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Pulsed Modulation in Electro-Hyperthermia

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Abstract

Modulated electro-hyperthermia (mEHT) is one of the novel oncological treatments with many preclinical and clinical results showing its advantages. The basis of the method is the synergy of thermal and nonthermal effects, similar to the thermal action of conventional hyperthermia combined with ionizing radiation (radiotherapy). The electric field and the radiofrequency current produced both the thermal and nonthermal processes. The thermal effects produce the elevated temperature as a thermal background to optimize the nonthermal impacts. The low frequency amplitude modulation ensures accurate targeting and promotes immunogenic cell death to develop the tumor specific memory T cells disrupting the malignant cells by immune surveillance. This process (abscopal effect) works like a vaccination. The low frequency amplitude modulation is combined in the new method with the high power pulses for short time, increasing the tumor distortion ability of the electric field. The new modulation combination has much deeper penetration triplicating the active thickness of the effective treatment. The short pulse absorption increases the safety and decreases the thermal toxicity of the treatment, making the treatment safer. The increased power allows for reduced treatment time with the prescribed dose.

Keywords

Tumor, Pulsed Modulation, Nonthermal Excitation, Thermal Homeostasis, Cell-Killing, RF Current, Immunogenic Cell Death, Apoptosis

1. Introduction

Hyperthermia as a treatment for cancer has been recognized since ancient medicine. Conventional hyperthermia aims to achieve uniform heating of the tumor mass. The desired uniform heating condition forms the basis for dose calculation in clinical practice. Heat has been continuously employed in oncology, expecting

to narrow the therapeutic gap caused by cellular distortion efficacy limitations and conventional treatments' toxicity (see **Figure 1**). Electromagnetic heating accelerated the application of oncologic hyperthermia, making it a significant tool in the oncotherapeutic arsenal. While electromagnetic heating revitalized hyperthermia methodologies, the underlying principle remained unchanged: exposing the tumor to high temperatures. This heat, coupled with the heightened metabolic demands of malignancies, leads to ATP depletion [1], severe hypoxia, and, ultimately, acidosis, which contributes to cancer cell death.

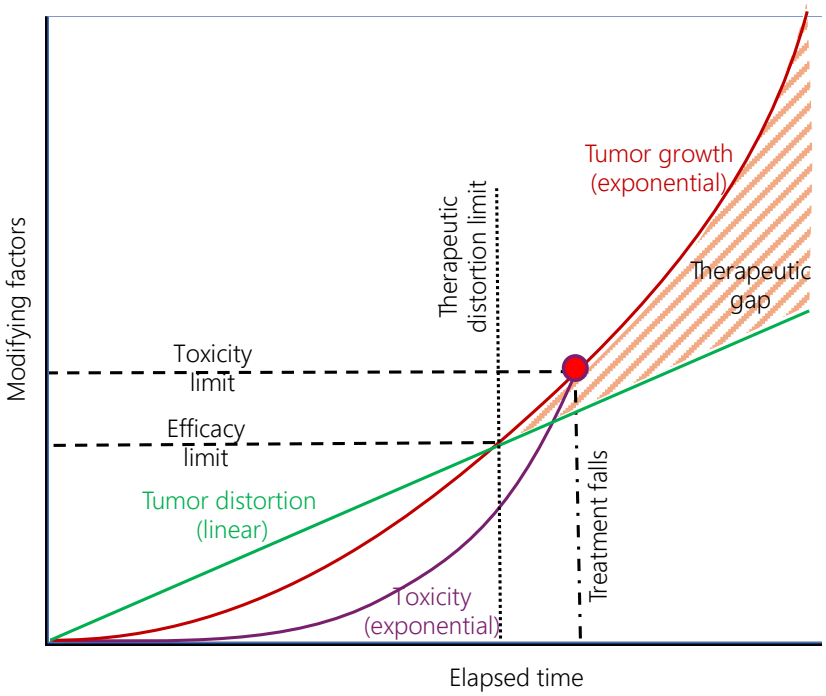


Figure 1. The development of tumors and their distortion. Cancer grows exponentially over time, while the distortion rate is linear. The exponential growth exceeds the distortion rate if the distortion cannot eliminate the tumor. Another limitation factor is toxicity, which is usually polynomial by time. The therapeutic gap is the missing therapy efficacy to the intensive growth of tumors.

The simple heating concept, however, does not consider thermal regulation and general homeostatic control of the body. The regulation mechanisms try to compensate for the unusual changes in the target and repair the damage caused. The stress proteins give a molecular basis of cellular protection [2], and numerous enzymatic reactions are activated to correct the unbalanced processes [3]. The primary regulating tool is the bloodstream [4], which tries to recool the heated volume, and the subcutaneous capillary bed works like an active heat exchanger to ensure the cooling capacity [5]. Oncologic hyperthermia can be a complementary tool to conventional therapies to reduce the therapeutic gap, increase the efficacy of traditional treatments, and expand patient survival with an acceptable quality of life. The increased blood flow can be used for reoxygenation to block the radiation-damaged DNA repair by enzymes, and it can also be used to deliver more

drugs to the tumor and increase their reaction rate by elevated temperatures. However, the therapy is limited by precise targeting (the heat spreads), thermal toxicity (burning in the healthy tissues), and the risk of malignancy by blood-stream-assisted malignant dissemination. Due to the complications, the heating task has a complex multilevel approach. The technical solutions must fit the biological and technical requirements and be optimal regarding quality assurance and medical regulations (Table 1).

Table 1. The biological, clinical, and technical tasks address different therapy requests, which must be optimized.

Tasks		
Biological	**Clinical**	**Technical**
Tumor cell destruction	Tolerable temperature	Appropriate energy delivery
Tumor-selective cell destruction	Destroy the malignance	Appropriate focus and tuning of the energy
Immunogenic cell death (ICD)	Antitumor immune-effects	Do not destroy the malignant info from the dying tumor cells
Complementary possibilities to conventional therapies	Improve the therapeutic effect, decrease the therapeutic gap	Make appropriate blood flow regulation
Kill the malignancy independently from its depth	Give also proper dose to deep-seated tumors	Increase the effective penetration depth of energy delivery

2. The Challenges of Oncological Hyperthermia

Hyperthermia with electromagnetic energy delivery faces multiple challenges.

1) Due to the regulation of thermal homeostasis, we quickly lose control of heat localization inside the body. The temperature is automatically spread in the neighborhood of the target. The growing temperature intensifies the thermal homeostatic regulation and tries to cool the heated volume primarily with increased blood flow.

2) In long-term treatment, heat diffusion and gradually heated blood heat the whole body to the fever range. This elevation could help the development of micrometastases due to the increased metabolic reaction rate. At the same time, due to the homeostatic regulation compensating for the increasing temperature, the metabolism decreases in the healthy parts.

3) The more extended thermal equilibrium (keeping the tumor at a fixed temperature) replaces only the heat loss primarily caused by the blood thermal exchange. This period has much less cell destruction than the heating phase when the SAR intensively forces the apoptotic processes [6].

4) Heat Shock Proteins (HSP) are intensively developed and play a key role in protecting the tumor against prolonged exposure to high temperatures. HSP requires 48 hours to return to the baseline [2].

5) The long thermal equilibrium helps the dissemination of cancer cells from the tumor, mainly because of the high blood flow gradient at the tumor boundary,

which has a high proliferation rate [7]. It increases the risk of metastases, drastically reducing the patient's survival time and quality of life.

6) The high blood flow may support tumor growth and increase the risk of dissemination. To reduce the blood flow, we have to limit the average temperature, while for the effectual cell distortion, we need higher than the physiological limit (>42˚C).

7) Growing hypoxia with more prolonged heating may increase the necrotic core of large tumors, which increases the risk of tumor lysis or cytokine storm. The goal is the apoptotic cell destruction of cancer, which needs to trigger proper signals and lower destructive heat.

