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The role of the cell volume-area ratio in thermodynamic analysis of the cancer growth control for *in vitro* experiments

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Abstract: From a thermodynamic point of view, living cell life is no more than a cyclic process. It starts with the newly separated daughter cells and restarts when the next generations grow as free entities. In this cycle the cell changes its entropy. In cancer the growth control is damaged. In this paper we analyze the role of the volume-area ratio in cell in relation to the heat exchange between cell and its environment in order to point out the effect on the cancer growth. The result holds to a possible control of the cancer growth based on the heat exchanged by the cancer towards its environment, and the membrane potential variation, with the consequence of controlling the ions fluxes and the related biochemical reactions. This second law approach could represent a starting point for a possible future support for the anticancer therapies, in order to improve their effectiveness for the untreatable cancers.

Keywords: Biothermodynamics; Complex systems; Thermodynamics of biological systems; Biophysical resonance.

1. Introduction

Living cells live in an environment which slowly changes its chemical and physical properties. In mammalian animals, organs and tissues are generally endowed with a homeostatic capability, which is characterized by internal thermal regulation.

A living system can be defined as an open system, so non-equilibrium thermodynamics, can be applied just to living systems [1].

Cancer is no more than a living cell with a different growth behaviour in relation to the cell we consider normal. At a definite phase of its life any cancer cell divides into two cells. The size of the single cell at the beginning of division can vary [2]; consequently, also the relative size of the two daughter cells can vary, but lower and upper bounds on cell volume have been observed [3].

Living cell life, cancer included, is a cyclic process, which starts with the newly separated daughter cells and restarts when the next generations grow as free entities. Consequently, thermodynamics of cycle processes can represent a good tool for the analysis of cancer. In thermodynamics is fundamental the definition of the control volume: in this context it could be useful to consider the single cell as the observed control volume, but the usual experimental setup doesn't allow us to introduce such approach, so we are forced to observe a multitude of single cells and consider this multitude as a complex cooperative system of single cells. This system, considered as a single cell or as the cooperative system of all cells in a culture, is no more than an open system from a thermodynamic point of view, so, energy and matter flow through the border of the system, while biochemical and biophysical transformations occur within the system, with a related net

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production of entropy. The environment of the system considered is composed by the suspending aqueous solution of cell nutrients, the substances discarded by the cells, the gaseous atmosphere above the suspending solution. Consequently, the bio-system results composed of:

- The cell membrane, which delimits the volume of the cell, controls inflows and outflows of molecules;
- The cytoplasm which is an aqueous solution of molecules that fills the cell interior;
- The organelles, suspended in the cytoplasm.

The system may contain many substances not initially present in its environment. Many enzymes found in fragments of cytoplasm membranes are often not directly accessible to system environment, while, in the environment, the concentrations of some molecular species decrease in time, and nutrients must flow into the system in order to allow the biochemical reactions to occur and produce macromolecular cell components, with a related increase of the living cell mass and volume [4]. These biochemical reactions require energy. This energy is obtained from the same reactions, which involve the nutrients, with a related waste of heat towards the living cell environment. The net effect of all these biochemical reactions is to reduce the entropy of the system, with an increase of the entropy generation in the environment [5].

From a thermodynamic point of view, the living system is an open system.

The analysis of the chemical species and of their reactions in the living cells have led to some sequences which start with nutrient molecules and end with the formation of living cells substance and waste molecules and waste heat. Too numerous reaction sequences are considered to exist. Moreover, many molecules can be part of more than one sequence, providing coupling of sequences.

The characteristics of the living systems have been determined by using batch cultures, grown at the optimum temperature for the species employed by the experimenter, even if these experimental method presents disadvantages because the living system is forced to live in a continually changing environment, due to supply and decrease of the environmental concentration of nutrients related to the cells growth, while both the environmental concentration of the waste molecules and the waste heat rise proportionally.

