Supporting Information

In Vivo Cancer Cells Elimination Guided by Aptamer-Functionalized Gold-Coated Magnetic Nanoparticles and Controlled with Low Frequency Alternating Magnetic Field

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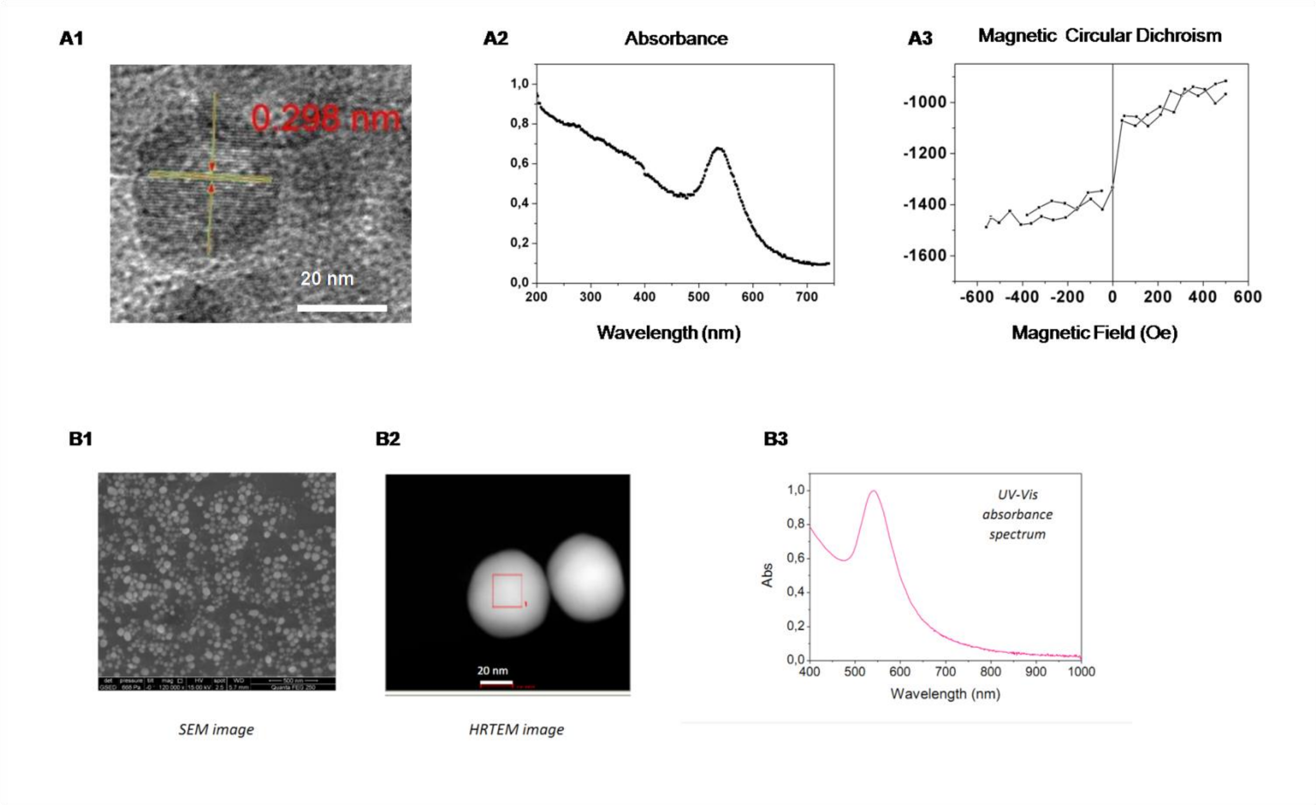

Figure S1. Properties of magnetic nanoparticles NITmagoldCit 50 nm, Nanoimmunotech, Spain.

(A) Scanning electron microscopy of aptamer modified GMNPs (1); absorbance (2); magnetic circular dichroism (3) before the experiment.

(B) Scanning electron microscopy (1); high-resolution transmission electron microscopy (2); absorbance from product datasheet.

