Is intracellular hyperthermia superior to extracellular hyperthermia in the thermal sense?

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Abstract

More than 20 years ago, it was hypothesized that intracellular hyperthermia is superior to extracellular hyperthermia. It was further hypothesized that even a single biological cell containing magnetic nanoparticles can be treated for hyperthermia by an AC magnetic field, independent of its surrounding cells. Since experimental investigation of the thermal effects of intracellular hyperthermia is not feasible, these hypotheses have been studied theoretically. The current report shows that nano scale heating effects are negligible. This study further shows that intracellular heat generation is sufficient to create the necessary conditions for hyperthermia only in a large group of cells loaded with nanoparticles, having an overall diameter of at least 1 mm. It is argued in this report that there is no reason to believe that intracellular hyperthermia is superior to extracellular hyperthermia in the thermal sense.

Keywords: Magnetic Heating, Nanoparticles, Hyperthermia, Intracellular Hyperthermia, Thermal Ablation, Thermal Analysis

Introduction

The potential of AC magnetic heating effects in a scale length smaller than the biological cell diameter was first addressed by Gordon and co-workers [1] and termed 'intracellular hyperthermia.' Gordon et al. hypothesized that intracellular hyperthermia is a more destructive heating mechanism than extracellular heating, causing the intracellular space to reach higher temperatures. It was rationalized that the cell membrane may act as a thermal insulator due to its low thermal conductivity, and it was suggested that only intracellular heating can overcome this thermal barrier. The potential of intracellular hyperthermia has been addressed by several researchers over the years [2-5]. Recently, Jordan and co-workers [6] showed that magnetic particles can selectively penetrate into target cells in very large quantities and therefore, heat can be generated selectively within target cells. Jordan et al. measured the uptake of iron particles by various cell types -- data which is applied for the purpose of the current report.

Hyperthermia by AC magnetic excitation involves heat transfer in three different scale levels: (i) nano scale, characterized by the size of the magnetic particles (typically, 5 to 100 nm); (ii) micro scale, characterized by the biological cells size (typically, 5 to 20 μm); and (iii) macro scale, characterized by the size of the target tumor or the tissue size to be treated (typically, up to

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20 mm). Due to technical difficulties in measuring nano and micro scale thermal effects, only macro scale effects are quantitatively addressed in the literature of hyperthermia, either theoretically or experimentally [7-9].

The purpose of the current study is to test the following hypotheses: (I) intracellular hyperthermia via magnetic heating can be achieved even in a single cell containing nanoparticles, when surrounded by a large cellular structure free of nanoparticles; and (II) the thermal effect of intracellular hyperthermia is superior to the thermal effect of extracellular hyperthermia. Since thermal experimentation at the cellular level is not feasible, this study is based on a theoretical analysis of heat transfer. For this purpose, a credible set of assumptions is presented, boundary cases are investigated, and order of magnitude analysis is presented.

Thermal Analysis

Since the various scales of magnetic heating presented above are a few orders of magnitude different from one another, it is deemed reasonable to discuss the effect of each scale independently. Unless otherwise specified, thermophysical properties of water are taken as representative values for biological solutions in the this report.

Nano scale bioheat transfer

At the nano scale, there exists a threshold below which traditional heat transfer by conduction (Fourier law) is not applicable and the ballistic energy transfer is typically considered [10]. In broad terms, the ballistic heat transfer approach is derived from the physics of electromagnetic wave propagation, which -- in the context of heat transfer -- is known as thermal radiation. This threshold is the mean free path of the material, which is defined as:

$$\Lambda = \frac{3k}{Cv} \tag{1}$$

where k is the thermal conductivity of the domain (body solutions in this study), C is the volumetric specific heat, and v is the average speed of sound in the material. The classical analysis of heat transfer by conduction can be applied only when the characteristic length for heat transfer is longer than the mean free path of the material. Assuming physical properties of biological tissues similar to those of water: k=0.64 W/m-ºC, C=4.18×10^6 J/m^3, and v=1.5×10^3 m/s, the mean free path is calculated to be 3×10^{-10} m (0.3 nm). Since the typical diameter of magnetic nanoparticles for medical applications is at least one order of magnitude larger than the mean free path, one can apply the classical Fourier law for nano heat transfer analysis around the magnetic particles. Note that predictions based on the ballistic heat transfer approach, where applicable, are likely to yield dramatically higher local temperatures when compared with predictions based on the classic Fourier law.

