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Abstract. In the third decade of XX century, Warburg pointed out that cancer cells follow a fermentative respiration process, as a consequence of a metabolic injury. In this paper, we consider this statement in the following way: any cell process requires energy, so, in the cell, a control of the energy conversion can represent a possible control of the cell processes. Engineering thermodynamics is the science that studies the conversion of energy into work. So, thermodynamics could represent a powerful approach to analyse of the energy conversion in the biosystems, for their control. Cells regulate their metabolisms by energy and mass (ions included) flows, and the heat flux occurs by the convective interaction with their environment. Here, we consider fluxes through the biosystems border, their shapes and the characteristic time of thermal interaction with the blood and water, in the cell environment. Moreover, just in relation to time, it is possible to consider the resonance phenomena. Resonance forces natural behaviours of systems, when a wave of a frequency, related to the characteristic time, income to a system. Here, we introduce the biothermodynamic characteristic frequency, which is the characteristic frequency of a biosystem, evaluated by a thermo-fluid dynamic approach, in order to control the fluxes through the cancer membrane, and to force it towards an optimal behaviour, by changing the concentrations of ions, inside and outside of the membrane itself. The result consists in a control of the cellular metabolic processes, and also of the energy available to cancer, for its growth. In this way, the cancer growth rate can be reduced.

Nomenclature

Latin letters

A	Area [m^2]
c	Specific heat [$J\ kg^{-1}K^{-1}$]
C	Capacitance [F]
G	Gibbs free energy [J]
H	Enthalpy [J]
Q	Heat [J]
$\dot{Q}$	Thermal power [W]
r^*	Volume/Area ratio [m]
R	Resistance [Ω]

S Entropy [J K^{-1}]
t Time [s]
T Temperature [K]
V Volume [m^3]

Greek letter
α Convection coefficient [W m^{-2} K^{-1}]
χ Ion concentration [mol m^3]
ϕ Electric potential [V]
ρ Mass density [kg m^{-3}]
τ Characteristic time [s]

Symbols
pH Potential of Hydrogen

Constants
R_u 8314 J $kg^{-1}K^{-1}$
F 96485 A s mol^{-1}

1. Introduction

In 2018, eighteen million people have contracted cancer worldwide, and more than four hundred million people are expected to contract cancer by 2025 [1].

In medicine and biology, cancer is defined as a disease caused by an uncontrolled division of abnormal cells, in a part of the body. So, it is an irregular behaviour of cells, and it is considered a disorder, caused by genetic or epigenetic alterations in the somatic cells. The unregulated growth of cells may also be spread in other parts of the body [2],[3].

A great number of behavioural risks allows the cancer to begin, even if its first cause is considered genetic [2]; indeed, human body contains about 10^{14} cells, and all kinds of cancer begin its growth, by changing some cells [4]. Cells in human body are specialized for different tasks, but they all have a similar structure, and use biochemical reactions for life and growth, proliferation, etc. At any cell division, mutation occurs. Mutation is the mechanism by which genes are copied twice, damaged or lost: mutation is a possible pathway for the cancer to start [2],[4]. But, up today, the trigger mechanism isn't still completely understood. What is clear is that interdisciplinary and holistic approach must be considered.

In any thermodynamic approach, a cell can be modelled as an open system which converts heat (its metabolic energy) into work. In this context, we must consider the Warburg results, as a starting point of our research. Indeed, in 1931, the Nobel laureate Otto Warburg showed that cancer cells, if compared with the normal ones, follow a different respiration pathway, which is characterized by a glucose fermentation, even when there is no lack of oxygen. He highlighted that the variation on the cancer cells metabolism was caused by a metabolic injury [5]. In healthy cells, mitosis and cell growth are phenomena which occur in synch, in order to preserve the cell size, during its replication. The Warburg result embodies the bioenergetic base to relate the lactic acid production, and the extracellular-intratumoral acidification, to the cancer growth and proliferation (including metastasis formation) [6]. Furthermore, the pH of the cytoplasmatic cells, and the extracellular environment, are directly linked to the cells membrane potential [7]. Comparing the polarization of quiescent cells with the one of the differentiated cells, the latter results hyperpolarized [8], and this hyperpolarization increases the outflow of some ions, such as Ca^{2+}, K^{+}, Zn^{2+}, etc. Consequently, recent researches have been addressed towards the adoption of specific channel inhibitors, as anticancer therapies [7],[9]. They have highlighted both the role of inhibition of proliferation in healthy and in neoplastic cells, and also the role of ion channels

in the signalling network [10]. One of the key issues of carcinogenesis is the study of the derangement of mitosis and of the mitosis/apoptosis ratio [11],[12]. However, all the cellular processes are driven by fluxes of mass and energy; indeed, in Nature, all the spatial and the temporal structures are the result of the fluxes optimization, related to the cell living conditions [13].