8) The further complication is to choose a power high enough for the excitation but not too high to cause necrosis and avoid thermal toxicity, primarily the burning of the skin and the fatty tissues.

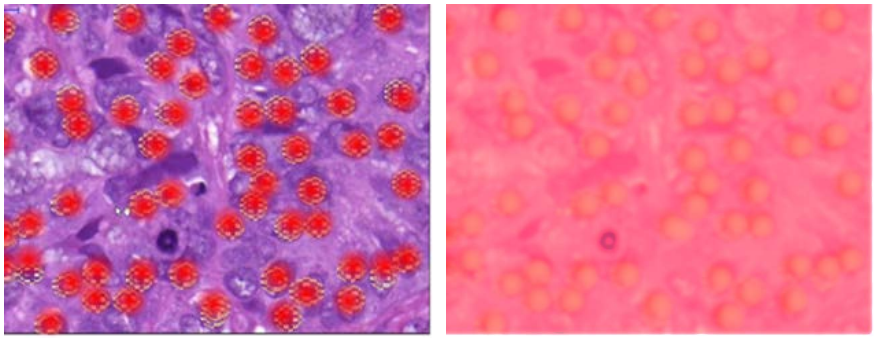
9) The electric field drives the energy absorption. The tumor is targeted by radiative, capacitive, or inductive coupling, which puts the energy into the tumor. Technically, the process is like the antenna matching at the radio broadcasts. However, the antenna has fixed parameters that do not change the coupling conditions. The electric parameters of the human body vary by patient and tumor location during the treatment, and the tumor's electric properties may change during the treatment, so the coupling must follow the changes.

10) From the immunogenic point of view, the average tumor temperature must remain under 40˚C. Higher temperature arrests immune activity [8]. The growing systemic temperature could give extra temperature to the heated tumor and go over the immunogenic limit, decreasing the mEHT efficacy.

11) The penetration of energy absorption sharply declines from the surface to depth into the body.

3. Method

To solve the above challenges, a novel method, the modulated electrohyperthermia (mEHT, oncothermia®), was developed [9]. The isothermal heating concept is unrealistic due to the massive thermal and bioelectromagnetic heterogeneity, and the energy absorption fits the structural peculiarities of the living organism. The technical solution of optimal energy absorption must consider the complexly interconnected micro and macro heterogeneities and be compatible with the physiologic parameters regulated by the organism's homeostatic control. The modulated electrohyperthermia (mEHT) changed the hyperthermia paradigm, using heterogenic, selective heating to target the death receptors of the malignant cells and induce apoptotic signals to destroy these cells [7]. In heterogenic heating, the specific absorption rate (SAR) changes by the position of the absorber ($\boldsymbol{x}$) and time (t), so the complete energy is the sum of SAR($\boldsymbol{x}$, t). The absorbed SAR is used to raise the temperature of the target by heating its environment by heat conduction and bloodstream-assisted heat convection. The point heating enlarges over time and heats the mass by the contained energy-absorbing molecules (**Figure 2**).

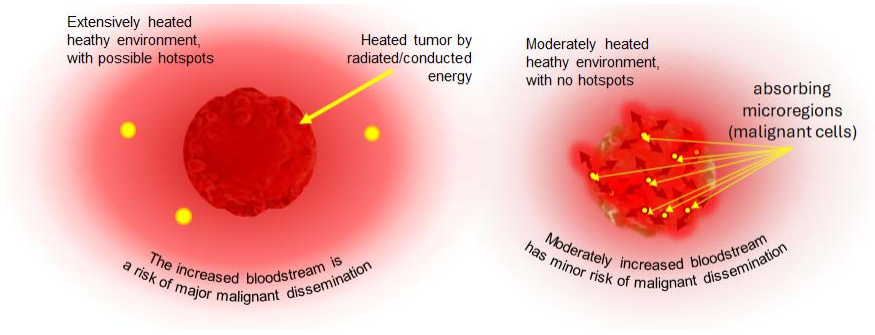


(a) (b)

Figure 2. mEHT heats heterogeneously in selected molecular clusters and transmembrane proteins (membrane rafts) on malignant cells. (a) The molecules' energy absorption heats at high temperatures (yellow dots), which conductively heats the cell (red circles), and the induced blood flow heats the complete mass convectively; (b) When the power is off, the mass starts to cool primarily by the blood flow, and in the end, it will be almost isothermal at a lower temperature.

The method uses amplitude-modulated 13.56 MHz carrier frequency [10] [11] to deliver the appropriate energy for hyperthermia processes. The key to proper focusing is the use of the electric and thermal heterogeneity of the tumor. The tumor specialties differ from the healthy tissues, and those allow selective heating of the cancer cells. The high metabolic rate of malignancy and the missing healthy cellular network can conduct the appropriately chosen radiofrequency (RF) current differently.

The cytoplasmic transmembrane proteins of malignant cells selectively absorb the RF current delivered energy [12]. The mEHT method selectively targets the membrane rafts of the malignant cells [13]. The malignant cells have a denser lipid-raft population on their membranes than their healthy counterparts [14] due to the high density of the unbounded transmembrane proteins from the broken intercellular network. The targeted energy absorption makes the precise and well-focused hyperthermic impact possible (**Figure 3**).



(a) (b)

Figure 3. The difference between conventional and heterogeneous heating. (a) The conventional hyperthermia aims to heat the tumor isothermally, homogeneously heating the tumor mass; (b) The naturally selected absorbers (clusters of transmembrane proteins, membrane rafts) heat heterogeneously, focusing the malignant cells only.

The thermal effect is proportional to the radius of the targeted rafts [15]. The weight heating of the nanoparticles is approximately. 100 - 1500 kW/kg [16]. The thermal effect of mEHT acts not only on the rafts but also heats the TME and the neighboring tissues to a less significant level. Thermal energy absorption of the rafts by mEHT is like the nanoparticles, and it is approximately 350 kW/kg, which is comparable to nanoparticle heating [11] (**Figure 4**). The energy absorption process is more complex in the case of rafts than in nanoparticles, having additional molecular excitations [14] and nonthermal processes that do not happen in nanoparticle heating. The nonthermal impact excites protein complexes, which thermal processes heat to an optimal chemical reaction rate. The thermal influence of this heterogenic heating is identical to conventional hyperthermia, but it targets, more precisely, the malignant cells.

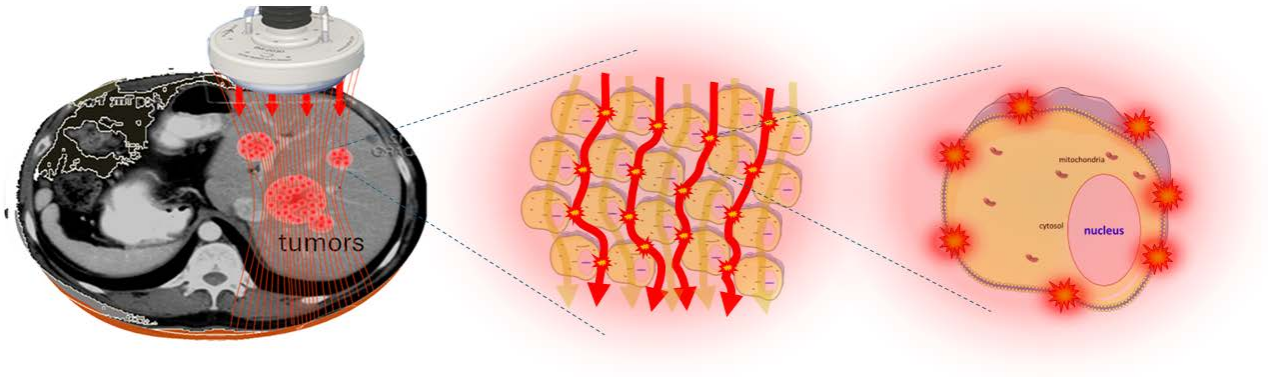


Figure 4. The applied special RF current choses the tumors. The majority of the current flows in the extracellular matrix, and excites the transmembrane proteins (rafts). The absorbed energy on rafts heats the cells and also triggers intracellular signals.

