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Seebeck–Peltier Transition Approach to Oncogenesis

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Abstract: In this paper, a non-equilibrium thermodynamic approach to cancer is developed. The thermo-electric effects in the cell membrane are analysed, in relation to the Seebeck-like and the Peltier-like effects. The role of the cell membrane electric potential is studied from a thermodynamic viewpoint, pointing out the relation between the proliferation rate and the membrane potential, the existence of a thermodynamic threshold for the mitotic activity, the relation between metastases and membrane potential and the comprehension of the role of ions fluxes in the cell behaviour.

Keywords: membrane electric potential; heat flux; ions fluxes; cancer; non-equilibrium thermodynamics

1. Introduction

Living cells membrane presents different permeability related to specific ions (Na^+, K^+, Cl^-, Ca^{2+}, etc.), which generate an electric potential difference $\Delta\phi$, between the cytoplasm and the extracellular environment, in relation to the environment itself [1]. A cell is defined as depolarized if its electric potential difference is relatively less negative in relation to the normal value of a living cell, which, on the contrary, is defined as hyperpolarised because it is more negative.

The membrane electric potential is evaluated by the Goldman–Hodgkin–Katz equation, which allows us to express $\Delta\phi$ as a function of the permeability P, the concentrations of ions, at the both side of the membrane and the temperature [2,3]. Moreover, it was pointed out how intercellular communications can also modify the membrane electric potential [1].

Since 1956, it emerged that cancer cells are electrically different from the normal cells [4]. In 1969, Cone Jr. highlighted that a hyperpolarisation state characterises the start of the M phase of the cell cycle, and he introduced the hypothesis of a possible relation between the cell cycle progression and the membrane electric potential changes [5]. Moreover, in 1970, he pointed out how the membrane hyperpolarisation could block reversibly the synthesis of DNA and mitosis [6]. Last, in 1971, generalising the experimental results, a lowered membrane potential was identified as a cause of an increase in proliferation of the cancer cells, in relation to the normal ones [7]. Until now, all of these results have always been confirmed [8–12].

The molecular organisation and electrical properties of the living cell membranes act as a diffusion barrier between the cytoplasm and the external medium. The cell membrane is a bi-molecular film of lipid molecules, in which are embedded functional proteins, used by the cell for a great number of functions, including energy transduction, signalling, transport of ions, etc. [13]. Recently, the fundamental role played by electrostatic interactions between macroions in aqueous electrolyte solutions has been highlighted [14], with particular regard to soft matter, interface physics and chemistry, biophysics and biochemistry, etc. The presence of macroions affects the distribution of the small mobile ions in the electrolyte, because their counterions are attracted to the macroion surface, while the coions are repelled from the macroion surface [14], with the result of generating the electric double layer [14–19]. The analytical and numerical models for the analysis and description of the structure and energy of the electric double layer have represented a fundamental development in the comprehension of their properties [14,20–31] also in relation to their application in medicine and biophysics. Concepts like charge neutrality, Debye length and double layer have been proven to be very useful to explain the electrical properties of a cellular membrane [32].

In the last years, the role of the membrane electric potential has been shown also in the control of the fundamental cell functions, such as proliferation, migration and differentiation [33–35]. In this context, many experimental evidences pointed out significant depolarisation during malignant transformation of normal cells [36? ,37], due to and increase of Na^+ intracellular concentration in tumour, in relation to the normal cells. The intracellular concentration of K^+ seems to maintain approximately the same values [38].

As a consequence of the previous results, some questions arose:

- The relation between the proliferation rate and the membrane potential.
- The possible existence of a threshold for the mitotic activity.
- The possible relation between metastases and membrane potential.
- The comprehension of the role of ions fluxes in the cell behaviour.

The aim of this paper was to develop a non-equilibrium thermodynamic analysis of the membrane electric potential, recently published in Ref. [39], in order to suggest a new approach to respond to these questions, by considering the link between ions and heat fluxes.