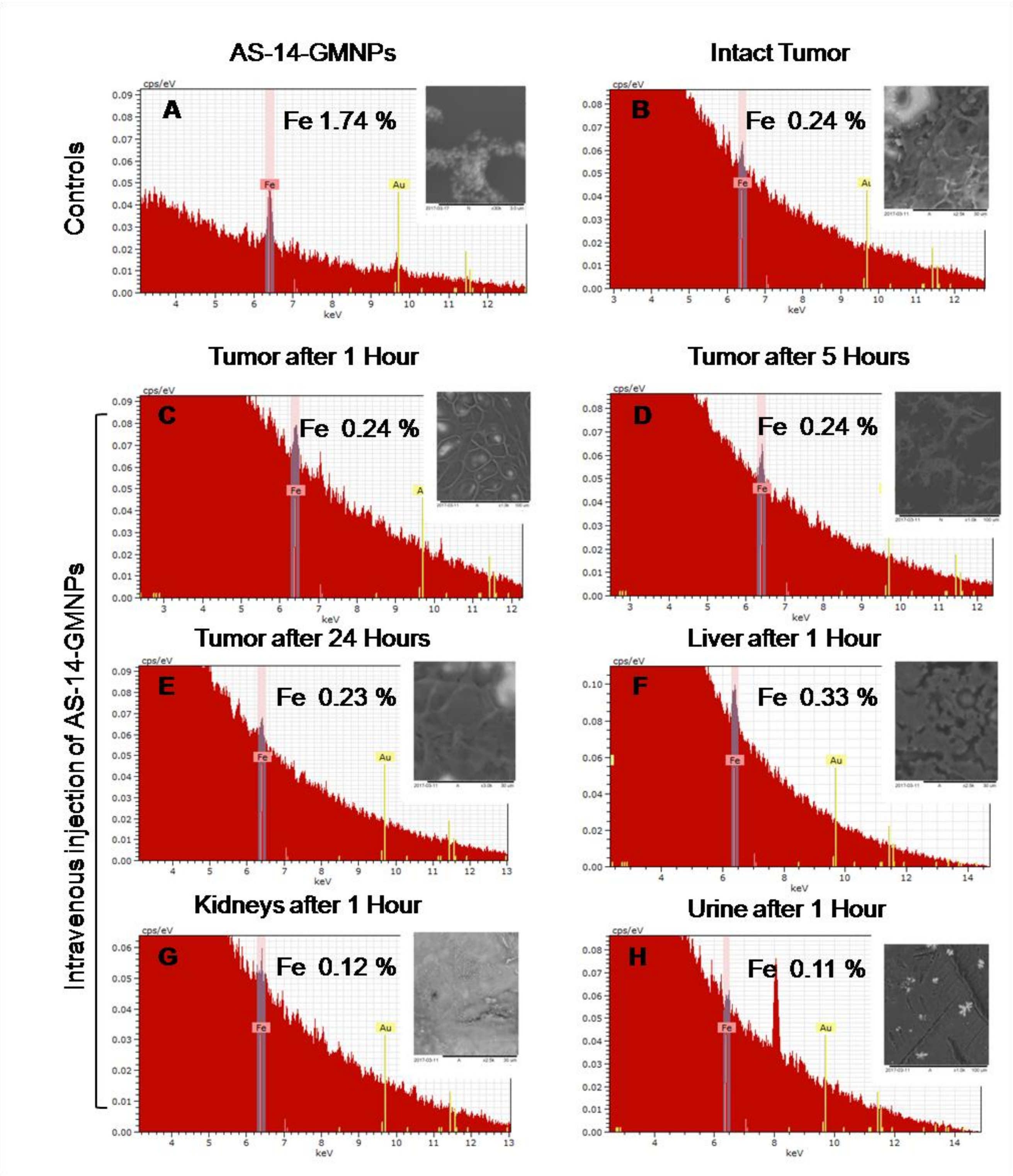

Figure S2. Distribution of iron and gold ions in organs after intravenous injection of AS-14GMNPs in 100 μL DPBS (1.6 μg kg^{-1}) determined by electron microscopy (EM). Spectral analyses of iron and gold content in AS-14-GMNPs (A); thin section of the intact tumor tissue (B); tumor after 1 (C), 5 hours (D) and 24 hours (E) after intravenous injection of AS-14-GMNPs; liver (F), kidneys (G), and urine (H) 1 hour after injection of AS-14-GMNPs. Inserts - contrast EM of the correspondent sample.