The rafts are heated to optimally high, but the tumor temperature remains under 40˚C. This relatively low average temperature avoids downregulating the cytotoxicity of immune impacts, which are inhibited in higher temperatures [8] [17] [18]. However, on the contrary, conventional hyperthermia intends to have direct cellular thermal damage, which needs higher temperatures (above 41˚C - 42˚C [19]). The heterogenic, selective heating matches both seemingly contradictory demands, having high temperatures (>40˚C) in nanoscopic absorbers, and the tumor mass average remains under 40˚C.

The mild average temperature (39˚C - 41˚C) intensifies blood perfusion [20], optimizing the reoxygenation for complementary radiotherapy and delivering chemo drugs in a targeted manner, enhancing their reaction rate. The preclinical experiences verify [21]-[23], and the clinical studies validate these effects [24] [25].

The delivered thermal effect is concomitantly applied with the nonthermal processes offered by the electric field (**Figure 5**). The heat drives the chemical reaction rate, and the thermal energy activates numerous temperature dependent molecular pathways. The nonthermal energy promotes such processes which are normally not excited by heat only, similar to the chemical "machinery" of the life, which needs different energy involvenet than the heat energy alone. The synergy of the thermal and nothermal energy absorption produces apoptosis of the

malignant cells.

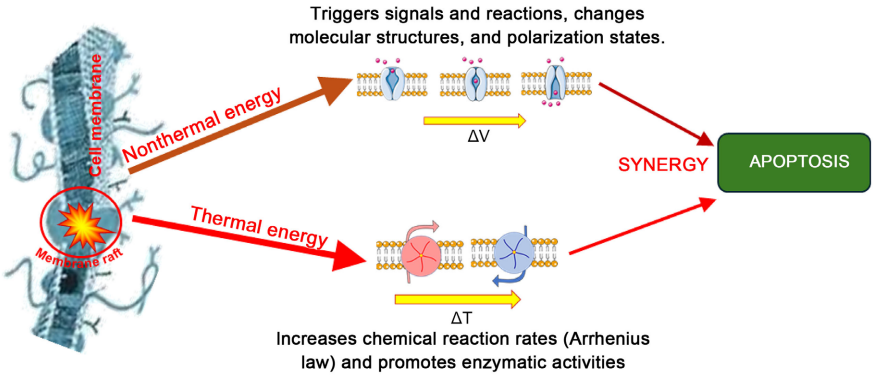


Figure 5. The synergy of thermal and nonthermal energy produces apoptosis of malignant cells.

The technical challenge of optimal and variable coupling is solved with precise and dynamic impedance-matching [26] [27]. The electronic solution is tuning the system to the measured impedance of the treated individual. The tuning seeks to form a touching situation as a purely metallic electrode would be fitted to the skin directly. This matching situation calculates the actual energy loss carefully, controls the reflected power, and matches the resonant compensation of the surface capacitor of the adipose tissue. The impedance-matched capacitive coupling produces the appropriate RF current density. The matching of the load in the RF circuit harmonizes the multiple interconnected parameters responsible for the conductivity [28]. The matching transforms the varying complex impedances at constant carrier frequency 13.56 MHz to nominal reference (conventionally real. 50 Ω) impedance [29]. The selection of the membrane rafts for energy absorption solves this challenge [13].

Our goal is to apply the pulsing technique for the mEHT method. The pulsing could be characterized by the duty cycle (D), which is the ratio of the pulse width (t_p) and the repetition time (t_r). This D time ratio is equal to the ratio of the mean power (P_m) and the pulse power (P_p):

$$\frac{t_p}{t_r} = \frac{P_m}{P_p} = D \tag{1}$$

The novel pulsing modulation and the standard modulation of mEHT [30] [31] give a unique mixture (**Figure 6**).

4. Results

The solution to optimize the ratio of the thermal and nonthermal components of the RF current needs a precise fit to the individual case, which changes in every treatment. The active impedance-guided capacitive solution (like mEHT) can use the bioelectromagnetic specialties of the malignant cells directly by RF current flow when it is matched to the optimized current. The apparent contradictory requirements (high frequency for selection and low frequency for action) are solved

with low-frequency modulation of the high-frequency carrier [31] [32]. With the high carrier frequency, the interfacial polarization effect [32] is optimal for the selection using β/δ frequency dispersion range [32] [33]. However, the optimal excitation, the polarization-dependent extrinsic molecular signal of selected membrane rafts, requires low frequency, which is in the α-dispersion range. The α-dispersion frequency in living tissues refers to the frequency range where the dielectric properties of the tissue exhibit significant changes due to the relaxation times of molecules and ions present in the tissue [34]. The cell membrane rectifies the modulated signal [35]-[37], cutting the high-frequency carrier and presenting a simple low-frequency spectrum of the modulation signal alone.

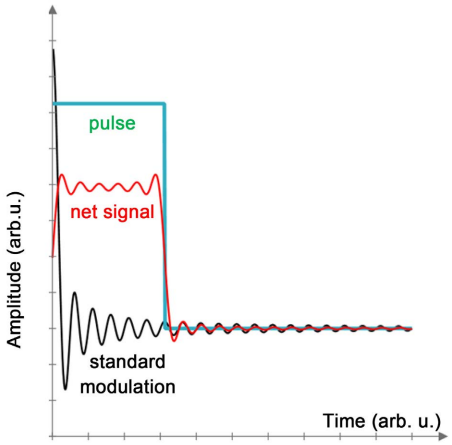


Figure 6. The modulation and temperature during a limited time pulse. The net signal of the modulation is the convolution of the mEHT standard and the pulsing. When no next pulse happens, the temperature returns to the baseline.

The precise impedance matching, adapted to all target changes, promotes a high penetration depth into the body [38]. The tuning adaptation constrains the spreading, but the depth does not exceed 20 cm [39].

The current density (j) is a characteristic dose in impedance-matched situations like mEHT. It does not depend on the technical losses outside of the target. When the matching is correct, the current that goes into the body in one electrode comes out in the other without loss. However, isodose of j is only an average in heterogeneous material. The statistical average of the current density over the whole target is similar to the dose in chemotherapy, where the drug distribution is also heterogeneous in the various tissues. Still, in the statistical average, we declare homogeneous drug delivery all over the body, giving the dose by the subject's size. The electric field ($\boldsymbol{E}$) generates the j: $\boldsymbol{j}=\sigma\boldsymbol{E}\left(\frac{\mathrm{A}}{\mathrm{m}^2}\right)$, where σ is the average conductivity of the tissues where the current flows through.

The electric field drives both the thermal and nonthermal processes. Consequently, keeping the current density constant provides the dose for the treatment. The j in first approximation does not depend on the A size of the electrodes. The $A=r^2\pi$ size of a circular electrode with a radius r defines the j current

density from the provided current (I): $\boldsymbol{j} = \frac{I}{A}$. According to the Ohm-law, the electrode voltage (V) and the resistivity (R) between the electrodes determines the I: $I = \frac{V}{R}$. In a first approximation, the current flows in a cylinder between the two circular electrodes, so $R = \frac{d}{\sigma A}$, where d is the distance between the electrodes, so the thickness of the body. Consequently, $\boldsymbol{j} = \frac{V\sigma}{d}$, constant when the applied voltage does not change on the same patient. The power (P) of the absorbed energy depends on the square of the field: $P = \sigma \boldsymbol{E}^2 = \frac{1}{\sigma}\boldsymbol{j}^2\left(\frac{\mathrm{W}}{\mathrm{kg}}\right)$. When the power kept constant, the $\boldsymbol{j} = r \cdot \sqrt{\frac{P \cdot \sigma \cdot \pi}{d}}$, so the current density increases by the electrode size when the power is constant. This surprising increase of $\boldsymbol{j}$ is approximately compensated with the growing radiation loss by the electrode size, so the simple dose monitoring by the power [39] [40] could be the dosing instead of by the local temperature high efficacy of current matching [41]. The higher power increases the current density dosing by $\boldsymbol{j} \sim \sqrt{P}$. Conventional hyperthermia heats the tumor mass and needs much higher incident power than mEHT, which heats only a tiny fraction of the membrane rafts of the selected malignant cells.

