

Why does thermomagnetic resonance affect cancer growth? A non-equilibrium thermophysical approach

Original

Why does thermomagnetic resonance affect cancer growth? A non-equilibrium thermophysical approach / Grisolia, G.; Lucia, U.. - In: JOURNAL OF THERMAL ANALYSIS AND CALORIMETRY. - ISSN 1388-6150. - ELETTRONICO. - 147:9(2022), pp. 5525-5531. [10.1007/s10973-022-11294-8]

Availability:

This version is available at: 11583/2964060 since: 2022-05-18T13:02:02Z

Publisher:

Springer Science and Business Media B.V.

Published

DOI:10.1007/s10973-022-11294-8

Terms of use:

openAccess

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

(Article begins on next page)



Why does thermomagnetic resonance affect cancer growth? A non-equilibrium thermophysical approach

Giulia Grisolia¹ · Umberto Lucia¹

Received: 2 November 2021 / Accepted: 24 February 2022 / Published online: 23 March 2022
© The Author(s) 2022

Abstract

Recently, the low frequency thermomagnetic effects on cancer cells have been analysed, both theoretically and experimentally. They have been explained by introducing an equilibrium thermodynamic approach. But, in this context, two related open problems have been highlighted: (1) Does there exist a magnetic interaction or do there exist any other processes? (2) Do there exist also thermal effects? Here, we introduce a non-equilibrium thermodynamic approach in order to address an answer to these questions. The results obtained point out that: (a) the effect produced by the electromagnetic wave is just a consequence of the interaction of the magnetic component of the electromagnetic wave with the biological matter; (b) the interaction of the electromagnetic wave causes also thermal effects, but related to heat transfer, even if there have been applied low frequency electromagnetic waves; (c) the presence of the magnetic field generates a symmetry breaking in the Onsager's coefficients, with a related perturbation of the cancer stationary state.

Keywords Biothermodynamics · Bio-thermomagnetism · Cancer · Symmetry breaking

List of symbols

Latin letters

A	Surface (m^2)
B	Magnetic field (T)
c	Specific heat ($\text{J kg}^{-1}\text{K}^{-1}$)
ELF	Extremely low frequency
IF	Intermediate frequency
F	Heat power surface density (W m^{-2})
\mathbf{J}_e	Current density (A m^{-2})
\mathbf{J}_Q	Heat flux (W m^{-2})
K_j	Thomson coefficient
L_{ij}	Phenomenological coefficients
ℓ	Length of the cell membrane (m)
\dot{Q}	Heat power (W)
$\langle R \rangle$	Cell characteristic length (radius) (m)
Re	Reynolds number
RF	Radio frequency
Pr	Prandtl number
s	Specific entropy ($\text{J m}^{-3}\text{K}^{-1}$)
t	Time (s)

T	Temperature (K)
u	Internal energy density (J m^{-3})
\mathbf{v}	Velocity (m s^{-1})
V	Volume (m^3)

Greek letters

α	Coefficient of convection ($\text{W m}^{-2}\text{K}^{-1}$)
ϕ	Membrane potential (V)
λ	Conductivity ($\text{W m}^{-1}\text{K}^{-1}$)
μ	Chemical potential (J mol^{-1})
μ_e	Electrochemical potential (J mol^{-1})
ν	Frequency (Hz)
σ	Entropy production ($\text{J K}^{-1}\text{m}^{-3}$)
τ	Finite time of the process (s)

Constants

c	Speed of light in vacuum = $3 \times 10^8 \text{ m s}^{-1}$
F	Faraday constant = $96485 \text{ A s mol}^{-1}$
R	Ideal gas constant = $8.314 \text{ J mol}^{-1}\text{K}^{-1}$
μ_0	Permeability = $4\pi \times 10^{-7} \text{ H m}^{-1}$

Subscripts

0	Environment
surf	Surface

✉ Giulia Grisolia
giulia.grisolia@polito.it

¹ Dipartimento Energia “Galileo Ferraris”, Politecnico di Torino, Corso Duca degli Abruzzi 24, Torino 10129, Italy

Introduction

During the 20th century, the electromagnetic fields (EMF) have increased their relevance in any application, biomedical applications included, in relation both to safety and direct use. So, over the last decades, the analysis and the study of the effects of electromagnetic fields, on human health, has gained increased attentions [1]. Indeed, electromagnetic-based treatments have achieved therapeutic potentials and effects, in a large number of medical fields [2].