Considering the chemical reaction at constant pressure and temperature the Gibbs Free Energy could seem the selected function for the study of the steady states of the living systems, but its decrease as a criterion for occurrence of a spontaneous evolution is limited to the complex phenomena which occur at constant temperature and pressure inside the living system. But, a general objective function for the analysis of the living systems is required. Moreover, considering that the system wastes energy, and mass, it generates irreversibility, so the general criterion for study its spontaneous evolution is the entropy generation related to the changes of the system. Entropy generation always increases in any spontaneous and irreversible evolution.

The aim of this paper is to analyze deeply the use of the heat role in the study of the cancer systems. Indeed, recently we have used the entropy generation to introduce a thermodynamic approach to the analysis of the cancer system in order to design a possible support to the present anticancer therapies. But, the thermodynamic approach introduces a new viewpoint for the analysis of the living systems. This paper wishes to explain and improve the thermodynamic formulation of this approach, with the second aim to highlight how to support the biomedical sciences in the comprehension of the possible thermodynamic support to the present anticancer therapies.

2. Materials and Methods

Life involves organisational and thermodynamic processes, which tend towards the maximum conversion of available energy [1-8]. The biochemical reactions produce or consume external metabolites, and connect internal metabolites, at constant concentrations in the cells at their steady states. In order to do so, cells must exchange energy and matter through their membrane. Indeed, many processes such as replication, transcription and translation, require fluxes of ions and molecules which are driven by the endogenous electric fields and accumulate in the nm-thin layer of water [11,14,15]. These fluxes of ions induce biochemical reactions within cells and tissues.

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Moreover, the 1931 Nobel laureate Otto Warburg proved that cancer cells are fermentative, pointing out that this was the consequence of a metabolic injury [16]. In normal cells, mitosis is synchronized with cell growth for cells to maintain their size during replication. Even if the tumor behavior is more complex and it is probably based also on genetic structures of the cells, the results of Warburg highlights the important role of energy conversion in cells. From a thermodynamic point of view, this result can represent the starting point of the analysis; indeed, the genetic processes have the consequence of regulation for the cell behavior, but, if we consider the cell as a black box, as usually done in thermodynamics, then the genetic regulation is no more than the "mind" of the cell, without any direct consequence on the thermodynamic balances. Indeed, the thermodynamic approach evaluates the life cycle of the cell by considering only the energy and mass fluxes balances during the whole cycle of cell life, and not considering the gene activities, but evaluating only their consequences expressed by the energy conversion in cell.

Living cells metabolism implies flows of matter and heat into and out of the cells. What are the consequences of these fluxes and metabolic reactions? To answer to this question, we consider the Gibbs free energy variation in time, due to the mass fluxes. It results [12,17-19]:

$$\frac{dG}{dt} = \dot{W} + \sum_{in} \dot{n}_{in} \tilde{\mu}_{in} - \sum_{out} \dot{n}_{out} \tilde{\mu}_{out} + T_0 \dot{S}_g \tag{1}$$

where G is the Gibbs free energy, $\dot{W}$ is the useful power done by the cell, $\dot{n}$ is the molar flow, $\tilde{\mu} = \mu + Ze\phi$ is the molar electrochemical potential, with μ chemical potential, Ze charge of the ion considered, and ϕ cell membrane electric potential, T_0 is the temperature of the cell environment, t is the time, in means inflow, out means outflow, and $\dot{S}_g$ is the entropy generation rate, defined as [12,17-19]:

$$\dot{S}_g = \frac{dS}{dt} - \frac{\dot{Q}}{T_0} - \sum_{in} \dot{n}_{in} \bar{s}_{in} + \sum_{out} \dot{n}_{out} \bar{s}_{out} + \sum_{f} \dot{n}_f \bar{s}_f \tag{2}$$

where $\dot{Q}$ is the heat power exchanged, $\bar{s}$ is the molar specific entropy, and f means formed. This equation highlights as these flows cause entropy variation; moreover, these fluxes imply also a great number of chemical reactions within the cells, accompanied by entropy generation. Now, we introduce the equation (2) into the equation (1) obtaining:

$$\frac{dG}{dt} = \dot{W} - \dot{Q} + \sum_{in} \dot{n}_{in} \left(\tilde{\mu}_{in} - T_0 \bar{s}_{in} \right) - \sum_{out} \dot{n}_{out} \left(\tilde{\mu}_{out} - T_0 \bar{s}_{out} \right) + \sum_{f} \dot{n}_f \bar{s}_f + T_0 \frac{dS}{dt} \tag{3}$$