Conventional clinical hyperthermia uses intensive surface cooling to avoid skin and subcutaneous burns caused by heating. The cooling takes away a part of the incident power, which has to be higher to provide the same effect, so the treatment current density increases by $\boldsymbol{j} \sim \sqrt{P}$. Due to the thermal mass heating, the complete penetrating power heats the target. However, the situation differs in mEHT practice. A high current is also desired in mEHT, but due to the precise dosing, the cooling of the surface has to be somewhat limited. The consequence of the relatively low power, which is optimal for clinical control, is that it does not allow for an increase in the current density. It was shown *in vitro* mEHT experiments that when the average thermal component is arrested by the cooling down of the cells back to the 36˚C baseline, the apoptosis grows by the incident power, which determines the RF current density. However, the applied cooling (energy loss) significantly modifies the incident power so that it cannot be used for dosing. The dose is the current which is forced to flow through the patient. The current flows through the patient independently from the energy loss, which characterizes only the absorbed SAR. The electrode size distributes the current, but the $\boldsymbol{j}$ current density does not depend on it when the voltage is kept constant.

The current density represents an isodose in the mEHT. The nonionizing RF radiation works like the isodose intensity of ionizing radiation in radiotherapy. From a hyperthermia point of view, it is a complementary therapy of nonthermal nonionizing radiation to conventional thermal treatment. The mEHT thermally generates optimal conditions for the concomitantly present nonthermal electric field. The combination improves the reaction rates and enzymatic reactions.

Thermal and nonthermal effects synergy is like conventional hyperthermia with radiotherapy [42]. The modulated RF signal of mEHT triggers resonant excitations of the proteins [43], driving an extrinsic signal pathway for apoptosis [44] in a dose-dependent way [6].

The rise in temperature has a typical curve in hyperthermia (**Figure 7**). The starting period is the same as for any other solid state; the temperature linearly grows by the constant power. The linear slope declines when the target starts to exchange heat with the intensified blood flow and surroundings. The temperature grows slower despite the same power and becomes steady when equilibrium with the cooling effects. In this period, the absorbed energy equals the heat loss, replacing only the energy exchanged with the environment. Cellular damage is controlled by the ratio of the heating temperature to the growing and keeping periods. The heating period when the temperature grows has essential importance. The increasing thermal effect and the intensive molecular excitations (primarily by polarization nonthermal effect) characterize the process due to the thermal improvement of the molecular reaction rates. The thermal and nonthermal factors are unchanged in the heating phase of a stable temperature. However, it is observed that mEHT significantly increases the apoptosis in the heating-up period, while the temperature-keeping equilibrium has only moderate cell destruction [6]. Applied step-up heating uses this difference to improve apoptotic processes [45].

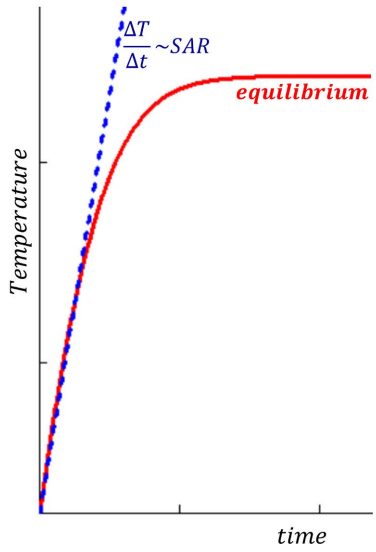


Figure 7. Typical pattern of temperature development. The initial linear growth heats the tumor, while the heat transfer to its neighborhood modifies it after a time lag. The formed equilibrium needs only such energy, which replaces the heat by exchanges, while the tumor remains on constant temperature.

In equilibrium, the absorbed heat replaces the lost energy, not heat further at higher temperatures. Equilibrium depends on the heat loss to the environment of the tumor. Primarily, the blood flow regulates the loss of energy [46] and secondary the heat conductivity of the host tissues, which is driven by the

temperature gradient between the tumor and the host. Usually, the SAR for equilibrium is less than that for temperature-grooving tumor heating (Figure 8). The timing of the decline of the initial linear slope depends on thermal conditions and *in vivo* on the physiologic feedback, which starts to cool the heated volume. The feedback differs by the tumor size, tumor location and the personal reaction abilities in treatments and of course, defined by the temperature limiting preset conditions (usually ≤ 42˚C) where the treatment continuously runs.

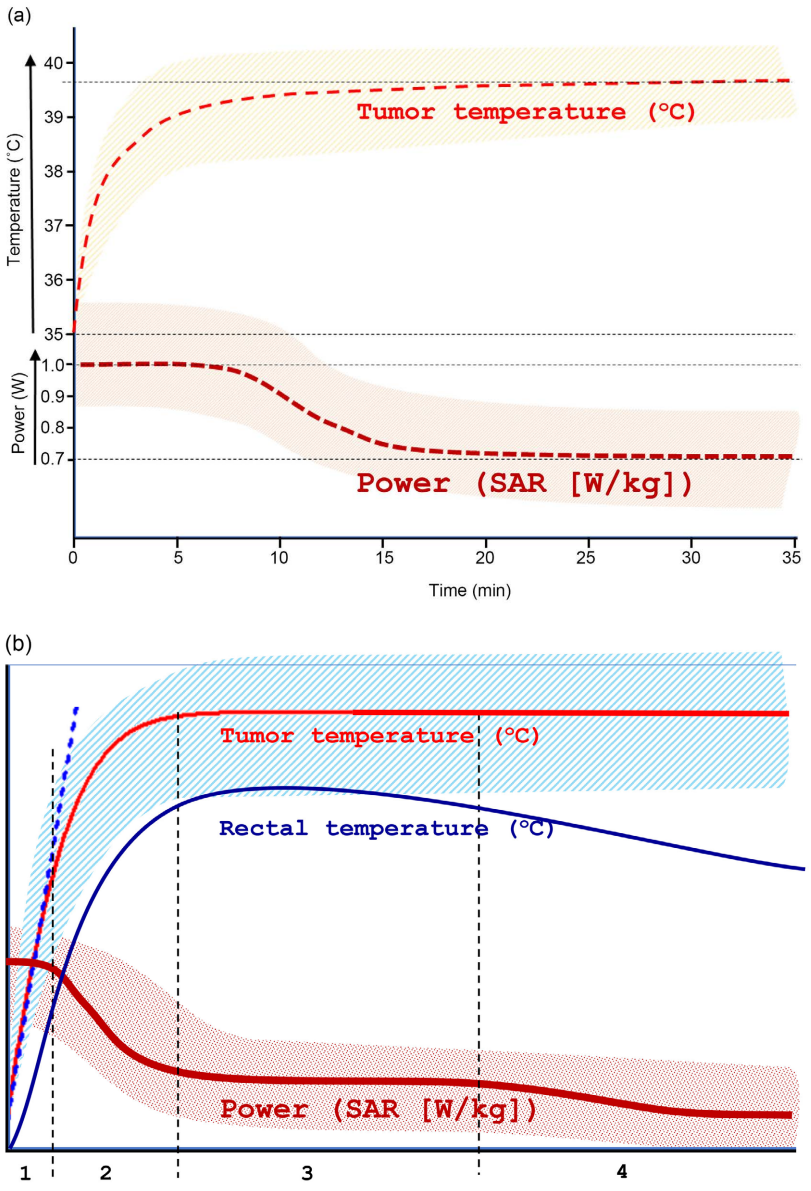


Figure 8. Relation of power and temperature patterns by time. The requested power is higher at the heating phase for temperature increase than in the phase of heat exchange equilibrium. (a) Both the power and the temperature are unchanged in the equilibrium (such shape is shown *in vitro* [47]); (b) While the tumor temperature is in equilibrium the power may be decreased in pulmonary heating, when the intensive breathing could not stabilize the heat-exchange but the final equilibrium needs less energy. (Such shape is shown *in vivo* pulmonary treatment [48].