The electromagnetic frequency spectrum is usually classified in [3]:

- Static, with a frequency $\nu = 0$ Hz;
- ELF (Extremely Low Frequency), with a frequency in the range $0 < \nu \leq 300$ Hz;
- IF (Intermediate Frequency), with a frequency in the range $300 < \nu \leq 1 \times 10^4$ Hz;
- RF (Radio Frequency), with a frequency in the range $1 \times 10^4 < \nu \leq 3 \times 10^{11}$ Hz;
- IR (Infrared), Visible and UV (Ultraviolet), with a frequency in the range $3 \times 10^{11} < \nu \leq 3 \times 10^{15}$ Hz;
- IR (Ionizing Radiation) for higher frequencies.

In this context, in particular, the ELF range has attracted the interest of the researchers, because it characterizes some physical phenomena, such as the geomagnetic fluctuations [4], the Schumann resonance [5], the cell pulsations [6], etc. In particular, the vibration of a cell membrane has been pointed out to play a key role in the regulation of cell shape, and in the behaviour of the cells. Moreover, new directions, based on bio-inspired approaches, have been highlighted to develop possible future therapies, based on the use of Electromagnetic Fields, for various diseases, including cancer, diabetes, neural diseases, immune diseases, etc. [6].

In recent papers [7–9], the thermomagnetic effects on cancer cells have been shown and explained from an equilibrium thermodynamic point of view. The experiments have been carried out by comparing the growth of some cancer cell lines, under the exposure of an ELF-EMF, at their proper characteristic resonant frequencies, with the same lines of untreated cells. The characteristic resonant frequency has been evaluated for each cell line, by considering the cells average geometric parameters as required by the theoretical results [10]. The ELF-EMF exposure system is constituted by two independent couples of coaxial coils, wound into a cylindrical frame, with external radius of 8 cm, and a distance between the couples of coils of 8 cm. The outer casing of the exposure system is constituted by a box, that shields it from the background magnetic field,

in order to expose the cells at their resonant frequencies. The treated cells plate was set in the centre of the shielded exposure system, inside an incubator, while the untreated cells (the control ones) of the same cell line were placed inside the incubator, without any shield.

Some results obtained are summarised in Table 1, where the resonant frequency has been calculated for each cancer cell line. The cells have been exposed to their proper characteristic resonant frequency, as derived by our previous thermodynamic approach [10]. The effect on the cancer cells growth has been compared with that of the untreated cells. Always in Table 1, it is possible to highlight that the cancer growth reduction depends on the frequency, which is a function of the cell lines shapes [10].

In particular, during the experiments, the frequencies of the magnetic field were in the range of the order of 1 – 30 Hz, with the maximum amplitude of the wave was always $B = 100 \mu\text{T}$.

Consequently, the power applied was of the order of $B^2 c A / \mu_0 \sim 10^{-3} - 10^{-2}$ W, where $c \approx 3 \times 10^8$ m s⁻¹, $A \sim 10^{-10}$ m² is the area of cell membrane external surface, and $\mu_0 \approx 4\pi \times 10^{-7}$ H m⁻¹.

The fundamental results obtained can be summarised as follows:

- the key role of the volume-area ratio has been highlighted in relation to the cells heat exchange;
- the thermomagnetic resonance effect has been characterised by its resonant frequency, obtained by a thermodynamic analysis of the cell system, based on the heat outflow from the cell towards its environment;
- the related consequences on the behaviour of cancer cell have theoretically been shown, and experimentally confirmed, too;

Table 1 Growth variation of some cancer cell lines after the exposure to the calculated resonant frequencies [8, 9]

Cell line	Frequency/Hz	Growth variation/%
A375P	31	-15
HT-29	24	-19
GTL16	14	-24
MCF7	5	-22
SKBR3	8	-18
MDA-MB-231	6	-18

In particular, A375P is a human melanoma cell line, HT29 is a human colorectal adenocarcinoma cell line, GTL16 is a human gastric cancer cell line, MCF7, MDA-MB-231 and SKBR3 are three different cell lines of human breast cancer

- a device, based on the use of the electromagnetic field, has been designed, in order to trigger the behaviour of the cancer cell.

These experimental results open a theoretical problem in biophysics: which is the biophysical process involved in the thermomagnetic effect? In order to address an answer to this open problem, other two questions must first be analysed:

- is the effect produced by the electromagnetic wave a consequence of the magnetic interaction?
- Does the interaction of the electromagnetic wave cause also a thermal effect?

Since 1956, cancer cells were proven to be electrically different from normal cells [11]. Indeed, the start of the M phase of the cell life cycle has been highlighted to be characterised by a hyperpolarized state; consequently, a hypothesis has been introduced on the correlation between the cell cycle advancement and the modifications of the membrane electric potential [12]. Moreover, membrane hyperpolarization was shown to stop in a reversible way the DNA synthesis and the mitosis [13]. So, the membrane potential has been found to be one of the causes of the increase of the cancer cells proliferation [14–18], with relation also to migration and differentiation [19–25]. In Table 2, the membrane potential - both for some healthy and cancerous tissues - has been summarised by considering the data in literature [25].