The case of a single spherical nanoparticle in a relatively large medium of body solutions is considered first. It is assumed that the nanoparticle (metallic material) can be considered a perfect conductor with respect to the surrounding biological material (the thermal conductivity of magnetic nanoparticles is at least one order of magnitude higher than that of biological solutions). The dimensionless Fourier law specialized, for 1D radial heat flow in the biological material surrounding the nanoparticle, is:

$$\frac{1}{\xi^2}\frac{\partial}{\partial\xi}\left(\xi^2\frac{\partial\theta}{\partial\xi}\right)=\frac{\partial\theta}{\partial Fo} \quad ; \quad \xi=\frac{r}{R_p}\geq 1 \quad ; \quad Fo=\frac{\alpha t}{R_p^2} \tag{2}$$

where θ is the temperature change from a uniform initial condition, r is the radius, R_p is the nanoparticle radius, ξ is a dimensionless radius, α is the thermal diffusivity of the surrounding domain, and Fo is a dimensionless time parameter, known as the Fourier number. Note that temperature distribution is considered in the surrounding medium only, while the nanoparticle is considered isothermal (a perfect conductor).

For large Fo values, say Fo>100, heat transfer can practically be considered steady state. It follows that for a typical thermal diffusivity value of water, α=1.3×10^{-7} m^2/s, and nanoparticle radius of up to 100 nm, heat transfer can be considered steady state after 10^{-5} s from activation of the AC magnetic field, which is negligible for hyperthermia. This time period for steady state is representative at the nano scale level only. Note that the time period for steady state in larger scales is longer. Further Fo number analysis is given below, in the macro scale analysis section.

Solving Eq. (2) for the nanoparticle temperature difference at steady state yields:

$$\theta_p=\frac{q\rho D_p^2}{12k} \tag{3}$$

where ρ is the density of the nanoparticles, D_p is the nanoparticle diameter, and q is the heat generation in the nanoparticles, which is typically in the order of 150 mW per mg of Fe [6]. For a typical density of 7,900 kg/m^3 (Fe) and particle diameter of up to 100 nm, θ_p is no greater than 10^{-5} ºC.

It can be concluded that nano heat transfer effects can be neglected for all practical cases during hyperthermia. The implication of this conclusion is that a single nanoparticle, which is remotely located in the body, cannot cause any thermal damage.

Micro scale bioheat transfer

In order to simplify the micro scale heat transfer analysis, a hypothetical case is assumed in which all the intracellular space of a single spherical cell is densely packed with nanoparticles, while the surrounding cells are free of nanoparticles. Although the probability for such an arrangement is very low, the rationale for studying this case is to analyze the upper boundary for magnetic heating. Analogous to the nano scale analysis presented above, it is now assumed that the analyzed cell is the only cell containing nanoparticles in a large cellular matrix free of nanoparticles. Under these conditions, Eqs. (1)-(3) are still valid when substituting the nanoparticle diameter, D_p, with the cell diameter, D_c. Equation (3) can be rearranged as follows:

$$D_c=\sqrt{\frac{12\theta_c k}{\rho q}} \tag{4}$$

where θ_c is the cell temperature difference between steady and initial condition.

It can be calculated from Eq. (4) that in order to elevate the cell temperature in 0.01, 0.1, and 1ºC, a minimal cell diameter of 8, 25, and 80 μm is required, respectively. For practical applications of hyperthermia, a minimal temperature of 43ºC is typically required, which is considered a threshold for significant cell destruction (Dewey, 1994). It follows that a minimal cell diameter of 200 μm is required, however, such huge hypothetical cell diameter is about one

order of magnitude larger than the typical human cell (typically, 10-20 μm). This diameter size is actually of the order of magnitude of a cell cluster, however the underlying assumptions for thermal analysis of a cell cluster are different, as is addressed below in the macro scale analysis section.

