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Umberto Lucia [1,*] and Giulia Grisolia [2,*]

[1] Dipartimento Energia "Galileo Ferraris", Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[2] Dipartimento di Ingegneria dell'Ambiente, del Territorio e delle Infrastrutture, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
* Correspondence: umberto.lucia@polito.it (U.L.); giulia.grisolia@polito.it (G.G.); Tel.: +39-011-090-4558 (U.L.)

Abstract

Cells operate as open thermodynamic systems where energy transformations and transport processes occur across membranes, exhibiting distinct thermo-electro-biochemical behaviours in healthy versus diseased states. Living organisms generate waste heat due to internal irreversibility, which dissipates into the environment and serves as an observable flow of information. By analysing this heat loss and its changes under external influences, new insights into cellular behaviour can be gained. This paper highlights recent advances in this thermodynamic approach, which frames living systems as black boxes, focusing on their input–output dynamics and introducing the emerging field of bioengineering thermodynamics. A key challenge in applying extremely low-frequency electromagnetic fields (ELF-EMF) to proliferative disorders has been the empirical selection of effective field parameters. To address this, we employed a bio-thermodynamic engineering model to calculate the ELF frequency that maximizes mean entropy changes based on cellular biophysical parameters. This entropy change corresponds to a metabolic shift that reduces cell proliferation. Experimental validation was performed on six human cancer cell lines, where proliferation rates served as indicators confirming the model's predictions. For the first time, this approach enabled the calculation and experimental validation of ELF frequencies selectively effective on different cell types, demonstrating a promising method for targeted therapeutic applications.

Keywords: bio-thermodynamics; mechano-biology; cancer; thermal resonance; bio-energy; thermo-magnetism

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1. Introduction

Cancer is one of the leading causes of death globally. According to the World Health Organization, in 2008, it was responsible for approximately 13% of all fatalities, equating to around 7.6 million individuals. Projections for 2030 suggest that cancer-related deaths could exceed 11 million people [1].

Mechanical phenomena play a crucial role in biological processes at various levels, including organs, tissues, cells, and biomolecular motors. These phenomena can impact genes such as c-MET and TP53. Many cell processes are influenced by the nano-mechanical properties of cellular membranes [2]. Research has shown that external forces or changes within cells or tissues can lead to developments and physiological changes, as well as the onset of diseases. In response, a new interdisciplinary approach called mechanobiology

has emerged, which combines principles from mechanical engineering with biological and medical insights.

Based on systems biology, this engineering perspective aims to enhance our understanding of cell behaviour by analysing how bio-molecular signals are transmitted in relation to their capacity to modify the structure, function, and dynamics of cellular systems. A key outcome of mechanobiology is its ability to explain the molecular mechanisms of mechano-transduction, which is the process through which cells detect and respond to mechanical signals and convert them into biochemical signals [3]. Numerous receptors, including extracellular matrix (ECM) molecules, transmembrane proteins, cytoskeletal components, nuclei, and lipid bilayers, are responsive to mechanical stimuli and engage in transduction processes that operate both from the inside out and outside in within the cell.

Life operates as a non-selective process, involving a cooperative interplay of interconnected mechanisms, since physiological stimuli are typically static and influenced by the intensity, occurrence rate, and length of external influences. Therefore, mechanobiology alone does not offer adequate understanding for researchers and clinicians in their investigations. Similar to principles applied in engineering, it is essential to consider thermal, chemical, and electromagnetic factors to gain a holistic understanding of biological systems. To achieve this, integrating thermodynamic analysis into mechanobiology, and, more broadly, into biomedical engineering, is crucial for developing a new scientific discipline tailored to biological systems that serves both biologists and physicians: the field of bioengineering thermodynamics is on the rise.

Engineering thermodynamics examines energy use and optimization in relation to available energy resources, particularly focusing on energy conversion processes such as power generation and refrigeration: energy is considered a thermodynamic characteristic of any system relative to a defined reference state. Although energy undergoes transformations during various processes, the total energy within the universe—encompassing both the system and its surroundings—remains conserved. This principle applies to the properties of matter, including living organisms. In cellular activities such as replication, transcription, and translation, the conversion of energy derived from molecular interactions, chemical bond cleavage, and electromagnetic gradients is vital for executing mechanical tasks that facilitate conformational shifts and molecular movements. Investigations into the biomechanics of DNA have uncovered significant relationships among mechanical forces, thermodynamic principles, the nano-mechanical and electromagnetic characteristics of biological structures, and their kinetic behaviours [4,5].

One could argue that incorporating thermodynamics into the mechanobiological approach could enhance our understanding of this field by examining biological systems from a thermal perspective. The significance of thermodynamics in biology is highlighted in several key studies. For instance, Wilson et al. [6] established thermodynamic relationships in mitochondrial oxidative phosphorylation, while Waldeck et al. [7] developed a nonequilibrium thermodynamics model to investigate the behavior of fully coupled Ca^{2+}-ATPase, with particular attention to how the coupling stoichiometry varies in response to changes in electrochemical gradient magnitude during ATP hydrolysis. Jin and Bethke [8] employed a nonequilibrium thermodynamic approach to the chemiosmotic model of proton translocation and energy conservation, demonstrating that the electron transport rate through the mitochondrial respiratory chain and in respiring prokaryotes is determined by three key factors: electron donation, electron acceptance, and the thermodynamic driving force. Their work is distinguished by its simultaneous consideration of both thermodynamic and kinetic influences on respiration rate, highlighting the nonlinear relationship between flux and electrical potential gradient, the hyperbolic dependence on substrate concentration, and the inhibitory impact of reaction products. Furthermore,

Turina et al. [9] performed a thermodynamic evaluation of ATP synthesis and hydrolysis coupled to proton transport. Demirel [10] presented a linear nonequilibrium thermodynamics framework under the assumption that the biosystem is near global equilibrium, deriving a non-isothermal reaction–diffusion system that governs various studies of transport and rate processes across physical, chemical, and biological systems encompasses phenomena such as pattern formation and chemical pumping mechanisms. It has been shown that flow can occur counter to the primary thermodynamic driving forces, which may include gradients in temperature, chemical potential, or reaction affinity. Analytical investigations have introduced new definitions for cross-coefficients that connect chemical reactions with heat and mass transport, framed in terms of kinetic parameters, transport coefficients, and coupling degrees. These developments have yielded novel parameters critical for accurately characterizing certain coupled reaction-transport systems. More recently, Chowdhury and Chanda [11] advanced the thermodynamic framework by focusing on the electro-mechanical coupling mechanisms within voltage-gated ion channels.

