterogeneity and anisotropy degrees$^{8,56}$, different degrees of macro- and micro-imperfections of physical and chemical origins$^{1,8}$, among others. These aspects confirm that the active endogenous bioelectricity of the untreated tumor is related to its environment, growth and metastasis, in accordance with previous studies$^{21,24,54,65,100,102,103,105}$.

The dynamic self-regulation of negativity of $\sigma_{12}$ at Σ may suggest that negatively charged carriers (for instance, electrons, molecules, ions, proteins) migrate from Σ towards the interior of the entire tumor volume, mainly in its central region. This may supposes that positive electrical charges migrate toward the surrounding healthy tissue, in agreement with computational results reported for diffusion of hydrogen (H⁺) ions, which migrate from tumor towards to the surrounding normal tissue$^{110,111}$.

Furthermore, these authors report that H⁺ ions damage the normal tissue. We explain this migration of H⁺ ions and other positives charged carriers through Σ to avoid that $\sigma_{12} = 0$ at Σ and weaken the electrostatic coupling among cancer cells (negatively charged) in tumor regions near Σ to favor
Surface charge density changes in tumor growth

metastasis of cancer cells. This may explain the acidification of the tumor microenvironment, which is related to the progression, invasion, metastasis, stimulation of many immunosuppressive processes, and resistance to therapeutics in several cancer histological varieties. The aforementioned may suggest that $\sigma_{12}$ at $\Sigma$ is related to hypocellular gap on the tumor-host interface; it is responsible of the differentiation between tumor electrical properties and the surrounding healthy tissue. Furthermore, negative carriers in the tumor may be related to its negative electric bio-potentials and permanent depolarization of cancer cells, whereas positive carriers in the surrounding healthy tissue may be linked to its positive electric bio-potentials and normal polarization/depolarization that occur in normal cells, in agreement with 1-16,18-54.

The migration of these negatively charged carriers implies the existence of an electric current density that flows into the tumor. Consequently, the electric current density of the positive carriers may be directed from the center towards the tumor periphery. This macroscopic electric current density of negative and positive carriers that appears in the entire tumor volume $V$ ($\int j_{f}dV \neq 0$) is a consequence of $\nabla \cdot \vec{E}_{f} \neq 0$ and it has been documented in 18,24. It should not be rejected that this macroscopic electric current density may create a macroscopic magnetic field into the entire tumor and therefore it has an endogenous magnetic energy (per unit volume) that grows rapidly with increasing size, unprecedented in the literature.

The existence of negatively charged electrical sources into the tumor may be justified because $\nabla \cdot \vec{E}_{f}$ is positive through its interior, corroborating the tumor electronegativity reported in 14,15,18-21. The highest electronegativity in the tumor center reported in 19 may be explained by the higher concentration of negative electrical charges in it due to $\vec{E}_{f}$ is very intense in $r = 0$ ($\nabla \cdot \vec{E}_{f} = 2/r$). In addition, the dynamic self-regulation of negativity of $\sigma_{12}$ at $\Sigma$ may justify the increase of the tumor electronegativity during its growth, as reported in 19, because negative carriers from $\Sigma$ towards the tumor interior increase in time until $\sigma_{12} = \sigma_{12-f}$.

Very intense values of $\vec{E}_{f}$ in the tumor center and its neighborhood may explain in part the endogenous central intra-tumor necrosis observed in some tumors and why tumor cells migrate towards $\Sigma$, in agreement with previous simulations. For this, $\vec{E}_{f}$ should be higher and equal than the endogenous physiological electric field related to the viability of cancer cells, one of the essential variables for tumor growth. Central intra-tumor necrosis explained here from the electrical point of view does not contradict explanations given to it related to the diffusion of cancer cells from the tumor center towards its periphery or the lack of oxygen and nutrients in the central region of the tumor during its growth, as documented in 56,100.

The higher electrophysiological activity of cancer cells into tumor regions near $\Sigma$ and weak electrical coupling between them are possible if $\vec{E}_{f}$ is weak in the tumor regions near $\Sigma$. This is theoretically corroborated here because $\nabla \cdot \vec{E}_{f} = 2/r$, which means that the divergence of $\vec{E}_{f}$ decreases when $r \to R_T$. Weak signals from biological systems are reported in 112. From the bioelectrical point of view, all these aspects and cancer cells negatively charged may favor the invasion and metastasis of the cancer cells towards other parts of the organism due to electrostatic repulsion between them. For this, bio-potentials have to be more negative in the central region of the tumor than in its periphery. Although this electrostatic repulsion is not considered in the literature, it may be the initial stage of the metastatic cascade reported in the literature 105,106.

The previously analyzed permits to suppose that the tumor interior, during its growth over time, behaves like a negatively charged heterogeneous endogenous electrical shield, whose electric field intensity changes dynamically over time and depends on $\phi_0 - \phi$, $\eta_1/\eta_2 - \varepsilon_1/\varepsilon_2$ and the change speed from $\sigma_{12-0}$ to $\sigma_{12-f}$ at $\Sigma$. From the bioelectrical point of view, the existence of this negatively charged
endogenous electrical shield may favor metastasis of the negatively charged cancer cells\textsuperscript{21} by electrostatic repulsion. We do not discard that this negatively charged endogenous electrical shield brings about that cancer electrostatically repels humoral and cellular components of the immune system, mainly those negatively charged (e.g., T lymphocytes, natural killer cells, among others)\textsuperscript{21}. Consequently, the immune system does not recognize the tumor.

