# Elongated Nanoparticle Aggregates in Cancer Cells for Mechanical Destruction with Low Frequency Rotating Magnetic Field

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**Supplementary Information**

S.1. Characterization of the magnetic nanoparticles

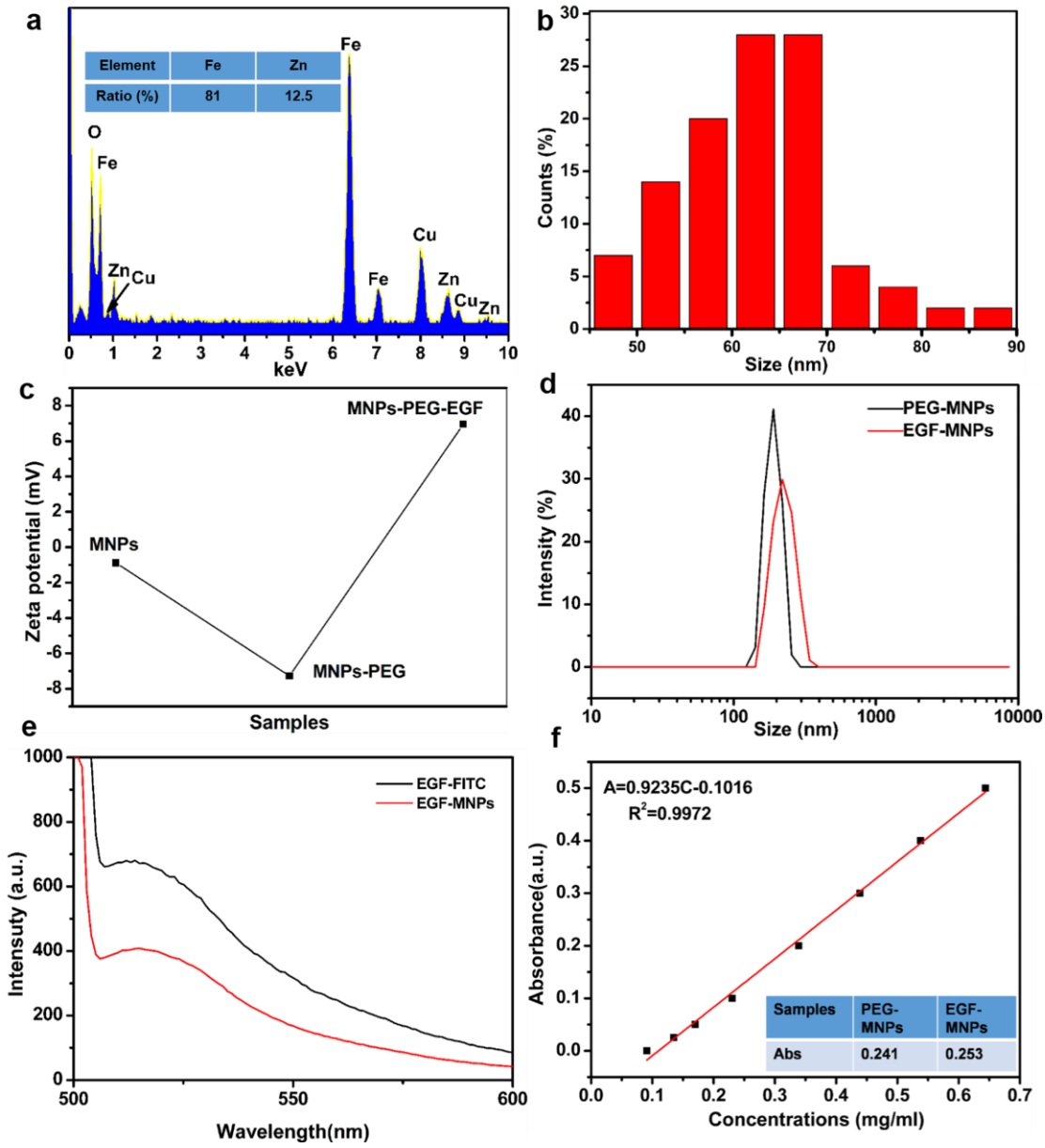


**Figure S1. Characterization of MNPs and EGF-MNPs.** (a) EDS analysis and (b) size distribution of MNPs. (c) Zeta-potentials and (d) hydrodynamic diameters distribution of PEG-MNPs and EGF-MNPs. (e)Fluorescence spectra of EGF-FITC with the concentration of free