These fundamental thermodynamic frameworks examine distinct biochemical and transport phenomena, laying the groundwork for a more integrative approach that encompasses all processes involved in the interactions between biological systems and their environments. Building upon these foundational concepts, there is a need to develop a more comprehensive and universal framework. According to the first law of thermodynamics, energy is conserved, while the second law asserts that entropy within the system and its surroundings invariably increases [12], also offering a statistical and informational perspective on global thermodynamic quantities. The third law further bridges the second law with principles of quantum mechanics and thermodynamics [13]. Consequently, thermodynamics emerges as a fundamental discipline for analysing open systems engaged in energy exchange and transformation. In the context of mechanobiology, the integration of thermodynamic principles represents a major advancement, proposing a novel scientific foundation for the engineering analysis and regulation of biological systems. This approach envisions a systems theory that unites engineering thermodynamics, mechanobiology, biomedical engineering, biothermodynamics, bioenergy, and systems biology.

This paper aims to present the physical principles underlying this innovative framework, discussing initial findings. The aim of this study lies in evaluating the homeostatic response of cells by analysing their thermo-biophysical output, specifically, the average entropy changes exchanged between the cell and its environment, rather than concentrating solely on individual affected pathways. In this research, we applied the principle of action and reaction to membrane flux variations and calculated the frequency that maximizes entropy production within the cell-environment system for each cell type. Our thermal biophysical model suggests that cell growth inhibition is driven by these average entropy changes; therefore, we use proliferation rate, obtained in previous studies [14,15] as a measurable parameter to validate the predictions of the thermodynamic model. Biophysical data from six different cell types are integrated into the physical–mathematical model to identify the optimal electromagnetic frequency of treatment for each cell line. Subsequent proliferation assays confirmed both the effectiveness and specificity of the chosen electromagnetic field parameters in inhibiting cell growth. Section 2 summarises thermodynamic fundamentals, while Section 3 explores how engineering thermodynamics results can be applied to biosystems.

2. Materials and Methods

From various perspectives, physical, biological, chemical, and mathematical, nature can be seen as a complex system, whereas from an engineering perspective, it stands as the original engineer. However, predicting the dynamics and evolution of these complex

systems poses challenges for scientists and engineers alike. A key method for studying such systems is thermodynamics, which is characterized by its clarity, simplicity, and ongoing evolution. This discipline offers global insights (engineering and irreversible thermodynamics), local insights (statistical thermodynamics), and approaches that incorporate linear (classical thermodynamics) and complex (non-equilibrium and non-linear thermodynamics) dynamics.

The myriad schools of thermodynamics can create communication barriers—not only between thermodynamicists and other scientists but also among thermodynamicists themselves. Therefore, pursuing a unified approach grounded in the fundamentals of physics is essential.

Carnot [16] emphasized the presence of a theoretical maximum efficiency for transforming heat into mechanical work, whereas Clausius introduced the concept of entropy to better understand irreversible and dissipative phenomena [17]. More recently, Gouy and Stodola independently presented a new thermodynamic perspective on evaluating lost exergy in 1889 and 1905 [18], respectively.

It has become increasingly evident that every effect in nature results from the dynamic balance of interactions between real systems and their environments. Energy balances result from the transfer of energy, more precisely, exergy, between a real system and its environment. The progression of real systems is fundamentally associated with a decrease in their free energy occurring in the shortest feasible time frame.

These critical insights provide a foundation for proposing a unified approach to the diverse schools of thermodynamics, emphasizing two fundamental concepts in physics: interactions and flows. This leads to the development of a flow-based analytical framework in thermodynamics, which could serve as a "rallying point" for these various schools. Notably, natural systems are invariably open, allowing for the exchange of heat and mass with their environment. Therefore, the relationship between a system and its surroundings emerges as a fundamental element in thermodynamic analysis.

In this section, we explore the bioengineering thermodynamics [19,20] of biological cells, focusing on how controlling ion transport across the cell membrane may influence cell growth. We start by acknowledging that cells naturally exchange heat, which connects to their biochemical and biophysical activities. This excess heat serves as a form of "spontaneous communication" between the cell and its surroundings, highlighting the importance of this interaction for a thermodynamic analysis of the cell. Given the complexity of cells, it is challenging to isolate the impact of each process on the overall outcome. Thus, treating cells as black boxes simplifies the analysis by concentrating on the balances of inflow and outflow.

2.1. The Entropy Approach to Living Cells

The key focus of this analysis is global variation of entropy, which can be considered the primary indicator for assessing changes within a system, particularly emphasizing the variation in entropy due to irreversibility, known as entropy generation. This aspect arises from the overall impact of entropy change that occurs through interactions with the environment (flows) and internally within the system (interaction). The concept of entropy generation is introduced to prevent physical–mathematical contradictions, as entropy functions as a state variable; thus, nothing is truly created or generated. Essentially, entropy acts as a parameter that defines the thermodynamic state, and the irreversibility component, S_g, quantifies the distance of the system from a state that could be achieved reversibly. It is important to note that S_g is always greater than or equal to zero. When examining natural systems, it becomes evident that they are open systems, capable of exchanging both heat

and mass with their surroundings highlight the importance of environmental interaction in thermodynamic analysis [19,20].