From a thermodynamic point of view, the cell life is no more than a thermodynamic stationary state, at constant environmental temperature and pressure, and this can be obtained by introducing the two conditions [12,17-19]:

$$\begin{cases} \dfrac{dG}{dt} = 0 \\ \dfrac{dS}{dt} = 0 \end{cases} \tag{4}$$

which hold to the equation:

$$\dot{Q} = \dot{W} + \sum_{in} \dot{n}_{in} \left(\tilde{\mu}_{in} - T_0 \bar{s}_{in} \right) - \sum_{out} \dot{n}_{out} \left(\tilde{\mu}_{out} - T_0 \bar{s}_{out} \right) + \sum_{f} \dot{n}_f \bar{s}_f \tag{5}$$

which expresses the fundamental role of heat fluxes between the cell and its environment.

The equation (5) is very difficult to be numerically evaluated, because it implies the knowledge of all the balance at the second member of the equation for each cell. So, we must find and alternative way to evaluate the cell heat flux.

The heat flux is no more than the heat power exchanged by the cell and its environment. We can consider that, inside the experimental setup usually used in the biophysical and biochemical analysis of cells, the heat flux is exchanged by convection with the suspending aqueous solution around any cell, so we can write:

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$$\dot{Q} = \alpha A\left(T_{cell} - T_0\right) = \alpha \frac{V}{\langle R \rangle}\left(T_{cell} - T_0\right) \tag{6}$$

where α is the coefficient of convection, $A = V / \langle R \rangle$ is the surface area of the cell, which changes with the phases of the development of the cell, V is the volume of the cell, $\langle R \rangle$ is the volume/area ratio, a parameter which influences the chemical reaction time and the fluxes through the cell membrane, and $\left(T_{cell} - T_0\right)$ is the difference of temperatures between the cell temperature and the environment temperature. The term $A = V / \langle R \rangle$ is the geometric shape of the cell in relation to convection. We must introduce this quantity because at a stage of his life a cell has a definite volume, but it can change its shape in relation to its duplication phase at that time. Indeed, in eukaryotic cells, the main control process occurs at the G1/S transition, in late S (DNA synthesis) phase, at mitosis (M) entry and at the metaphase to anaphase transition. Any process is controlled by the cyclin-dependent kinases, regulated by the oscillatory expression of G1 and G1/S-cyclins, S-cyclins, and M-cyclins. The transition between metaphase to anaphase is triggered by the anaphase-promoting complex/cyclosome (APC/C). Mitogens stimulate the entry into the cell cycle from a quiescent (G0) phase. Exit from mitosis can lead to differentiation, apoptosis, or return to quiescence [20]. These mechanisms are altered in neoplastic cells.

Now, introducing this last equation (6) in the equation (5) we can obtain:

$$\langle R \rangle = \frac{\alpha\left(T_{cell} - T_0\right)V}{\dot{W} + \sum_{in} \dot{n}_{in}\left(\tilde{\mu}_{in} - T_0 \bar{s}_{in}\right) - \sum_{out} \dot{n}_{out}\left(\tilde{\mu}_{out} - T_0 \bar{s}_{out}\right) + \sum_{f} \dot{n}_f \bar{s}_f} \tag{7}$$

which highlights how the cell adapts its volume/area rate in order to optimize the cell membrane fluxes to obtain the work it needs. But, conversely, this geometric rate controls also the heat exchange. Indeed, we consider the heat balance for the system under analysis as follows:

$$\rho_{cell} V c_{cell} \frac{dT_{cell}}{dt} = A\alpha\left(T_{cell} - T_0\right) \tag{8}$$

where ρ_{cell} is the cell mass density, and c_{cell} is the specific heat of the cell. It follows that:

$$\frac{d \ln\left(T_{cell} - T_0\right)}{dt} = \frac{\alpha}{\rho_{cell} c_{cell}} \frac{1}{\langle R \rangle} \tag{9}$$

with the result that greater is the volume-area ratio lower is the thermal exchange, being α, ρ_{cell} and c_{cell} approximately constant.