Rigorously speaking, $\phi_o$, $\phi_s$, $\sigma_1$ and $\varepsilon_1$ should change in time that also lead to time changes of $\sigma_{12}$ at $\Sigma$, being a possible limitation of this model; nevertheless, we do not explicitly know how? Therefore, we assume constants these physical parameters in our approximation and give special attention to ($\phi_o - \phi_s$) instead of $\phi_o$ and $\phi_s$ values separately. We believe that these physical parameters change in time due to self-regulated migration of negative carriers from $\Sigma$ towards the tumor center during TGK. Additionally, in the tumor may emerge biophysics-chemical mechanisms that guarantee that $\phi_s$ at $\Sigma$ to be constant. For instance, a possible mechanism may be that the tumor itself generates more negative charges by different redox processes and/or duplicate more cancer cells in regions near $\Sigma$.

If $\sigma_{12,f}$ were more negative than $\sigma_{12,0}$ at $\Sigma$, carriers of negative electrical charges would essentially concentrate at $\Sigma$, being noticeable for the greater negativity of $\sigma_{12,f}$. This high concentration of negative charge carriers at $\Sigma$ would mean that this interface behaves as an electrical barrier, preventing the entry and exit of different substances (for example, nutrients, cancer cells, among others) through it. In this case, the tumor would behave as an isolated system and therefore would completely self-destruct. As a result, the tumor does not grow over time, in contrast with the experiment.

If $\sigma_{12} = 0$ at $\Sigma$, the cellular elements of the immune system would enter the interior of the tumor and cause its destruction, in contrast with the experiment. This corroborates the essential role of the tumor contour in its growth, in agreement with other results\textsuperscript{1,8,9}. Endogenous angiogenesis may be the emerging physiological mechanism to avoid $\sigma_{12} = 0$ at $\Sigma$. This is expected because $\sigma_{12}$, TGK shape and the fractal dimension of the vascular network show a sigmoidal behavior during tumor growth, in agreement with\textsuperscript{97}. Although there are no experimental and theoretical evidences $\sigma_{12}$ at $\Sigma$, the aforementioned may explain why $\sigma_{12}$ at $\Sigma$ should decrease in absolute value as the tumor increases in size, being marked for the highest values of $\phi_o - \phi_s$ and $\eta_1/\eta_2 - \varepsilon_1/\varepsilon_2$. This is necessary for the formation of new blood vessels to facilitate the migration of cancer towards the surrounding healthy tissue and the entry of nutrients into the tumor during its growth, as it occurs mainly in aggressive tumors. This corroborates the essential role of dynamical changes in $\sigma_{12}$ during tumor growth and the close relationship between electrical and physiological parameters.

We hypothesize that the possible migration of negative charges from cancer boundary towards its interior may be related to dynamical alterations in physical (i.e., electronegativity and bioelectricity) and biological processes in cancer, as report in previous studies\textsuperscript{17-21,24-44}. Change of $\sigma_{12,0}$ to $\sigma_{12,f}$ at $\Sigma$ may be related to instabilities of cancer cells into the tumor and cancer spatiotemporal dynamics at $\Sigma$ (using fractal anomalous diffusion model with microenvironment plasticity), as reported in\textsuperscript{113}. This cancer spatiotemporal dynamics may connect with $\sigma_{12}$ at $\Sigma$ if we express $\sigma_{12}$ in terms of the diffusion matrix of the cancer cells\textsuperscript{7,113}, tumor mass fractal dimension and tumor contour fractal dimension\textsuperscript{8,9}, corroborating the close relationship between electrical and mechanical properties of the tumor contour.

Anti-cancer therapies should have into account the previously mentioned aspects because cancer cells and $\sigma_{12}$ at $\Sigma$ are negative, whereas many molecules used in chemotherapy and immunotherapy are positively/negatively charged. Then, two questions arise immediately. Are the negative charged molecules across $\sigma_{12}$ at $\Sigma$ (from surrounding cancer tissue) more easily than the positive ones? Do the negatively charged molecules that across $\sigma_{12}$ at $\Sigma$ induce the highest antitumor effectiveness than
those positively charged? A meta-analysis may be carried out to give answer to these questions and others related to them.

As $\nabla \cdot \mathbf{E}_f$ is different from zero, tumor heterogeneity is implicit in the model, but not for the intra-tumor anisotropy. The intra-tumor heterogeneity and anisotropy have a role in tumor growth and metastasis\(^1\text{-}^2,^8,^9,^9,^99,^9,^92\). If the tumor and surrounding healthy tissue are assumed anisotropic, electrical properties of these two tissues should be replaced by their corresponding tensors. As a result, equations must be replaced by more complicated ones, being cumbersome the calculation procedure for obtaining the analytical solution of the problem (Eq. (8)).

Although malignant tumors are not generally spherical\(^1\text{-}^3,^5,^6,^8,^12,^19,^100\), results of this study confirm the usefulness of the spheroidal model of a tumor to reveal intrinsic findings in its TGK, in accordance with\(^7,^17,^92-^94\). As the ellipsoidal geometry of the solid tumor is often observed in the experiment\(^1\text{-}^3,^8,^12\), the problem (Eq. (8)) has to be solved in elliptical coordinates. Furthermore, if we consider that boundary condition depends on $(r, \hat{\theta}, \varphi)$ in problem (Eq. (8)), $\phi_1$, $E_1$ and $\sigma_{12}$ would depend on the spherical coordinates $(r, \hat{\theta}, \varphi)$, which means that $\sigma_{12}$ is not uniform at the entire $\Sigma$. We hypothesize that non-uniform distribution of $\sigma_{12}$ values at $\Sigma$ means that fractal dimension, amount of pores (link to endogenous angiogenesis process\(^8\)), spicule size (relate to tumor mechanical properties\(^1,^97-^99\)) are unevenly distributed along the tumor surface, aspects that may be related with the main tumor growth direction (prevalence of a tumor diameter during its growth), as reported in\(^1\text{-}^3,^5,^6,^8\). For this, an analysis of $m$ (degree of the polynomial) and $n$ (order of the polynomial) in (Eq. (8)) will be required, which may provide further characterization of the tumor surface (e.g., shape and orientation of the $\sigma_{12}$ deformation) due to endogenous (as unperturbed tumors) and exogenous (as treated tumors) perturbations.

