

Manipulation of cellular orientation and migration by internalized magnetic particles

Jiaojiao Liu,[†] Xiaodong Tian,[†] Meimei Bao, Jingliang Li, Yujiang Dou, Bing Yuan,^{*} Kai Yang,^{*} Yuqiang Ma

Supplementary Information

S1. Experimental procedures

S1.1 Materials

Aqueous dispersion of carboxyl-modified Fe₃O₄ particles with a size of 100–200 nm (0.1 mg mL⁻¹) was purchased from Suzhou Nord Derivatives Pharm-tech Co., Ltd (China). Rhodamine phalloidin, for F-actin labeling, was purchased from Life Technologies. LysoTracker Red DND-99 (1 mM) and DAPI, for lysosome and nucleus fluorescence localizations respectively, were purchased from Shanghai Beyotime Bio-tech Co., Ltd (China). Paraformaldehyde (8%) was purchased from Electron Microscopy Sciences. Human gastric carcinoma cells (MGC-803) were purchased from the cell bank of the Chinese Academy of Sciences in Shanghai and cultured in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C and equilibrated in 4% CO₂ and air.

S1.2 Cellular endocytosis of Fe₃O₄ particles

An amount of ~60k MGC-803 cells were pre-seeded onto the cover-glass substrate of the chamber cell. It was then incubated in 2.5 mL culture medium containing 0.277, 1.385 or 2.770 μg mL⁻¹ Fe₃O₄ particles at 37 °C for 24 h for the following experiments. Each time prior to use, the particle dispersion was diluted with cell medium and sonicated step by step, to escape from precipitation before cellular uptake.

S1.3 Cell modulation under magnetic field direction

After incubation, the cells in the chamber was washed carefully with PBS to remove the excess Fe₃O₄ particles and re-filled with fresh medium again. (Besides the MNPs those have been uptaken by the cells, there are still some particles or particle aggregates adsorbed onto the substrate which can not be removed by washing.) The whole chamber was transferred into the incubator of Cell Cultivation Systems equipped on the Zeiss confocal microscope. Two pieces of bar magnet (with surface

magnetic field of around 2,000 G each) were placed at one end of the chamber, being 15 mm away from observation area, to provide a magnetic field in the vertical direction. The behavior of cells was monitored in situ under the time-lapse confocal microscopy.

S1.4 Fluorescence labeling of cells after magnetic modulation

For the fluorescence labeling of lysosome, the cells were cultured with LysoTracker Red DND-99 (at 50 nM) at 37 °C for 60 min. It was then washed with PBS for 3 times and fixed with 3.7% paraformaldehyde for 10 min. After washing 3 times with PBS and one more time with distilled water, the cells were dried in air flow for further confocal and AFM observations to estimate the localization of Fe₃O₄ particles within cells.

Cells both before and after magnetic modulation were F-actin labeled following the traditional procedures. For short, the cells were first fixed with 3.7% paraformaldehyde for 10 min, washed 3 times with PBS, followed by 0.1% Triton X-100 permeation for 5 min and PBS washing. Rhodamine phalloidin (5 μL diluted in 200 μL 50 mg mL⁻¹ BSA), followed by 10 μg mL⁻¹ DAPI, was added and incubated in dark for 20 and 10 min, respectively, for F-actin and nucleus staining. The cells were then washed 3 times with PBS and one more time with water, and dried in air flow for observation.

S1.5 Other characterizations

The size distribution and charges of the Fe₃O₄ particles were confirmed through dynamic light scattering and zeta potential tests by the Zetasizer Nano ZS90 equipment (Malvern Instrument Ltd., UK). Optical observation was performed on a LSM 710 inverted confocal laser scanning microscope (Zeiss, Germany) equipped with a 63× oil objective. The microscope is also equipped with a Cell Cultivation System including an incubator with the temperature, humidity and CO₂ controlling modules. DAPI stained nucleus was excited through the EX G 365 nm filter, and the fluorescence was collected through the EM BP 445/50 filter. The excitation and emission for observing the Rhodamine phalloidin labeled F-actin and LysoTracker Red DND-99 labeled lysosome were using the EX BP 546/12 nm and EM BP 575-640 nm filters. The transmission channel illuminated with a Halogen lamp was acquired meanwhile.