In this study, a response to the previous open problems is suggested, by introducing the role of the membrane electric potential into the previous thermodynamic approach. To this purpose, an approach, based on the non-equilibrium thermodynamics, recently introduced in biophysics [10], is considered. The fundamental result of this research consists in the analysis of the resonant approach in heat transfer, when low frequency electromagnetic fields are applied to cancer cells.

Table 2 Some cell membrane potential in different tissues [25]

Tissue	Membrane potential	
	Normal/mV	Cancer/mV
Liver	-51	-44
Thyroid	-47	-25
Muscle	-89	-16
Gastric	-34	-24

Theory

Here, a non-equilibrium thermodynamic analysis of the membrane heat and mass transfer is introduced [10, 26–34], in order to improve the analysis of the thermomagnetic effects on cancer, in agreement with the experimental results reported in literature [11–21, 35–59].

In non-equilibrium thermodynamics, it is accepted to follow the Onsager’s approach [60], for which every flow \mathbf{J}_i is linearly dependent on all the forces \mathbf{X}_j operative in the system, such that:

$$\mathbf{J}_i = \sum_j L_{ij} \mathbf{X}_j \tag{1}$$

where the coefficients L_{ij} are always positive, independent of the forces and satisfy the reciprocal relations $L_{ij} = L_{ji}$. So, in relation to the analysis of heat and mass transport through the cell membrane, the Onsager relations can be written in relation to the phenomena which cause the fluxes themselves. They are the temperature gradient in relation to heat transfer and the gradient of chemical potential in relation to ion transport (mass transport). Consequently, the relation (1) results [26, 27, 31, 61–63]:

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

where \mathbf{J}_e represents the current density per surface area, \mathbf{J}_Q is the heat flux per surface area, $\mu_e = \mu + Ze\phi$ denotes the electrochemical potential, with μ the chemical potential, ze the electric charge per mole, and ϕ the membrane potential, T is the living cell temperature, and L_{ij} are the Onsager coefficients, with [64] $L_{12}(\mathbf{B}) = L_{21}(-\mathbf{B})$ (Onsager-Casimir relation [65]), and $L_{11} \geq 0$ and $L_{22} \geq 0$, and [64] $L_{11}L_{22} - L_{12}L_{21} > 0$. Now, when there are ion and metabolites fluxes, $\mathbf{J}_e \neq \mathbf{0}$ and $\mathbf{J}_Q = \mathbf{0}$, it follows [26, 61, 62]:

$$\frac{d\mu_e}{dT} = -\frac{L_{21}}{L_{11}} \frac{1}{T} \tag{3}$$

with [61, 62]:

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_Q \tag{4}$$

where u is the internal energy density.

Living cells outflow heat power to the environment by convection, thus, we can write [10]

$$\frac{du}{dt} dV = \delta \dot{Q} = -\alpha (T - T_0) dA \tag{5}$$

where $\alpha \approx 0.023Re^{0.8}Pr^{0.35}\lambda/\langle R \rangle$ is the coefficient of convection, with $\lambda \approx 0.6 \text{ W m}^{-1}\text{K}^{-1}$ conductivity, $Re \approx 0.2$ the Reynolds number and $Pr \approx 0.7$ the Prandtl number [66], A is the area of the external surface of the cell membrane, V is the cell volume, T depicts the mean temperature of the external surface of the cell's membrane, and T_0 is the temperature of the cell environment.

So, considering Equations (4) and (5), and the Divergence Theorem [67], the heat flux can be written as:

$$J_Q = \alpha (T - T_0) \tag{6}$$

and the related power flux yields:

$$\dot{Q} = \int_A \mathbf{J}_Q \cdot \hat{\mathbf{n}} dA = \alpha A (T - T_0) \tag{7}$$

Furthermore, considering Equation (2), together with $\mathbf{J}_e = \mathbf{0}$ and $\mathbf{J}_Q \neq \mathbf{0}$, it follows [26]:

$$\frac{d\mu_e}{d\ell} = \frac{T J_Q}{\left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right)} = - \frac{\alpha T (T - T_0)}{\left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right)} \tag{8}$$

with ℓ the length of a cell membrane and $|\nabla\mu_e| \approx d\mu_e/d\ell$. This relation is the link between the cell membrane electric potential and the temperature of the cell itself.