The main consequences of this study

- Regulations of dynamical changes of $\sigma_{12}$ at $\Sigma$ may be a possible biophysical mechanism to explain dynamical alterations in cancer bioelectricity (e.g., $V_{\text{mem}}$, spatiotemporal ionic and faradic currents, tumor electronegativity (migration of negative carriers from $\Sigma$ towards its interior), redox processes, metabolic deregulations, among others), which are involved in the growth, progression, metastasis, and physiochemical microenvironment during the untreated solid tumor.

- Time changes of $\sigma_{12}$ at $\Sigma$ and negative bioelectricity are self-regulated by the solid tumor for its protection against the attack of the immune system and anticancer therapies. This later supposes to improve the design of conventional therapies (e.g., chemotherapy or immunotherapy) by estimating better the polarity of the substances in therapy or designing completely novel therapies.

- Results of this study allows to relate electrical, thermal, biological and mechanical properties of cancer, taking into account the results of this study and those reported in\(^1,^7,^9,^24,^59\).

The first point of section “The main consequences of this study” may be questionable because the following question may appear: Does time changes of $R_T$ lead to time alterations in $\sigma_{12}$ at $\Sigma$ or vice versa? The first part of this question supposes that the increase of $R_T$ in time (due to dynamical changes in biological aspects) lead to bioelectrical changes in cancer, as reported in the literature\(^24-^54\). In contrast, we believe that dynamical bioelectrical changes (due to changes in physical parameter and electrical state) are primary mechanisms involved in cancer that lead to modifications in biological aspects, as well as, an increase of $R_T$ (secondary mechanisms).

Insights for cancer therapy

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Although the exact mechanism is poorly understood, different cancer types generate specific galvanotaxis responses to low direct current electric fields. The results of this study confirm that anodes (positive electrodes) should be inserted in tumor regions near $\Sigma$ to avoid metastasis of cancer cells (negatively charged) when electrochemical therapy is applied to solid tumors, as reported. This may be explained because anodes generate positively charged carriers (e.g., $H^+$ ions) that may intensify electrostatic interactions between negatively charged carriers (e.g., cancer cells) by means of the formation of ionic bridges. Consequently, a possible anti-cancer therapy that inhibits the exit of positively charged carriers from the tumor may be suggested. Furthermore, knowledge of shape and orientation of $\sigma_{12}$ may be essential to elucidate if anodes should be inserted in regions near $\Sigma$ with higher or smaller $\sigma_{12}$ values to maximize tumor volume destruction with the minimum damage to the surrounding healthy tissue. This suggests that size, $\eta_1$, $\varepsilon_1$, and $\sigma_{12}$ should be measured previously in electrochemical therapy application in mice and patients with cancer.

The results of this study corroborates that the ionic and faradic currents should not be analyzed separately, in agreement with Robinson et al., who report that these two types of currents are closely related and explain why bioelectronic medicine is suggested to correct alterations in the electrical communication system of cancer by manipulating its bioelectrical properties. Consequently, new bioelectronic devices are designed to monitor these bioelectric changes, aspect that may be relevant for rapid and early detection of cancer. Furthermore, they document that non-invasively detect bioelectricity with high accuracy may be possible taking into account advances in new platforms integrating electronics with biology.

Deep understanding of results of this study may suggest the use of therapies that reestablish the bioelectrical states and $V_{m_{\text{mem}}}$ of cancer cells within the physiological range, in agreement with Cervera et al., who recommend that the use of non-physiological perturbations would not be necessary for cancer.

This study opens new questions that may be relevant to understand TGK and how electrophysiological variables of the untreated tumor change during its growth. Among the questions these arise. Does the tumor growth bring about change from $\sigma_{12,0}$ to $\sigma_{12,f}$ at $\Sigma$ or does this change lead to the tumor growth? What relationship exists between $\sigma_{12}$ and the tumor contour fractal dimension reported in? What implication non-homogeneous distribution of $\sigma_{12}$ at $\Sigma$ has during tumor growth? What expression adopts $\sigma_{12}$ when a heterogeneous tumor and nonlinear $\phi_1$ are considered? Can electrochemical therapy with low-level of direct current re-establish bioelectrical disorders that happen in an untreated tumor? How do the endogenous magnetic field and the ellipsoidal geometry influence the untreated tumor growth? How does $\sigma_{12}$ relate to other biophysical-chemical processes that occur in the tumor? How does $\sigma_{12}$ at $\Sigma$ change experimentally over time during the growth of untreated and treated solid tumors using any experimental techniques reported in (e.g., electrostatic force microscopy)? Future studies may be carried out to answer these questions or others. The elucidation of these aspects may be of importance for the proposal of an individualized electrochemical therapy addressed to cancer.

**CONCLUSION**

In conclusion, graphic strategies corroborate the correspondence between the electrical and physiological parameters in the untreated cancer, which may have an essential role in its growth, progression, metastasis and protection against immune system attack and anti-cancer therapies. In addition, knowledge of $\sigma_{12}$ at $\Sigma$ may be relevant in the redesign of chemotherapy and immunotherapy that have into account the polarity of the substances or in the design completely novel therapies.
AUTHOR CONTRIBUTIONS
H.B.P., N.A.V.G., A.A.S.J., Y.I.F., E.J.R.O., M.M.G., N.H.M., V.G.S.G., J.I.M. and L.E.B.C. participated in the conceptualization, organization and writing-original draft; H.B.P., J.A.H.K., E.J.R.O., J.I.M. and L.E.B.C. contributed to physics-mathematical models; H.B.P., J.I.M., and L.E.B.C. performed the simulations; H.B.P., A.A.S.J., Y.I.F., E.J.R.O., J.I.M., and L.E.B.C. revised the manuscript and supervised the entire work. All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTERESTS
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not required.