Jordan and co-workers (1999) have measured the uptake of nanoparticles by various malignant and benign cells. In their study, a maximal uptake of 5×10^{-10} gram Fe per cell is reported for human mammary carcinoma cells, which are typically 15 μm in diameter. Taking into account Fe density of 7,900 kg/m^3, it can be estimated that only about $1/30^{th}$ of the cell volume was occupied by nanoparticles in those experiments. This suggests a lower heating power by a factor of $1/30^{th}$, compared with the hypothetical extreme case presented above. If follows that significantly larger hypothetical cell diameter would be required for such lower heat generation rate. Of course, the assumption of a perfect thermal conductor is not valid in the case of a biological cell loaded with only $1/30^{th}$ of its volume with magnetic nanoparticles.

Assuming that Fo=100 represents steady state, and considering a typical cell diameter of 15 μm, the time period from initiation of heating to steady state is less than 0.05 s. It can be concluded that the transient effect at the micro scale can be neglected for all practical cases of hyperthermia.

From the micro scale analysis of heat transfer one can conclude that it is highly unlikely that the conditions for magnetic hyperthermia can be met in the case of a single cell loaded with nanoparticles and surrounded by a large cellular structure free of nanoparticles (i.e., hypothesis I is rejected). The rate of heat generation within the cell is insufficient, even in the hypothetical case, where the entire intracellular space is filled with nanoparticles. It follows that hyperthermic conditions can be met in a larger cellular structure only. However, the assumption of a cellular structure having similar properties to these of the nanoparticles is far from being valid when only a fraction of the volume of the treated region is occupied by nanoparticles. This observation requires a more advanced mathematical tool for analysis, as described in the macro scale analysis below.

For magnetic nanoparticle design purposes, Eq. (4) can be rearranged to find the minimal heating power required for hyperthermia in a single cell, which yields the minimal volumetric heat generation required in the particles. For example, for a typical cell diameter of 15 μm and temperature raise of 6°C, a minimal volumetric heating power of 2.05×10^{11} W/m^3 is required ($\rho\times q$). However, only about 1.2×10^{9} W/m^3 can be generated in a single iron nanoparticle, and on average, far less in a mixture of intracellular solutions and nanoparticles. To the best of this author's knowledge, nanoparticles capable of generating heating power in the order of 10^{11} W/m^3 are not available.

The analysis presented above referred to magnetic heating in the absence of blood perfusion. Blood perfusion can generate an overwhelming cooling effect on hyperthermia by a mechanism of heat convection from the heated region. Higher heating power is required for hyperthermia in the presence of blood perfusion. Therefore, the micro scale analysis presented in the absence of blood perfusion is a conservative one: the cooling effect of blood perfusion decreases the probability of intracellular hyperthermia.

Macro scale bioheat transfer

It has been shown in this study that heating a single nanoparticle, or heating a single cell loaded with nanoparticles, is highly unlikely to lead to hyperthermic conditions. The purpose of the macro scale analysis presented below is to identify the minimal cells volume required for

practical hyperthermic conditions. For that purpose a temperature threshold of 43°C is assumed. The reason that such minimal volume exists is that single cells cannot generate a heat rate high enough, and the cumulative effect of closely packed cells containing nanoparticles is required.

For the purpose of simplicity, the macro scale analysis addresses a spherical target region (a 'spherical' tumor), containing uniformly distributed magnetic nanoparticles. The ideally spherical geometry is chosen in order to simplify the analysis and to enable generation of a closed form solution of the process. Under the above assumption, the governing equation for heat transfer is:

$$\frac{1}{\xi^2}\frac{\partial}{\partial\xi}\left(\xi^2\frac{\partial\theta}{\partial\xi}\right)+\frac{q'}{k}=\frac{1}{\alpha}\frac{\partial\theta}{\partial Fo}\quad;\quad\xi=\frac{r}{R_t}\quad;\quad q'=\begin{cases}\rho q & 0<\xi\le 1\\ 0 & 1<\xi<\infty\end{cases} \tag{5}$$

where R_t is the radius of the thermally treated region, and q' is the volumetric magnetic heating rate. The average values of the thermal conductivity, k, and the thermal diffusivity, α, are expected to be higher at the thermally treated area, $r < R_t$, due to the presence of magnetic nanoparticles. However, due to the relatively low ratio of the nanoparticles to cell volume (up to one third, as discussed above), due to the high uncertainty of the actual value of these properties in the tissue, and in order to provide an order of magnitude analysis, it is logical to assume the same property values for the entire domain without significantly affecting the outcome of the analysis.