FITC-EGF and FITC-EGF modified MNPs (EGF-MNPs), respectively ($\lambda_{ex}$ = 488 nm). (f) UV-Vis absorbance vs. concentration as characterized by BCA assay and linear fitting (A and C represent absorbance in arbitrary units and concentration in mg/mL, respectively).

S.2. Effect of the treatment with magnetic nanoparticles and RMF on brain cancer cells

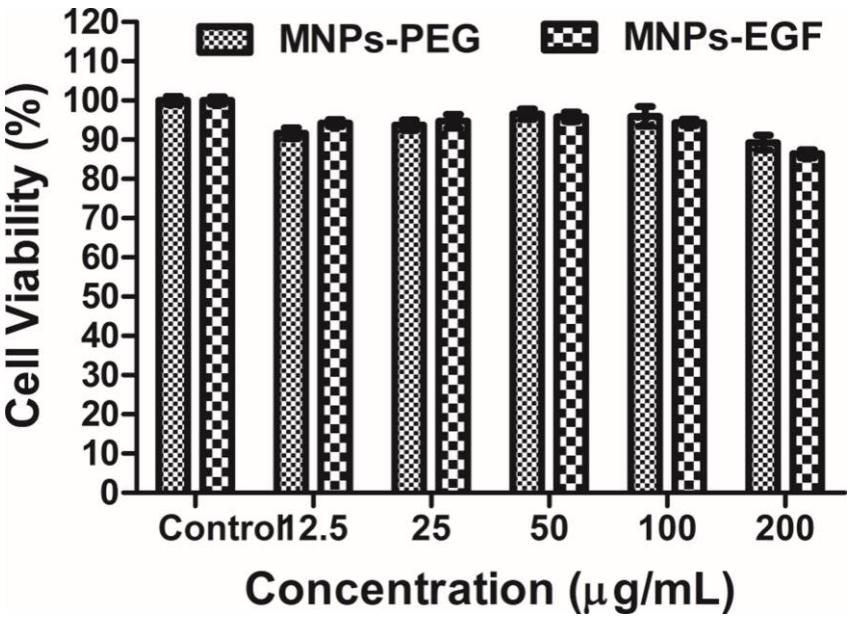


**Figure S2. Cell viability**. U87 cells treated with different concentrations (12.5, 25, 50, 100 and 200 μg/mL) of PEG -MNPs and EGF- MNPs for 24 h, respectively.