When the power is switched off, the target is cooled by the bloodstream and by heat conduction to neighboring tissues. The physiological feedback of homeostasis has a time lag of a few minutes. Until this time, the rafts and cells are heated quasi-adiabatically without heat loss by the effectively cooling bloodstream. During this period, the temperature (T) rise solely depends on the SAR only, allowing the measurement of the effective SAR with the $\frac{\Delta T}{\Delta t}$ Afterward, the absorbed energy does not increase the temperature. The temperature development has a saturated curve. The physiological self-time in humans, which determines the time lag of the heating modification, is ~6 min [46] (**Figure 9**). The temperature change follows the thermal washout. The original application of the washout time was to characterize the drug washout [49] (like chemo-drugs, radiofarmacons, tracers, blood-delivered molecules, and nanoparticles) from the tissues, but in general, it is also used for the "wash out" of excess heat energy from the target. The primary difference between the thermal washout and the washout of various blood-delivered particles or molecules is the heat diffusion, which depends on the tumor and the host thermal properties, depends on the blood flow in the tumor and its neighborhoods, and depends on the temperature difference (gradient) between the heated tumor and its host tissue. The metabolic heat also affects the thermal washout.

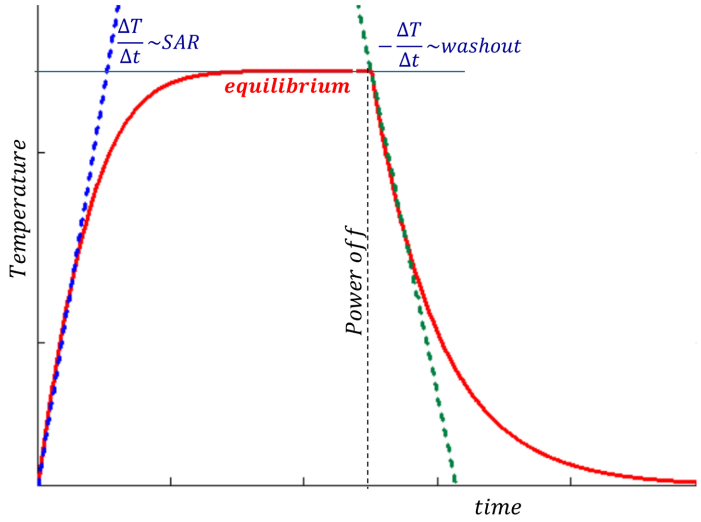


Figure 9. When power is off, the wash out by blood flow intensively cools down the tumor to the baseline.

The living system has a self-time which depends on the individual complexity [50]. Thermodynamic optimizing varies the self-time [51], which is connected to allometry structure [52], and it scales with the allometric factor $\alpha \approx \frac{3}{4}$ [53]. The time of thermal washout can be modified by changing the metabolic rate by lowering the temperature, and it will cause a longer tail to the washout function in time. Consequently, metabolic modification is an addition to the dynamism of

cooling. The physiological self-time of the thermal washout is ~6 min in healthy condition. For example, the thermal washout of a tissue from 42˚C after 30 - 60 min microwave heating is ~6.5 min [54].

The apoptotic cellular degradation could be used for dosing in the active heating period [6], and it shows the efficacy of the therapy *in vitro* (U937 cell line) [55]. The current is proportional to the percentage of apoptosis [56], because the necrosis remains low (<2%). The standard mEHT treatment has a synergy of thermal (41˚C) and nonthermal effects at 8W without cooling energy loss, while the samples that were forced to remain in the temperature baseline produced only nonthermal effects depending on the applied power [56] (**Figure 10**).

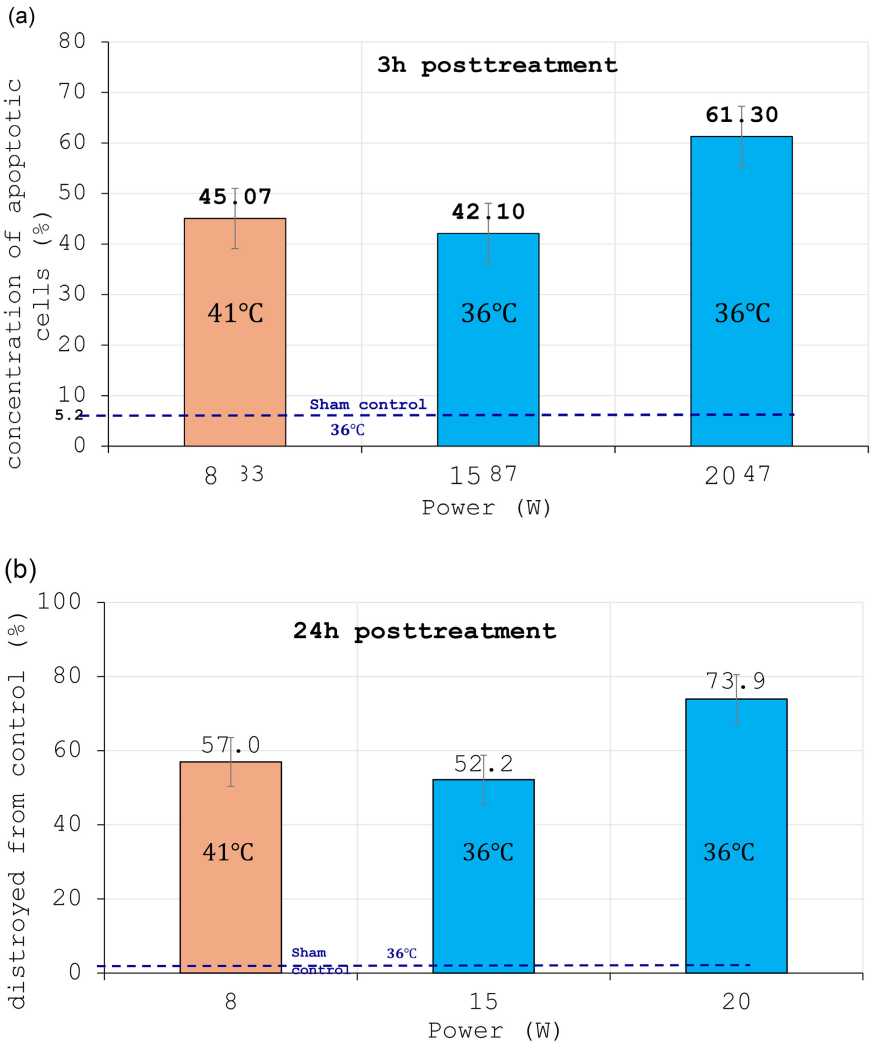


Figure 10. The cellular distortion of mEHT *in vitro* U937 cell line [57]. Intensive cooling of the sample kept the cell-contained medium at 36˚C, while the standard treatment was at 41˚C. Due to the energy taken by cooling, higher power was requested to maintain thermal balance. (a) 3 h after treatment the standard mEHT shows apoptosis (necrotic cells < 2%), with various power (the applied current is $I \sim \sqrt{P}$), and temperatures. The cooling forced samples remain in the temperature baseline, so show the impact of the nonthermal processes only. (b) 24 h posttreatment results with same conditions as (a).

The *in vivo* experiments on HT29 tumors in xenograft mouse model also proves the difference between the thermal and nonthermal components of the incident power [57] (**Figure 11**).

