Supplementary Materials

Article

Low-Frequency Dynamic Magnetic Fields Decrease Cellular Uptake of Magnetic Nanoparticles

Anna V. Ivanova [1,†], Nelly S. Chmelyuk [1,†], Aleksey A. Nikitin [1,2], Alexander G. Majouga [3], Vladimir P. Chekhonin [1,4] and Maxim A. Abakumov [1,*]

[1] Department of Medical Nanobiotechnology, N.I. Pirogov Russian National Research Medical University, 117997 Moscow, Russia; super.fosforit@yandex.ru (A.V.I.); nellichmelyuk@yandex.ru (N.S.C.); nikitin.aa93@mail.ru (A.A.N.); chekhoninnew@yandex.ru (V.P.C.)
[2] Department of General and Inorganic Chemistry, Mendeleev University of Chemical Technology of Russia, 125047 Moscow, Russia
[3] Laboratory of Biomedical Nanomaterials, National University of Science and Technology (MISIS), 119049 Moscow, Russia; alexander.majouga@gmail.com
[4] Department of Basic and Applied Neurobiology, Serbsky National Medical Research Center for Psychiatry and Narcology, 119991 Moscow, Russia
* Correspondence: abakumov1988@gmail.com; Tel.: +7-9035864777
† These authors contributed equally to this work.

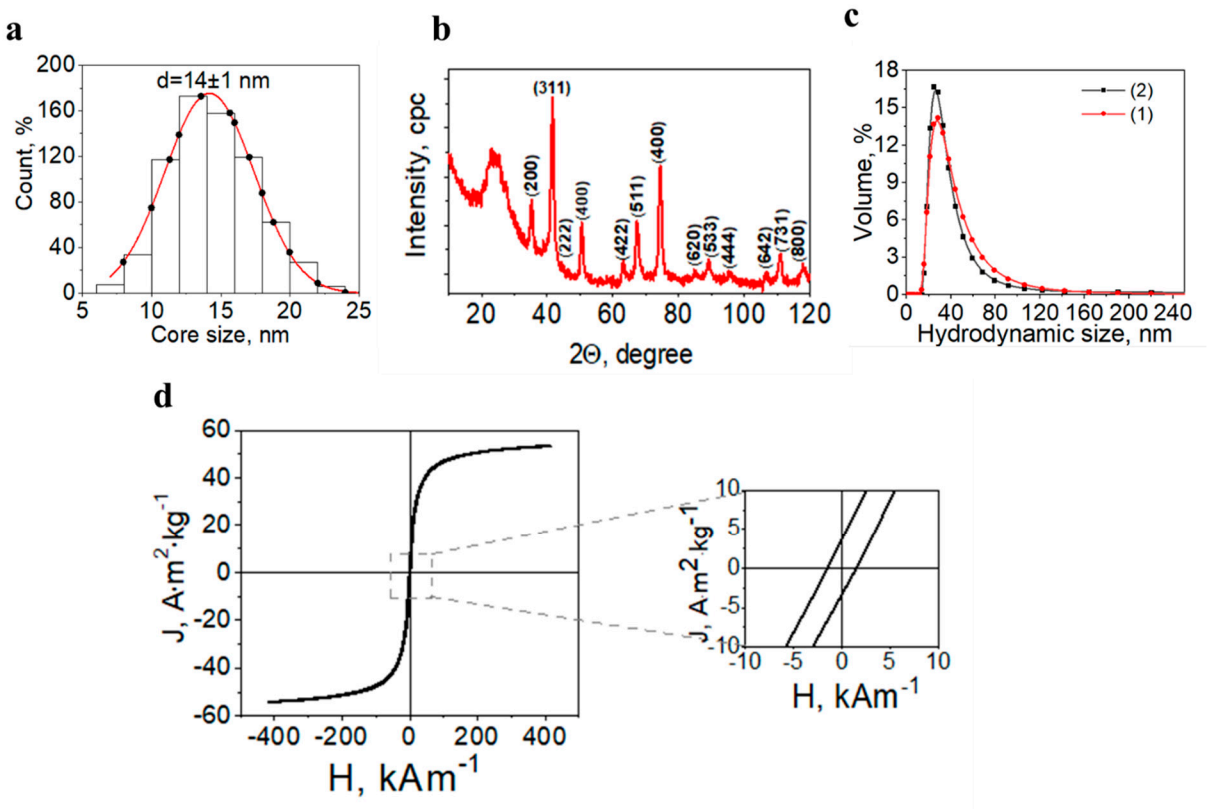

Figure S1. (a) Histogram of MNPs core size distribution. (b) XRD pattern of Fe_3O_4@OA MNPs powder with Miller indices of the Bragg peaks in an inverse spinel structure (cps, counts per second). (c) Hydrodynamic diameter of Fe3O4-DOPAC-PEG-Cy5 and Fe3O4-DOPAC-HSA-PEG-Cy5 in PBS at pH=7.4. d) Hysteresis loop of MNP at T=300 K. The specific magnetization was calculated using the Fe3O4 mass and the Fe content determined by the thermogravimetric analysis and AES analysis.