But, energy conversion always causes irreversible production of entropy [14], as well known from the second law of thermodynamics, also in biological systems [15]. In this context, in the history of thermodynamics, two scientists introduced a new viewpoint for the evaluation of irreversibility, introducing a global analysis of a general thermodynamic system: the French physicist Louis Georges Gouy (1854-1926) [16] and the Slovak engineer and physicist Aurel Boleslav Stodola (1859-1942) [17], who, independently, proved that the loss of exergy can be evaluated by the product of the environmental temperature and the entropy generation [14], the entropy due to irreversibility. Today, this theorem represents out powerful way to evaluate irreversibility in real processes and systems; indeed, it is the basis of thermodynamic optimization in engineering design.

Resonance is defined as the phenomenon in which an oscillating system drives another system to oscillate with a greater amplitude at specific frequencies, the resonant ones. At these frequencies, also small harmonic forces can produce large effects, due to the storage of vibration energy. Recently, at a frequency of about 1 MHz, the neurons microtubules have been shown to oscillate spontaneously, generating the characteristic shape of the electrical oscillations of the brain, at the electroencephalographic signal of 4-40 Hz nested gestalts, called beat frequencies [18],[19]. This result highlights the fundamental role of resonance in cell behaviour.

In relation to the previous considerations on the energy conversion in a cell, we highlight that the fundamental thermodynamic aspect of life is the reorganization. It can be achieved by dissipating heat towards environment. In this context, cells can exchange heat with the fluid flows around them, and this can be studied by the thermo-kinetic lumped model. This model considers the cell as an isothermal system, which exchanges heat only at its surface [20],[21]. In this way, we try to control the behaviour of the cell by a thermo-magnetic resonance. This is a thermodynamic resonance, induced by an electromagnetic wave, at the frequency of the thermal characteristic time. In this paper, we review our previous theoretical and experimental results [22],[23],[24],[25].

2. The thermo-kinetic lumped biophysical model

Life is characterized by organizational and thermodynamic biophysical processes, based on the "natural" principle of the maximum conversion of available energy [21]. Indeed, the cellular biochemical reactions involve external metabolites to perform work (replication, transcription and translation), and related heat outflow. Thus, a cell exchanges energy and matter through its membrane [26], driven by endogenous electric fields [27] and source of other reactions within cells and tissues.

The Warburg result, related to the metabolic injury, highlights the fundamental role of the energy conversion in cells, and it allows us to introduce the thermo-kinetic lumped model in biophysical analysis of cells. Indeed, we can analyze the cell system as a black box, which is the usual thermal physical approach for the open systems, and consider the genetic regulation as a control system of the cell. Moreover, the genetic regulation cannot affect the thermodynamic balances. Cells exchange heat power with their environment and, the heat flux is related to their metabolism. This heat outflux occurs by convection with the fluids around any cell, and it results [13]:

$$\dot{Q} = \rho_{cell} V c_{cell} \frac{dT_{cell}}{dt} = \alpha A \left(T_{cell} - T_0 \right) = \alpha \frac{V}{\langle r \rangle} \left(T_{cell} - T_0 \right) \tag{1}$$

where ρ_{cell} is the mass density of the cell, V is its volume, c_{cell} is its specific heat, T_{cell} is the cell temperature, α is the convection coefficient, $A = V \langle r \rangle^{-1}$ is the surface area of the cell, which varies during the phases of the cellular development, $\langle r \rangle = V/A$ is the volume-area ratio, that is a characteristic

parameter of the heat exchange through the cell membrane, and ($T_{cell} - T_0$) is the temperature difference between the cell temperature and the environment temperature (T_0). Now, introducing the first principle of thermodynamics:

$$\dot{Q} = \rho_{cell} V c_{cell} \frac{dT_{cell}}{dt} \tag{2}$$

into the Equation (1), it follows the differential equation:

$$\frac{1}{T_{cell} - T_0} \frac{d\left(T_{cell} - T_0\right)}{dt} = \frac{\alpha}{\rho_{cell} c_{cell}} \frac{1}{\langle r \rangle} \tag{3}$$

with

$$\frac{\alpha}{\rho_{cell} c_{cell}} \frac{1}{\langle r \rangle} = \frac{1}{\tau} \tag{4}$$

where τ is the characteristic time of the convective heat exchange [13].