3. Results

The results obtained highlight the role of the volume-area ratio of the cells in relation to their heath exchange in *in vitro* experiments. This effect is fundamental when the heat exchange plays a fundamental role in the analysis of the experimental data.

In particular, we wish to highlight that this effect is particularly interesting in the study of the cancer growth compared with normal cell growth, because cancer presents a different metabolic cycle. This is important when we study the cancer growth control. Indeed, we can point out that the equation (9) is also the equation which links a frequency, i.e. the inverse of the time $\nu = 1/\tau = \left(\alpha / \rho_{cell} c_{cell} \langle R \rangle\right)$, to a structural and geometrical properties of the cell and its environment, in relation to the heat exchange. But, what is this frequency?

It is difficult to find an answer without considering the thermodynamic approach. Indeed, each system presents a proper time of answer to the external thermal perturbation. We suggest that this frequency is no more than the inverse of the cell proper time of answering to the external thermal perturbation, or the heat exchange rate. Indeed, the heat flow can be written as:

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$$\dot{Q} = \frac{Q}{\tau} = Q\nu \tag{10}$$

where Q is the heat wasted during the cell life. Moreover, we can consider also that the ions fluxes are controlled by the cell membrane potential, which is related to the Gibbs free energy by the relation [17-19]:

$$dG = d\phi - 2.3\frac{RT_0}{F}d\mathrm{pH} \tag{11}$$

where R is the universal constant of gasses and *F* is the Faraday constant. At the stationary states, remembering the relations (4), it follows:

$$d\phi = 2.3\frac{RT_0}{F}d\mathrm{pH} \tag{12}$$

which links the variation of the cell membrane electric potential to the variation of the pH, related to the ions fluxes. Conversely, we can try to force a variation of the pH by a variation of the cell membrane electric potential.

How can we try to do so? We can induce a variation in the cell membrane electric potential by using an electromagnetic wave, with a frequency just equal to the proper frequency of our system.

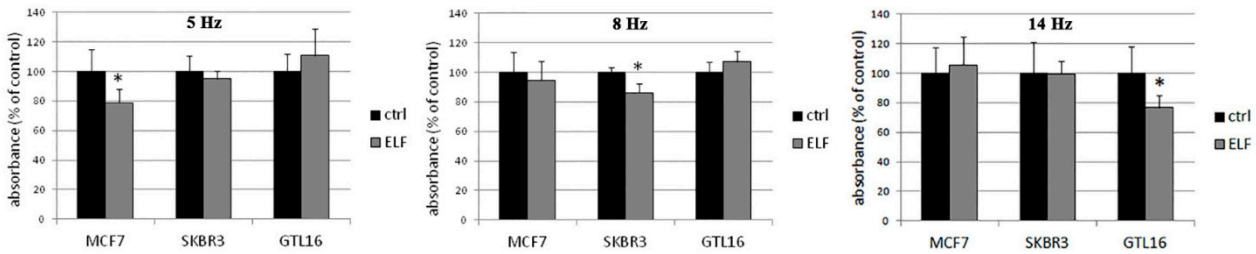
The results are summarized in the Table 1. It is possible to highlight that the electromagnetic waves induce a different behavior in the cancer cells considered; indeed, they decrease their growth if compared with the cancer cells outside of the electromagnetic field, proving that there exists a forcing phenomena of heat flux control which controls the related ions fluxes and, consequently, induce a different behavior to the biosystem.

Table 1. Cell parameters and calculated ELF-EMF frequencies.