From Equations (6) and (8) follows:

$$J_Q = \alpha (T - T_0) = - \frac{1}{T} \left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right) \frac{d\mu_e}{d\ell} \tag{9}$$

where:

$$\left(L_{22} - L_{21} \frac{L_{12}}{L_{11}} \right) = K_J T^2 \tag{10}$$

with $K_J = \lambda$ being the Thomson coefficient. Consequently, it follows:

$$\frac{\partial\mu_e}{\partial\ell} = \frac{\partial\mu_e}{\partial T} \frac{\alpha}{K_J} (T_{\text{surf}} - T_0) \tag{11}$$

from which, taking into account that $\mu_e = \mu + ze\phi$, becomes:

$$\frac{\partial\mu}{\partial\ell} = -ze \frac{d\phi}{d\ell} + \frac{\partial\mu_e}{\partial T} \frac{\alpha}{K_J} (T_{\text{surf}} - T_0) \tag{12}$$

So, we can obtain:

$$\frac{\partial\mu_e}{\partial T} = \frac{K_J}{\alpha} \frac{F + ze}{T_{\text{surf}} - T_0} \frac{d\phi}{d\ell} - \frac{K_J}{\alpha} \frac{2.3RT_0}{T_{\text{surf}} - T_0} \frac{dpH}{d\ell} \tag{13}$$

which links the electrochemical potential to the pH.

The effect of the application of an electromagnetic wave with the thermal resonant frequency is to force the heat transfer, with a related thermal and electric perturbation.

Thus, cancer cell must activate ions fluxes in order to restore its initial condition, so $\mathbf{J}_e \neq \mathbf{0}$, and the concentration of ions varies in time [61, 62]:

$$\frac{dc_i}{dt} = -\nabla \cdot \mathbf{J}_i \tag{14}$$

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. In this condition, considering the Eq. (2), it follows the Thomson's Second Relation [61, 62]:

$$\frac{d\phi}{dT} = - \frac{L_{21}}{L_{11}} \frac{1}{T} \tag{15}$$

Therefore, the ion fluxes generate a variation in the electric potential and, consequently, a temperature variation and a heat flux occurs, too [61, 62, 68]:

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_u \tag{16}$$

where u is the specific internal energy. As a consequence of this temperature change, the specific entropy rate occurs [69]:

$$T \frac{ds}{dt} = \nabla \cdot \left(\mathbf{J}_u - \sum_{i=1}^N \mu_i \mathbf{J}_i \right) - \sum_{i=1}^N \mathbf{J}_i \cdot \nabla \mu_i \tag{17}$$

where T is the temperature, s is the specific entropy, and μ is the chemical potential, $\mathbf{J}_s = \mathbf{J}_u - \sum_{i=1}^N \mu_i \mathbf{J}_i$ represents 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 [61].

The contribution of the dissipation function is to generate a continuous entropy outflow, which generates order from disorder inside the cancer cell, as Schrödinger himself pointed out [70]. We must highlight that all these equations can be analytically solved; indeed, any term presents coefficients that are constant, as a consequence of the lamped model, introduced for the temperature values, and any gradient is constant, if we consider any particular cell line, because of their defined membrane electric potential, ion concentrations and the length of the membrane.

Discussion and conclusions

In this paper, we have analysed the thermomagnetic effect on cancer, by introducing the non-equilibrium thermodynamics.

We have pointed out the emerging of two thermoelectric effects on the cell membrane: a Seebeck- and a Peltier-like effects, related to the heat and the ion fluxes. Both these effects are related to the membrane electric potential, that results modified.

In relation to our previous experimental results [8], the findings, here obtained, allow us to suggest that:

- the effect produced by the electromagnetic wave is just a consequence of the magnetic interaction, due to the low frequencies used, as the optical properties of the biological matter suggest [71];
- the interaction of the electromagnetic wave cause also a thermal effect, which are obtained as Seebeck- and Peltier-like effects;
- the presence of the magnetic field generates a symmetry breaking in the Onsager's coefficients ($L_{12} \neq L_{21}$), which perturbs the stationary state of the cancer.

Now, some considerations can be added, in order to highlight a possible use of the low frequency electromagnetic waves in medicine, as a possible support therapy to the actual anticancer treatments. Indeed, we wish to highlight that, in our experiments, only a decrease in growth has been shown [8, 9]. This is due to the interaction of the electromagnetic fields with the cancer cell membrane. Indeed, following Blank et al. [72], membrane Na/K-ATPase activity is affected in opposite ways by electric and magnetic fields. Under optimal conditions, enzyme activity is inhibited by electric fields and stimulated by magnetic fields. Magnetic fields (in the range 0-70 Hz) were shown to increase Na/K-ATPase activity of around 5-10%, with little dependence on field intensity. The effect of an electric field is similar to an increase in binding of activating ions on the enzyme surface. Ion activation depends on the electric field effect, but also on the frequencies. Magnetic fields influence charge flow within the enzyme during the reaction.