In the framework presented here, the environment is modeled as a thermostat. Together, the system and its environment form an adiabatic closed system [17], within which the total entropy invariably increases, consistent with the second law of thermodynamics [21]. This total entropy can be mathematically represented as follows [17]:

$$\frac{dS}{dt} = \frac{d_i S}{dt} + \frac{d_e S}{dt} \tag{1}$$

where dS indicates the differential change in total entropy, t is time, $d_e S$ represents the entropy change due to interactions between the open system and its environment, while $d_i S$ signifies the entropy change resulting from irreversibility, such that:

$$\begin{aligned} \frac{dS}{dt} &\geq 0 \\ \frac{d_i S}{dt} &\geq 0 \end{aligned} \tag{2}$$

We can reformulate Equation (1) as [20]:

$$\frac{dS}{dt} = \int_V \left[-\nabla \cdot \left(\frac{\mathbf{q}}{T} \right) + \dot{s}_g \right] dV \tag{3}$$

In this representation, $\mathbf{q}$ is the heat flow, i.e., heat transferred per unit area and time, T is the temperature, V stands for volume, t represents time, and s_g denotes the entropy generation rate density, defines as [22]:

$$\dot{s}_g = \sum_i \mathbf{J}_i \cdot \mathbf{X}_i \tag{4}$$

In this context, $\mathbf{J}_i$ denotes the flux of the i-th quantity involved in the process under consideration, while $\mathbf{X}_i$ represents the corresponding thermodynamic force. According to the Curie principle, which states that fluxes and forces with different tensorial properties do not couple, the vectors $\mathbf{J}$ and $\mathbf{X}$ can be treated as mutually independent [22].

Furthermore, it is recognized that the stationary states of an open system correspond to the equilibrium states of the associated adiabatic closed system. By examining the system together with its environment, it follows that for an adiabatic closed system, the entropy change within the specified volume attains its maximum value at equilibrium [21]:

$$dS = 0 \implies -\nabla \cdot \left(\frac{\mathbf{q}}{T} \right) + \dot{s}_g = 0 \implies \nabla \cdot \left(\frac{\mathbf{q}}{T} \right) = \dot{s}_g \implies \nabla \cdot \left(\frac{\mathbf{q}}{T} \right) = \sum_i \mathbf{J}_i \cdot \mathbf{X}_i \tag{5}$$

This concluding relationship demonstrates that the exchanges between an open system and its surroundings cause an increase in the rate density of entropy generation. Therefore, irreversibility arises specifically from the interaction between the system and its environment; in the absence of such interaction, irreversibility would not appear. This last relation can also be managed and rewritten as follows:

$$\nabla \cdot \left(\frac{\mathbf{q}}{T} \right) = \frac{\nabla \cdot \mathbf{q}}{T} + \mathbf{q} \cdot \nabla \left(\frac{1}{T} \right) = \frac{\nabla \cdot \mathbf{q}}{T} - \mathbf{q} \cdot \frac{\nabla T}{T^2} \implies \frac{\nabla \cdot \mathbf{q}}{T} = \sum_i \mathbf{J}_i \cdot \mathbf{X}_i + \mathbf{q} \cdot \frac{\nabla T}{T^2} \tag{6}$$

that can be written as:

$$\int_V \frac{\nabla \cdot \mathbf{q}}{T} \, dV = \int_V \sum_i \mathbf{J}_i \cdot \mathbf{X}_i \, dV + \int_V \mathbf{q} \cdot \frac{\nabla T}{T^2} \, dV \implies \dot{S}_e = \dot{S}_{ion} + \dot{S}_i \tag{7}$$

From an engineering thermodynamics viewpoint, cells act as biological engines, characterized by a series of specifically arranged chemical reactions, generated by metabolites and ion fluxes through the cell membrane, i.e., the border of our thermodynamic control volume. During these processes, some energy is released to the cell's environment as heat. While we can observe the end products of these biochemical reactions, the individual steps are often hidden from direct analysis. Thus, studying cells can be approached using a usual engineering thermodynamic approach, which focuses solely on the spontaneous ion and heat flows occurring within the cell. Any effort to control cell behaviour involves managing these flows, which are linked to the generation of entropy due to energy loss from interactions with external forces and fields, such as electric, magnetic, and mechanical influences. On the bases of this bio-engineering thermodynamic approach, Equation (7) can be written as

$$\dot{S}_e = \dot{S}_{ion} + \dot{S}_i \tag{8}$$

and its (bio-)physical meaning can be obtaining following the approach of non-equilibrium thermodynamics [22]: the entropy variation, $\dot{S}_e$, for interaction between the open system considered and its environment is the result of the entropy variation due to matter (in particular ions, on the bases of the Julius Bernstein's membrane theory [23]) flux, $\dot{S}_{ion}$ and the entropy variation due to heat flux (irreversibility), $\dot{S}_i$.