S1.6 Data analysis

Cell viability analysis. The analysis of cell viability under different conditions was realized through counting the number of cells before and after the treatment. An amount of 60k cells was initially collected for all the experiments. After the particle incubation and/or magnet treatment, the cells were trypsinized and stained with a 0.4% trypan blue solution followed by counting the number again to calculate the viability of cells.

Cell orientation analysis. For a certain cell, the orientation angle, θ , was determined through analyzing the direction of the cell in respect to that of the magnetic field with an in-house developed program, based on the microscopy images. Cells, with internalized MNPs, were exposed to the magnetic field at the time of 0 h. An amount of approximately 30 cells were monitored and tracked. Time-lapse images were taken at the time of 2, 4, 6, 8 and 10 h. In each image, the orientation angle of these cells was determined, and the percentage of cells within three angle ranges (i.e. $0 < \theta \leq 30^\circ$, $30^\circ < \theta \leq 60^\circ$ or $60^\circ < \theta \leq 90^\circ$) was calculated respectively. The time-dependent distribution of such percentages was plotted, to demonstrate the influence from magnet on cellular orientation. Four repeated experiments were carried out, and error bars were obtained based on the SD of these parallel systems.

Cell migration analysis. A real-time video recording the behavior of cells under magnet treatment, at 3 min per frame and for a total time of over 12 h, was acquired by the time-lapse microscopy observation. The cells were pre-treated with Fe_3O_4 NPs at different concentrations and magnet was applied at the time of 0 h. The morphology and position of each cell were tracking, and more than 30 cells from four independent experiments were analyzed. During the whole duration, the migration behavior of the cells was recorded and divided into two types: moving towards the magnetic direction, and others (including those maintaining the original direction of the filopodium, moving in complex directions, or do not move at all). The percentage for each type was obtained and the time-dependent distribution of the percentages was plotted.

S2. Characterization and cellular uptake of Fe₃O₄ NPs

Hydrodynamic diameter measured in water for the Fe₃O₄ NPs as shown in Fig. S1(a) is determined to be 170±50 nm. The particles are negatively charged due to the carboxyl-decorated surface. Fig. S1(b) shows the AFM image of the NPs spin-coated on a mica surface, and the height profile in (c) corresponds to the red line in (b). More than 20 particles were analyzed and an average size of the particles was determined to be approximately 150 nm.

The MGC-803 cells were cultured with Fe₃O₄ NPs at 37 °C for 24 h. After the particle treatment, a large amount of particles was taken up probably through endocytosis by the cells. Fig. S1(d) and (e) are AFM images of cells, with and without particle treatment, followed by air-flow drying on the glass substrate. From the image, rough sketch of the cells can be distinguished. The prominently raised spheres within the cellular area refer to the nuclei of cells. In addition, there are many little spots separated around the nucleus in image (d), which can not be observed in (e). Fig. S1(f) presents the height profile referring to the red line in (d), showing an average height of the spots to be around 200-250 nm, being quite approaching the size of the Fe₃O₄ particles.

Based on the previous reports, the NPs endocytosed by a cell are probably localized in the lysosomes within the cell. To further confirm the position of the internalized Fe₃O₄ NPs, the cells, after particle treatment, were lysosome-labeled with fluorescence and observed under confocal microscope. Lyso-Tracker Red is one of the frequently used molecular probes based on DND 99, which would specifically stay in the acidic lysosomes for the targeted fluorescence staining of them. Fig. S1(g) shows the confocal images, in transmission, fluorescence and overlaid channels, of model cells treated with Fe₃O₄ NPs followed by lysosome labeling. The red spots refer to the fluorescence-labeled lysosomes, and the black particles in the transmission channel stand for the Fe₃O₄ NPs or aggregates. The co-localization of particles with lysosomes in the overlaid image confirms the localization of endocytosised particles in the lysosomes of a cell. This further confirms that the spots in (d) should stand for the particle-encapsulated lysosomes.