Our results point out how the electromagnetic wave generates changes in the membrane electric potential at the resonant frequency, with the consequence of inducing a hyperpolarization of the membrane. Therefore, some related fluxes could be activated. When an active flux occurs against the membrane electrochemical potential, the required energy is obtained by hydrolysis of ATP, or by the movement of a co-transported, or coupled ion, along its electrochemical gradient. Within this framework, the H⁺-ATPase plays a central role, because it transfers positive charges into the cell, generating a membrane voltage (negative inside the cell), and pH gradients [73–76]. Protein phosphorylation is a fundamental cellular regulatory mechanism; indeed, it activates or deactivates many enzymes and receptors [77, 78], by involving kinases and phosphatases, both involved in the cellular transduction signalling [79].

In the analysis of the mitotic activities in sarcoma cells, the membrane potential was found to undergo hyperpolarization before entering M phase. It suggests that the level of membrane potential is correlated with cell cycle progression.

Moreover, membrane hyperpolarization was shown to block reversibly DNA synthesis and mitosis and to be correlated with the level of differentiation [12–14]. Consequently, the membrane electric potential represents a fundamental quantity to control critical cell functions, with particular regards to proliferation, migration and differentiation. Lastly, cell migration is controlled by the movement of ions and water [35], because an acidic environment furthers this phenomenon. This environmental pH is regulated by the H⁺ concentration, which is related to the H⁺-ATPase functions. In addition, the membrane potential is considered an indirect factor of cell migration, strictly related to the electrical driving force for Ca²⁺ whereas a hyperpolarized membrane potential increases intracellular Ca²⁺ through the Transient Receptor Potential (TRP) channels; in contrast, membrane depolarization activates the Ca²⁺ channels [21]. Notably, migrating cells have a high intracellular Ca²⁺ concentration gradient [80].

In this context, the results, here obtained, introduce a non-equilibrium viewpoint, by pointing out the fundamental role of the thermoelectric phenomena in the comprehension of the thermomagnetic effects on cancer cells, by considering the key role of the membrane electric potential, of the heat and ion fluxes, and of the pH changes.