2.2. Hypotheses on Biophysical Consequences

In our thermal biophysical framework, we propose that ion fluxes induced by resonant frequencies of extremely low-frequency electromagnetic fields (ELF-EMF) trigger a homeostatic response aimed at counteracting and minimising disturbances. The restoration of ionic gradients occurs via primary or secondary active transport mechanisms, both of which rely on adenosine triphosphate (ATP) hydrolysis for energy. Consequently, the cell must increase mitochondrial ATP production through enhanced mitochondrial respiration. This oxidative metabolism within mitochondria supports the respiratory chain, where the energy from electron transfer is converted into a proton gradient essential for ATP synthesis. However, a portion of this energy is lost through uncoupling processes that dissipate it as heat. In particular, Warburg demonstrated the presence of metabolic dysfunction in cancer, emphasising the critical role of energy transformation within biological systems [24]. He highlighted that cellular biochemical processes convert external metabolites into various cellular functions, such as cell replication, protein synthesis, and the transcription and translation of DNA and RNA, while simultaneously releasing heat into the surrounding environment [25,26]. From a thermodynamic perspective, cellular metabolism can be modelled as an energy inflow analogous to the heat input in a thermal engine cycle, which transforms this energy into useful thermodynamic work alongside dissipated heat. Cells regulate the exchange of energy and matter across their membranes [27], a process influenced by endogenous electric fields [28]. The cell membrane, composed of a bilayer of lipids, acts as a barrier separating the cytoplasm from the extracellular milieu. Embedded within this membrane, specific proteins function as channels that facilitate the controlled transport of mass and ions in and out of the cell. The cell membrane can be represented as an electric RC circuit [29], which exhibits both transient and resonant responses depending on whether step or harmonic input signals are applied [30,31]. Focusing on the RC circuit, the transient response can be described by the current flowing through the resistor of resistance R during the capacitor's charging and discharging phases [30,31]:

$$i(t) = \frac{V_0}{R}\, e^{-t/\tau_{el}} \tag{9}$$

where $i(t)$ denotes the current, V_0 is the applied electric potential across the capacitor, R is the resistance, and τ_{el} defines the system's characteristic time. This characteristic time, which governs the transient electrical behaviour, is also linked to the resonant frequency, given by [30,31]:

$$\nu_{el} = \frac{1}{2\pi\,\tau_{el}} \tag{10}$$

This characteristic time corresponds to the electric resonance behavior of the system, in this case, the membrane. Specifically, when an electromagnetic wave interacts with a system possessing a defined electric characteristic time, a resonance phenomenon arises. We have demonstrated that a comparable resonance effect also manifests in the thermal domain [32]. In the Result section, we use our previous findings to point out a thermodynamic-based biophysical interpretation.

Within this thermal biophysical model, the connection between changes in entropy and cellular proliferation is represented by a metabolic shift prompted by ELF-EMF exposure. Indeed, electromagnetic waves have been shown to influence intracellular metabolism across various cell types [33], including yeast [34], mammalian cells [35], and organisms such as *Caenorhabditis elegans* [36] and *Saccharomyces cerevisiae* [37]. Additionally, coherent domains may initiate specific thermal biophysical reactions via resonance, a mechanism that contributes to the precise regulation of gene expression [38].

2.3. The Experimental Approach to Support the Thermodynamic Theory

We conducted six independent experiments [14] to evaluate the thermal biophysical thermodynamic model, with all results aligning with the model's predictions. We selected five distinct tumour-derived cell types, each differing in morphology, growth rate, and oncogene expression, and fibroblasts as control cells. After characterizing certain biophysical properties of these cells, we incorporated the data into our mathematical model to determine the frequency most effective for each cell type. In every experiment, three different cell lines were exposed to an electromagnetic field at a frequency and intensity specifically chosen to maximise the average entropy change in only one of the three cell lines. The amplitude of the electromagnetic wave was considered less critical, as it primarily represented the number of photons initiating the biological response. Since our hypothesis focuses on the electromagnetic field eliciting a resonant response, frequency was identified as the crucial parameter rather than wave intensity. Accordingly, we selected an intensity level deemed sufficient to generate a statistically significant number of interaction events during energy analysis. Following four days of exposure, cell proliferation was assessed using a colorimetric assay. Notably, a reduction in cell number was observed exclusively in the cell line for which the applied frequency was predicted to be optimal under each experimental condition. Data were analysed statistically using an unpaired, two-tailed Student's t-test, with a significance threshold set at $p < 0.05$ [14].

2.3.1. Cell Lines Considered

The selected cellular models included primary fibroblasts as the control cell system, along with the MCF7, SKBR3, GTL16, HT29, and A375P cell lines. The primary fibroblasts represent normal differentiated tissue, while the latter cell lines serve as models of transformed, proliferating precancerous tissue. MCF7 and SKBR3 are two human breast cancer cell lines that differ in their reliance on estrogen stimulation and oncogene expression, as well as in cell size, although both exhibit growth in epithelial-like clusters. GTL16 is a human gastric cancer cell line characterized by overexpression of the MET oncogene, rapid proliferation, and a rounded cellular morphology. HT29 is a human colorectal adenocarci-

noma cell line displaying typical epithelial morphology. A375P is a human melanoma cell line distinguished by variations in morphology, growth rate, and malignancy.

2.3.2. The Experimental Set-Up

The experimental setup consisted of two independent pairs of coaxial coils, each made of 200 loops of copper wire with a diameter of 0.3 mm. Each loop measured 2.5 cm in length, resulting in a loop density of 8000 loops per meter, and was tightly wound into a plastic frame. The frame was cylindrical with an outer radius of 8 cm, and the distance between the two pairs of coaxial coils was 8 cm. The cell culture dish was positioned at the center of the apparatus for magnetic irradiation. The entire setup was housed inside a magnetically shielded box, where the residual magnetic field was measured at approximately 1–2 μT. The box was constructed from stainless steel and featured a shielding system composed of a 2 mm thick inner layer of mu-metal and an outer layer made from a special aluminum-free alloy supplied by G-Iron. The inner coils, with a radius of 7.5 cm, were connected to an AC current generator. The AC current was a square wave starting from zero to a selected positive value, operating at 50 Hz with a 50% duty cycle. The amplitude was set to produce an AC magnetic field of up to 12 μT rms within the irradiation volume. The outer coils were powered by a DC current, generating a constant magnetic field of 45 μT. This value was chosen to replicate the average environmental magnetic field in the Turin area, particularly in the building where the experiments took place, which is approximately 45 ± 5 μT. Thus, the DC magnetic field inside the box simulated the environmental magnetic field in a controlled manner. Within the irradiation volume, the total magnetic field varied point-to-point by up to ±5% of the nominal value. The temperature inside the box was maintained at a constant 37 °C, with fluctuations limited to ±0.1 °C. All measurements were conducted under controlled pressure and humidity conditions.

