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Abstract

Cell membranes are the reason of the cell energy transfer. In cells energy transfer, thermo-electro-chemical processes and transports phenomena occur through their membranes. Cells can actively modify their behaviours in relation to any change of their environment. They waste heat into their environment. The analysis of irreversibility related to this wasted heat, to the ions transport and the related cell-environment pH changes represents a new useful approach to the study of the cells behaviour. This analysis allows also the explanation of the effects of electromagnetic fields on the cell behaviour, and to suggest how low intensity electromagnetic fields could represent a useful support to the present anticancer therapies.

Keywords: Constructal law; Cancer; Irreversible biochemical thermodynamics; living cells ions transport; open systems; cancer invasion; cancer metastasis.

Introduction

Breast cancer is the leading cause for cancer incidence among women worldwide [1]. The present therapeutic scheme consists of surgery followed by radio- or/and chemotherapy to help prevent or contain the development of metastases. But, these two adjuvant therapies have always also unintended side effects on normal cells.

Consequently, other kinds of adjuvant therapies should be considered as well. This includes low energy non-ionizing radiation which has been shown to promisingly [2-8] support the aforementioned therapies by modulating their intensity and, therefore reducing their collateral effects [1]. Indeed, recently, electromagnetic fields have been applied successfully in the areas of bone healing [9,10], wound repair [9-11], neural regeneration [11-16], as support to myocardial therapies [17], and in ischemia reperfusion [17-19].

Moreover, studying the significance of effect and efficacy [1], the authors note that pulse electromagnetic fields can yield a therapeutic effect if their frequency is less than 300 Hz and their intensity is less than 200 mT, with particular results obtained in bone repair at 500 μT.

Although electromagnetic technology is well known since 1865, when Maxwell obtained his results on electromagnetism, its clinical applications have been developed only since the 1980s. Interest in the use of low frequency and intensity electromagnetic fields as complementary therapy in clinical oncology is continuously growing [5,20-25], driven by encouraging results both *in vitro* and *in vivo* that support it to lead to a decrease in cancer growth [1]. Recently, it was highlighted that low frequency electromagnetic fields can support conventional anticancer therapies, rather than represent an autonomous therapy themselves because such electromagnetic fields cause a reduction in tumor growth, rather than complete growth inhibition [26-31].

To improve clinical applicability of this new adjuvant anti-cancer method, it is critical to better understand the interactions between these low frequency electromagnetic waves and living

cells. We therefore use a recently proposed thermodynamic approach which combines the constructal law with biochemical thermodynamics, with the result of obtaining an irreversible biochemical engineering thermodynamic method. Indeed, Schrödinger, in *What's life* [32], introduced the hypothesis that irreversibility is the result of the interactions and fluxes. Moreover, processes in non-equilibrium regions and the resulting systems can be described in terms of gradients maintaining systems away from equilibrium [33-36], and of information content of Gibbs free energy [37], related to the entropy variation for irreversibility. So, we have studied the bio-systems as adaptive bio-chemical thermodynamic non linear open systems, able to convert energy from one form to another by coupling fluxes, physical and chemical reactions with transport processes [38], and consuming irreversibly free energy for thermal, chemical and physical processes, for transport of matter, energy and ions. So, fluxes across the system border represent the fundamental quantities for any physical analysis. To develop this approach, some considerations must be introduced [39,40]:

1. The energy lost by the system is gained by the environment. This energy lost can be considered as the information lost by the system, but also the information obtained by the environment. As such, the information exchanged is no more than a communication between the system and its environment. Therefore, if we would be able to codify this information we could increase our knowledge on the system's behaviour;
2. The environment is always completely accessible by any observer. Consequently, we can always observe and collect data on this communication between systems and their environment;
3. The flows cause entropy generation variations. Entropy generation is a measure of the irreversibility related to the flows across the system border and the interactions between system and environment;
4. Entropy generation is a global quantity. As such, we can obtain information about the behaviour of the whole system, so that we can analyze the cooperative effect of the different energetic processes inside the systems.

Consequently, we consider that complex systems attain their "optimal" performance, as constructal law suggests [41-47], by a selection process driven by the interactions with their environment; this effects the redistribution of the energy, ions and mass flows in their energetic network, by involving regulatory subsystems.