The solution of Eq. (5) for zero initial condition in an infinite domain is given by Carslaw and Jaeger [12], which has been recompiled in the following dimensionless form:

$$\begin{aligned}\frac{\theta k}{\rho q R_t^2}=\frac{2Fo}{\xi}\Bigg[&p_1\frac{\xi}{2}+p_2\left(\frac{fx^3}{3}-\frac{x^2}{2}+\frac{fx}{2}-\frac{1}{4}\right)erfc(p_2x)-\left(\frac{fx^2}{3}-\frac{x}{2}+\frac{f}{3}\right)\frac{e^{-x^2}}{\sqrt{\pi}}\\&-\left(\frac{fy^3}{3}-\frac{y^2}{2}+\frac{fy}{2}-\frac{1}{4}\right)erfc(y)+\left(\frac{fy^2}{3}-\frac{y}{2}+\frac{f}{3}\right)\frac{e^{-y^2}}{\sqrt{\pi}}\Bigg]\end{aligned} \tag{6}$$

where *erfc* is the complimentary error function, and where:

$$Fo=\frac{\alpha t}{R_t^2}\equiv f^2\quad;\quad x=\frac{1-\xi}{2f}\quad;\quad y=\frac{1+\xi}{2f} \tag{7}$$

$$p_1=\begin{cases}1 & 0\le r<R_t\\ 0 & R_t\le r\end{cases}\quad;\quad p_2=\begin{cases}1 & 0\le r<R_t\\ -1 & R_t\le r\end{cases} \tag{8}$$

Equation (6) can be simplified at the center of the heated region (ξ=0) to the form:

$$\frac{\theta_t k}{\rho q R_t^2}=\frac{1}{2}+\left(Fo-\frac{1}{2}\right)erf\left(\sqrt{\frac{1}{4Fo}}\right)-\sqrt{\frac{Fo}{\pi}}\exp\left(-\frac{1}{4Fo}\right) \tag{9}$$

where θ_t is the temperature at the center of the thermally treated tissue (maximal temperature), and *erf* is the error function. Note that the right hand side term of Eq. (9) reaches a maximal

value of ½ as *Fo* tends to infinity, which defines a steady state condition. Using a value of ½, the upper boundary for the temperature to rise at the center of the treated region after a very long time is given by:

$$\theta_t = \frac{\rho q D_t^2}{8k} \tag{10}$$

where D_t is the diameter of the thermally treated region.

It can be calculated from Eq. (10), that a minimal diameter of 0.9 mm of the region occupied by nanoparticles is required, for an average heating power of $1/30^{th}$ of the maximum possible heating power (i.e., 4×10^7 W/m^3 for iron particles), and a center temperature of 43°C. It can further be calculated from Eq. (6) that a minimal diameter of 1.1 mm is required in order to ensure a temperature rise of 6°C at the edge of the region occupied by nanoparticles (raising the temperature of the entire target region above 43°C). Clearly, these minimal diameter values are two orders of magnitude larger than the typical size of a typical target cell, but in the same order of magnitude of small cancer tumors. Furthermore, a larger target region is required in order to achieve higher hyperthermic temperatures, where the temperature raise is proportional to the diameter to the second power. A larger target region is also required in the presence of blood perfusion, due to its convective cooling effect.

The above example is related to a steady state condition. For the purpose of future analysis of similar cases, Eq. (6) is presented graphically in Fig. 1. The special solution at the center of the target region, Eq. (9), is presented graphically in Fig. 2. With respect to the initial temperature, it can be seen from Fig. 2 that 60, 70, 80, and 90% of the steady state temperature raise is achieved after *Fo* values of 0.8, 1.5, 3.5, and 14, respectively. For the above example, it follows that a time period of 33 seconds is required for achieving 90% of the temperature rise between the initial condition and steady state for a target region diameter of 1.1 mm. Note that the time period to steady state is dependent on the diameter to the second power. This means that double and triple the target region diameter requires four and nine times longer periods to reach the same response, respectively.