2.3.3. Experimental Procedure

The impact of ELF-EMF on the proliferation of various human cell lines was evaluated using a colorimetric assay based on crystal violet staining to quantify cell numbers. Cells were seeded in 96-well plates at densities of 2000, 1000, or 500 per well, adjusted according to their respective proliferation rates. They were then cultured for four days under standard conditions (control) or subjected to ELF-EMF exposure. After this incubation period, cells were fixed with 11% glutaraldehyde for 15 min, followed by three washes. Plates were air-dried and stained with a 0.1% crystal violet solution for 20 min. Subsequently, the wells were thoroughly rinsed and air-dried again before the bound dye was solubilized using a 10% acetic acid solution. Absorbance was measured at 595 nm to determine cell density. For each experimental condition, data from twelve replicate wells were averaged, and the entire experiment was performed in triplicate [14].

The cell culture plate was positioned at the center of the magnetic exposure apparatus, which consisted of two separate pairs of coaxial coils arranged within a cylindrical frame. This frame had an outer radius of 8 cm, and the distance between the two pairs of coils was also 8 cm. The entire setup was housed inside an incubator within a shielding box designed to protect the apparatus from ambient magnetic fields. The alternating current (AC) signal was generated as a sine wave at frequencies of $1/\tau$, $2/\tau$ and $4/\tau$ to account for variability in cell volume. The intensity of the fundamental frequency $1/\tau$ was set at 70 μT for all experiments, a level deemed sufficient to yield a statistically significant number of interaction events based on energy analysis. Since the amplitudes correspond to the relative weight of the resonant frequency distribution in relation to cell volume, the intensities for $2/\tau$ and $4/\tau$ were adjusted to 22 μT and 11 μT, respectively. Control cells were incubated without shielding, thus exposed to the typical background electromagnetic

fields encountered in daily life. In contrast, cells subjected to ELF-EMF exposure were shielded to isolate the effects of the specific frequencies and field intensities applied.

3. Results

A living system is an adaptive open system where the size of the initial cell can vary, affecting the sizes of the daughter cells [39]. Living cells undergo thermochemical processes that convert energy into work, leading to energy and matter flow across their boundaries, while biochemical transformations generate net entropy [40]. Consequently, any cell is modelled as a thermodynamic engine; consequently, the system must dissipate heat to its surroundings. Within this framework, the principle of entropy generation minimization, as applied in finite-time thermodynamics or thermodynamic optimization, becomes relevant for cellular systems. Building on Schrödinger's insights [26], this approach focuses on optimizing real, irreversible systems and processes while considering constraints related to finite size and lifetime.

In living systems, analysing the exchange of entropy resulting from heat flux is particularly interesting because it is essential for comprehending irreversible chemical reactions, as shown by Zivieri et al. [41].

Furthermore, the bio-engineering thermodynamic approach treats fluxes as fundamental physical quantities, considering both the geometrical properties and the timing of heat exchange processes. Moreover, while healthy cells maintain order, the biochemical reactions in cancer cells, as shown by Otto Warburg, lead to metabolic injuries and altered cellular cycles [24].

3.1. Thermodynamic Results on Ion Fluxes Through Membrane

To study this process, the cell membrane can be modelled as an equivalent electrical circuit. It is important to note that such circuits may exhibit both transient and resonant behaviours. Thus, we need to explore the potential equivalent behaviours in heat transfer from the cell to its surroundings.

Considering Equation (8), life results in an organisational process driven by biochemical reactions and the flow of energy and matter (ions). Our thermodynamic approach focuses on energy and mass flux balances, while excluding gene activities and evaluating their consequences in terms of energy conversion. Living cells' metabolism involves the exchange of matter and heat with their environment, with heat flux representing the energy lost to the surroundings. Therefore, we can consider that the heat flux is exchanged by convection with the suspending aqueous solution around each cell.

Thus, following the usual approach in biophysics in parallel to the RC model of membrane, considering heat transfer across the membrane, a thermo-kinetic lumped parameter model can be introduced, supported by the homeostasis of the living cell [25,32] and experimental evidence [42,43]. The lumped model simplifies biological systems by disregarding the detailed compartmentalisation within cells. This approach focuses on capturing the overall, average system response rather than the intricate spatial distribution of components. Such simplification is justified when the transport processes, such as diffusion or membrane crossing, occur on a much faster timescale compared to metabolic reactions. Additionally, lumped models are appropriate when the primary interest lies in understanding the global system behaviour instead of localised regulatory mechanisms [44]. The cell exchanges heat power with its surroundings, where this heat outflow is directly associated with cellular metabolism. In relation to heat transfer, the characteristic thermal time constant τ can be introduced. To obtain it, we introduce some considerations from our previous results [13,19,20].