S3. Supplementary images

Fig. S1 (a, b), DLS profile and AFM image of the Fe_3O_4 NPs in dispersion or deposited on a mica surface, respectively. (c) presents the height profile along the red line in panel (b). (d), AFM image of a model MGC-803 cell after Fe_3O_4 treatment. Cells are incubated with $0.277 \mu\text{g mL}^{-1}$ Fe_3O_4 NPs at 37°C for 24 h. As control experiment, model cells without Fe_3O_4 treatment were also shown in panel (e). (f), height profile along the red line in panel (d), demonstrating the size of particle-encapsulated lysosomes, as marked with black arrows in (d). (g), confocal micrographs, in the transmission, red and overlaid channels, of typical cells showing the intracellular distribution of Fe_3O_4 particles in MGC-803 cells. Cells are treated by Fe_3O_4 NPs (at $1.385 \mu\text{g mL}^{-1}$) at 37°C for 24 h followed by lysosome fluorescence labeling with LysoTracker Red DND-99. Scale bars refer to $10 \mu\text{m}$.

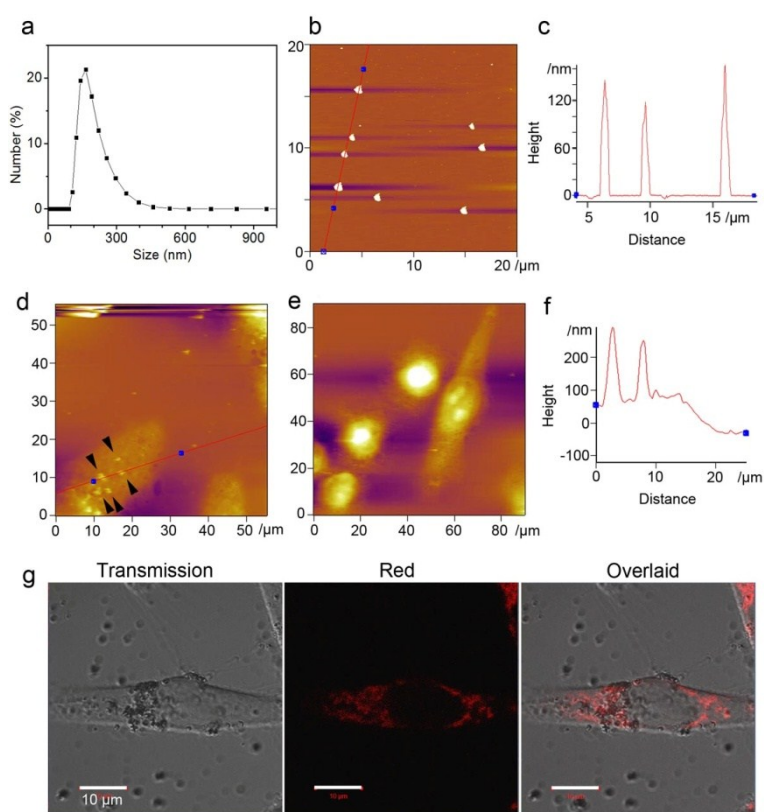


Fig. S2 Side view of magnetic field simulations in Comsol software. Rectangle and circle in black demonstrate the position of magnet and petri dish, respectively. Red arrows represent field direction, intensity is color coded (with logarithmic coordinate; low intensity in dark blue, high intensity in red).