The above example is given for thermophysical properties of water in the target region. If a higher thermal conductivity value is taken for calculations, as a representative value of the solution-nanoparticles mixture, even larger heated region would be required in order to meet the same level of hyperthermic condition at steady state. The reason being that a higher thermal conductivity leads to a more moderate temperature distribution within the thermally treated region, as can be seen in Fig. 1. A similar effect is observed at the transient stage if a higher thermal diffusivity value is taken into account within the target region.

Cell membrane as a thermal barrier

Gordon et al. [1] hypothesized that intracellular hyperthermia is a more destructive heating mechanism than extracellular heating, causing the intracellular space to reach higher temperatures. It was rationalized that the cell membrane may act as a thermal insulator due to its low thermal conductivity, and it was suggested that only intracellular heating can overcome this thermal barrier. The thickness of the cell membrane is in the range of micro scale or less. It has been shown in the current report that micro scale heat transfer effects are negligible. It follows that even if indeed the cell membrane has a low thermal conductivity value, its thermal effect is negligible. This conclusion rejects the major underlying assumption made by Gordon et al.,

suggesting that intracellular hyperthermia is superior to extracellular hyperthermia.

Summary and Conclusions

It has been hypothesized more than 20 years ago that intracellular hyperthermia is superior to extracellular hyperthermia. It was further hypothesized that even a single biological cell containing magnetic nanoparticles can be treated for hyperthermia by an AC magnetic field, independent of its surrounding cells. Since experimental investigation of the thermal effects of intracellular hyperthermia is not feasible, these hypotheses have been examined theoretically in the current study.

It was found in this study that nano scale heating effects are negligible, which indicates that a single magnetic nanoparticle has no practical effect on hyperthermia. It was further found that the heating generation in a single cell, which is densely packed with nanoparticles, is not sufficient to create the conditions for hyperthermia unless it is a part of a larger cellular structure of similar cells (rejection of hypothesis I). Although hypothesized in the literature and rejected as a conclusion of this study, it is unlikely that one would encounter a clinical situation in which one cell is significantly loaded with magnetic particles, while its surrounding cells remain completely unaffected by nanoparticles.

The most conservative calculation indicates that the region occupied by nanoparticles must be at least 1.1 mm in diameter, in order to reach the threshold for hyperthermic conditions. This value is expected to increase dramatically in the presence of blood perfusion. This diameter is also expected to increase with the elevation of the desired temperature level for hyperthermia, and especially in the case of thermal ablation. This value of minimal diameter is at least two orders of magnitude larger than the typical size of a typical cancer cell.

Since the thermal effects at the cellular level are found negligible, and since the thermally treated region diameter is expected to be more than ten times the diameter of a single cell, it is argued that there is no reason to believe that intracellular hyperthermia is superior to extracellular hyperthermia in the thermal sense, providing that the same average amount of nanoparticles are present in both cases (rejection of hypothesis II).

If experimental observations are made proving that higher destruction is obtained in a cell containing nanoparticles when compared with cells surrounded only by nanoparticles, it is suggested here that these observations are related either to chemical effects triggered by the presence of the nanoparticles, or mechanical damage caused to the cell by intracellular vibrations and rotations of the nanoparticles.

Although this report suggests that intracellular hyperthermia is not superior to extracellular hyperthermia in the thermal sense, it is by no means suggested that selective coating for nanoparticles is not advantageous. The advantage of selective coating is in the nanoparticles delivering technique. With selective coating, nanoparticles can be introduced intravenously and penetrate independently into the target cell population. In the absence of selective coating, nanoparticles can only be introduced by injection, where the concentration, and therefore the heat generation potential, are expected to be highly time dependent. When limitations are well appreciated, selective coating is a noble method of introducing nanoparticles into cancer tumors.