2. Materials and Methods

The membrane potential of a living cell can be evaluated by using the modified Goldman–Hodgkin–Katz equation [2,3]:

$$\Delta\phi = \frac{RT}{F}\ln\left(\frac{P_{Na^+}[Na^+]_{\text{outside}} + P_{K^+}[K^+]_{\text{outside}} + P_{Cl^-}[Cl^-]_{\text{outside}}}{P_{Na^+}[Na^+]_{\text{inside}} + P_{K^+}[K]_{\text{inside}} + P_{Cl^-}[Cl^-]_{\text{inside}}}\right) \tag{1}$$

where [A] is the concentration of the ion A, $R = 8.314$ J mol^{-1}K^{-1} is the universal constant of ideal gasses, T is the absolute temperature, F is the Faraday constant and P is the relative permeability such that $P_{Na^+} = 0.04$, $P_{K^+} = 1$ and $P_{Cl^-} = 0.45$.

Our aim was to introduce a non-equilibrium thermodynamic approach; therefore, we must use the general phenomenological relations [40–42]:

$$\begin{cases} \mathbf{J}_e = -L_{11}\frac{\nabla\phi}{T} - L_{12}\frac{\nabla T}{T^2} \\ \mathbf{J}_Q = -L_{21}\frac{\nabla\phi}{T} - L_{22}\frac{\nabla T}{T^2} \end{cases} \tag{2}$$

where $\mathbf{J}_e$ is the current density [A m^{-2}], $\mathbf{J}_Q$ is the heat flux [W m^{-2}], T is the living cell temperature and L_{ij} are the phenomenological coefficients, such that [42] $L_{12} = L_{21}$ in absence of magnetic fields, and $L_{11} \geq 0$ and $L_{22} \geq 0$, and [42] $L_{11}L_{22} - L_{12}^2 > 0$.

At the stationary state, the net ion fluxes is null, $\mathbf{J}_e = \mathbf{0}$, so the previous equations hold to:

$$\begin{cases} \frac{\nabla T}{T} = -\frac{L_{11}}{L_{12}}\nabla\phi \\ \mathbf{J}_Q = \left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)\frac{\nabla\phi}{T} \end{cases} \tag{3}$$

In relation to the first equation, it is possible to highlight that a Seebeck-like effect occurs in the cell membrane [39], while the other equation expresses an analytical, but also biophysical, relation between the heat flux towards the environment and the membrane electric potential gradient. Considering that:

$$\dot{Q} = \int_A \mathbf{J}_Q \cdot \hat{\mathbf{n}}\, dA \tag{4}$$

where A is the area of the membrane external surface, we can write:

$$\delta\dot{Q} = \left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)\frac{\nabla\phi}{T} \cdot \hat{\mathbf{n}}\, dA = \frac{k}{T}\,\nabla\phi \cdot \hat{\mathbf{n}}\, dA \tag{5}$$

where $k = (L_{22}L_{11}/L_{12}) - L_{12}$ is a thermoelectric property of the cell, which links the heat flux to the membrane electric gradient. Equation (5) relates the heat power, exchanged with the cell environment, to the cell membrane potential gradient.

Cells exchange heat power with their environment by convection [43]:

$$\delta\dot{Q} = \rho\, c\,\frac{dT}{dt}\, dV = -\alpha\,(T - T_0)\, dA \tag{6}$$

where $\rho \approx 10^3$ kg m^{-3} is the cell density, $c \approx 4186$ J kg^{-1} K^{-1} is the specific heat of the cell, $\alpha \approx 0.023\, Re^{0.8} Pr^{0.35} \lambda / \langle R \rangle$ is the coefficient of convection, with $\lambda \approx 0.6$ W m^{-1}K^{-1} conductivity, $Re \approx 0.2$ the Reynolds number and $Pr \approx 0.7$ the Prandtl number [43], A area of the cell membrane, V is the cell volume and $\langle R \rangle = dV/dA \approx V/A$ is the mean radius of the cell. So, it follows that:

$$\frac{d\phi}{d\ell} = -\frac{\alpha}{\left(L_{22}\frac{L_{11}}{L_{12}} - L_{12}\right)}\, T\,(T - T_0) = -\frac{\alpha}{k}\, T\,(T - T_0) \tag{7}$$

which highlights the relation between the membrane gradient of the membrane electric potential and the temperature of the cell, being ℓ the length of the membrane.