Some data of the cells are the mass density $\rho \approx 10^3$ kg m^{-3}, the specific heat $c \approx 0.6$ J kg^{-1}K^{-1}, and the convective heat transfer coefficient $h = 0.023\,\mathrm{Re}^{0.8}\mathrm{Pr}^{0.35}\,\lambda/< \mathrm{R} >$, where $\lambda = 0.6$ W m^{-1}K^{-1} is the conductivity, Re $= 0.2$ is the Reynolds' number, Pr $= 7$ the Prandtl's number, with a Biot number Bi $= 0.0056$ lower than 0.1 allowing the use of lamped elemnt model in heat transfer analysis, so we can write the first law as follows:

$$\dot{Q} = \frac{dU}{dt} \implies -h\,A\,(T - T_0) = \rho\,V\,c\,\frac{dT}{dt} \implies \frac{d\ln(T - T_0)}{dt} = -\frac{h}{\rho\,c\,< R >} \tag{11}$$

where h is the coefficient of convection, A is the surface area of the cell, which changes during the cell growth, V is the volume of the cell, $< R >$ is the volume/area ratio, a parameter which influences the fluxes through the cell's membrane, ρ is the cell mass density, c is the specific heat of the cell, and $(T - T_0)$ is the difference of temperatures between the cell temperature T and the environment temperature T_0. We highlight that the term $< R >$ is the geometric shape of the cell related to convection, and it plays a fundamental role in heat transfer, and:

$$\tau = \frac{\rho\,c\,< R >}{h} \tag{12}$$

it represents the characteristic time of the biosystem. The result emphasizes the volume–area ratio of cells in relation to heat exchange; indeed, Equation (12) links frequency to the structural and geometrical properties of the cell and its interactions with the environment. Each system has a specific response time to external thermal changes, i.e., the inverse of the cell's response time or the heat exchange rate that causes a variation in the entropy:

$$\Delta_i S = \frac{\dot{Q}\,\tau}{T_0} \tag{13}$$

considering the cell's environment as a thermostat.

In accordance with Schrödinger [26], in order to live, cells maximise the entropy outflow with the consequence that $d_e S \geq 0$. Consequently, in relation to Equation (7), an entropy variation related to mass (ion) fluxes is generated:

$$\Delta_{(ion)} S = -\Delta_i S = -\frac{\dot{Q}\,\tau}{T_0} \tag{14}$$

Now, we can consider that the ion fluxes are controlled by the cell membrane electric potential ϕ [25]:

$$d\phi = 2.3\,\frac{RT_0}{F}\,d\mathrm{pH} \tag{15}$$

where R is the universal ideal gas constant and F is the Faraday constant. Consequently, any change in ion fluxes determine changes in heat transfer and vice versa. However, any change in ion fluxes has consequences on the behaviour of the cell, as a consequence of the changes in its membrane electric potential; indeed, in 1969, Cone Jr. discovered that the onset of the M phase in the cell cycle is marked by a state of hyperpolarization, leading to the hypothesis that there is a connection between cell cycle progression and changes in membrane electric potential [45]. By 1970, he showed that membrane hyperpolarization can reversibly inhibit DNA synthesis and mitosis [46].

3.2. Biophysical Interpretation

In 1971, based on various experimental findings, Cone proposed a hypothesis regarding the essential role of the cell membrane potential. More recently, the critical importance of membrane electric potential has been emphasized in regulating vital cellular functions

such as proliferation, migration, and differentiation [47]. As a result, the pivotal role of membrane electric potential has been underscored in relation to various cellular functions.

As a consequence of the bio-engineering thermodynamic results and the findings in medicine, we conjectured that by applying electromagnetic waves at the system's resonant frequency $\nu = 1/\tau$, we can induce pH changes through adjustments in the membrane potential. An in vitro study using a specialised device applying Extremely Low Frequencies (ELF-EMF) at resonant frequencies to tumoural cell lines confirmed our conjecture. Indeed, Table 1 shows that cells exposed to these frequencies demonstrated reduced proliferation compared to control cells. In support to our theoretical and experimental results, in literature, ELF magnetic fields have been reported to affect the ion transfer, e.g., in the ethanol production of *Saccharomyces cerevisiae* a mechanism involving modifications in ion transport has been pointed out to stimulate cellular metabolic activity [37]. Our approach suggests a comparable effect of electromagnetic waves, conjecturing that ELF-EMF induced ion fluxes enhance catabolic pathways to preserve ionic gradients, potentially at the cost of biosynthetic processes required for proliferation.

Based on thermophysical investigations [48–50], the distribution and mobility of charged species, local field intensities, and dipolar arrangements are sensitive to variations in the external electric field. These shifts are converted into conformational transitions that directly impact protein functionality. Specifically, these electromagnetic waves influence the transport of mass, energy, and ions across the lipid bilayer. To clarify the regulation of these trans-membrane fluxes, we must examine the ionic concentration gradients on either side of the membrane. These gradients necessitate a cellular redistribution of both chemical species and energy, subsequently triggering various biochemical and biophysical pathways essential for healthy cell function, which can potentially remediate oncogenic behaviour. The primary biological mechanism identified in the literature [33,51] involves the capacity of electromagnetic waves to modify voltage-gated anion channels in both the plasma and mitochondrial membranes. This alteration likely triggers cysteine proteases, specifically caspases, via the translocation of cytochrome c from the mitochondria into the cytosol. The clinical ramifications of these pathways include [52,53]:

- Induction of apoptosis in malignant cells;
- A systemic decrease in tumour progression.