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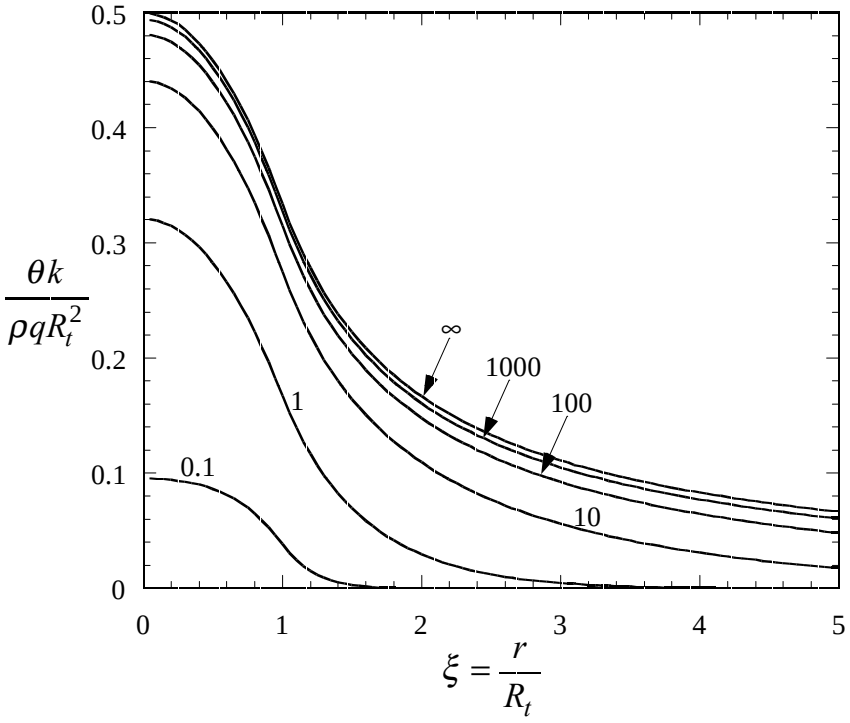

Figure 1: Dimensionless temperature distribution; numbers represent the *Fo* value for each curve

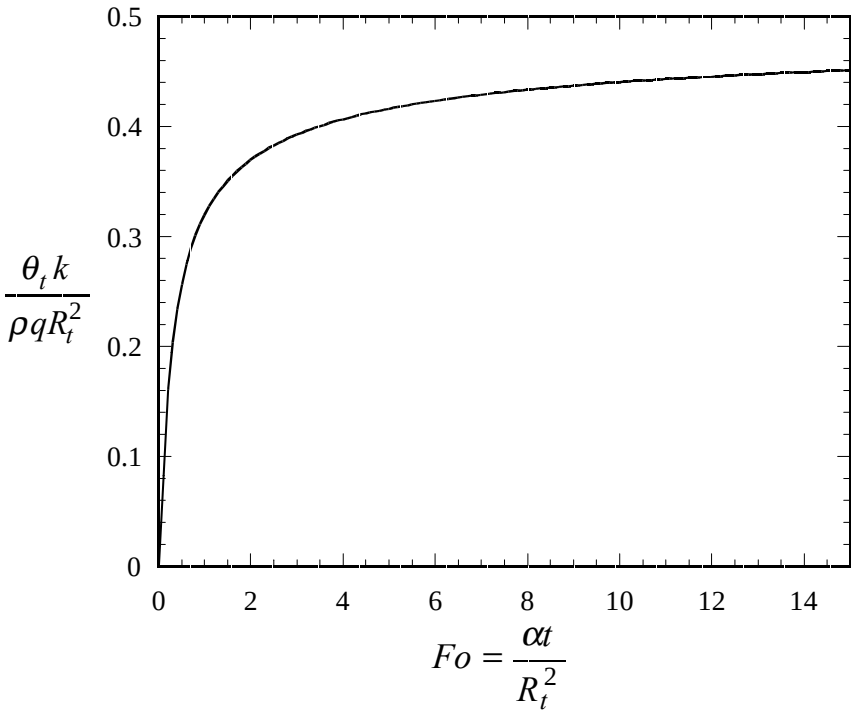

Figure 2: Macro scale dimensionless temperature variation with *Fo* number at the center of the hyperthermic region