The mechanical action of the magnetic particles on cells

If a magnetite particle has a spherical shape of radius R_p, then its magnetic moment in a uniform external magnetic field $\vec{H}_0$ equals to

$$d = 4\pi R_p^3 \frac{\mu - 1}{\mu + 2} H_0,$$

where μ - relative magnetic permeability, which value for magnetite is close to 5 according to the data [3]. This formula follows from the known exact solution for the problem of magnetization of a homogeneous ball [2]. Magnetic moment of a ball is directed along the field $\vec{H}_0$, and therefore no torque appears. Any field inhomogeneity leads to magnetophores that we do not consider here.

The shape of our magnetic particles is substantially different from a ball. In such a case, the magnetic moment is large, if the body is elongated along the field $\vec{H}_0$, and small for another orientation. For bodies of particular shapes, these parameters can be calculated. We assume that our particle is a prolate ellipsoid of revolution, and the long half-axis $a = 12$ nm is twice longer than the short ones $b = 6$ nm. The exact solution for the problem of magnetization of the ellipsoid is known [2]. For μ = 5 it gives the magnetic moments in the direction of elongation and normal one

$$d_{1,2} \approx (1 \pm 0.17) 8ab^2 H_{1,2}.$$

If there is an angle ϑ between the magnetic field $\vec{H}_0$and the direction of elongation of the body, the magnetic moment is not parallel to the field. The projections of the moment on the field H_0 and normal to it equal $d_1 \cos\vartheta$ and $d_2 \sin\vartheta$. Hence, there is a torque

$$N = (d_1 - d_2)\mu_0 H_0 \sin(\vartheta)\cos(\vartheta) \approx 1.4\mu_0 ab^2 H_0^2 \sin(2\vartheta),$$

where μ_0 - magnetic permeability of vacuum. If the magnetic field strength $H_0 = 8$ kA/m, which corresponds to 100 Oe, the torque depending on the angle ϑ can reach $0.5 \cdot 10^{-22}$ N·m.

Figure 3 is a diagram of the mechanical interaction of the magnetic particle with the cell. We use superparamagnetic particles which are covered by a thick layer of gold, so that from the outside they look like balls of radius $R_p = 25$ nm. Their surface is covered with aptamers which cling to fibronectin filaments located in the intercellular space. These filaments are attached at their ends to integrins located in cell membranes. We approximately simulate the filaments as inextensible ones and we consider integrins as solid cylinders of radius $R = 2$ nm.

When under the influence of the magnetic field the particle is rotated clockwise, as shown in Figure 3, it pulls the left filament up and pulls the right one down. The left filament above the particle can be bent, and therefore does not transmit efforts at its upper end. The lower part of this filament pulls integrin with the force $\vec{F}$. Similarly, the right filament does not act on the integrin shown in Figure 3, but pulls the other integrin, located somewhere above the drawing area.

For evaluation of the elastic forces generated by pulling integrin to the height h, we approximately assume that all the elastic forces are determined by deformation of the membrane. According to [1] the mammalian cell membranes in the normal state are stretched so that the order of magnitude of the tension $\tau = 10^{-5}$ N/m. It may be noted that this tension of the membrane is balanced by the pressure inside the cells increased by $\Delta P = 2\tau / R_c$ as compared with the pressure in surrounding environment. For the cells with radius $R_c = 10\,\mu$m, this difference $\Delta P \approx 2$ P.

We are interested in the phenomenon with a scale much smaller than the radius of the cell R_c. So we neglect the curvature of the membrane. We consider a thin membrane, and we suppose that it is fixed at the circle of some large radius R_∞. In our model the integrin looks like a solid circle of radius R centered at the same point as the center of the selected circle of the membrane.

This object is rotationally symmetric. Hence, we use the polar coordinates r, φ of the points in the plane of the non-deformed membrane. Points do no move in direction of φ because of the symmetry. For small strains the displacement in direction of r has a higher order of smallness than the deflection in the direction normal to the membrane. The latter is denoted as $w(r)$. Therefore, the displacement of the membrane points is described by one function $w(r)$. The following boundary conditions correspond to the rise to the height h at the circle $r = R$ and zero displacement at $r = R_\infty$

$$w(R) = h, \quad w(R_\infty) = 0\,. \qquad (1)$$

The membrane takes such a form that the elastic energy J reaches a minimum. In our case of axial symmetry in accordance with [4], we have

$$J = \pi\tau \int_R^{R_\infty} \left(\frac{dw}{dr}\right)^2 r dr\,.$$

The condition of its minimum is the equation

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{dw}{dr}\right) = 0\,.$$

Its solution is the function

$$w(r) = A \ln(r / B)\,,$$

where A, B - arbitrary constants. Their values can be found from the boundary conditions (1). The result is

$$w(r) = h \ln(R_\infty / \mathrm{r}) / \ln(R_\infty / R)\,. \qquad (2)$$

The graph of this solution is shown in qualitative manner in Figure 3.