If the ion fluxes persists to be null, the cell cannot develop biochemical reaction to sustain the cell life [39,44]; consequently, $\mathbf{J}_e \neq \mathbf{0}$, so [40,41]:

$$\frac{dc_i}{dt} = -\nabla \cdot \mathbf{J}_i \tag{8}$$

where c_i is the concentration of the i-th ion (Na^+, K^+, Ca^{2+}, Cl^-, etc.), t is the time and $\mathbf{J}_i$ is the current density of the i-th ion. Therefore, using Equation (2), it follows [40,41]:

$$\frac{d\phi}{dT} = -\frac{L_{21}}{L_{11}}\,\frac{1}{T} \tag{9}$$

which allows us to point out that a Peltier-like effect occurs, a temperature variation is caused by the variation of the membrane electric potential, as a consequence of the ions fluxes, and a related heat flux is generated [40,41,45]:

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_u \tag{10}$$

Consequently, the specific entropy rate can be obtained as follows [46–48]:

$$T\,\frac{ds}{dt} = \nabla \cdot \left(\mathbf{J}_u - \sum_{i=1}^{N} \mu_i\, \mathbf{J}_i\right) - \sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla\mu_i \tag{11}$$

where s is the specific entropy, T is the temperature and μ is the chemical potential. $\mathbf{J}_S = \mathbf{J}_u - \sum_{i=1}^{N} \mu_i \mathbf{J}_i$ is the contribution of the inflows and outflows, and $T\sigma = -\sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla \mu_i$ is the dissipation function [40]. We can highlight that diffusion is caused by the gradient of the chemical potential:

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,p,n_{k\neq i}} \tag{12}$$

where G is the Gibbs energy, n is the number of moles and p is the pressure. Until now, we have considered a flux in accordance with the concentration gradient. In the case of fluxes against the concentration gradients, it is possible to introduce [40,41]:

$$\mathbf{J}_i = -\sum_{k=1}^{N} L_{ik} \nabla \mu_k \tag{13}$$

together with the Gibbs–Duhem relation [41]:

$$\nabla \mu_N = \sum_{i=1}^{N-1} \frac{c_i}{c_N} \nabla \mu_i \tag{14}$$

Consequently, Onsager's reciprocity relations are not satisfied, but, if we consider:

$$\mathcal{L}_{ik} = L_{ik} - \frac{c_i}{c_N} L_{iN} \tag{15}$$

where $\mathcal{L}$ are the real measurable quantities, it is possible to obtain [40]:

$$\mathbf{J}_i = -\sum_{k=1}^{N-1} \mathcal{L}_{ik} \nabla \mu_k \tag{16}$$

with $\mathcal{L}_{ik} = \mathcal{L}_{ki}$ and

$$T\sigma = -\sum_{i=1}^{N-1} \mathbf{J}_i \cdot \nabla \mu_i \tag{17}$$

The entropy outflow is fundamental in order to generate order from disorder [49], as Schrödinger himself pointed out [44].

3. Results

In this paper, we have developed a non-equilibrium thermodynamic analysis of the cell membrane electric potential in order to explain, analytically, the role of the ions fluxes in relation to cancer behaviour, but also to the thermoelectric properties of the membrane itself. Indeed, the Equation (7) allows us to state that an increase in the cell temperature (development of any inflammation) implies a decrease in the membrane electric potential.

Always in relation to Equation (7), we can highlight that the values of α, T and T_0 are approximately the same for cancer and normal cells; therefore, from a physical viewpoint, the difference between cancer and normal cells must be expressed in terms of the thermoelectric coefficient k. In particular, experimental results point out that the membrane of cancer cells is depolarized [1,12]; consequently, in relation to our Equation (7), the thermoelectric coefficient for cancer results greater than the one of a normal cell.