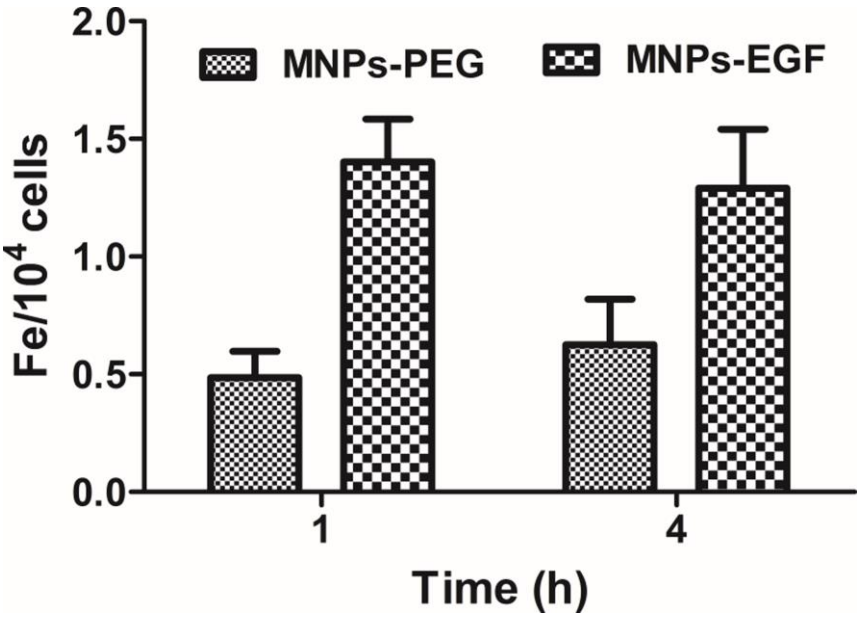


**Figure S3.** Iron concentration in U87 cells cultured with 100 μg/mL of PEG-MNPs and EGF-MNPs 1h and 4h, respectively. The iron content was quantified via ICP-OES**.**