Author Contributions Conceptualization, UL; methodology, UL and GG; software, GG; validation, UL and GG; formal analysis, UL; investigation, GG; resources, UL; data curation, GG; writing—original draft preparation, UL and GG; writing—review and editing, UL and GG; visualization, GG; supervision, UL; project administration, UL; funding acquisition, UL. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Barnes FS, Greenebaum B. Biological and medical aspects of electromagnetic fields. Boca Raton: Taylor & Francis; 2007.
- Liu X, Yan X, Zhang S, Liu Z, Win TTY, Ren L. The Effects of Electromagnetic Fields on Human Health: recent Advances and Future. *J Bionic Eng.* 2021;18:210. <https://doi.org/10.1007/s42235-021-0015-1>.
- Marino C, Galloni P, Merla C. In: Hashmi S, editors. Reference module in materials science and materials engineering. Oxford: Elsevier; 2016
- Krylov VV. Biological effects related to geomagnetic activity and possible mechanisms. *Bioelectromagnetics.* 2017;38:497. <https://doi.org/10.1002/bem.22062>.
- Cherry N. Schumann resonances, a plausible biophysical mechanism for the human health effects of solar. *Nat Hazards.* 2002;26:279. <https://doi.org/10.1023/A:1015637127504>.
- Chen BB, Lv J, Wang XY, Qian RC. Probing the membrane vibration of single living cells by using nanopipettes. *ChemBioChem.* 2019;21:650. <https://doi.org/10.1002/cbic.201900385>.
- 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. *Sci Rep.* 2015;5:11587. <https://doi.org/10.1038/srep11587>.
- Lucia U, Grisolia G, Ponzetto A, Bergandi L, Silvagno F, Soc R. Thermomagnetic resonance affects cancer growth and motility. *Open Sci.* 2020;7. <https://doi.org/10.1098/rsos.200299>.
- Bergandi L, Lucia UGG, Granata R, Gesmundo I, Ponzetto A, Paolucci E, Borchiellini R, Ghigo E, Silvagno F. The extremely low frequency electromagnetic stimulation selective for cancer cells elicits growth arrest through a metabolic shift. *Biochimica et Biophysica Acta (BBA). Mol Cell Res* 2019;1866: 1389. <https://doi.org/10.1016/j.bbamcr.2019.05.006>
- Lucia U, Grisolia G. Thermal resonance and cell behavior. *Entropy.* 2020;22:774. <https://doi.org/10.3390/e22070774>.
- Ambrose EJ, James AM, Lowick JH. Differences between the electrical charge carried by normal and homologous tumour cells. *Nature.* 1956;177:576 (printed in 1956)
- Cone CD, Trans NY. Electroosmotic interactions accompanying mitosis initiation in sarcoma cells in vitro. *Acad Sci* 1969;31:404. <https://doi.org/10.1111/j.2164-0947.1969.tb02926.x>
- Cone CD. Variation of the transmembrane potential level as a basic mechanism of mitosis control. *Oncology.* 1970;24:438. <https://doi.org/10.1159/000224545>.
- Cone CD. Unified theory on the basic mechanism of normal mitotic control and oncogenesis. *J Theor Biol* 1971;30:151. [https://doi.org/10.1016/0022-5193\(71\)90042-7](https://doi.org/10.1016/0022-5193(71)90042-7)
- Tokuoka S, Marioka H. The membrane potential of the human cancer and related cells (I). *Gann* 1957;48:353 (printed in 1956)
- Altman PL, Katz D. Biological handbook vol. 1: cell biology. Bethesda: Federation of American Society for Experimental Biology; 1976
- Balitsky KP, Shuba EP. Resting potential of malignant tumour cells. *Acta Unio Int Contra Cancrum.* 1964;20:1391 (printed in 1964)
- Jamakosmanovic A, Loewenstein W. Intracellular communication and tissue growth. III. Thyroid cancer. *J Cell Biol* 1968;38:556. <https://doi.org/10.1083/jcb.38.3.556>
- Sundelacruz S, Levin M, Kaplan DL. Role of the membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev.* 2009;5:231. <https://doi.org/10.1007/s12015-009-9080-2>.
- Lobikin M, Chernet B, Lobo D, Levin M. Resting potential, oncogene-induced tumorigenesis, and metastasis: the bioelectric basis of cancer *in vivo.* *Phys Biol.* 2012;9. <https://doi.org/10.1088/1478-3759/9/6/065002>
- Schwab A, Fabian A, Hanley PJ, Stock C. Role of the ion channels and transporters in cell migration. *Physiol Rev.* 2012;92:1865. <https://doi.org/10.1152/physrev.00018.2011>
- Tokuoka S, Morioka H, Gan I. The membrane potential of the human cancer and related cells. 1957;48:353 (printed in 1957)
- Johnstone RM. Microelectrode penetration of ascites tumour cells. *Nature.* 1959;183:411. <https://doi.org/10.1038/183411a0>
- Marino AA, Morris DM, Schwalke MA, Iliev IG, Rogers S. Electrical potential measurements in human breast cancer and benign lesions. *Tumour Biol.* 1994;15:147. <https://doi.org/10.1159/000217885>
- Binggelli R, Cameron IL. Cellular potential of normal and cancerous fibroblasts and hepatocytes. *Cancer Research.* 1980;40:1830
- Lucia U, Grisolia G. How life works—a continuous Seebeck-Peltier transition in cell membrane? *Entropy.* 2020;22:960
- Lucia U, Grisolia G. Non-equilibrium thermodynamic approach to Ca²⁺-fluxes in cancer. *Appl Sci.* 2020;10:6737
- Lucia U. Bioengineering thermodynamics: an engineering science for thermodynamics of biosystems. *Int J Thermodyn.* 2015;18:254
- Lucia U. Bioengineering thermodynamics of biological cells. *Theor Biol Med Modell.* 2015;29:254. <https://doi.org/10.1186/s12976-015-0024-z>
- Lucia U, Grisolia G. Resonance in thermal fluxes through cancer membrane. *Atti dell'Accademia Peloritana dei Pericolanti.* 2020;98:SC1. <https://doi.org/10.1478/AAPP981SC1>
- Lucia U, Grisolia G. Thermal physics and glaucoma: from thermodynamic to biophysical considerations to design future therapies. *Appl Sci* (accepted in printing) (2020)
- Lucia U, Grisolia G, Dolcino D, Astori MR, Massa E, Ponzetto A. Constructal approach to bio-engineering: the ocular anterior chamber temperature. *Sci. Rep.* 2016;6:31099. <https://doi.org/10.1038/srep31099>
- Lucia U, Grisolia G, Astori MR. Constructal law analysis of Cl⁻ transport in eyes aqueous humor. *Sci Rep.* 2017;7:6856. <https://doi.org/10.1038/s41598-017-07357-8>
- Lucia U, Grisolia G, Francia S, Astori MR. Theoretical biophysical approach to cross-linking effects on eyes pressure. *Physica A.* 2019;534:122163. <https://doi.org/10.1016/j.physa.2019.122163>
- Yang M, Brackenbury WJ. Membrane potential and cancer progression. *Front Physiol.* 2013;4:185. <https://doi.org/10.3389/fphys.2013.00185>
- Rizzuto R, Marchi S, Bonora M, Aguiari P, Bononi A, Stefani DD, Giorgi C, Leo S, Rimessi A, Siviero R, Zecchini E, Pinton P. Ca(2+) transfer from the ER to mitochondria: when, how and why. *Biochim Biophys Acta.* 2009;1787:1342
- Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol.* 2003;4:517
- Giorgi C, Missiroli S, Patergnani S, Duszynski J, Wieckowski MR, Pinton P. Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. *Antioxid Redox Sig.* 2015;22:995
- Pinton P, Ferrari D, Rapizzi E, Virgilio FD, Pozzan T, Rizzuto R. The Ca²⁺ concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. *EMBO.* 2001;20:2690
- Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Virgilio FD, Pozzan T, Rizzuto R. Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells. *J Cell Biol.* 2000;148:857

41. Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demaurex N, Krause KH. Bcl-2 decreases the free Ca²⁺ concentration within the endoplasmic reticulum. *Proc Natl Acad Sci USA*. 2000;97:5723
42. Akl H, Vervloessem T, Kiviluoto S, Bittremieux M, Parys JB, Smedt HD, Bultynck G. A dual role for the anti-apoptotic Bcl-2 protein in cancer: mitochondria versus endoplasmic reticulum. *Biochim Biophys Acta*. 2014;1843:2240
43. Akl H, Bultynck G. Altered Ca(2+) signaling in cancer cells: proto-oncogenes and tumor suppressors targeting IP3 receptors. *Biochim Biophys Acta*. 2013;1835:180
44. Marchi S, Marinello M, Bononi A, Bonora M, Giorgi C, Rimessi A, Pinton P. Selective modulation of subtype III IP(3)R by Akt regulates ER Ca²⁺ release and apoptosis. *Cell Death Dis*. 2012;3:e304
45. Giorgi C, Ito K, Lin HK, Santangelo C, Wieckowski MR, Lebedzinska M, Bononi A, Bonora M, Duszynski J, Bernardi R, Rizzuto R, Tacchetti C, Pinton P, Pandolfi PP. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science*. 2019;330:1247
46. Stewart TA, Yapa KT, Monteith GR. Altered calcium signaling in cancer cells. *Biochim Biophys Acta*. 2015;1848:2502
47. Bononi A, Bonora M, Marchi S, Missiroli S, Poletti F, Giorgi C, Pandolfi PP, Pinton P. Identification of PTEN at the ER and MAMs and its regulation of Ca²⁺ signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ*. 2013;20:1631
48. C. Giorgi, M. Bonora, G. Sorrentino, S. Missiroli, F. Poletti, J.M. Suski, F. Galindo Ramirez, R. Rizzuto, F. Di Virgilio, E. Zito, P.P. Pandolfi, M.R. Wieckowski, F. Mammano, G. Del Sal, P. Pinton, p53 at the endoplasmic reticulum regulates apoptosis in a Ca²⁺-dependent manner. *Proc Natl Acad Sci USA*. 2015;112:1779
49. Giorgi C, Bonora M, Missiroli S, Poletti F, Ramirez FG, Morciano G, Morganti C, Pandolfi PP, Mammano F, Pinton P. Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling. *Oncotarget*. 2015;6:1435
50. Rimessi A, Marchi S, Patergnani S, Pinton P. H-Ras-driven tumoral maintenance is sustained through caveolin-1-dependent alterations in calcium signaling. *Oncogene*. 2014;33:2329
51. Parsadaniantz SM, le Goazigo AR, Sapienza A, Habas C, Baudouin C. Glaucoma: A degenerative optic neuropathy related to neuroinflammation? *Cells*. 2020;9:535. <https://doi.org/10.3390/cells9030535>
52. Soto I, Howell GR. The complex role of neuroinflammation in glaucoma. *Cold Spring Harb Perspect Med*. 2014;4. <https://doi.org/10.1101/cshperspect.a017269>
53. Tezel G. Immune regulation toward immunomodulation for neuroprotection in glaucoma. *Curr Opin Pharmacol*. 2013;13:23. <https://doi.org/10.1016/j.coph.2012.09.013>
54. Wax MB, Tezel G, Yang J, Peng G, Patil RV, Agarwal N, Sappington RM, Calkins DJ. Induced autoimmunity to heat shock proteins elicits glaucomatous loss of retinal ganglion cell neurons via activated T-cell-derived fas-ligand. *J Neurosci* 2008;28:12085. <https://doi.org/10.1523/JNEUROSCI.3200-08.2008>
55. Gupta N, Yucel YH. Glaucoma as a neurodegenerative disease. *Curr Opin Ophthalmol*. 2007;18:110. <https://doi.org/10.1097/ICU.0b013e3280895aea>
56. Yucel YH, Gupta N. Glaucoma of the brain: A disease model for the study of transsynaptic neural degeneration. *Prog Brain Res*. 2008;173:465. [https://doi.org/10.1016/S0079-6123\(08\)01132-1](https://doi.org/10.1016/S0079-6123(08)01132-1)
57. Imamura K, Onoe H, Shimazawa M, Wada SNY, Kato K, Nakajima H, Mizuma H, Onoe K, Taniguchi T, Sasaoka M, Hara H, Tanaka S, Araie M, Watanabe Y. Molecular imaging reveals unique degenerative changes in experimental glaucoma. *Neuroreport*. 2009;20:139. <https://doi.org/10.1097/WNR.0b013e3283281d7f82>