Moreover, during its life cycle, cell membranes have continue transitions between Seebeck-like and Peltier-like effects to sustain heat and mass fluxes. These continuous transitions are the thermo-electric cycle responsible for respiration, metabolism, reorganisation, proliferation, communication and all the biophysical and biochemical processes inside the cells [39].

During the Seebeck-like effect, the membrane exchanges heat towards the environment, with a related decrease in its entropy, in accordance with the Schrödinger approach. During the Peltier-like effect, the membrane exchanges ions, metabolites and waste molecules with the environment in order to realise the biochemical processes for life and proliferation.

Here, we develop a numerical evaluation in relation to the Ca^{2+}-flux. This example is very important due to the fundamental role played by the Ca^{2+} ion in the regulation of a great number of cell functions [50–54].

To do so, we write the Equation (11) as follows:

$$T\,\frac{ds}{dt} = -\nabla \cdot \Big(\mathbf{J}_u - \mu_{\text{Ca}}\,\mathbf{J}_{\text{Ca}}\Big) \tag{18}$$

This last equation, considering T constant, and following Prigogine ($ds/dt = 0$) [55], becomes

$$\nabla \cdot \Big(\mathbf{J}_u - \mu_{\text{Ca}}\,\mathbf{J}_{\text{Ca}}\Big) = 0 \tag{19}$$

Now, we introduce the First Law of Thermodynamics for the cell membrane, so we can write [50]

$$\frac{du}{dt}\,dV = \rho\,c\,\frac{dT}{dt}\,dV = \delta\dot{Q} = -\alpha\,(T - T_0)\,dA \tag{20}$$

where $\rho \approx 10^3$ kg m^{-3} is the cell density, $c \approx 4186$ J kg^{-1} K^{-1} is the specific heat of the cell, $\alpha \approx 0.023 Re^{0.8} Pr^{0.35} \lambda / \langle R \rangle$ is the coefficient of convection, with $\lambda \approx 0.6$ W m^{-1}K^{-1} conductivity, $Re \approx 0.2$ the Reynolds number and $Pr \approx 0.7$ the Prandtl number [43], A area of the cell membrane, V is the cell volume and $\beta = \alpha\, dA/dV$ is constant. Now, considering Equation (10), we can write:

$$\nabla \cdot \mathbf{J}_u = \alpha\,\frac{dA}{dV}\,(T - T_0) \tag{21}$$

and, it follows [50]:

$$\nabla \cdot (\mu_{\text{Ca}}\mathbf{J}_{\text{Ca}}) = \frac{\delta\dot{Q}}{dV} = \alpha\,\frac{dA}{dV}\,(T - T_0) \tag{22}$$

$$J_{\text{Ca}} = \frac{\ell \cdot \alpha}{\mu_{\text{Ca}} \cdot \langle R \rangle}\,(T - T_0) = \frac{4\times10^{-9}\times0.023\times0.2^{0.8}\times0.7^{0.35}\times0.6\times0.4}{-552.79\times10^3\cdot\langle R\rangle^2} = \frac{-0.97\times10^{-17}}{\langle R\rangle^2}\ [\text{mol s}^{-1}\text{m}^{-2}] \tag{23}$$

where $\ell \approx 0.004$ µm is the depth of the cell membrane [56] and $\langle R \rangle$ is the mean radius of the cell, considered, in the first approximation, as a sphere, $\mu_{\text{Ca}} = -552.79$ kJ mol^{-1} and $T - T_0 \approx 0.4$ °C [57]. The numerical result depends on the mean size of the cell. Considering that the mean radius for a human cell is of the order of 10^{-6}–10^{-5} m, it follows that the Ca^{2+}-flux is of the order of 21–450 mmol s^{-1}m^{-2}, which can be expressed as ∼0.010 mol s^{-1}kg^{-1}, in agreement with the experimental results obtained in [58].