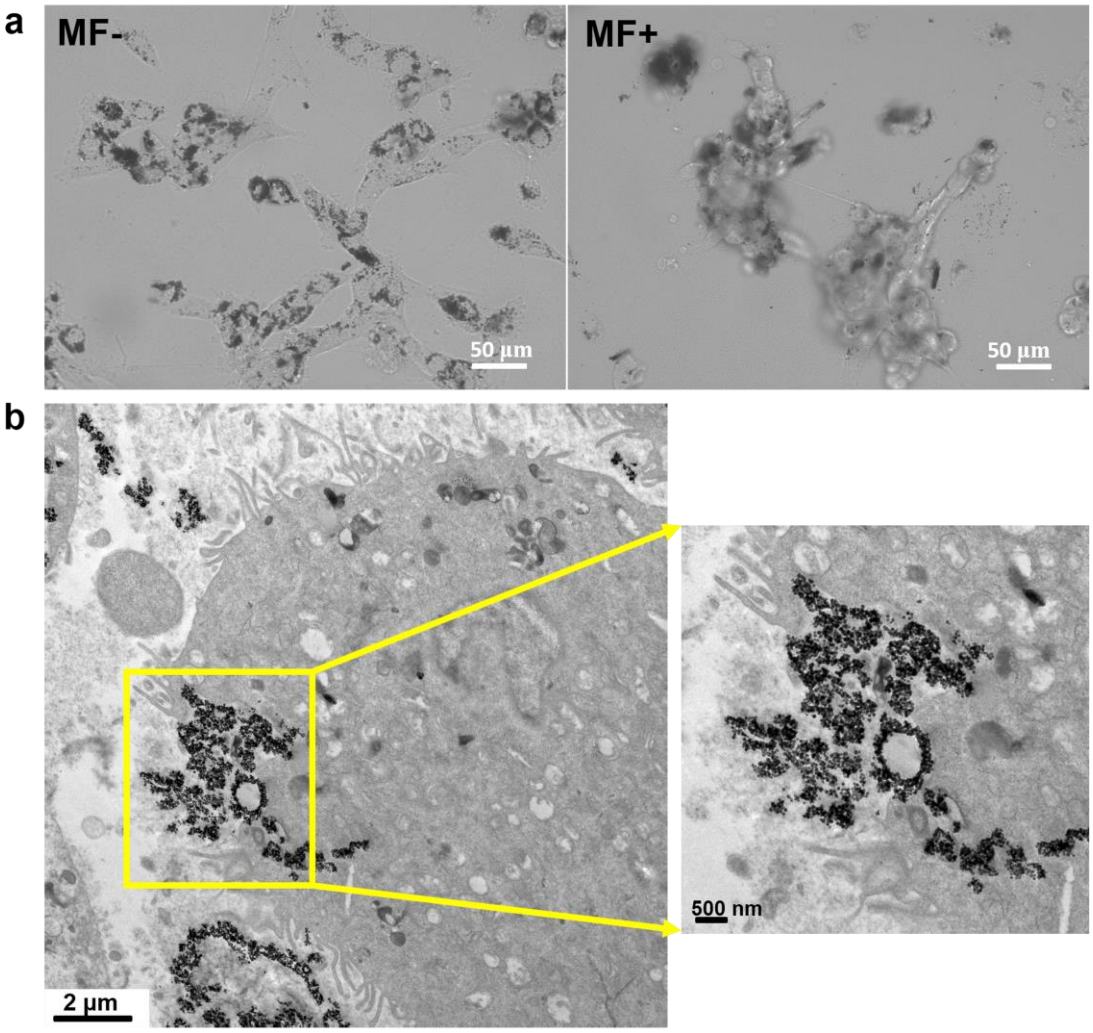


**Figure S4. Micrograph images of U87 cells were incubated with 100 µg/mL for 24 h**. (a) without RMF (all the scale bars are 50 µm), (b) with RMF treatment for 30 min.

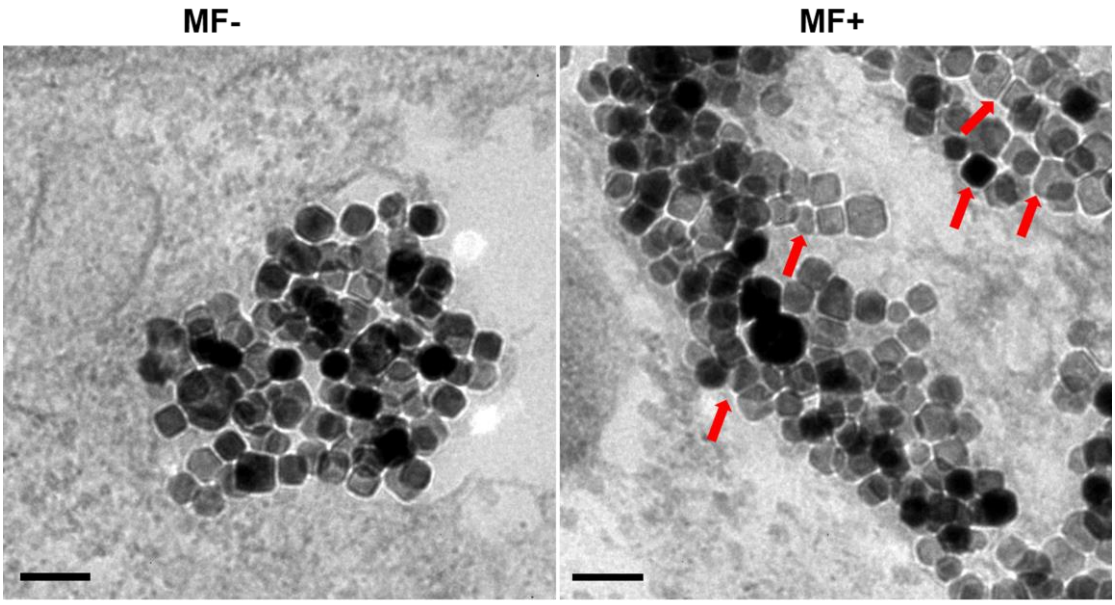


**Figure S5.** TEM images showing EGF-MNPs in lysosomes of U87 cells after incubation for 24 h (MF-) and after a subsequent 30 minutes, 15 Hz, RMF treatment (MF+). (Scale bar: 100 nm)