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REFERENCES
1. González, M. M. et al. Is cancer a pure growth curve or does it follow a kinetics of dynamical structural transformation? BMC Cancer 17, 174 (2017). doi: 10.1186/s12885-017-3159-y
2. Goris, N. A. V. et al. Efficacy of direct current generated by multiple-electrode arrays on F3II mammary carcinoma: Experiment and mathematical modeling. J. Transl. Med. 18, 1-17 (2020). doi: 10.1186/s12967-020-02352-6
3. Cabrales, L. E. B. et al. Mathematical modeling of tumor growth in mice following low-level direct electric current. Math. Comput. Simul. 78, 112-120 (2008). doi: 10.1016/j.matcom.2007.06.004
4. Waliszewski, P. & Konarski, J. The Gompertzian curve reveals fractal properties of tumor growth. Chaos, Solitons Fractals 16, 665-674 (2003). doi: 10.1016/S0960-0779(02)00469-1
5. Vaidya, V. G. & Alexandro, F. J. Evaluation of some mathematical models for tumor growth. Int. J. Bio. Med. Comput. 13, 19-35 (1982). doi: 10.1016/0020-7101(82)90048-4
6. Marušić, M. Mathematical models of tumor growth. Math. Commun. 1, 175-192 (1996). Available from: https://hrcak.srce.hr/file/2874
7. Cabrales, L. E. B., Montijano, J. I., Schonbek, M. & Castañeda, A. R. S. A viscous modified Gompertz model for the analysis of the kinetics of tumors under electrochemical therapy. Math. Comput. Simul. 151, 96-110 (2018). doi:10.1016/j.matcom.2018.03.005
8. Castañeda, A. R. S. et al. New formulation of the Gompertz equation to describe the kinetics of untreated tumors. PloS One 14, e0224978 (2019). doi: 10.1371/journal.pone.0224978
9. Goris, N. V. et al. Correspondence between formulations of Avrami and Gompertz equations for untreated tumor growth kinetics. Rev. Mex. Fis. 66, 632-636 (2020). doi: 10.31349/RevMexFis.66.632
10. Bera, T. K. Bioelectrical impedance and the frequency dependent current conduction through biological tissues: A short review. IOP Conf. Ser.: Mater. Sci. Eng. 331, 012005 (2018). doi: 10.1088/1757-899X/331/1/012005
11. Khalil, S. F., Mohktar, M. S. & Ibrahim, F. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. Sensors 14, 10895-10928 (2014). doi: 10.3390/s140610895
Surface charge density changes in tumor growth

12. Serša, I. et al. Electric current density imaging of mice tumors. *Magn. Reson. Med.* **37**, 404-409 (1997). doi: 10.1002/mrm.1910370318
13. Foster, K. R. & Schwan, H. P. Dielectric properties of tissues. In *Handbook of Biological Effects of Electromagnetic Fields* (eds Polk, C. & Postow, E.) 68-70 (CRC Press LLC, ISBN 0-8493-06418, Boca Raton, Florida, 1996).
14. Smith, S. R., Foster, K. R. & Wolf, G. L. Dielectric properties of VX-2 carcinoma versus normal liver tissue. *IEEE Trans. Biomed.* **BME-33**, 522-524 (1986). doi: 10.1109/TBME.1986.325740
15. Miklavčič, D., Pavšelj, N. & Hart, F. X. Electric properties of tissues. *Wiley Encyclopedia of Biomedical Engineering* 3578-3589 (John Wiley & Sons, Inc., NJ, USA, 2006).
16. Haemmerich, D., Schutt, D. J., Wright, A. S., Webster, J. G. & Mahvi, D. M. Electrical conductivity measurement of excised human metastatic liver tumours before and after thermal ablation. *Physiol. Meas.* **30**, 459-466 (2009). doi: 10.1088/0967-3334/30/5/003
17. Shimonov, G., Koren, A., Sivek, G. & Socher, E. Electromagnetic property characterization of biological tissues at D-band. *IEEE Trans. Terahertz Sci. Technol.* **8**, 155-160 (2018). doi: 10.1109/TTHZ.2018.2789357
18. Hassan, A. M. & El-Shenawee, M. Modeling biopotential signals and current densities of multiple breast cancerous cells. *IEEE Trans. Biomed.* **57**, 2099-2106 (2010), doi: 10.1109/TBME.2010.2049575
19. Miklavčič, D., Serša, G., Novaković, S. & Rebersek, S. Tumor bioelectric potential and its possible exploitation for tumor growth retardation. *J. Bioelectricity* **9**, 133-149 (1990). doi: 10.3109/15368379009119801
20. Habal, M. B. & Schauble, M. K. Electropotential differentiation of normal and tumor tissue. *Surg. Forum* **18**, 88-90 (1967).
21. Haltiwanger, S. The electrical properties of cancer cells. (2008). Available from: http://www.royalrife.com/haltiwanger1.pdf [Last update: April 2nd 2008]
22. Chernet, B. T., Fields, C. & Levin, M. Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in Xenopus laevis embryos. *Front. Physiol.* **5**, (2015), doi: 10.3389/fphys.2014.00519
23. Ribeiro-Rodrigues, T. M., Martins-Marques, T., Morel, S., Kwak, B. R. & Girão, H. Role of connexin 43 in different forms of intercellular communication-gap junctions, extracellular vesicles and tunnelling nanotubes. *J. Cell Sci.* **130**, 3619-3630 (2017). doi: 10.1242/jcs.200667
24. Robinson, A. J. et al. Toward hijacking bioelectricity in cancer to develop new bioelectronic medicine. *Adv. Ther.* **4**, 2000248 (2021). doi:10.1002/adtp.202000248
25. Zerfaß, C., Asally, M. & Soyer, O. S. Interrogating metabolism as an electron flow system. *Curr. Opin. Syst. Bio.* **13**, 59-67 (2019). doi: 10.1016/j.coisb.2018.10.001
26. Wang, Y. et al. Correlation between electrical characteristics and biomarkers in breast cancer cells. *Sci. Rep.* **11**, 1-11 (2021). doi: 10.1038/s41598-021-93793-6
27. Iorio, J., Petroni, G., Duranti, C. & Lastraioi, E. Potassium and sodium channels and the Warburg effect: Biophysical regulation of cancer metabolism. *Bioelectricity* **1**, 188-200 (2019). doi: 10.1089/bioe.2019.0017
28. Abdul Kadir, L., Stacey, M. & Barrett-Jolley, R. Emerging roles of the membrane potential: Action beyond the action potential. *Front. Physiol.* **9**, 1661 (2018). doi: 10.3389/fphys.2018.01661
29. Payne, S. L., Levin, M. & Oudin, M. J. Bioelectric control of metastasis in solid tumors. *Bioelectricity* **1**, 114-130 (2019). doi: 10.1089/bioe.2019.0013
30. Yang, M. & Brackenbury, W. J. Membrane potential and cancer progression. *Front. Physiol.* **4**, 185 (2013). doi: 10.3389/fphys.2013.00185
Surface charge density changes in tumor growth