Now, we can evaluate the electric field at the living cell membrane. The electric potential at the membrane is of the order of 10–100 mV. The thickness of the membrane is of the order of 0.004 µm. Consequently, the electric field of the living cell membrane is of the order of 10^7–10^8 V m^{-1}. Therefore, for the Ca^{2+} fluxes, the power generated by the fluxes through the membrane electric fields results in:

$$\dot{W}_{el,\text{Ca}} = J_{\text{Ca}} \cdot \mathcal{N} \cdot 2e \cdot E \cdot 4\pi\,\langle R \rangle^2 \tag{24}$$

where $\mathcal{N} = 6.022 \times 10^{23}$ mol^{-1}, $e = 1.6 \times 10^{-19}$ C is the value of the elementary electric charge, 2 is the valence of the Calcium and E is the electric field. Therefore, the power results are of the order of $(2.4 \times 10^{-4}$–$2.4 \times 10^{-3})$ [W].

As a consequence of these results, we are able to propose the following responses to the questions introduced in the previous section:

- The relation between the proliferation rate and the membrane potential can be explained by the modification of the cytosolic proteins functions due to phospholyration or dephospholyration, related to the hydrolysis of ATP necessary for the coupled ion along its electrochemical gradient.
- The possible existence of a threshold for the mitotic activity is shown in Equation (7), which presents a thermal threshold ($T > T_0$) for the cell membrane electric potential gradient, which has been experimentally shown to be related to the mitotic activity.
- The possible relation between metastases and membrane potential can be explained by considering that the metastasis is related to the ability of the cell to move through the intercellular space, but this ability is related to the water outflow and the Ca^{2+}-K^+ channel activation, related to the hyperpolarisation of the membrane itself.
- The comprehension of the role of ions fluxes in the cell behaviour can be explained by considering that the depolarization was experimentally shown to be a characteristic of cancer, and our approach highlights just the differences in the thermo-electric properties of the cell membrane in cancer and normal cells.

4. Discussion and Conclusions

A decrease of Cl^- intracellular concentration has been showed to be directly linked to malignant proliferation [12], and a decrease of Na^+ intracellular concentration is able to hyperpolarise the cell membrane, with a determinant consequence on the mitotic arrest [12,51–54,59–72]. Hyperpolarisation determines an activation of the Ca^{2+}-K^+ channel which increases the Ca^{2+} intracellular concentration [12,73], with the consequence that the Ca^{2+}-K^+ channel results a fundamental controller of the membrane electric potential. In this context, also water outflow was shown to play a fundamental role in metastasis activity [12,74].

Proteins play a fundamental role in ion transport. Proteins in the cytosolic can be modified in their functions by phosphorylation or dephosphorylation. An ion actively crosses the membrane against its electrochemical potential, whereby the necessary energy is derived either from the hydrolysis of ATP, or from the movement of a co-transported, or coupled ion along its electrochemical gradient. In this context, the role played by the H^+-ATPase is fundamental, because it moves positive charges into the cell, while it generates large membrane voltage (inside negative and outside positive) and a pH gradient [75–78]. Protein phosphorylation is an important cellular regulatory mechanism, because many enzymes and receptors [50,79,80] are activated or deactivated by phosphorylation by involving kinases and phosphatases. Moreover, kinases are responsible for cellular transduction signalling [81–84].

In this paper, the theoretical explanation of the ions role in relation to the cell membrane potential has been obtained by introducing the non-equilibrium thermodynamic approach. It is the first fundamental step for a new approach in cancer researches. Indeed, it highlights:

- The relation between cell membrane potential and temperature.
- The relation between cell membrane potential and ions fluxes.
- The spontaneous symmetry breaking in the Onsager relations as a fundamental transition between the Seebeck-like effect and the Peltier-like effect in the cell membrane.
- The link between life and the transition from a Seebeck-like effect to a Peltier-like effect and viceversa.

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