Such a membrane is inclined relative to the plane at the angle β, such that

$$tg\,(\beta(r)) = \frac{dw(r)}{dr} = \frac{-h}{\ln(R_\infty / \mathrm{R})}\frac{1}{r}\,. \qquad (3)$$

This slope on the border with the solid circle defines the force with which the membrane draws the circle dawn in the vertical direction

$$F = 2\pi R\tau \cdot \sin\beta \approx 2\pi\tau h / \ln(R_\infty / R)\,. \qquad (4)$$

The formula is simplified for small angle β, when $tg\,\beta \approx \beta \approx \sin\beta$. This expression contains one parameter R_∞, whose value we have identified only by the inequalities

$$R_c \gg R_\infty \gg R\,.$$

The uncertainty of R_∞ can be solved only within a more general model that takes into account spherical membrane. We do not do this, since the logarithm $\ln(R_\infty / R)$ in (2-4) is only slight varied when R_∞ changes in a wide range. Take the seemingly reasonable average value $R_\infty = \sqrt{R_c R} \approx 150$ nm when $\ln(R_\infty / R) \approx 4$. Expression (4) reduces to

$$F \approx 1.5\tau h\,. \tag{5}$$

The maximum value of torque $N = 0.5\cdot10^{-22}$ N·m due to the magnetic field acting on the magnetic particle was obtained above. It is easy to show that since we use low frequencies, the inertia during the rotation of the particle can be neglected. The friction of the surrounding liquid is more important, but the friction torque is also a few orders of magnitude smaller than the torque N. Therefore, the pair of the elastic force (5) and having the same module elastic force applied to the right-hand filament in Figure 3, balances the torque N. It gives the equality

$$N \approx 3\tau h R_p\,,$$

that permits to evaluatethe height to which integrins can be drawn by the magnetite particles which we use, $h \approx 0.07$ nm. Possibly, the angle $\beta(R) \approx 0.5^0$ of rotation of the membrane in the place of its attachment to the integrin (3) is of value. The force (5), that pulls the integrin from the membrane can be up to $F \approx 10^{-15}$ N. It can be mentioned that the magnetic particle rotates h / R_p angle that is of about 0.15^0 when $h = 0.07$ nm.

Note that these values of h, F are obtained only for the magnetic particles oriented in a certain way with respect to the direction of the external magnetic field $\vec{H}_0$. Since the particles when attached to the cells are randomly oriented, the specified parameter values ought to be reduced several times to estimate the average impact on a cell.

The obtained limit values could be achieved when varying over time magnetic field $\vec{H}_0$ has amplitude value, that occurs twice during the period. The frequency is 50 Hz in our experiments. When the magnetic field $\vec{H}_0$ is reversed, the magnetic moment of each magnetite particle does the same. The torque keeps sign since it equals to their vector product. Consequently, during both half cycles the particle rotates in the same direction when the field strength increases, and returns to its free position when the field is weakened. Therefore, during 0.01 sec integrin is pulled out of the cell to a height about 0.07 nm and returns to its original position. Perhaps, just these twitches with frequency 100 Hz damage cells in our experiments, whereas the stationary membrane deformations of the same scale could be not so effective.

Heating

Let us show that the thermal energy released in magnetic particles is distributed throughout the liquid. In our experiments, the particles have a concentration of the order of $10^{14}\,\text{m}^{-3}$, so the distance between adjacent particles $L \simeq 2\cdot10^{-5}$ m. By the heat conduction equation typical time of heat propagation for the distance L is assessed as

$$\tau \simeq \frac{C}{\lambda}\cdot L^2\,,$$

where C - heat capacity λ - thermal conductivity. For water, $C = 4.2\cdot10^6$ J/(m^{3}K), $\lambda = 0.6$ W/(m $\cdot$K). We get the characteristic time $\tau \simeq 0.003$ seconds. Such a small τ ensures uniform heating throughout the liquid and particles, not only for total time of the experiment $t = 10$ minutes, but even during the period of the magnetic field variation that equals $1/f = 0.02$ seconds.