As to these subsystems, we note that cellular phenotypes are determined by the expression levels of many genes and their products, i.e. proteins. Gene expression analysis represent a fundamental approach to track down the cellular phenotypic diversity. In the last two decades, the interest in MET (Hepatocyte Growth Factor Receptor) tyrosine kinase has grown considerably. It is the receptor for HGF/SF (Hepatocyte Growth Factor/Scatter Factor), a mesenchyme-derived pleiotropic growth factor. It has been proved that it is active on a variety of target cells as, for example, hepatocytes, melanocytes, keratinocytes, cells of the mammary, renal, gastric and biliary epithelium, as well as hematopoietic precursors, vascular endothelial cells, and neurons [48-50]. Moreover, *in vivo*, HGF/SF has been highlighted as a very potent angiogenic factor [51-53]. Last, it is also involved in regeneration of organs as, for example, liver, kidney and lung [54-56]. The role of the alteration of the MET gene expression and its receptor was highlighted by dentification of activating mutations in the MET kinase in both hereditary and sporadic human apillary kidney carcinoma [57-59].

Recently, a thermodynamic approach to biosystems has been deeply developed [60-71]. This approach consider that cells are open complex thermodynamic systems. They can be also regarded as complex engines that execute a series of chemical reactions. Energy transformations, thermo-electro-chemical processes and transports phenomena can occur across the cells membranes. Moreover, cells can also actively modify their behaviours in relation to changes in their environment. Moreover, different thermo-electro-biochemical behaviours occur between health and disease states. But, all the living systems waste heat, which is no more than the result of their internal irreversibility. This heat is dissipated into the environment. But, this wasted heat represents also a sort of information, which outflows from the cell toward its environment, completely

accessible to any observer. Consequently, the analysis of irreversibility related to this wasted heat can represent a useful approach to study the behaviour of the cells themselves and to control their behaviours. So, this approach allows us to consider the living systems as black boxes and analyze only the inflows and outflows and their changes in relation to the modification of the environment. Therefore, information on the systems can be obtained by analyzing the changes in the cell heat wasted in relation to external perturbations. So, this bio-chemical engineering thermodynamic approach can be used to analyse the interaction between the low frequency electromagnetic waves and the cell systems, in order to try the identification of the bio-molecules involved in this interaction. The final aim is the use of the low frequency electromagnetic waves for the control of the ions fluxes across the cells membranes, and, consequently, of the cancer growth. To achieve this aim we must understand a great number of biophysical and biochemical processes. So, in this paper, we develop this irreversible biochemical thermodynamic analysis to analyse the cancer invasion and metastasis.

The thermodynamic approach

During their evolution, living systems increase their structure in organization, with a related increase of their entropy [62]. To do so, they must improve their efficiency in converting energy into entropy, in order to reduce the energy gradients. This reorganisation [72] against gradients determines energy, mass, and ions fluxes with a consequent variation of the entropy generation rate:

$$\dot{S}_g = \frac{d_i S}{dt} = \sum_i J_i X_i \tag{1}$$

where d_iS is the entropy variation due to irreversibility, J_i are the fluxes, and X_i are the conjugated generalized forces.

The cells are separated from their environment by the lipid bilayer membrane which behaves as a dielectric capacitor, able to maintain the ionic gradients between the two membrane's surfaces.

As a consequence, the membrane maintains different concentrations of specific ion species (H^+, Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}, etc.), on both sides, i.e. inside and outside the cell:

$$c_{outside} = c_{inside} \exp\left(\frac{\Phi_{outside} - \Phi_{inside}}{RT} \right) \tag{2}$$

where c stands for the molar concentration of the chemical species, R is the universal constant of gas, T is the temperature and Φ is the electric potential energy. Consequently, there is a charge separation across the membrane, with a negative potential inside the cell membrane of around -70 to –100 mV; an electro-diffusion of ions via their electrochemical gradient can occur, with an ion drift velocity v_{drift} obtained by using the classical kinetic theory as [62]:

$$v_{drift} = \frac{Ze}{m} \frac{\phi}{d} \tau_{drift} \tag{3}$$

where Ze is the electric charge of the ion, m is the ion mass, ϕ is the electric potential across the membrane, d is the length of the membrane and τ_{drift} is the mean time between two collisions [50]:

$$\tau_{drift} = \frac{m\sigma}{n(Ze)^2} \tag{4}$$

where σ is the electric conductivity and n is the density number of ions. As a consequence, the following mass flow, $\dot{m}$, is generated:

$$\dot{m} = \rho v A = \rho\, A\, N_{coll} v_{drif} \tag{5}$$

where ρ is the mass density, A is the surface area of the cell membrane, N_{coll} is the number of collisions of the ion when it crosses the cell membrane, which can be evaluated as the ratio between the time τ required to cross the membrane and the time of drift τ_{drift}:

$$N_{coll} = \frac{\tau}{\tau_{drift}} = \frac{n(Ze)^2}{m\sigma} \tau \tag{6}$$

As such, the mass flow results [62]:

$$\dot{m} = \rho v A = \rho\, A \frac{Ze}{m} \frac{\phi}{d} \tau \tag{7}$$

Now, we consider the second law for the open systems [42]:

$$S_g = \int_0^{\tau} \dot{S}_g dt = \Delta S - \sum_i \frac{Q_i}{T_i} - \int_0^{\tau} \left(\sum_{in} \dot{m}_{in} s_{in} - \sum_{out} \dot{m}_{out} s_{out} \right) dt \tag{8}$$

where Q is the heat exchanged, T is the temperature of the thermal source, s is the specific entropy, $\dot{m}$ is the mass flow and τ is the lifetime of the process. Introducing relations (3)-(7) into the relation (8), it follows:

$$S_g = \Delta S - \sum_i \frac{Q_i}{T_i} - \frac{\phi}{d} A \sum_i \Delta\rho_i \frac{Z_i e}{m_i} \tau_i s_i \tag{9}$$

where i = H^+, Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}, ...

From this analytical results, it is possible to argue that the cell functions are regulated by membrane proteins, sensitive to the electric field. Changes in the electric field are transduced into a conformational change of the biological molecules, and this allosteric effect achieves the function of the membrane protein, with consequences for the regulation of cell functions. Consequently, considering the role of the electrostatic potential in regulating normal cell migration, differentiation, and proliferation, its control, or rather loss of control, is fundamental for the development of cancer as well. This result can be obtained simply by the control of the ion fluxes. Indeed, the voltage-responsive transduction mechanisms on the cell membrane allow bioelectric signals to regulate the polarization of the cell molecules. Last, the biochemical reactions that enable cell life produce or consume external metabolites, and connect with internal metabolites. The fundamental phenomena used by cells to reach their optimal function, consist of a redistribution of the flow patterns through their metabolic network.

Thermodynamic stationary is fundamental to quantify the steady state concentration of mRNA. Indeed, it is possible to evaluate the chemical potential μ_i of the i-th species as follows [36,71]:

$$\mu_i = \left(\frac{\partial G_i}{\partial c_i} \right)_{T,p} = -\frac{RT}{c_i} \tag{10}$$

where G is the Gibbs' potential, and c is the concentration of the i-th species, at constant temperature T, and pressure p, and R is the constant of the perfect gas (R = 8314 J mol^{-1} K^{-1}). Now,

considering the relations (2) and (10) in the explicit relation for the entropy generation as obtained in non-equilibrium thermodynamics, it follows that, as a consequence of any concentration variation there exists an entropy generation variation [39,40]:

$$\delta S_{g,dc} = \frac{V_m}{T}\sum_i \dot{\mathbf{x}}_{th}\cdot\nabla\mu_i \approx \frac{V_m\dot{x}_{th}}{T}\,\frac{\mu_{i,outside}-\mu_{i,inside}}{d_m} = \frac{RV_m\dot{x}_{th}}{d_m}T\left(\frac{1}{c_{i,outside}}-\frac{1}{c_{i,inside}}\right) = \\ = \frac{RV_m\dot{x}_{th}}{d_m}\frac{T}{c_{i,outside}}\left(1-\frac{c_{i,outside}}{c_{i,inside}}\right) = \frac{RV_m\dot{x}_{th}}{d_m}\frac{T}{c_{i,outside}}\left[1-\exp\left(\frac{\Phi_{outside}-\Phi_{inside}}{RT}\right)\right] \tag{11}$$