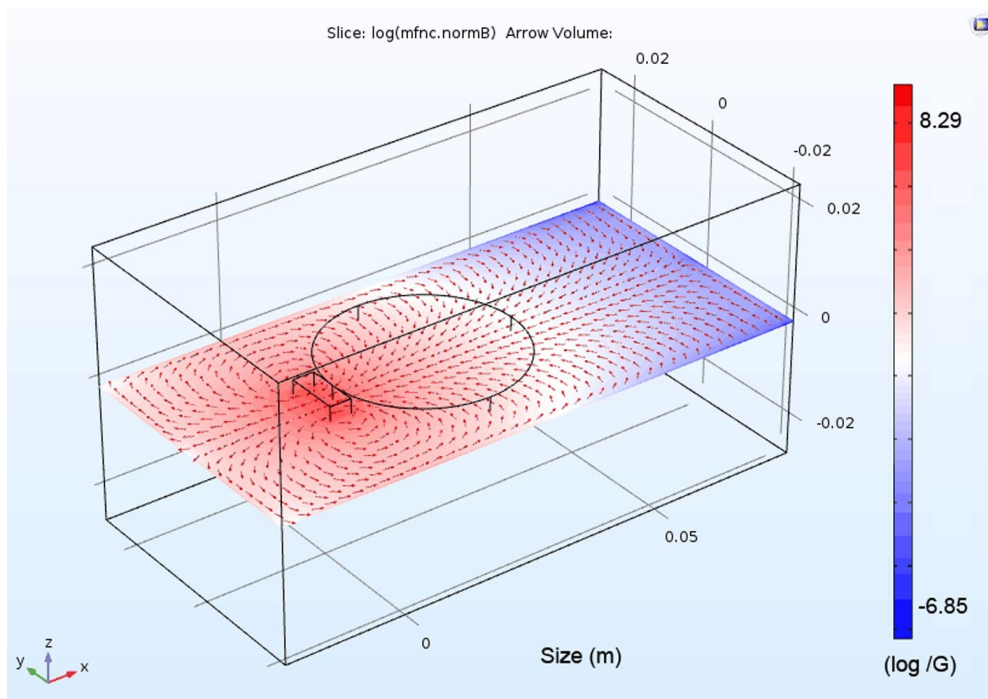
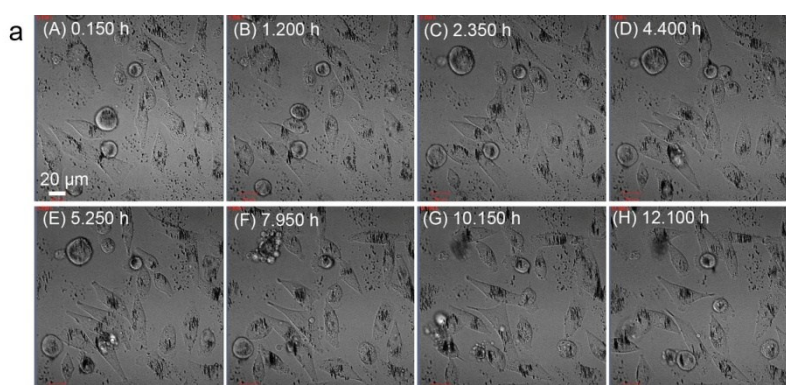


Fig. S3 Time-lapse microscopy images tracking the movement of cells in magnetic field. The cells are pre-treated with Fe_3O_4 NPs at 0.277 (a) and $1.385 \mu\text{g mL}^{-1}$ (b) at 37°C for 24 h. The magnet orientates downward in the image. The black clusters refer to Fe_3O_4 particles or particle aggregates. The time taking the image was marked in the top-left, and at the time of 0 h the magnet was applied.