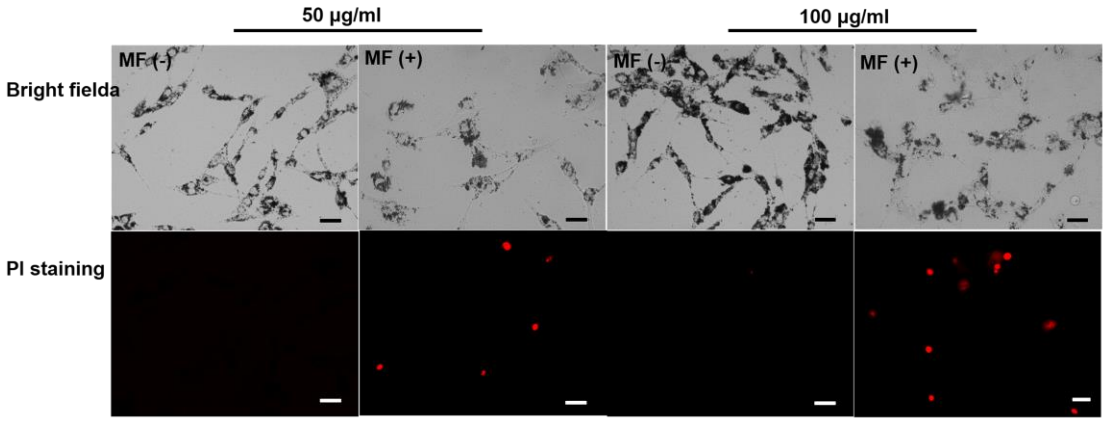


**Figure S6. Live cell Imaging System images.** U87 cells were incubated with 0, 50, 100 μg/ml EGF-MNPs for 24 h and exposed to a 15 Hz RMF for 10 min with PI staining. (Scale bar: 50 μm).

S.3. Magnetic field in the spinning device and torque and force on the aggregates of magnetic nanoparticles

**Formation of aggregates of magnetic nanoparticles**. As explained in the article, both the initial interaction of nanoparticles and the effect of the magnetic field results in the formation of aggregates of elongated shape (observed in particular in the lysosomes as shown in the Results section of the article). The TEM images in the article show large aggregates containing $10^2$ – $10^3$ magnetic nanoparticles in the two-dimensional image. For instance, it is possible to estimate the number of

MNPs in the big aggregate in Figure 4b, boxed in iii, dividing the total area by the area of a single MNP. The boxed area included in total about $1.65 \times 10^3$ EGF-MNPs. Therefore, in our calculations, we will consider a reference value of $10^3 - 10^4$ nanoparticles in the aggregates.

**Torque on the aggregates of magnetic nanoparticles**. In the videos showing the rotation of the aggregates of nanoparticles in our system, it is observed that in the cell-growth medium typically some aggregates perform a synchronous rotation with the frequency of the rotating magnetic field. The nanoparticle aggregates are magnetically anisotropic, with easier magnetization along the direction of larger size [S1]. Because of the anisotropic magnetization of the nanoparticle aggregates, which is higher along the longitudinal direction (i.e. the direction of larger size), their magnetization if higher in that direction that in the transversal direction and the magnetic moment is nearly aligned in the longitudinal direction, even if the magnetic field is oriented in a relatively different direction (further discussed below). The torque on an aggregate is given by

$$\vec{\tau} = \vec{m} \times \vec{B}$$

Being $\vec{m}$ the total magnetic moment of the aggregate and $\vec{B}$ the external magnetic field (in our system produced by the rotating magnet). For steady rotation, the modulus of the torque is then given by $m_0 B_0 \sin[(\omega - \Omega)t + \delta_0]$, being $m_0$ the modulus of the total magnetic moment of the magnetic nanoparticles, $B_0$ the modulus of the rotating magnetic field, $(\omega - \Omega)$ the difference in angular frequency of the rotating magnet $(\Omega)$ and the aggregate $(\omega)$, and $\delta_0$ the initial phase difference between the two magnitudes. Therefore the magnitude of the torque is not higher than $m_0 B_0$.

For many aggregates of moderately large size, it is observed in the microcope that the rotation is synchronous with the rotating magnetic field, i.e. $(\omega - \Omega) = 0$. Therefore, the torque is given by $m_0 B_0 \sin[\delta_0]$. If the viscosity of the surrounding medium is sufficiently small, the torque is small and also the angle $\delta_0$, being the long direction of the aggregates practically aligned with the magnetic field. If $\delta_0$ is relatively small, the magnetic moment is practically oriented in the direction of larger size of the aggregates, due to their magnetic anisotropy, as explained above.