For each cell line, thirty cells in different fields were evaluated in their size. Cell volumes were estimated and used to calculate the forcing frequencies of ELF-EMF.

cell line	cell size [µm²]	cell volume [µm³]	Volume-area ratio	mean frequency [Hz]
MCF7	1,993 ± 16	16,468 ± 793	8.26 ± 0.46	5.0 ± 0.7
	1,051 ± 13	17,303 ± 1,040	16.45 ± 1.18	
	2,604 ± 21	42,284 ± 2,068	16.24 ± 0.93	
SKBR3	1,033 ± 11	1,795 ± 97	1.74 ± 0.11	8.0 ± 2.0
	2,066 ± 17	29,048 ± 1,301	14.06 ± 0.74	
	2,454 ± 20	47,594 ± 2,168	20.21 ± 1.05	
GTL16	1,042 ± 11	1,300 ± 80	1.25 ± 0.09	14.0 ± 3.0
	1,873 ± 15	2,630 ± 140	1.40 ± 0.09	
	1,059 ± 12	1,260 ± 77	1.19 ± 0.09	

Figure 1. Decreasing of growth at the proper frequency.



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The experimental results confirm the results here obtained, in particular that the cancer cells modify their behavior only if irradiated by an electromagnetic field at the proper frequency for the cell line considered [21].

4. Discussion

The results, here obtained, point out the fundamental role of the cell volume-are ratio in relation to the fluxes control.

Indeed, there is a temperature difference between the interior of a living cell and its environment. This is a thermodynamic necessity for life. Sensible heat is exchanged between inside and outside of the cell due to this temperature difference. This heat flow contribute to entropy generation. Part of the entropy generated appears outside the cell as sensible heat. The fraction of all entropy generation that appears in this form depends on the nature and number of processes occurring in the cell. Consideration of the temperature difference between environment and cell interior allows the introduction of non-equilibrium thermodynamics for the analysis of cells behavior. Brock suggested that the stability of thermophilic organisms can be attributed to membrane structure properties of these organisms [22].

The temperature gradient contribution to the flow of substances through the cell membranes of the cell with a consequent influence on metabolic processes. The approach here suggested allows us to evaluate the homeostatic cellular response to external perturbations. This answer is no more than a thermo-chemical output of the cell in the environment. So, we can suggest that the thermodynamic approach holds to a model of analysis of the action and reaction in terms of membrane flux variation. This approach could represent a new approach to design possible support to the present anticancer therapies, by introducing external fields variation, at the proper answer time, in the therapeutic protocols. From the experimental results it is clear a reduction of the growth of the cancer with the consequence of improving the effects of the present therapies.

The growth rate of cells, cancer included, at a fixed temperature is a function of both composition of the medium and chemical potentials of the component substances. This represents a sort of control to the growth, because there is a maximum rate at which each bio-chemical reaction can occur under the existing constraints. This rate is conditioned by the volume-are ratio, because it controls the ions fluxes, i.e. the fluxes of the chemical reactants.

So, our results show a method for the design of therapies and experiments for their analysis. Indeed, the specific effect of the single frequencies has two important consequences:

- In every cell type different parameters of electromagnetic waves impact differently the energy utilization and proliferation, with different inhibition effects on the cell growth;
- The same electromagnetic wave has distinct effects on different cells, with a selectivity behavior.

5. Conclusions

In this paper, we have developed the analysis of a thermodynamic approach to cancer cells, with particular regards to the role of the volume-area ratio in the heat exchange and the consequences to the cancer cells behavior.

We have pointed out the existence of a proper time of answer of any cell line to the heat exchange. This time results related to the cells volume-area ratio, a geometrical parameter fundamental for the considerations on the fluxes and cells membrane electric potential variation.

Then, starting from some previous experimental results [23-26], we have obtained also an experimental proof of the present results.

The results highlight how the irreversibility plays a fundamental role also in biophysical systems; indeed, the geometrical rate is completely related to the entropy generation as it is clear by introducing the relation (6) into the relation (2). This holds to a new approach to biological physics, based on the first and second law of thermodynamics.