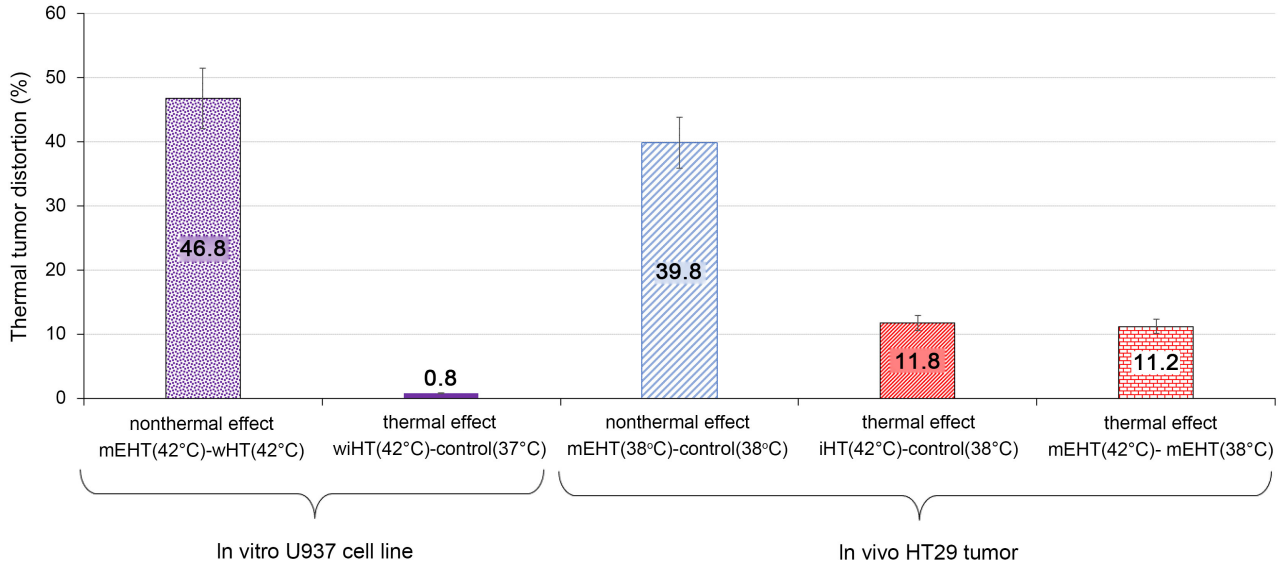


Figure 11. The nonthermal effect of mEHT could be measured by its difference from wHT at the same temperature (42˚C) *in vitro* [56]. The *in vivo* experiments show a similarly high nonthermal effect in HT29 tumor xenograft [58]. The thermal effect is measured in two ways: the thermal destruction of the infrared heated tumor (iHT), deducting the value of the untreated control, and showing it on the same deduction with mEHT. The obtained thermal effects are the same as those measured using these two methods.

The heating pattern does not follow the rectangular pulses. The spreading of the heat extends the temperature growth to the neighboring volumes. The result depends on the pulse width and the heating pattern of the target (**Figure 12**).

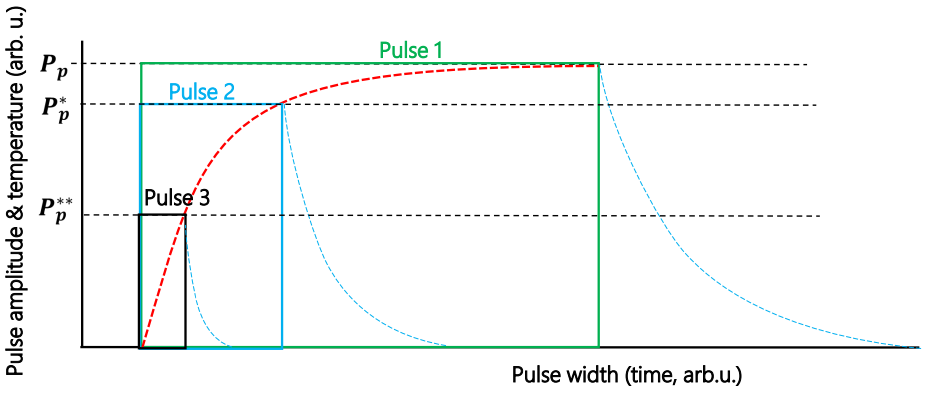


Figure 12. Various pulses have different temperature patterns. The short pulse is active in the linear part of the temperature growth (pulse 3), and quickly cools back to the baseline from this little temperature increase. The wide pulse contains the equilibrium phase (pulse 1), while in the middle range the pulse cuts the temperature curve in indefinite position (pulse 2).

The temperature of the tumor depends on the target's thermal parameters (**Figure 13**).

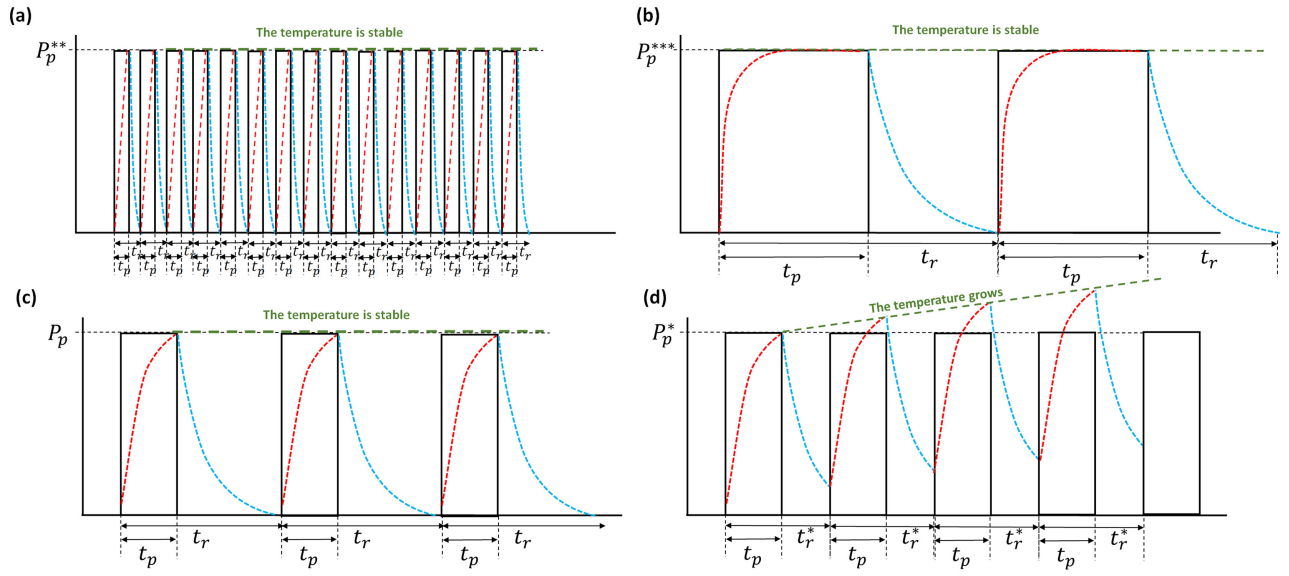


Figure 13. The electric pulsing and its thermal reaction differ and depend on the duty cycle $D = \frac{t_p}{t_r} = \frac{P_m}{P_p}$. (a) The pulse heats the material only in its starting (quasi-adiabatic) period when the physiological feedback is not active (pulse 3 in **Figure 9**), and the break period of the pulsing is long enough to cool the energy-absorbing microdomains back to baseline from the relatively small temperature growth. The temperature is practically unchanged, but the nonthermal effects could be activated when their characteristic time is shorter than the t_p pulse width, despite the possible high repetition frequency. (b) When the pulse is long enough till the stable temperature (pulse 1 in **Figure 9**), and the break time is enough to cool the target; the temperature remains in the saturated equilibrium. (c) When the pulse length is in the transition phase of the temperature development (starting physiological feedback, pulse 2 in **Figure 9**), and the break is enough for cooling to the baseline, the temperature will be stable but will not reach the same value as the thermal equilibrium in (b). (d) When the pulse repetition is quicker than necessary to cool down to the baseline, the temperature rises, despite the constant P_p power. The change of t_0 may drastically change the thermal pulse at the same electric pulsing. The temperature growth can happen in all the pulsing in **Figure 9**, it depends on only the necessary time to return to baseline temperature.