In our experiments, performed at a distance from the rotating magnets (z = 5mm) for which $B_0 \approx 40$ mT, the expected magnetization of the nanoparticles is of the order of $M \approx 0.02$ Am$^2$/g

(see Figure 1 of the article). The total magnetic moment is $m_0 = \text{mass} \times M$. For a total number of $10^3$ magnetic nanoparticles (of cubic shape with thickness of 60 nm, i.e. the mass of each NP is $1.12 \times 10^{-15}$ g), $m_0 \approx 2 \cdot 10^{-14}$ Am$^2$, being therefore the torque not higher than $m_0 B_0 \approx 8 \cdot 10^2$ pNμm. The torque may be much smaller for small values of the angle $\delta_0$ between $\vec{m}$ and $\vec{B}$, when the aggregate rotates in an environment of low viscosity. For large values of the angle between $\vec{m}$ and $\vec{B}$, the total magnetic moment may not be aligned in the direction of the long axis of the aggregate, being also its magnitude smaller due to the lower magnetization of the aggregate in the transversal direction (higher demagnetizing factor) .

An estimate of the maximum value of the torque may be obtained by assuming that the magnetization of the aggregate is negligible in the transversal direction (compared to the longitudinal direction), and that the magnetic moment in the longitudinal direction varies linearly with the component of the magnetic field in that direction, between the value given by the retentiveness $m_r$ and the value for 40 mT, $m_0$. If $\theta$ is the angle between the magnetic field and the longitudinal direction of the aggregate, the modulus of the torque is then

$$\tau = B_0(m_r + (m_0 - m_r)\cos(\theta))\sin(\theta)$$

From the magnetization curve of the nanoparticles, $m_r/m_0$ = 0.0036 Am$^2$/g / 0.02 Am$^2$/g. The maximum value of the modulus of the torque is obtained for $\theta = 40°$ an is equal to

$$\tau_{max} = 0.52\ B_0 m_0$$

Therefore, $\tau_{max} \approx 4 \cdot 10^2$ pNμm for an aggregate containing $10^3$ nanoparticles.

**Maximum forces associated to the magnetic torque**. One simplistic approach to estimate the order of magnitude of the forces exerted by the aggregate of nanoparticles, due to the magnetic torque, is to consider a linear distribution of friction force per unit length during the rotation of the nanoparticle in a viscous medium. Then, as represented in Figure S7, the total forces (equal to the equivalent point forces) would be $F = \tau / \frac{2L}{3}$. The TEM images in the article show that the length of the large aggregates is of several microns. For $L$ = 5 μm, the maximum force would be of the order of $10^2$ pN.