31. Chen, B. et al. Targeting negative surface charges of cancer cells by multifunctional nanoparticles. *Theranostics* **6**, 1887 (2016). doi: 10.7150/thno.16358
32. Bhavsar, M. B., Leppik, L., Oliveira, K. M. C. & Barker, J. H. Role of bioelectricity during cell proliferation in different cell types. *Front. Bioeng. Biotechnol.* **8**, 603 (2020). doi: 10.3389/fbioe.2020.00603
33. Leslie, T. K. et al. Sodium homeostasis in the tumour microenvironment. *Biochim. Biophys. Acta Rev. Cancer* **1872**, 188304 (2019). doi: 10.1016/j.bbcan.2019.07.001
34. Venkataramani, V. et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature* **573**, 532-538 (2019). doi: 10.1038/s41586-019-1564-x
35. Venkatesh, H. S. et al. Electrical and synaptic integration of glioma into neural circuits. *Nature* **573**, 539-545 (2019). doi: 10.1038/s41586-019-1563-y
36. Zeng, Q. et al. Synaptic proximity enables NMDAR signalling to promote brain metastasis. *Nature* **573**, 526-531 (2019). doi: 10.1038/s41586-019-1576-6
37. Cervera, J., Pietak, A., Levin, M. & Mafe, S. Bioelectrical coupling in multicellular domains regulated by gap junctions: A conceptual approach. *Bioelectrochemistry* **123**, 45-61 (2018). doi: 10.1016/j.bioelechem.2018.04.013
38. Lucia, U. & Grisolia, G. How life works—a continuous seebeck-peltier transition in cell membrane? *Entropy* **22**, 960 (2020). doi: 10.3390/e22090960
39. Lucia, U. & Grisolia, G. Constructal law and ion transfer in normal and cancer cells. *Proc. Rom. Acad. A Special Issue* 213-218 (2018). Available from: https://core.ac.uk/download/pdf/234923555.pdf
40. Pietak, A. & Levin, M. Bioelectric gene and reaction networks: Computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation. *J. R. Soc. Interface* **14**, 20170425 (2017). doi: 10.1098/rsif.2017.0425
41. Pietak, A. & Levin, M. Bioelectrical control of positional information in development and regeneration: A review of conceptual and computational advances. *Prog. Biophys. Mol. Biol.* **137**, 52-68 (2018). doi: 10.1016/j.pbiomolbio.2018.03.008
42. Chang, F. & Minc, N. Electrochemical control of cell and tissue polarity. *Annu. Rev. Cell Dev. Biol.* **30**, 317-336 (2014). doi: 10.1146/annurev-cellbio-100913-013357
43. Dhar, G., Sen, S. & Chaudhuri, G. Acid gradient across plasma membrane can drive phosphate bond synthesis in cancer cells: Acidic tumor milieu as a potential energy source. *PloS One* **10**, e0124070 (2015). doi: 10.1371/journal.pone.0124070
44. Wang, Y., Han, X., Cui, Z. & Shi, D. Bioelectricity, its fundamentals, characterization methodology, and applications in nano-bioprobing and cancer diagnosis. *Adv. Biosyst.* **3**, 1900101 (2019). doi: 10.1002/adb.201900101
45. Seyfried, T. N. & Chinopoulos, C. Can the mitochondrial metabolic theory explain better the origin and management of cancer than can the somatic mutation theory? *Metabolites* **11**, 572 (2021). doi: 10.3390/metabo11090572
46. Bălăeț, C., Manole, G., Bălăeț, T. & Coculescu, B. I. Determining the electric potential of blood—a possible screening method at the population level. *Biotechnol. Lett.* **24**, 499-505 (2019). doi: 10.25083/rbl/24.3/499.505
47. Burgos-Panadero, R. et al. The tumour microenvironment as an integrated framework to understand cancer biology. *Cancer Lett.* **461**, 112-122 (2019). doi: 10.1016/j.canlet.2019.07.010
48. Levin, M. & Martyniuk, C. J. The bioelectric code: An ancient computational medium for dynamic control of growth and form. *Biosystems* **164**, 76-93 (2018). doi: 10.1016/j.biosystems.2017.08.009