The small duty cycle may keep the temperature at the baseline. Noteworthy, the micro absorbers experience growth and drop in temperature, while the average mass temperature is unchanged (**Figures 13(a)-(c)**). When the t_r repetition period is longer than completing the physiological feedback (about 6 min), then the continuous and pulsed heating do not differ in the measured temperature. However, at larger duty cycles, the cooling period is not enough to reach the baseline again, and the temperature of the mass average grows repeatedly (**Figure 13(d)**).

Learning that the heating phase has the highest SAR (see **Figure 5**) *in vitro* experiment showed that higher SAR produces higher apoptosis [6]. Using this observation a considerable increase of apoptosis was shown in pulsing experiments *in vitro* [6] and *in vivo* [58]. Another *in vivo* experiment strongly supports the pulsing idea [59] (**Figure 14**). The experiments show the importance of the pulse amplitude, which has to be high enough producing better efficacy than the continuous mEHT.

Power determines the response. When the pulse's power is in the continuous power range, the response of pulsed mEHT does not differ from the standard mEHT efficacy. When the pulsed power is more than double than the continuous one, the pulsed treatment shows significantly increased response (**Figure 15**).

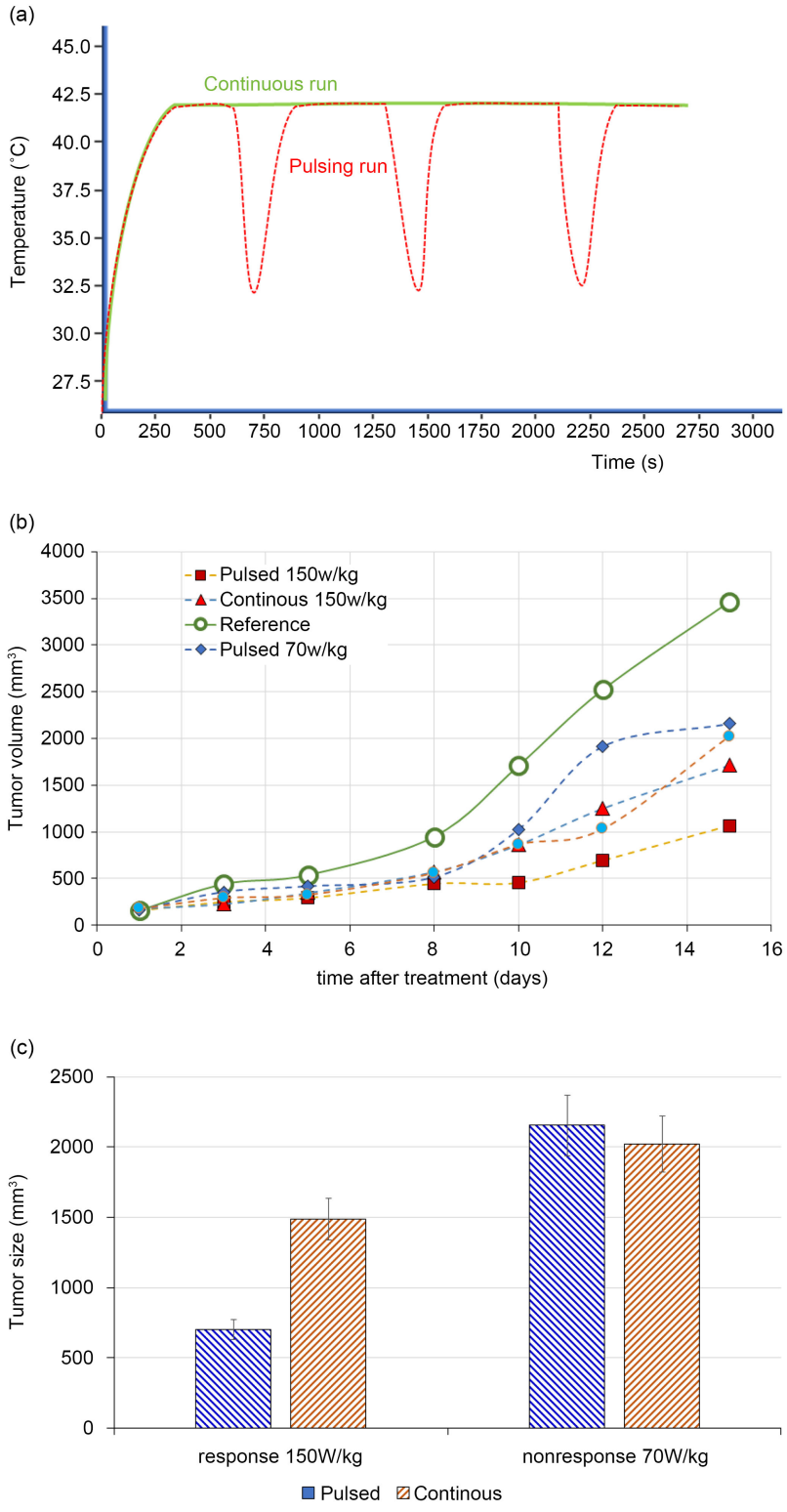


Figure 14. Pulsing *in vivo* experiment (HepG2 tumor in mice [60]). (a) temperature change in pulsed and in continuous mEHT treatment; (b) The tumor volume after treatment is continuous and pulsed treatment with standard SAR (70 W/kg) and high SAR (150 W/kg) measured by the initial slope of the temperature growth; (c) the results on the 16th day post-treatment.