Remembering that [63]

$$\Delta G_P = \Delta\phi - 2.3\frac{RT}{F}\Delta\mathrm{pH} \tag{12}$$

with $\Delta\phi$ being the membrane potential, R is the gas constant (8.314 J mol^{-1}K^{-1}), F is the Faraday constant (96.485×10^3 A s mol^{-1}), and 2.3 ΔpH is the physiological concentration gradient, it follows that:

$$\delta S_{g,dc} = \sum_i \frac{RV_m\dot{x}_{th}}{d_m}\frac{T}{c_{i,outside}}\left[1-\exp\left(\frac{\Delta G_P + 2.3\frac{RT}{F}\Delta\mathrm{pH}}{RT}\right)\right] = \\ = \frac{RV_m\dot{x}_{th}}{d_m}T\left[1-\exp\left(\frac{\Delta G_P}{RT}+\frac{2.3}{F}\Delta\mathrm{pH}\right)\right]\sum_i\frac{1}{c_{i,outside}} \tag{13}$$

which allows us to state that any variation in the entropy generation, as a result of the interaction between the electromagnetic waves and the biological system[39,40], determines the variation of the Gibbs' potential, of the concentration of the different chemical species, and of the pH.

Discussion

The mean geomagnetic field is in the order of 50 μT. Some every-day house hold devices that generate, for short time, up to 1 mT at 3-30 cm from the human body, while for instance the electric blanket generates 60 Hz fields up to 5 μT for a usually longer time exposure. However, all these applications are considered safe. In this context, we note that the possible biomedical applications

discussed here refer to low frequency and low intensity electromagnetic fields, and that they have been evaluated as safe in multiple experimental studies [66,67].

The recent development of our thermodynamic approach [26,30,68], which has just been experimentally verified [60], is following the need to verify the theoretical optimal frequency [30] and intensity [69] in order to find the optimal therapeutic regimen to induce cancer cell apoptosis and promote tumor growth inihibition. Indeed, from these experimental results, it has been pointed out that the long-term exposure to low frequency and intensity electromagnetic fields reduces the proliferation of several cancer cell lines and this effect is associated with an increased mitochondrial activity without evident changes in ATP levels [64]. This result can be explained as a consequence of the biochemical and biophysical effects of the thermal flux across the membrane [38-40,63], a concept that has just been verified by an *in vitro* experiment [64]. But, the mitochondria behaviour in relation to the consumption of ATP is related to the oxidative phosphorylation $ADP + P_i + H^+ \rightarrow ATP$, where ADP stands for Adenosine-di-phosphate, P is the Phosphorus atom, H^+ the Hydrogen ion and ATP Adenosine-tri-phosphate. This reaction involves the Hydrogen ion which can exit the cell across the membrane by V-ATPsynthase [68].

The electromagnetic waves interact with the bio-molecular structures of the cell systems. In particular, it has been highlighted [1] that the tyrosine is one of the targets of this interaction.This has consequences on many proteins, in particular on MET and HGF/SF, whose tight regulation and signalling is lost in cancer [70]. This change often causes transcriptional deregulation, inadequate degradation, receptor crosstalk, and synergies in downstream signalling [70].

Conclusions

The biochemical thermodynamic analysis highlights that the charged species, their arrangements, the local field strength, charges and dipole disposition and movements can vary as a result of changing the electric field, which is transduced into a conformational change related to the protein

functions themselves. These considerations suggest that control and regulation of the electric field of cell membranes could represent a new therapeutic approach against diseases such as cancer [29] , with the possibility of an irradiation of a larger region because low frequency and intensity electromagnetic fields have been proven to be safe for normal tissues[2]. Specifically, the low frequency and intensity electromagnetic waves act on the energy, mass, and ions fluxes across cell membranes. To understand how to control these fluxes across the membrane, we consider the concentration of ions on opposite sides of the membrane; these fluxes generate gradients of concentration of energy and chemical species, inducing in the cell the need of redistributing both, energy and these concentrations. Consequently, many bio-chemical reactions and biophysical phenomena related to normal cell behaviour are induced, with the consequence of correcting the cancer behaviour [26-31]. The principal biological effect, that we are able to point out [1,3-9,11-25], is that the electromagnetic waves can alter the membranes' voltage anion channels, mitochondria's ones included, with the consequence of the activation of the cysteine proteases, probably the caspases activated by the release of cytochrome c just from mitochondria into cytoplasm. The medical consequences [11-25] of these biological processes are:

1. The apoptotic death of the cancer cells, and thus;
2. Globally, a reduction in cancer growth.

In previous works, we have targeted the energy deficit [60], the apoptosis effect [28], and the positive ion transport [61-63]. Here, we focus on evaluating the results obtained from an *in vitro* experiment [64], that was designed to investigate the effects of low frequency electromagnetic fields on mitochondria metabolism. Specifically, the effects of low frequency and intensity electromagnetic fields on cancer growth have been studied by crystal violet assay, while the modulation of mitochondrial activity was assessed by cytofluorimetric evaluation of membrane potential and by real-time quantification of mitochondrial transcription. The electromagnetic frequency used was 50 Hz. Here, we consider these very same two cancer cell lines [64] and we develop a theoretical evaluation by using the entropy variation, and by employing relation (13) and the parameter just obtained from

previous results [26-28,38-40,63]; for the gastric carcinoma cell line (GTL16): its volume is 1.20×10^{-15} m^3, its membrane volume is 3.46×10^{-16} m^3, while for the breast adenocarcinoma cell line (SKBR3): its volume is 2.90×10^{-14} m^3, and the membrane volume is 2.88×10^{-15} m^3. Now, in order to prove our results, we must consider that (i) the mitochondria behaviour induced by the electromagnetic field produces a variation in the entropy generation due to thermal flux [29-31], and that (ii) the behaviour of the mitochondria can occur only as a consequence of H^+ flux across the cell membrane. In relation to the mitochondria activity this reaction produces a variation in pH equal to -1.4, in the membrane electric potential equal to 0.14 V, with a Gibbs potential variation equal to 21.5 kJ mol^{-1}. We note that in order to obtain phosphorylation of 1 mol of ADP, 3 mol of H^+ are required. Consequently, we assess the two values of the entropy generation and we must obtain the same result. It follows that the entropy generation due to H+-ATPsynthetase results in 34.7 J mol^{-1} K^{-1}, for the GTL16 entropy generation due to thermal flux results in 33.9 J mol^{-1} K^{-1}, and, for the SKBR3, entropy generation due to thermal flux results in 34.2 J mol^{-1} K^{-1}, confirming the theoretical results here developed, and recently introduced as a thermodynamic hypothesis [26,29-31].

In Figure 1 we have represented the entropy generation as a function of the temperature for the above two cell lines, GTL16 (Figure 1a) and SKBR3 (Figure 1b). We can deduce that the entropy generation is a decreasing function of the cell temperature. The consequence of this entropy generation is the entropy variation due to irreversibility, which refers to heat (T_0 S_g, with T_0 temperature of the cell environment) wasted across the cell membrane. Cells are able to waste a finite and constant quantity of heat across the membrane for convective phenomena and infrared radiation, processes independent from the cell. So, if some cell internal energy generation process changes, the cell internal temperature grows. Consequently, the cell needs to consume this surplus of energy. The only way is to build molecular structures, to grow and to replicate. Why do cancer cells accumulate energy? Cancer cells involve the Warburg cycle, which is less efficient than the Krebs one that is used by normal cells. As such, cancer adopts a non efficient way to manage

energy, which leads to an increase in its internal energy, and the concomitant need to dissipate it. Indeed, considering relation (13), such entropy generation depends also on the pH variation. For instance, if the interior of the cell becomes acid ($\Delta pH < 0$), the entropy generation decreases, and, as a consequence, the Krebs cycle cannot occur, leaving the cell to use the less efficient Warburg cycle, assuming a cancer phenotype.

Additional Information

The authors declare no competing financial interests.

Author contribution statement.

U.L. developed the irreversible bio-chemical engineering thermodynamic model, introduced the Constructal law in our approach, and developed the biochemical and biomedical engineering considerations. A.P. developed the biomedical considerations on the low frequency and low intensity electromagnetic waves on biological tissues, and the biomedical considerations on clinical application of the low frequency electromagnetic waves, with the related clinical considerations as anticancer clinical therapy support. UL wrote the main manuscript text. UL reviewed the manuscript.

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Figure 1. Entropy generation vs Temperature for (a) GTL16 cell line and (b) SKBR3 cell line.