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Surface charge density changes in tumor growth

49. Fan, J. J. & Huang, X. Ion channels in cancer: Orchestrators of electrical signaling and cellular crosstalk. In *Reviews of Physiology, Biochemistry and Pharmacology* 1-31 (Springer, Berlin, Heidelberg, 2020). doi: 10.1007/112_2020_48

50. Georgikou, C. et al. Inhibition of miR30a-3p by sulforaphane enhances gap junction intercellular communication in pancreatic cancer. *Cancer Lett.* **469**, 238-245 (2020). doi: 10.1016/j.canlet.2019.10.042

51. Yang, Q. et al. Integration of electrotaxis and durotaxis in cancer cells: Subtle nonlinear responses to electromechanical coupling cues. *Biosens. Bioelectron.* **186**, 113289 (2021). doi: 10.1016/j.bios.2021.113289

52. Pulikkathodi, A. K. et al. Dynamic monitoring of transmembrane potential changes: A study of ion channels using an electrical double layer-gated FET biosensor. *Lab. Chip* **18**, 1047-1056 (2018). doi: 10.1039/C7LC01305A

53. Zhu, K. et al. Electric fields at breast cancer and cancer cell collective galvanotaxis. *Sci. Rep.* **10**, 1-11 (2020). doi: 10.1038/s41598-020-65566-0

54. Soucy, J. R., Bindas, A. J., Koppes, A. N. & Koppes, R. A. Instrumented microphysiological systems for real-time measurement and manipulation of cellular electrochemical processes. *iScience* **21**, 521-548 (2019). doi: 10.1016/j.isci.2019.10.052

55. Landau, L. D. & Lifshitz, E. M. Electrodynamics of continuous media. *Course of theoretical physics 3rd ed.* (Pergamon Press, New York, 1984).

56. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144**, 646-674 (2011). doi: 10.1016/j.cell.2011.02.013

57. Kremer, F. & Schönhals, A. *Broadband dielectric spectroscopy 1st ed.* (Springer-Verlag, Berlin, 2003).

58. Sekino, M., Ohsaki, H., Yamaguchi-Sekino, S., Iriguchi, N. & Ueno, S. Low-frequency conductivity tensor of rat brain tissues inferred from diffusion MRI. *Bioelectromagnetics* **30**, 489-499 (2009). doi: 10.1002/bem.20505

59. Oria, E. J. R., Cabrales, L. E. B. & Reyes, J. B. Analytical solution of the bioheat equation for thermal response induced by any electrode array in anisotropic tissues with arbitrary shapes containing multiple-tumor nodules. *Rev. Mex. Fis.* **65**, 284-290 (2019). doi: 10.31349/revmexfis.65.284

60. Dash, S. et al. Differentiating between cancer and normal tissue samples using multi-hit combinations of genetic mutations. *Sci. Rep.* **9**, 1005 (2019). doi: 10.1038/s41598-018-37835-6

61. Martinsen, O. G., Grimnes, S. & Schwan, H. P. Interface phenomena and dielectric properties of biological tissue. in *Encyclopedia of surface and colloid science* 2643-2653 (Marcel Dekker, New York, 2002).

62. Das, S. & Gupta, N. Interfacial charge behaviour at dielectric-dielectric interfaces. *IEEE Trans. Dielectr. Electr. Insul.* **21**, 1302-1311 (2014). doi: 10.1109/TDEI.2014.6832278

63. Rogti, F. & Ferhat, M. Maxwell-Wagner polarization and interfacial charge at the multilayers of thermoplastic polymers. *J. Electrostat.* **72**, 91-97 (2014). doi: 10.1016/j.elstat.2013.11.012

64. Kuang, W. & Nelson, S. O. Low-frequency dielectric properties of biological tissues: A review with some new insights. *Transactions of the ASAE* **41**, 173-184 (1998). Available from: https://pubag.nal.usda.gov/download/34220/PDF

65. Huang, Y.-J. et al. Cellular microenvironment modulates the galvanotaxis of brain tumor initiating cells. *Sci. Rep.* **6**, 21583 (2016). doi: 10.1038/srep21583

66. Fabregas, R. & Gomila, G. Dielectric nanotomography based on electrostatic force microscopy: A numerical analysis. *J. Appl. Phys.* **127**, 024301 (2020). doi: 10.1063/1.5122984
Surface charge density changes in tumor growth

67. Wang, K., Lu, Y., Cheng, J., Zhu, X. & Ji, L. Quantitative electrostatic force measurement and characterization based on oscillation amplitude using atomic force microscopy. *AIP Advances* **10**, 015143 (2020). doi: 10.1063/1.5136332

68. Casuso, I., Redondo-Morata, L. & Rico, F. Biological physics by high-speed atomic force microscopy. *Philos. Trans. Royal Soc. A Math. Phys. Eng. Sci.* **378**, 20190604 (2020). doi: 10.1098/rsta.2019.0604

69. Mateus, R., Fuhrmann, J. F. & Dye, N. A. Growth across scales: Dynamic signaling impacts tissue size and shape. *Curr. Opin. Cell Biol.* **73**, 50-57 (2021). doi: 10.1016/j.cceb.2021.05.002

70. Bradley, R. Bio-electrical engineering: A promising frontier for synthetic biology. *The Biochem.* **41**, 10-13 (2019). doi: 10.1042/BIO04103010

71. Moore, D., Walker, S. I. & Levin, M. Cancer as a disorder of patterning information: Computational and biophysical perspectives on the cancer problem. *Converg. Sci. Phys. Oncol.* **3**, 043001 (2017). doi: 10.1088/2057-1739/aa8548

72. Stock, C. & Schwab, A. Ion channels and transporters in metastasis. *Biochim. Biophys. Acta Biomembr.* **1848**, 2638-2646 (2015). doi: 10.1016/j.bbamem.2014.11.012