First, we study the heating of the metal particles in an alternating magnetic field.

In our experiments, we use an alternating magnetic field with a strength of about 100 Oe or 8 kA/m, that corresponds to the magnetic induction $B = 0.01$ T. This field varies with time at a frequency $f = 50$ Hz.

Living cells and magnetic particles can be in different liquids. All of these liquids have salinity not exceeding seawater salinity. Therefore, to estimate maximum effect, consider seawater which conductivity is $\sigma_l = 3$ S/m. The main part of the used magnetic particles takes gold which conductivity is $\sigma_p = 0.5 \cdot 10^7$S/m.

The nature of the influence of the alternating magnetic field on a substance is determined by such a parameter as the thickness of the skin layer $\delta = 1/\sqrt{\pi\sigma\mu_0 f}$, and $\delta_l = 40$ m for the liquid and $\delta_p = 1$ cm for gold. Here μ_0 is magnetic permeability of vacuum. Because these parameters are many orders of magnitude greater than the characteristic size of the region occupied by the liquid, and the size of the particles, respectively, the magnetic field freely permeates without being distorted into the liquid and into the particles.

By virtue of the law of electromagnetic induction the variation of the magnetic field creates a vortex electric field $\vec{E}$ which satisfy the equation

$$\oint \vec{E} d\vec{l} = \frac{\partial}{\partial t} \int \vec{B} d\vec{s}, \tag{6}$$

where the integration in the left side is made over an arbitrary closed circuit, and in the right side - over the surface bounded by this circuit, t - time.

To simplify estimates, assume that liquid occupies a region which is symmetric with respect to rotation about the same axis as that of the solenoid which generates a magnetic field. The magnetic field is assumed homogeneous, and its induction is defined as $B\cos(2\pi f t)$. Then the electric field is also axially symmetric and has only an azimuthal component $-E\sin(2\pi f t)$, and the integration in (6) for a circle of radius r is simple. Get

$$-2\pi r E \sin(2\pi f t) = \frac{\partial}{\partial t}(B\cos(2\pi f t)\, \pi r^2).$$

We express E, and for all points of the fluid at a distance of less than 5 mm from the axis, we obtain the estimate

$$E = r\pi f B < 0.01 \text{ V/m}.$$

The electric current produced by this field has a density $j = \sigma_l E < 0.03$ A/m^2, and is accompanied by Joule dissipation which density equals $jE = \sigma_l E^2 < 3 \cdot 10^{-4}$ W/m^3. This energy heats the liquid. The temperature rises from the initial value T_0 to

$$T = T_0 + t \cdot jE/C,$$

after the time t. C - heat capacity per unit volume. For water, $C = 4.2 \cdot 10^6$ J/(K·m^3). For $t = 10$ minutes the temperature is increased by

$$T - T_0 < 5 \cdot 10^{-8}\text{K}, \tag{7}$$

that is negligible.

Now consider the heating of the gold ball, placed in the liquid. Since the radius of the ball, $R = 25$ nm, is much smaller than the distance between the balls, each ball can be considered separately, as being in an infinite domain with a uniform electric field with strength $\vec{E}$, which module is E.

We know the exact solution of this problem of electrical conductivity. The electric potential V in spherical coordinates r, ϑ, φ, with the axis $\vartheta = 0$ directed along $\vec{E}$, has the form

$$V = \begin{cases} -E_p r \cos\vartheta\,, & r < R \\ (A/r^2 - Er)\cos\vartheta\,, & r > R, \end{cases}$$

where the constants

$$A = ER^3\big(\sigma_p/\sigma_l + 1\big)/\big(\sigma_p/\sigma_l + 2\big),$$

$$E_p = 3E/\big(\sigma_p/\sigma_l + 2\big).$$

Linear dependence of the electric potential on the coordinate $z = r\cos\vartheta$ inside the ball means a uniform electric field with strength $E_p \approx 3E\sigma_l/\sigma_p$, because $\sigma_p/\sigma_l \gg 1$. Accordingly, the density of Joule dissipation inside the ball equals $\sigma_p E_p^2 \approx 9E^2\sigma_l/\sigma_p$, that differs $9\sigma_l/\sigma_p \ll 1$ times from dissipation in the liquid. The electric field strength in the vicinity of the ball as compared to E do not increase more than threefold, and returns to the value E with the distance from the ball. Accordingly, the density of Joule dissipation increases only in a small neighborhood of the ball and no more than 9 times.