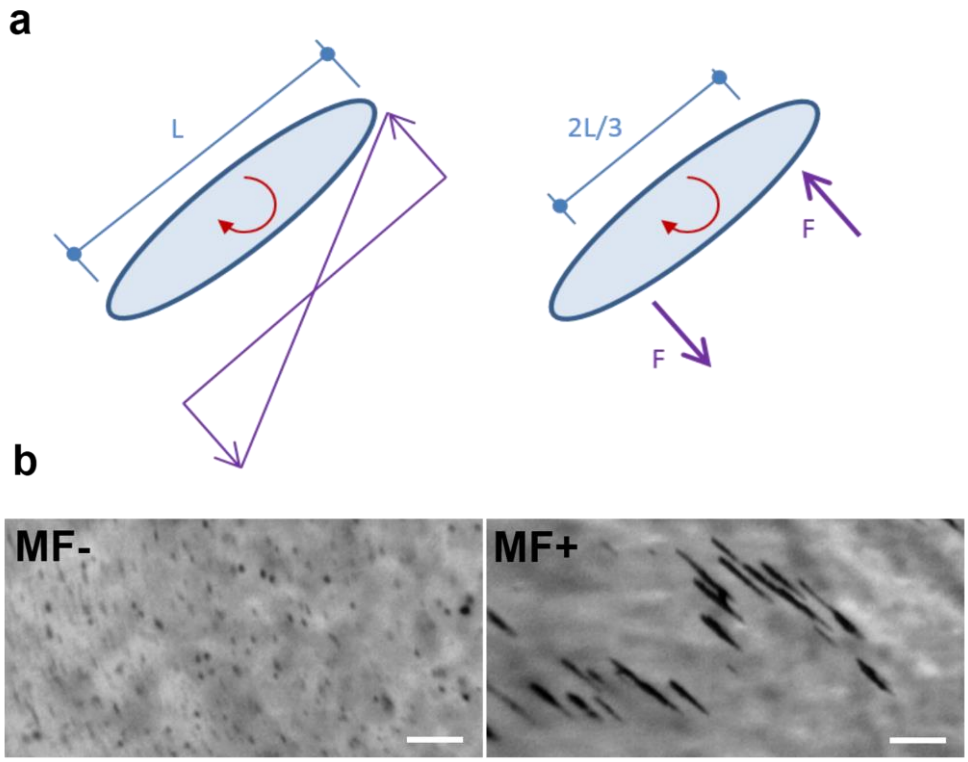


**Figure S7**. a. Scheme of a linear distribution of friction force per unit length on a rotating aggregate of nanoparticles and equivalent point forces, b. Microscope images of EGF-MNPs in matrigel before and after MF exposure. (Scale bar: 2 μm).

**Magnetic force, due to the gradient of magnetic field, on the aggregates of magnetic nanoparticles**. Due to the gradient of the magnetic field created by the magnets, there is a magnetic force acting on the aggregates of nanoparticles. The force is, in principle, an alternating force due to the rotation of the magnetic field.

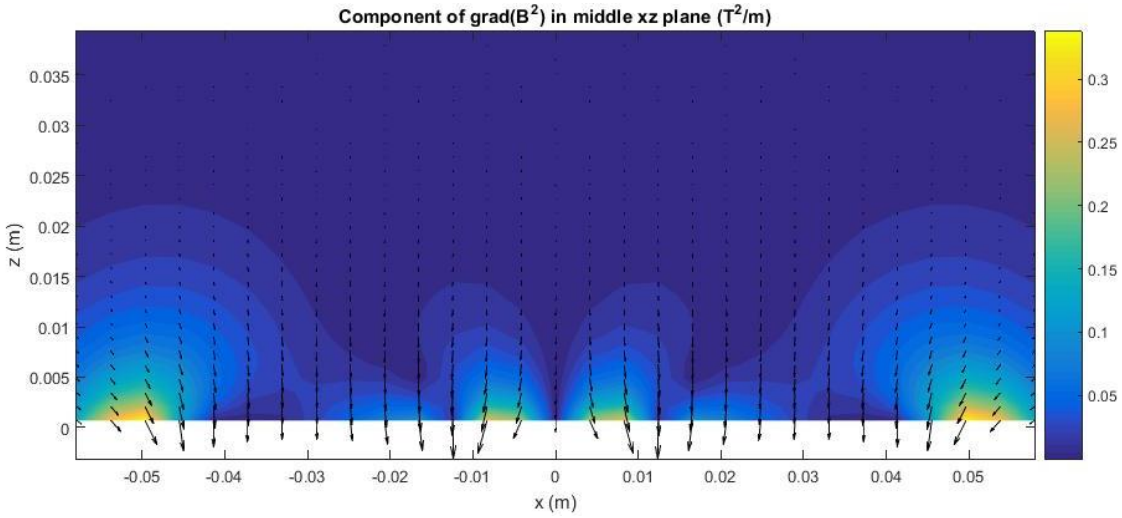


**Figure S8**. Field of $\vec{\nabla}(B^2)$ in xz plane.

The calculation of the force depends on how the aggregates are magnetized, and in the case of the aggregates rotating synchronously with the magnetic field, an estimate may be obtained assuming that $\vec{m}$ is nearly proportional to $\vec{B}$: $\vec{m} = C\vec{B}$ (an also $m_0 = CB_0$).