Author Contributions: UL developed the theoretical model, and the thermodynamic considerations. GG developed the experiments and the data analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Miller, D.G. Thermodynamics of irreversible processes: The experimental verification of the Onsager reciprocal relations. *Chem. Rev.* **1960**, *60*, 15-37.
2. Koch, A.L.; Schaechter, M. A model for statistics of the cell division process. *J. Gen. Microbiol.* **1962**, *29*, 435-454.
3. Errington, F.P.; Pwell, E.O.; Thompson, N. Growth characteristics of some gram-negative bacteria. *J. Gen. Microbiol.* **1965**, *39*, 109-123.
4. Lamanna, C.; Mallette, M.F. *Basic bacteriology*. 3rd Ed.; The Williams & Wilkins Company: Baltimore, USA, 1965.
5. Dean, A.C.R.; Hinshelwood, C. Growth, function and regulation of bacteridl cells. Oxford University Press: London, UK, 1966.
6. Demirel, Y.; Sandler, S.I. Thermodynamics and bioenergetics. *Biophysical Chemistry* **2002**, *97*, 87-111.
7. Toussaint, O.; Schneider, E.D. The thermodynamic and evolution of complexity in biological systems. *Comparative Biochemical Physiology A* **1998**, *120*, 3-9.
8. Caplan, S.R.; Essig, A. *Bioenergetics and Linear Nonequilibrium Thermodynamics. The Steady State*; Harvard University Press: Cambridge, 1983.
9. Lucia, U. Entropy generation approach to cell systems. *Physica A* **2014**, *406*, 1-11.
10. Lucia, U. Bioengineering thermodynamics: an engineering science for thermodynamics of biosystems. *IJoT* **2015**, *18*, 254-265.
11. Lucia, U. Bioengineering thermodynamics of biological cells. *Theor. Biol. Med. Model.* **2015**, *12*, 29-44.
12. Katchalsky, A.; Curran, P.F. *Nonequilibrium Thermodynamics in Biophysics*; Harvard University Press: Cambridge, 1967.
13. Lucia, U. Irreversibility in biophysical and biochemical engineering. *Physica A* **2012**, *391*, 5997-6007.
14. Bustamante, C.; Chemla, Y.R.; Forde, N.R.; Izhaky, D. Mechanical processes in biochemistry. *Annual Review of Biochemistry* **2004**, *73*, 705-748.
15. Lucia, U. Thermodynamics and cancer stationary states. *Physica A* **2013**, *392*, 3648-3653.
16. Warburg, O.; Wind, F.; Negelein, E. The metabolism of tumors in the body. *J. Gen. Phys* **1927**, *8*, 519-530.
17. Morowitz, H.J. *Energy flow in Biology*; Ox Bow Press: Woodbridge, 1979.
18. Santillàn, M. *Chemical Kinetics, Stochastic Processes, and Irreversible Thermodynamics*; Springer: Berlin, 2014.
19. Sears, F.W.; Salinger, G.L. *Thermodynamics, Kinetic Theory, and Statistical Thermodynamics*; Norosa Addison-Weslay: New York, 1975.
20. Becchetti, A. Ion channels and transporters in cancer. 1. Ion channels and cell proliferation in cancer. *Am. J. Physiol. Cell Physiol.* **2011**, *301*, C255-C265.
21. Lucia, U.; Grisolia, G.; Ponzetto A.; Silvagno F. An engineering thermodynamic approach to select the electromagnetic wave effective on cell growth. *Journal of Theoretical Biology* **2017**, *429*, 181-189
22. Brock, T.D. Life at high temperatures. *Science* **1967**, *158*, 1012-1019.
23. Lucia, U.; Grisolia, G. Constructal Law and Ion Transfer in Normal and Cancer Cells. *Proceedings of the Romanian Academy A Special Issue* **2018**, 213-218
24. Lucia, U.; Grisolia, G.; Ponzetto A.; Deisboeck, T.S. Thermodynamic considerations on the role of heat and mass transfer in biochemical causes of carcinogenesis. *Physica A* **2018**, *490*, 1164-1170.
25. Lucia, U.; Grisolia G. Second law efficiency for living cells. *Frontiers of Bioscience* **2017**, *9*, 270-275.
26. Lucia, U.; Grazzini, G.; Montrucchio, B.; Grisolia, G.; Borchiellini, R.; Gervino, G.; Castagnoli, C.; Ponzetto, A.; Silvagno, F. Constructal thermodynamics combined with infrared experiments to evaluate temperature differences in cells. *Scientific Reports* **2015**, *5*, 11587.