The cell membrane is a double lipid layer that separates the cytoplasm from the cell environment. In real cell membranes, some proteins perform a function of channels, in order to allow the inflows and outflows of mass and ions. But, this function reduces the value of the electric resistance of the membrane. So, it is possible to develop an electric model of cell membranes. It is the *RC* circuit analogy, as represented in Figure 1.

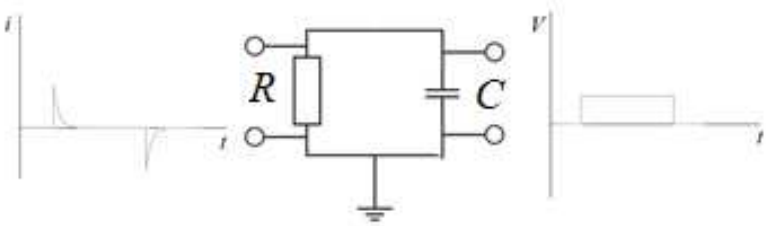

Figure 1. Electric analogy of a cell membrane. The cell membrane can be considered as a parallel *RC* circuit [28].

If the *RC* circuit analogy is considered [28], the current flows across the resistor of resistance *R* can be evaluated during the charge and the discharge of the capacitor of capacity *C* [29]:

$$I\left(t\right) = \frac{\Delta\phi}{R} e^{-t/\tau_{circ}} \tag{5}$$

where $I(t)$ is the current, $\Delta\phi$ is the value of electric potential applied to the capacitor, *R* is the value of the electric resistance, and $\tau_{circ} = RC$ is the characteristic time of the system (the circuit *RC*). If the circuit interacts with a harmonic wave, it presents a resonant frequency $\nu \sim \tau_{circ}^{-1} = (RC)^{-1}$ [29], to which is

related the heat power $\dot{Q}$, dissipated by the Joule effect in the resistor, during the resonant phenomenon, as follows:

$$\dot{Q}(t) = \frac{R\,\phi_M^2}{\sqrt{R^2 + \tau_{circ}^2 \left(2\pi C\right)^{-2}}} \sin^2\left(\frac{2\pi}{\tau_{circ}} t\right) \tag{6}$$

where ϕ_M is the maximum value of the electric potential of the electromagnetic wave.

In accordance with the *RC* analogy for the cell membrane, we consider that, in the thermal interaction with its environment (Eq. (4)), the cell presents also a characteristic time. Consequently, we can argue that an electromagnetic wave could generate a resonant effect, at a frequency proportional to this time. So, we could obtain the maximum heat exchange of the cell towards the environment. In accordance with the Warburg approach, the result is a more efficient management of the energy conversion. Indeed, in this way, the cell doesn't need to storage energy in lipidic molecules, with the result of decreasing its proliferation.

So, we evaluate the characteristic time τ in Equation (4) and generate an electromagnetic wave at a frequency τ^{-1}, named characteristic frequency. The related heat flow results:

$$\dot{Q} = \frac{Q}{\tau} = T_0 \frac{\Delta S_{cell}}{\tau} \tag{7}$$

where Q is the heat wasted by the cell towards its environment, T_0 is the environmental temperature and ΔS_{cell} is the entropy variation of the cell. As a consequence of the definition of Gibbs free energy *G* [30]:

$$\frac{\Delta G}{\tau} = \frac{\Delta H}{\tau} - T_0 \frac{\Delta S_{cell}}{\tau} \Rightarrow \Delta G = \Delta H - \dot{Q}\,\tau \tag{8}$$

where *H* is the enthalpy, and T_0 is the environmental temperature. But, the cell membrane potential can be related to the Gibbs free energy by the following equation [31],[32],[33]:

$$\Delta G = \Delta\phi - 2.3 \frac{R_u T_0}{F} \Delta\mathrm{pH} \tag{9}$$

where ϕ is the cell membrane electric potential, R_u is the universal gas constant, *F* is the Faraday constant, and pH is the potential of hydrogen. Consequently, the application of an electromagnetic wave, at the characteristic frequency, determines a change in the membrane electric potential:

$$\Delta\phi = \Delta H - \dot{Q}\,\tau + 2.3 \frac{R_u T_0}{F} \Delta\mathrm{pH} \tag{10}$$

with a related variation in the ion concentration at the membrane forced in accordance to the relation [34]:

$$\chi_{out} = \chi_{in} \exp\left(\frac{\Delta\phi}{R_u T_0}\right) \tag{11}$$

where χ_{out} and χ_{in} are the concentrations of any ion species outside and inside of the cell membrane. Consequently, a regulation of the cell functions is related to the regulation of the membrane electric potential.