Thus, the ball with high electric conductivity increases heating of some surrounding liquid, while the ball itself heats much less than liquid would be heated without it. The heating is negligible in view of the inequality (7).

One more important mechanism of energy transfer from the magnetic field to the medium is the work done by rotating particles.

Upon rotation of the particle the magnetic field does work $A = N\,\delta\varphi$, where $\delta\varphi$ - the angle of rotation, N - torque. Above, we obtain an estimate $\delta\varphi \simeq 0.15^0 \simeq 0.003$ radians. It was used the assumption that fibronectin filaments may be regarded as inextensible ones. If, on the contrary, they are easily stretched, they have virtually no influence on the rotation of the particles, which would turn to the ellipsoid orientation along the magnetic field, therefore, $\delta\varphi$ may be of the order of one radian. Of course, in such a case the cell membrane is not deformed, and all resistance would be determined by a rotation viscosity of the liquid. To evaluate the work from above, we use the limit $\delta\varphi \simeq 1$ radian and obtain $A < N$. All this work is ultimately converted into heat. The particle has a volume much smaller volume of fluid surrounding it, and this thermal energy is rapidly distributed throughout the liquid. Therefore, the law of conservation of energy can be written as

$$C\,L^3\,(T - T_0) = 2\,f\,t\,A,$$

where the frequency f is doubled, as the turns occur twice in the period of the field variation, and each time the magnetic field does the work A. Substituting the above estimates $A < N$, $N = 0.5\cdot 10^{-22}$ N·m, obtains an estimate of the temperature change

$$T - T_0 < \frac{2\,f\,N}{CL^3}\,t \simeq 10^{-10}\,K\,. \qquad (8)$$

This heating is negligible.

Another heating mechanism is associated with losses in reverses of magnetization of particles. Hysteresis for the magnetite particles is so small that it is difficult to find the magnetization curves at the amplitude of the field $H_0 = 100$ Oe = 8 kA/m, as in our experiments. Upper estimate can be obtained using the curves plotted in [3] at the amplitude of the field around 3000 Oe. The difference per unit mass of the magnetization with increasing and decreasing field is of about 2 A $\cdot$m/kg. For magnetic density of about 5000 kg/m^3, we get $\delta M = 10^4$ A/m. The hysteresis loop at low fields is definitely inside the loop obtained for large amplitude of the field, so its area

$$-\oint \vec{H} d\vec{M} = \oint \vec{M} d\vec{H} < 2\vec{H}_0 \delta\vec{M} .$$

The heat release in each cycle of magnetization is obtained by multiplying by the volume of the magnet and by μ_0. We get energy

$$2\mu_0 \frac{4}{3}\pi\, ab^2 H_0\, \delta M \simeq 4\cdot 10^{-22}\,\mathrm{J}.$$

Since this energy is four times more than the energy dissipation of turning particles, heating also increases four times compared with (8), and hence it is also negligible. This is consistent with the statement [3] about the possibility to neglect by hysteresis.

The same article [3] also considered other mechanisms of medium heating due to exposure to a magnetic field, such as Néel and Brownian relaxations. We do not analyze them in detail, and use the obtained theoretical estimates in [3], backed up by their experimental data. For magnetite particles with similar particle sizes in the field $H_0 = 150$ Oe varying with frequency 100 kHz the total heat release does not exceed 50 kW/kg. Their data demonstrate a linear dependence of the frequency and quadratic dependence of H_0. Under our $f = 50$ Hz and $H_0 = 100$ Oe we receive less than 10 W/kg. The mass of our magnetic particle is of about 10^{-20} kg. Thus, the released thermal energy is of about 10^{-19} W or 10^{-21} J per half-cycle of the magnetic field variation.

Since this energy is 10 times more than the energy dissipation of turning particles, heating is also increased 10 times as compared with (8), and hence it is also negligible.

Absence of heating in our experiments, as opposed to experiments on hyperthermia, is due to a lower magnetic field frequency and the smallness of the concentrations of magnetite particles. Our values of these parameters are three and eight orders of magnitude, respectively, less compared with the parameters used in [3] when a substantial heating was observed, up to 10 K per hour.

Thus, the alternating magnetic field has no thermal effect on the cells in our experiments.

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