From a thermodynamic perspective, these biophysical effects can be interpreted through two lenses: (i) the EMF-induced mitochondrial response results in fluctuations in entropy production linked to thermal flux, and (ii) mitochondrial dynamics are intrinsically tied to H^+-flux across cellular boundaries. This activity leads to shifts in local pH and corresponding fluctuations in the membrane's electrical potential. Experimental data indicate that prolonged exposure to low-frequency, low-intensity EMFs inhibits the growth of various cancer cell lines. This phenomenon is linked to heightened mitochondrial activity without a significant rise in ATP concentrations [35], which can be attributed to the biophysical consequences of trans-membrane thermal flux, as corroborated by recent in vitro studies [35]. Furthermore, mitochondrial ATP consumption is governed by oxidative phosphorylation: ADP + P_i + $H^+ \rightarrow$ ATP (where ADP denotes adenosine diphosphate, P represents phosphorus, H^+ is the hydrogen ion, and ATP signifies adenosine triphosphate). This process involves hydrogen ions, which may be exported from the cell via V-ATPsynthase [54]. Electromagnetic waves interact directly with cellular biomolecular frameworks; notably, tyrosine has been identified as a primary target. This interaction impacts several proteins, such as MET and HGF/SF, whose regulatory signalling pathways are typically compromised in malignant states [55], often leading to transcriptional errors, impaired degradation, and aberrant downstream signalling crosstalk [55].

Table 1. Experimental results obtained concerning the growth variation of some cancer cell lines after exposure to the calculated resonant frequencies under a sinusoidal magnetic field of 100 μT maximum amplitude [14,15].

Human Cancer	Cell Line	Theoretical Frequency [Hz]	Experimental Frequency [Hz]	Growth Change [%]
Melanoma cell line	A375P	31.0 ± 4.6	31.0 ± 1.6	−15
Colorectal adenocarcinoma	HT-29	50.0 ± 5.5	50.0 ± 2.5	−19
Gastric cancer	GTL16	14.0 ± 3.0	14.0 ± 0.7	−24
Breast cancer	MCF7	5.0 ± 0.7	5.0 ± 0.3	−22
Breast cancer	SKBR3	8.0 ± 2.0	8.0 ± 0.4	−18

3.3. Assessment of the Integration of Thermal Resonant Model Within the Entropy Framework

This paper presents improvements that integrate our previous approaches, one based on entropy analysis and the other on heat transfer. Indeed, this paper presents an integrated synthesis of thermodynamic and thermal approaches to biosystems, with a particular focus on cancer. The key points are summarized as follows:

- In 2012, we have develops a link between two fundamental thermodynamic approaches in complex systems: the minimum entropy production rate, applied to various processes in physics, chemistry, and biology, and the maximum entropy production principle, which is employed in engineering contexts and is now regarded as more general. Furthermore, this integrated framework was theoretically proven and experimentally applied to ATP synthesis during anaerobic fermentation [56].
- In 2013 we introduced these results in the thermodynamic approach to cancer, characterising it as an open, complex, dynamic, and self-organizing system [57]. This theoretical result was recently used in different applications and simulations [58–60].
- In 2014, this model was applied to analyze transport processes in cancer [61], with a particular focus on membrane behaviour [62], yielding numerical results that are consistent with experimental findings [2].
- In 2020, this thermophysical approach has been refined and experimentally validated for analysing the growth behaviour of cancer cells, highlighting the resonant behaviour of heat exchange in living cells [63].

In addition to the previously reported experimental results [14,15], we conducted further experiments, the outcomes of which are summarized here. For more detailed information, the reader is referred to the original texts, quoted in the following summary:

- In 2015 [42], we evaluated the differences in energy flows between normal and immortalized cells when these distinct biological systems were exposed to environmental stimuli. These differences were initially assessed through theoretical analysis and subsequently confirmed experimentally. Notably, temperature variations between cells exhibiting different behaviours were found to be amplified by the interaction between the cells and external magnetic fields. Furthermore, experimental validation of the lumped model approach was conducted on various cellular models exposed to electromagnetic fields. Using infrared thermography, we were able to detect subtle changes in heat dissipation, measured as variations in the cells' internal energy, which aligned with theoretical predictions. This is evidenced by distinct thermal dispersion patterns observed when normal and immortalized cells are subjected to electromagnetic fields. We propose infrared thermography as an effective technique for discriminating between different thermal dispersion patterns, thereby enabling the distinction between normal and transformed phenotypes.
- In 2017 [14], to achieve selective interaction between electromagnetic fields and living cells, we applied our thermodynamic principles to analyze the thermo-chemical

output generated by cells in their environment. This combined biophysical approach considered changes in entropy as indicative of a metabolic shift that leads to reduced cell growth. The proliferation of six human cancer cell lines was measured as an output signal to validate the theoretical frequencies of the electromagnetic field that are specifically effective on distinct cell types.

- In 2019 [64], we demonstrated that the frequency capable of inhibiting proliferation in each cell type selectively reduced cancer cell growth without affecting healthy cells. This effect was abolished when calcium fluxes were inhibited. Our findings provide evidence of enhanced respiratory activity, characterized by increased expression of respiratory chain components and oxidative phosphorylation elements at both the mRNA and protein levels. Although this respiratory burst amplified the production of reactive oxygen species, it was not accompanied by increased ATP levels, suggesting that the energy was rapidly consumed in the adaptive response to the electromagnetic field. Taken together, our data demonstrate that, regardless of individual molecular defects, cancer cells can be controlled through specific irradiation that induces a mitochondrial metabolic switch, regulates calcium fluxes, and ultimately impairs cancer growth.
- In 2020 [65], a correlation was established between cellular parameters, such as the volume-to-area ratio, and electromagnetic frequency, demonstrating their influence on resonant intracellular molecular oscillations. These findings were further validated in 2022 [43] using three-dimensional (3D) culture models that closely mimic the characteristics of tumours in vivo. The cell membrane was modelled as a resistor–capacitor circuit, allowing calculation of the specific thermal resonant frequency, which was subsequently confirmed through experiments on both two-dimensional (2D) and 3D cell cultures of human pancreatic cancer, glioblastoma, and breast cancer. Assessments included cell proliferation, transcription levels of respiratory chain components, adenosine triphosphate synthase subunits, and uncoupling proteins. For the first time, we demonstrate that extremely low-frequency electromagnetic fields (ELF-EMF) inhibit growth while enhancing both coupled and uncoupled respiration across all analysed models. Notably, this metabolic shift was evident even in 3D cell aggregates.
- Lastly, in 2024 [15], we evaluated the effectiveness of the thermal-resonant-ELF applications against osteosarcoma by applying a targeted electromagnetic field frequency to three human osteosarcoma cell lines grown as both adherent cultures and spheroids. We measured the antitumour effects of the irradiation by examining the cells' responses to chemotherapeutic agents, which are generally ineffective against this cancer type. Notably, our findings revealed that this innovative combinatorial approach significantly enhances the efficacy of multiple chemotherapeutic drugs in both two-dimensional and three-dimensional cancer models.