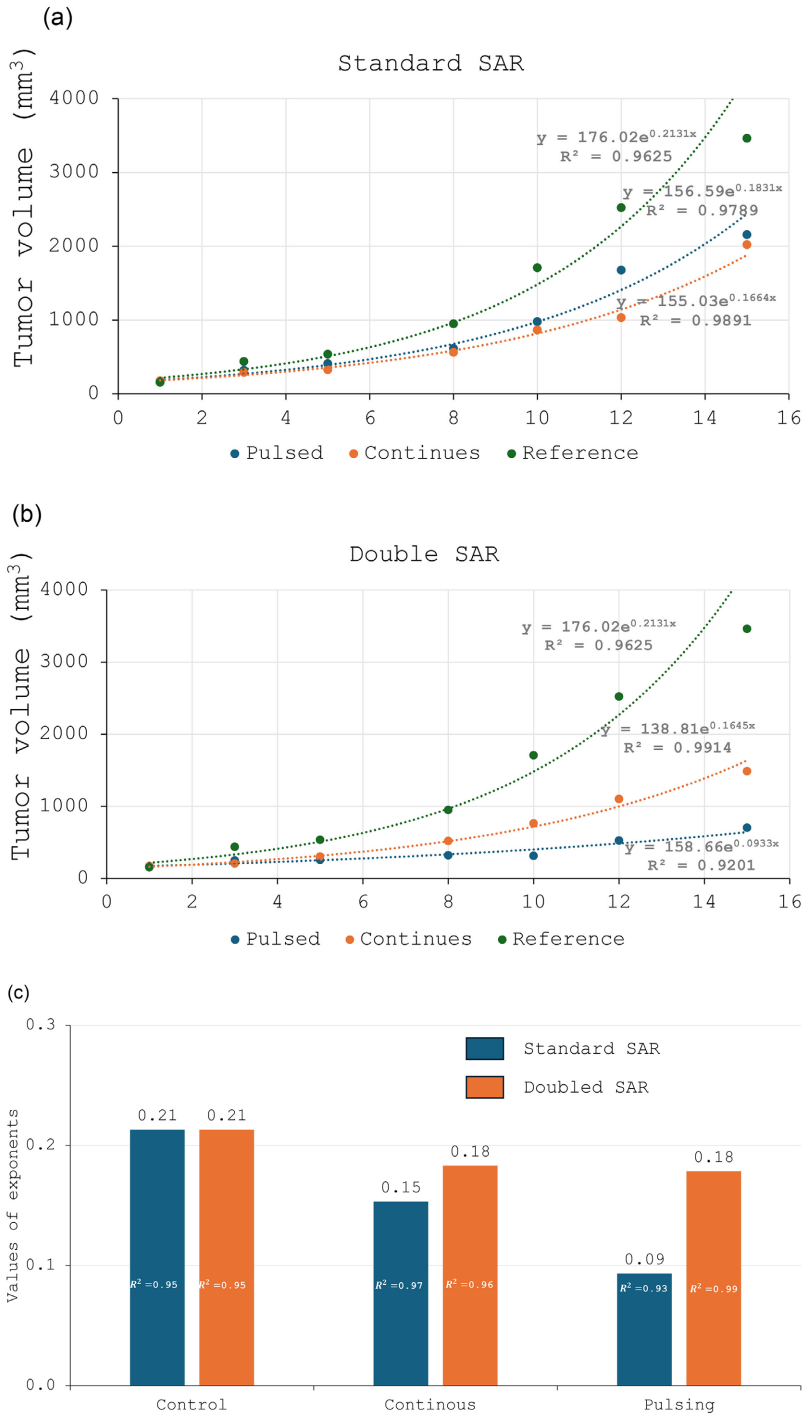


Figure 15. Comparison of the pulsing and continuous mEHT. (a) The continuous and the same SAR pulses do not differ significantly in tumor destruction results; (b) Increase the SAR by 2+, the pulsed efficacy has significantly better tumor destructive potential. The exponents and their correlation of the growing tumor after different mEHT treatments.

5. Discussion

Different studies were performed to check the benefits of pulsing in hyperthermia

applications. The theoretical estimation of the thermal effect of external heating with laser pulses in local thermal non-equilibrium approach [60]. The theoretical considerations in the bioheat model show that pulse heating is better for avoiding the thermal toxicity of healthy tissues [61]. Pulsing electromagnetic treatment is often used for electroporation, showing its better performance with higher pulse amplitude in combination with chemotherapy Bleomicyn, which is shown in chemoresistant cancer stem cells *in vitro* [61]. The multicomponent complementary treatment (electro-radio-chemotherapy) also indicates the superiority of the pulsing application, studied *in vivo* (SCCVII tumor, C3H mice) [62]. The hyperthermia with magnetic pulses and high duty cycle (0.8 - 0.9) showed optimal results for breast cancer with minimized adverse effects [63] inducing damage to the tumor microcirculation. A low frequency, low amplitude alternating current makes electric stimulation, which massively and significantly enhances the efficacy of the complementary doxorubicin chemotherapy in numerous cell lines *in vitro* [64]. The pulsing targeting allows high temperatures in pulse with minimal loads of the healthy tissues around in malignant melanoma treatment [65]. All information in the pulsed hyperthermia approach emphasizes the method's benefits independently of the other energy source.

When the tumor temperature grows linearly in the initial heating period, the starting energy delivery creates noticeably different heating processes than when the temperature grows nonlinearly and slower. During the linear period, the target solely absorbs the energy, which is why, later, the different types of heat transports modify the temperature growth, and a part of the heat energy is lost for the heating of the tumor. The nonthermal effect also decreases in the nonlinear heating period because the current density decreases by spreading the RF current to wider areas by depth and time. The thermal optimization for nonthermal processes also decreases in the target. The pulsed electromagnetic energy shows a massive nonthermal effect when the pulsing power is high [66]. The experimental skin layers also may suffer pulsing heat damage [67]. The chemical and thermal process in pulsing may activate the TRPV3 vanillin receptor [68], which participates in immunogenic effects in mEHT applications [12]. The pulsed electric treatment induces transient reversible opening of the blood-brain barrier (BBB) [69]-[71], promoting drug delivery to the brain. The BBB opening mechanism could impact nonthermally, involving the tight junctions [72]. The pain management of the pulsed electric field is an active pain killer [73], which increases the quality of life of patients suffering from severe pain. Thermal cycling shows benefits in conventional hyperthermia, enhancing the anticancer processes shown in pancreatic malignant cells [74] and treating humans [75]. The oncological applications of pulsed electromagnetic sources have a prosperous future [76].

The pulsed hyperthermia applies heat in short and intensive absorption with controlled bursts with constant amplitude. While continuous hyperthermia has uncontrolled heat transfers to healthy surroundings, pulsed targeting is controllable and gives great advantages to precise therapy. It reduces the risk of thermal

toxicity in healthy tissues, promoted by the usually higher blood perfusion in the healthy tissues than in the tumors. Pulsed heating has a higher potential for tumor distraction due to the higher power maximum in the pulse, while the tolerability by patients increases due to the reduced overall heat coverage.

The new mEHT pulsing modulation has multiple additional benefits to the continuous mEHT operation. The RF current is an active factor of mEHT. The current selectively flows through the target. In pulsing conditions, the extreme power gives a proportionally sizeable current density, which makes an effect. Pulsed heating can be more effective at killing cancer cells than continuous heating at the same average temperature.

Pulsing during treatment starts a gradual temperature increase, which is crucial for inducing cell death and triggering immune responses. Increased blood flow to tumors between pulses delivers more oxygen and nutrients, enhancing heat's cell-damaging effects. High-power pulses can also create temporary cell openings, improving how well complementary therapies work together. Unlike continuous heating, pulsed heating better preserves natural body functions, promoting more effective treatment. It can also effectively reduce pain and improve patients' daily lives.

It is technically important that the pulsed heating allows precise control over treatment temperature and duration. Adjusting pulse duration, frequency, and power helps achieve therapeutic effects while minimizing harm to surrounding tissues. Constant pulse power ensures consistent energy delivery, akin to digital technology, making dosing more predictable and reducing side effects. The method's short, low-duty cycles decrease the likelihood of skin and fat tissue burns, ensuring surface temperatures remain safe. Pulsed heating's potentially higher effectiveness might allow lower overall heat doses than continuous heating, potentially shortening treatment times. Benefits vary depending on cancer type, tumor size, and location. In pulsing applications, simplified electrode applicators are possible with reduced cooling needs. The method automatically adjusts to start the semi-adiabatic heating phase without requiring stepped-up heating. Maintaining constant power throughout simplifies tuning. In practice, this method treats all depths of tissue equally.

Like all treatments, pulsed mEHT has limitations and potential drawbacks. While it reduces overall heating time, localized tissue within the treatment target can still experience high temperatures, potentially causing damage. Depending on individual factors, nerve stimulations and systemic reactions like thirst, fever, and fatigue are possible. High-power pulses, such as rapid tumor breakdown (lysis) or cytokine storm syndrome, may also pose risks.

The periodic repetition of the pulses thermally averaged by the heat-conduction of the tissues, but the nonthermal component is periodically active, there is no mechanism of averaging. This increases the safety of the mEHT treatment, avoid the thermal overloading with providing only short load of energy, and a longer relaxing period, but in the same time the nonthermal molecular activity could be

increased without extra thermal load. Another factor of the safety increase is the minor thermal load on adipose tissues and skin structures, while the efficacy was considerable increase in depth of the body. The lowered thermal toxicity increases the patient's comfort during the treatment, and gives better quality of life parameters afterward.

6. Conclusion

The pulsed modulation mEHT increases the standard modulated electro-hyperthermia efficacy and provides safer and easier treatment. The additional high-power pulsing increases the penetration depth and, at the same time, decreases the thermal load on the fatty tissues supporting the patient's safety and quality of life. The new pulsed mEHT is a newer step forward in the increasing of safety and effectiveness of oncological hyperthermia methods. The pulsing modulation opens a new research for further optimization of mEHT using the facility of the large nonthermal electric current for electrophoretic combination. Further research may clarify the possible shortening of the treatment time due to the increased nonthermal efficacy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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