73. Silver, B. B. & Nelson, C. M. The bioelectric code: Reprogramming cancer and aging from the interface of mechanical and chemical microenvironments. *Front. Cell Dev. Biol.* **6**, 21 (2018). doi: 10.3389/fcell.2018.00021

74. Pethő, Z. et al. pH-channeling in cancer: How pH-dependence of cation channels shapes cancer pathophysiology. *Cancers* **12**, 2484 (2020). doi: 10.3390/cancers12092484

75. Jiménez, R. P., et al. 3D stationary electric current density in a spherical tumor treated with low direct current: An analytical solution. *Bioelectromagnetics* **32**, 120-130 (2011). doi: 10.1002/bem.20611

76. Yu, X., et al. Dielectric properties of normal and metastatic lymph nodes ex vivo from lung cancer surgeries. *Bioelectromagnetics* **41**, 148-155 (2020). doi: 10.1002/bem.22246

77. Guardiola, M., et al. Dielectric properties of colon polyps, cancer, and normal mucosa: Ex vivo measurements from 0.5 to 20 GHz. *Med. Phys.* **45**, 3768-3782 (2018). doi: 10.1002/mp.13016

78. Murphy, E. K., Mahara, A., Wu, X. & Halter, R. J. Phantom experiments using soft-prior regularization EIT for breast cancer imaging. *Physiol. Meas.* **38**, 1262 (2017). doi: 10.1088/1361-6579/aa91b

79. Smolyanskaya, O. A. et al. Terahertz biophotonics as a tool for studies of dielectric and spectral properties of biological tissues and liquids. *Prog. Quantum. Electron.* **62**, 1-77 (2018). doi: 10.1016/j.pquantelec.2018.10.001

80. Campelo, S. et al. An evaluation of irreversible electroporation thresholds in human prostate cancer and potential correlations to physiological measurements. *APL Bioengineering* **1**, 016101 (2017). doi: 10.1063/1.5005828

81. Yun, J., Hong, Y. T., Hong, K. H. & Lee, J. H. Ex vivo identification of thyroid cancer tissue using electrical impedance spectroscopy on a needle. *Sens. Actuators B Chem.* **261**, 537-544 (2018). doi: 10.1016/j.snb.2018.01.155

82. Ain, K., Kurniadi, D., Suprijanto, S. & Santoso, O. Dual modality tran-admittance mammography and ultrasound reflection to improve accuracy of breast cancer detection. *J. Teknol.* **82** (2020). doi: 10.1113/jt.v82i1491

83. Shawki, M. M., Azmy, M. M., Salama, M. & Shawki, S. Mathematical and deep learning analysis based on tissue dielectric properties at low frequencies predict outcome in human breast cancer. *Technol. Health Care*, 1-13 (2021). Available from: https://content.iospress.com/articles/technology-and-health-care/thc213096(preprint posted July 30, 2021).
84. Limkin, E. J. et al. The complexity of tumor shape, spiculatedness, correlates with tumor radiomic shape features. *Sci. Rep.* **9**, 1-12 (2019). doi: 10.1038/s41598-019-40437-5
85. Pupo, A. E. B., Jiménez, R. P. & Cabrales, L. E. B. Electrotherapy on cancer: Experiment and mathematical modeling. in Current cancer treatment—novel beyond conventional approaches. (ed Özdémir, Ö.) 585-620 (InTech-Open Access Publisher, Rijeka, Croatia 2011). Available from: https://www.intechopen.com/chapters/24598
86. Verjans, E. T., Doijen, J., Luyten, W., Landuyt, B. & Schoofs, L. Three-dimensional cell culture models for anticancer drug screening: Worth the effort? *J. Cell Physiol.* **233**, 2993-3003 (2018). doi: 10.1002/jcp.26052
87. Gilazieva, Z., Ponomarev, A., Rutland, C., Rizvanov, A. & Solovyeva, V. Promising applications of tumor spheroids and organoids for personalized medicine. *Cancers* **12**, 2727 (2020). doi: 10.3390/cancers12102727
88. Zhang, L. et al. Tumor chemo-radiotherapy with rod-shaped and spherical gold nanoprobes: Shape and active targeting both matter. *Theranostics* **9**, 1893 (2019). doi: 10.7150/thno.30523
89. Zhang, C. et al. 3D culture technologies of cancer stem cells: Promising ex vivo tumor models. *J. Tissue Eng.* **11**, 2041731420933407 (2020). doi: 10.1177/2041731420933407
90. Zhu, X., Vo, C., Taylor, M. & Smith, B. R. Non-spherical micro- and nanoparticles in nanomedicine. *Mater. Horiz.* **6**, 1094-1121 (2019). doi: 10.1039/C8MH01527A
91. Karolak, A., Markov, D. A., McCawley, L. J. & Rejniak, K. A. Towards personalized computational oncology: from spatial models of tumour spheroids, to organoids, to tissues. *J. R. Soc. Interface* **15**, 20170703 (2018). doi: 10.1098/rsif.2017.0703
92. Korshoej, A. R., Hansen, F. L., Thielscher, A., von Oettingen, G. B. & Sørensen, J. C. H. Impact of tumor position, conductivity distribution and tissue homogeneity on the distribution of tumor treating fields in a human brain: A computer modeling study. *PloS One* **12**, e0179214 (2017). doi: 10.1371/journal.pone.0179214
93. Gomes, A., Defaux, M., Leme, R. M., Lobjois, V. & Ducommun, B. Reversible growth arrest of 3D tumor spheroids stored in oxygen absorber-induced anoxia. *Oncol. Lett.* **15**, 2006-2009 (2018). doi: 10.3892/ol.2017.7465
94. Vu, T. T. N., Teyssedre, G., Roy, S. L. & Laurent, C. Maxwell-Wagner effect in multilayered dielectrics: Interfacial charge measurement and modelling. *Technologies* **5**, 27 (2017). doi: 10.3390/technologies5020027
95. Saez, P. On the theories and numerics of continuum models for adaptation processes in biological tissues. *Arch. Comput. Methods Eng.* **23**, 301-322 (2016). doi: 10.1007/s11831-014-9142-8
96. Rianna, C. & Radmacher, M. Influence of microenvironment topography and stiffness on the mechanics and motility of normal and cancer renal cells. *Nanoscale* **9**, 11222-11230 (2017). doi: 10.1039/C7NR02940C
97. Shim, E. B., Kim, Y. S. & Deisboeck, T. S. Analyzing the dynamic relationship between tumor growth and angiogenesis in a two dimensional finite element model. Preprint at https://arxiv.org/abs/q-bio/0703015 (2007).
98. Bhandari, S., Choudannavar, S., Avery, E. R., Sahay, P. & Pradhan, P. Detection of colon cancer stages via fractal analysis of optical transmission imaging of tissue microarrays (TMA). *Biomed. Phys. Eng. Express* **4**, 065020 (2018). doi: 10.1088/2057-1976/aae1e9
99. Song, E. J., Sohn, Y.-M. & Seo, M. Tumor stiffness measured by quantitative and qualitative shear wave elastography of breast cancer. *Br. J. Radiol.* **91**, 20170830 (2018). doi: 10.1259/bjr.20170830
Surface charge density changes in tumor growth