4. Discussion

This work has provided a concise overview of the bio-engineering thermodynamic framework for understanding cell behaviour, aiming to control heat and ion fluxes for future possible support for the present anticancer therapies. This framework emphasizes the interactions between open systems and their external environments, highlighting how these interactions lead to variations in entropy generation. Such variations are influenced by the flows of mass (ions) and heat through the system's boundaries. This methodology establishes a connection among engineering thermodynamics, mechano-biology, biomedical engineering, biology, and medicine, positioning it as a new perspective for examining biomedical systems. Therefore, it represents an initial step toward exploring new avenues in thermodynamics, marking the beginning of an interdisciplinary field focused on the

engineering and physical analysis of biological systems. If the community of thermodynamicists pursues this approach to biological systems, it could unlock new research possibilities in biomedical and pharmaceutical fields, further integrating thermal sciences and engineering. Indeed, in relation to tumour, various cancer treatment methods are available, including chemotherapy, radiation therapy, surgery, immunotherapy, and monoclonal antibody therapy, among others. The selection of the most suitable treatment for a patient is determined by assessing their overall health, the specific type of cancer, the tumour's location, its grade, and the stage of the disease. Some forms of cancer require a combination of treatments because of their intricate nature. These therapeutic approaches often come with undesirable side effects, such as fatigue, nausea and vomiting, reduced appetite, pain, hair loss, and effects on nerves and muscles, along with potential metastasis and more. Therefore, the main objective of promising possible therapeutic research is to enhance treatment effectiveness while minimizing adverse effects and enabling clinicians to address cancers that are currently untreatable. Electromagnetic fields (EMFs) might serve as a low-cost, safe, complementary treatment alongside existing cancer therapies. There is evidence indicating that EMFs can influence cancer. Our considerations are supported by existing clinical studies in the literature [66–68], to which we refer for further details.

Many experimental studies support our conclusions and the proposed approach, but additional considerations must be addressed. The proposed thermodynamic approach enables us to describe how external fields affect the entire cell concerning its metabolism and energy management, particularly emphasizing the flows across its membrane, or the interaction between the cell and its environment. As a result, it facilitates the evaluation of the frequency of the electromagnetic field in relation to each cell's geometric and biophysical characteristics.

The findings underscore the significant role of the cell volume-to-area ratio in regulating flux. These insights could pave the way for novel strategies to enhance current anticancer therapies by adjusting external fields at resonant times, as experimental evidence suggests a reduction in cancer cell proliferation under such circumstances. The conjecture of a potential future therapeutic approach is based on recent findings showing that cell and tissue behaviours are governed by mesoscale physical principles, which can be described using physical variables such as force, density, shape, adhesion, etc. These physical factors combine to produce emergent phenomena like cell jamming, topological defects, and underdamped waves [69,70]. Importantly, these mesoscale physical properties arise before and guide key biological functions including cell division, extrusion, invasion, and gradient sensing. While related to biomolecular properties, these behaviours cannot be fully predicted by biochemical principles alone. Instead, biological functions emerge from mesoscale physical states that can be anticipated through a straightforward set of physical parameters. Indeed, experimental evidence [69,70] has shown that a universal feature in the flow patterns spontaneously arises in groups of collectively behaving cells. Specifically, from experimental approach it was shown that flow patterns in dog kidney cells, human breast cancer cells, and two strains of pathogenic bacteria all exhibit robust conformal invariance. The discovery of universal conformal invariance highlights that macroscopic features of living biological matter display universal symmetries—translational, rotational, and scale—that do not depend on the microscopic details of the individual cells or organisms. These findings demonstrate that flow patterns are highly conserved across different biological systems and suggest that such systems can serve as experimental platforms to test theoretical predictions about conformally invariant structures [69,70].

5. Conclusions

The novelty of this study resides in assessing the homeostatic response of cells by analysing the thermo-biophysical output—specifically, the mean entropy changes exchanged between the cell and its environment—rather than focusing solely on individual affected pathways. In this work, we applied the principle of action and reaction in terms of membrane flux variations and calculated the frequency that maximizes entropy generation within the cell-environment system for each cell type. According to our thermal biophysical model, cell growth inhibition results from these mean entropy changes; thus, we used proliferation rate as a measurable output to validate the thermodynamic model's predictions. Biophysical parameters derived from six distinct cell types were incorporated into the mathematical model to determine the optimal frequency for each cell line. Subsequent proliferation analyses confirmed both the efficacy and specificity of the selected electromagnetic field parameters in suppressing cell growth.

In summary, we propose a strategy to identify the ELF-electromagnetic wave parameters most effective at reducing proliferation in specific cell lines. Our approach employs a thermal biophysical thermodynamic framework to quantify the thermochemical output of cells exposed to ELF-EMF and to interpret the resulting biological effect—namely, decreased proliferation. Selecting tailored field parameters could provide significant therapeutic advantages in combating proliferative disorders, particularly cancer. This method presents a promising solution to the challenge of optimizing wavelength and intensity for different tissues and even for heterogeneous subpopulations within the same diseased tissue, a common scenario in cancer.

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