The force on the magnetic nanoparticle is given by [S2]:

$$\vec{F} = m_k \vec{\nabla} B_k$$

Introducing in this equation the proportionality relationship between magnetization an magnetic moment,

$$\vec{F} = CB_k \vec{\nabla} B_k = \frac{1}{2} C \vec{\nabla}\left(\vec{B} \cdot \vec{B}\right) = \frac{1}{2} C \vec{\nabla}(B^2)$$

The slope $C$ of the magnetization curve is, about $B_0 = 40$ mT, $C = \frac{m_0}{B_0} \approx 0.5$ Am$^2$/gT. The vector field $\vec{\nabla}(B^2)$ is shown in Fig. S8 for xz plane. At the distance z = 5mm, and about the axis of rotation, where the cells are placed, the magnitude of this gradient is 0.03 T$^2$/m and it is directed along the rotation axis towards the rotating cylinder, i.e. to the base of the cell-culture dish. The magnitude of the force is given by 0.5×0.5 Am$^2$/gT× 0.03 T$^2$/m = 7.5mN/g. Considering again an aggregate of $10^3$ magnetic nanoparticles (each nanoparticle with a mass of $1.12\times10^{-15}$ g), the total force would be ≈ 0.008 pN. In the case of aggregates non rotating synchronously, or when the angle between the longer axis of the aggregate and the magnetic field is higher, the magnetization of the nanoparticle would be lower. Therefore, the order of the resultant force on the aggregates, due to the gradient of the magnetic field, is relatively small and negligible compared to the forces associated to the rotation.

S.4. Distribution of MNPs in the cells analyzed by confocal microscopy and viability by MTT assay

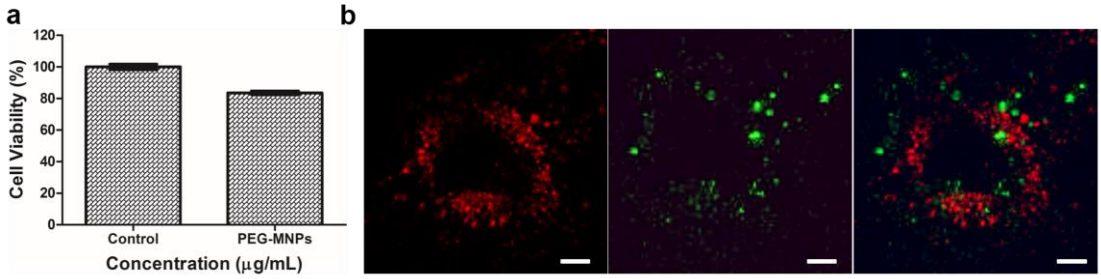


Figure S9. (a) Viability of U87 cells with RMF treatment for 30 min and incubation for additional 24 h, assayed by MTT assay. One group without PEG-MNPs as the control and the other was treated with PEG-MNPs for 24 h. (b) Confocal images of U87 cells incubated with 100 μg/mL of PEG-MNPs during 24 h at 37 °C. Cell lysosomes were stained by lysosome tracker red and the magnetic nanoparticles were labeled by FITC. The images (from left to right) were taken immediately after lysosome tracker red staining for 30 min, at excitations of 488 and 577 nm, respectively. The Pearson's correlation coefficient of PEG-MNPs nanoparticles and lysotracker red is 0.05. The size of the scale bars is 10 μm.

Supplementary references

S1. Coey J.M.D. *Magnetism and Magnetic Materials*, Cambridge University Press (2010).

S2. Zangwill A. *Modern Electrodynamics*. 1st Edition. Cambridge University Press (2012).

**Video S1**. Movement of aggregates of MNPs in cells under the action of the RMF at 0.67 Hz. In the experiment, the matrigel with a final concentration of 1 mg/mL was added after U87 cells incubated with EGF-MNPs for 24 h, then exposed to RMF; likely due to inhomogeneities in the intercellular environment and in the interface between the matrigel and the plate, the average viscosity of the surrounding material is different in different aggregates, resulting in a range of movements of the aggregates, from rotation synchronous with the magnetic field to inappreciable movement.