This effect, here theoretically derived, has been experimentally confirmed in Refs. [22],[23],[24],[25]. In Table 1, we summarize only some results, in order to show the effect of the

exposure, of each cancer cell line, to its characteristic resonant frequency. The setup, used to develop the experimental verification of the model, consists of two independent couples of coaxial coils wound into a frame of cylindrical shape, with an outer radius of 8 cm and a distance between the two coaxial coil couples of 8 cm. The experimental setup was placed inside an incubator, in order to maintain the cells living conditions. The cells were placed in the center of this apparatus. The volume/area ratio of any cell line, involved in the experimental analysis, was measured by microscope imaging in order to evaluate the characteristic frequency. The cells were exposed to an electromagnetic wave, generated by the device itself, at their characteristic frequency. In Table 1, three independent cancer cell lines are presented, originated from different tumors, different from each other in morphology, growth rate and oncogene expression. In each experiment, three different cell lines were exposed all together to the electromagnetic field, but at the characteristic frequency of only one of them, in order to prove the resonance phenomenon. Moreover, control samples of each cell line were incubated, without being exposed to the electro-magnetic field. After four days, the proliferation of untreated and exposed cells was analyzed by a colorimetric assay. In each experimental condition, a reduced cell number (growth variation, compared with the control samples) was found only for the cell line for which the applied frequency was the characteristic one, for the line itself. So, all the experiments have confirmed the resonance phenomenon.

Table 1. ELF-EMF frequencies and the cellular biophysical quantities.
For each cell line, the evaluation of 30 cells in different fields was carried out. Cell resonant frequency was used to calculate the frequency of the forcing Extremely Low Frequency Electric and Magnetic Fields (ELF-EMF). The result is the decrease in the growth of the cancer cells [22].

Cell line	**Evaluated frequency** [Hz]	**Applied frequency** [Hz]	**Growth variation** [%]
MCF7 (breast cancer)	5.0 ± 0.7	5 ± 1	-22 ± 2
SKBR3 (breast cancer)	8.0 ± 2.0	8 ± 1	-18 ± 3
GTL16 (gastric carcinoma)	14.0 ± 3.0	14 ± 1	-24 ± 1

3. Discussion

The cell cycle has been pointed out to be monitored by a check system, which controls DNA integrity, before the cell has any transition to its next living phase. In eukaryotic cells, the main control processes occur at the G1/S transition, in late S (DNA synthesis) phase, at mitosis (M) entry and at the metaphase to anaphase transition. All the processes are controlled by the cyclin-dependent kinases, which is regulated by the oscillatory expression of G1 and G1/S-cyclins, S-cyclins, and M-cyclins. The anaphase-promoting complex/cyclosome (APC/C) triggers the transition between metaphase to anaphase. The entry into the cell cycle from a quiescent (G0) phase is stimulated by mitogens. The exit from mitosis can bring to differentiation, apoptosis, or return to quiescence [8]. These mechanisms result altered in neoplastic cells.

All the biochemical processes require energy, and any energy conversion process generates outflows of energy, by virtue to the second law of thermodynamics. Thus, it is possible to analyse the cells system behaviour, by following a thermodynamic approach, considering the inflows and outflows of energy and masses, ions included.

Consequently, it follows that the surface phenomena are transduced by ion channels and energy transfer, to the protein machineries in the cytosol, by combining the sensitivity to electrical and chemical

signals - proper of cooperative allosteric proteins - and the ability to exploit the energy release, that occurs during the exchanges of mass and energy across the membrane, which amplify the biochemical signalling. In cancer cells, an alteration on some processes related to energy and ion channelling occurs, reducing their proliferation control.

We can control the variation of the biochemical quantities, and consequently the behaviour of the cancer forcing heat exchange, and the related concentrations of ions inside and outside the membrane.

In summary, a characteristic frequency wave interacts with the cancer system with the effect of forcing ions fluxes, which change the membrane electric potential and the pH, conditioning the biochemical reactions, and, consequently, forcing them towards a normal behaviour.

4. Conclusions

In this paper we have introduced a thermodynamic approach to cancer, based on the energy balance related to the Warburg results on metabolism. The basis of this approach is the electric analogy of the living cell membrane and the resonant effect in relation to thermal outflow from the cell towards its environment. The model proposed has also been experimentally confirmed by *in vitro* experiments.

The result could be useful as support to present anticancer therapies because it allows the control of the cancer growth, improving the effect of the anticancer therapies.

The limit of this approach is the knowledge of the volume/area ratio of the cell: this is a limit, because it is difficult to obtain this information in clinical applications and it is not unique for all the cells, but its value is in a range for any cancer cell line. However, this quantity is fundamental to obtain the characteristic frequency of the therapeutic electromagnetic field.

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