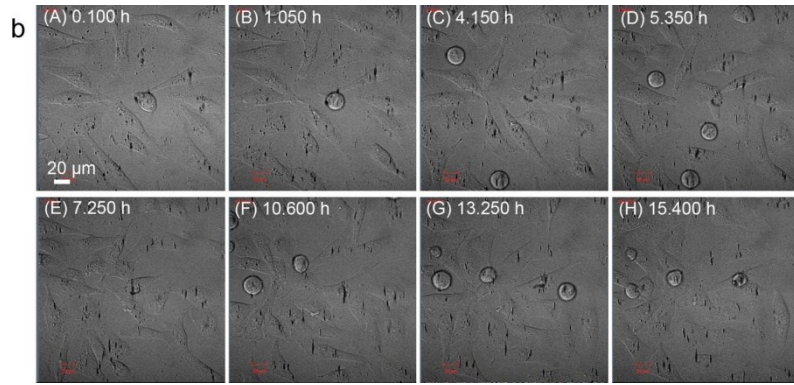


Fig. S4 Microscopy images showing the spatial distribution of cells with loaded MNPs, before (a) and after (b) magnet treatment. The cells were pre-cultured with MNPs at $2.770 \mu\text{g mL}^{-1}$ and the magnet was applied in a downward direction. (Right before taking each image, the magnet was withdrawn for a while and the chamber was gently shaken to disperse the particles (or particle aggregates) in order to avoid eye disturbance. This operation would not influence the cell distribution.)

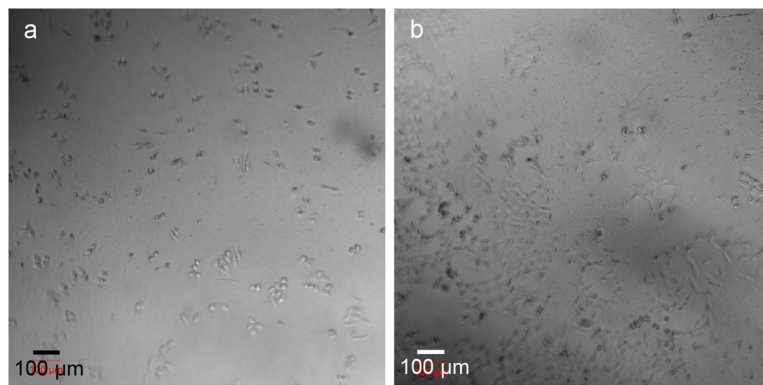
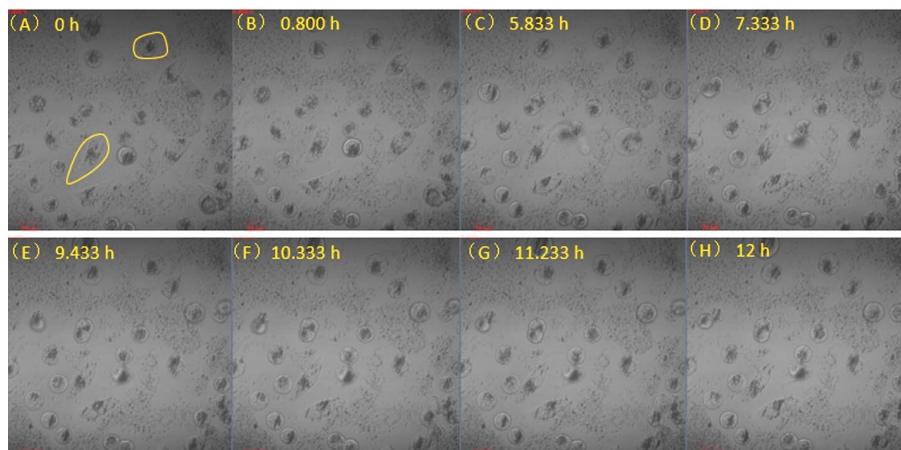


Fig. S5 Time-lapse microscopy images of HeLa cells exposed to magnetic field. The cells were pre-incubated with Fe_3O_4 particles at $2.770 \mu\text{g mL}^{-1}$. The magnet was applied in a vertical direction. MNP modulation on orientation or migration of cells was barely perceptible in this system.



S4. Supplementary video

Video S1. Time-lapse tracking of cells in magnet under microscopy. The cells were pre-treated with $2.770 \mu\text{g mL}^{-1}$ Fe_3O_4 MNPs at $37 \text{ }^\circ\text{C}$ for 24 h and the magnet was applied vertically upward.