100. Levin, M., Pezzulo, G. & Finkelstein, J. M. Endogenous bioelectric signaling networks: Exploiting voltage gradients for control of growth and form. *Annu. Rev. Biomed. Eng.* **19**, 353-387 (2017). doi: 10.1146/annurev-bioceng-071114-040647

101. Chapman, C. H. et al. Deformable image registration-based contour propagation yields clinically acceptable plans for MRI-based cervical cancer brachytherapy planning. *Brachytherapy* **17**, 360-367 (2018). doi: 10.1016/j.brachy.2017.11.019

102. Golberg, A., Bruinsma, B. G., Uygun, B. E. & Yarmush, M. L. Tissue heterogeneity in structure and conductivity contribute to cell survival during irreversible electroporation ablation by “electric field sinks”. *Sci. Rep.* **5**, 8485 (2015). doi: 10.1038/srep08485

103. Levin, M. Molecular bioelectricity: How endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol. Biol. Cell* **25**, 3835-3850 (2014). doi: 10.1091/mbc.e13-12-0708

104. McGranahan, N. & Swanton, C. Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell* **168**, 613-628 (2017). doi: 10.1016/j.cell.2017.01.018

105. Funk, R. H. W. Endogenous electric fields as guiding cue for cell migration. *Front. Physiol.* **6**, (2015). doi: 10.3389/fphys.2015.00143

106. Mittal, V. Epithelial mesenchymal transition in tumor metastasis. *Annu. Rev. Pathol. Mech. Dis.* **13**, 395-412 (2018). doi: 10.1146/annurev-pathol-020117-043854

107. Ariyan, C. E. et al. Robust antitumor responses result from local chemotherapy and CTLA-4 blockade. *Cancer Immunol. Res.* **6**, 189 (2018). doi: 10.1158/2326-6066.CIR-17-0356

108. Wu, M.-Z. et al. miR-25/93 mediates hypoxia-induced immunosuppression by repressing cGAS. *Nat. Cell Biol.* **19**, 1286-1296 (2017). doi: 10.1038/ncb3615

109. Gun, L., Ning, D. & Liang, Z. Effective permittivity of biological tissue: Comparison of theoretical model and experiment. *Math. Probl. Eng.* **2017**, 7249672 (2017). doi: 10.1155/2017/7249672

110. Enderling, H. & Chaplain, M. A. J. Mathematical modeling of tumor growth and treatment. *Curr. Pharm. Des.* **20**, 4934-4940 (2014). Available from: https://research-repository.st-andrews.ac.uk/bitstream/handle/10023/7710/enderling_chaplain_Mathematical_modeling_of_tumor_growth_and_treatment_rev2_majc.pdf?sequence=1&isAllowed=y

111. Gatenby, R. A. & Gawlinski E. T. A reaction-diffusion model of cancer invasion. *Cancer Res.* **56**, 5745 (1996). Available from: https://cancerres.aacrjournals.org/content/canres/56/24/5745.full.pdf

112. Huang, D., Yang, J., Zhou, D., Sanjuán, M. A. & Liu, H. Recovering an unknown signal completely submerged in strong noise by a new stochastic resonance method. *Commun. Nonlinear Sci. Numer. Simul.* **66**, 156-166 (2019). doi: 10.1016/j.cnsns.2018.06.011

113. Tsai, F.-C., Wang, M.-C., Lo, J.-F., Chou, C.-M. & Lin, Y.-L. Spatiotemporal dynamics of the biological interface between cancer and the microenvironment: A fractal anomalous diffusion model with microenvironment plasticity. *Theor. Biol. Med. Model.* **9**, 36 (2012). doi: 10.1186/1742-4682-9-36

114. Pupo, A. E. B. et al. 3d current density in tumors and surrounding healthy tissues generated by a system of straight electrode arrays. *Math. Comput. Simul.* **138**, 49-64 (2017). doi: 10.1016/j.matcom.2017.01.004

115. Huang, C. W., Cheng, J. Y., Yen, M. H. & Young, T. H. Electrotaxis of lung cancer cells in a multiple-electric-field chip. *Biosens. Bioelectron.* **24**, 3510-3516 (2009). doi: 10.1016/j.bios.2009.05.001