58. Shum JWH, Liu K, So KF. The progress in optic nerve regeneration, where are we? *Neural Regen Res*. 11, 32 (2016). <https://doi.org/10.4103/1673-5374.175038>
59. Bejan A. The golden ratio predicted: Vision, cognition and locomotion as a single design in nature. *International J Des Nat Ecol Dyn*. 2009;4:97. <https://doi.org/10.2495/DNE-V4-N2-97-104>
60. Onsager L. Reciprocal relations in irreversible processes. *Phys Rev*. 1931;37:405. <https://doi.org/10.1103/PhysRev.37.405>
61. Yourgrau W, van der Merwe A, Raw G. Treatise on irreversible and statistical thermodynamics. New York: Dover; 1982.
62. Callen HB. Thermodynamics. New York: Wiley; 1960.
63. Goupil C. In: Mizutani T, editor. Thermodynamics. IntechOpen, Shanghai; 2011
64. Katchalsky A, Curran PF. Nonequilibrium thermodynamics in biophysics. Boston: Harvard University Press; 1965
65. Degroot SR, Mazur P. Non-equilibrium thermodynamics. Amsterdam: North-Holland Publishing Company; 1962.
66. Lauga E. The fluid dynamics of cell motility. Cambridge: Cambridge University Press; 2020
67. Apostol TS. Calculus. volume 2: multi-variable calculus and linear algebra with applications to differential equations and probability. Hoboken: Wiley; 1969
68. Lucia U, Grisolia G, Ponzetto A, Deisboeck TS. Thermodynamic considerations on the role of heat and mass transfer in biochemical causes of carcinogenesis. *Physica A*. 2018;490:1164. <https://doi.org/10.1016/j.physa.2017.08.075>
69. Lucia U, Grisolia G. Second law efficiency for living cells. *Front Biosci*. 2017;9:270. <https://doi.org/10.2741/s487>
70. Schrödinger E. What's life? The physical aspect of the living Cell. Cambridge: Cambridge University Press; 1944.
71. Cheong WF, Prahl SA, Welch AJ. A Review of the Optical Properties of Biological Tissues. *IEEE - J Quant Electron* 1990;26:2166
72. Blank M, Soo L, Papstein V. Effects of low frequency magnetic fields on Na,K-ATPase activity. *Phys Rev*. 1995;38:267. [https://doi.org/10.1016/0302-4598\(95\)05032-4](https://doi.org/10.1016/0302-4598(95)05032-4)
73. Nakanishi-Matsui M, Sekiya M, Futai RKNM. The mechanism of rotating proton pumping ATPases. *BBA Bioenerg*. 2010;1797:1343. <https://doi.org/10.1016/j.bbabi.2010.02.014>
74. Stevens TH, Forgac M. Structure, function and regulation of the vacuolar (H⁺)-ATPase. *Annu Rev Cell Dev Bi*. 1997;13:779. [https://doi.org/10.1016/s0014-5793\(98\)01425-2](https://doi.org/10.1016/s0014-5793(98)01425-2)
75. Tuszynski JA, Kurzynski M. Introduction to molecular biophysics. Boca Raton: CRC Press; 2003
76. Lucia U, Ponzetto A, Deisboeck TS. A thermo-physical analysis of the proton pump vacuolar-ATPase: The constructal approach. *Sci Rep*. 2014;4:1. <https://doi.org/10.1038/srep06763>
77. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol*. 2006;24:419. <https://doi.org/10.1146/annurev.immunol.23.021704.115658>
78. Strong RK. Asymmetric ligand recognition by the activating natural killer cell receptor NKG2D, a symmetric homodimer. *Mol Immunol*. 2002;38:1029. [https://doi.org/10.1016/s0161-5890\(02\)00032-9](https://doi.org/10.1016/s0161-5890(02)00032-9)
79. Ardito F, Giuliani M, Perrone D, Troiano G, Muzio LL. The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy. *Int J Mol Med*. 2017;40:271. <https://doi.org/10.3892/ijmm.2017.3036>
80. Brundage R, Fogarty K, Tuft R, Fay F. Calcium gradients underlying polarization and chemotaxis of eosinophils. *Science*. 1991;254:703. <https://doi.org/10.1126/science.1948048>