Table S1. The hydrodynamic parameters of MNPs.

	Hydrodynamic size, nm	Polydispersity	Solvents

	Volume	Intensity	Index	
Fe3O4-OA	20±1	24±2	0,059	Hexane
Fe3O4-DOPAC	25±1	30±1	0,279	dH2O
Fe3O4-DOPAC-PEG	37±1	46±2	0,283	PBS
Fe3O4-DOPAC-HSA	40±1	43±2	0,268	PBS
Fe3O4-DOPAC-HSA-PEG	37±3	63±1	0,250	PBS
Fe3O4-DOPAC-HSA-PEG-Cy5	38±2	48±2	0,246	PBS
Fe3O4-DOPAC-HSA-PEG-Cy5	39±1	44±3	0,389	PBS

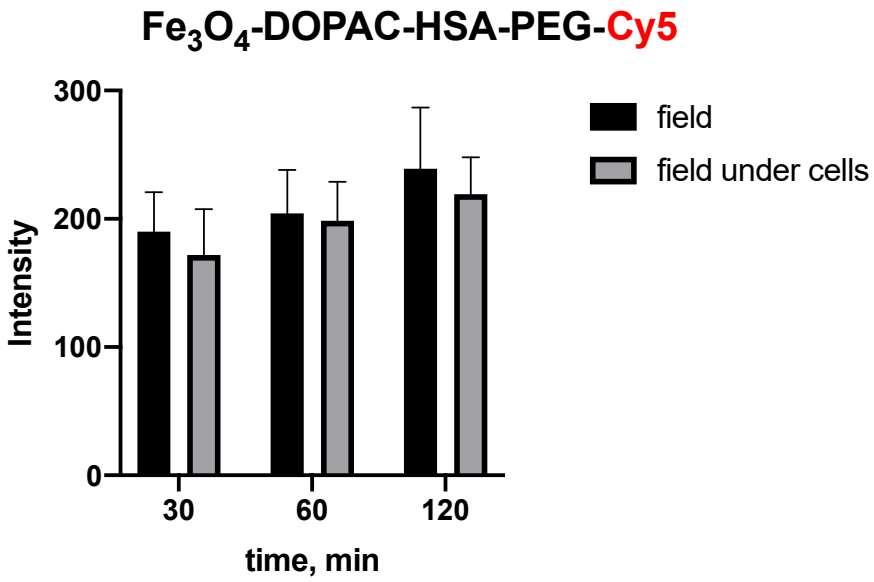

Figure S2. (a) Average intensity values of confocal images of SH-SY5Y cells areas in various incubation time with Fe_3O_4-DOPAC-HSA-PEG-Cy5 when magnetic field was applied from the top of the cells or under the cells, [Fe] = 100 ug/mL. *—*t*-test, significantly different ($p < 0.05$).

For cytotoxicity assay $10*10^3$ SH-SY5Y cells and $15*10^3$ RAW 264.7 cells were seeded in 100uL growth medium in 96 cell well. 24 h after incubation at 37 C, 5 % CO_2, MNPs were added to the cells in various concentrations. After 48 h incubation, the cells were washed with PBS, and a fresh growth medium with MTS-reagent was added (100 ul of growth medium and 20 ul of MTS-reagent in each well). Cells without MNPs were used as a control. The cells were incubated with MTS-reagent for 4 h at 37 °C and 5 % CO_2 in the humid atmosphere. The test was done in six replicates. Statistical analysis using "Unpaired t test" was performed using GraphPad Prism 9.0. Optical density was measured with a Multiscan GO plate reader (Thermo Scientific), $\lambda = 490$ nm. Cell viability was calculated as:

$$\text{Cell viability}(\%) = (As - Ab) / (Ac - Ab) \times 100, \text{ where} \quad (1)$$

As – mean optical density in sample wells,
Ab – mean optical density in blank wells,
Ac – mean optical density in control wells.

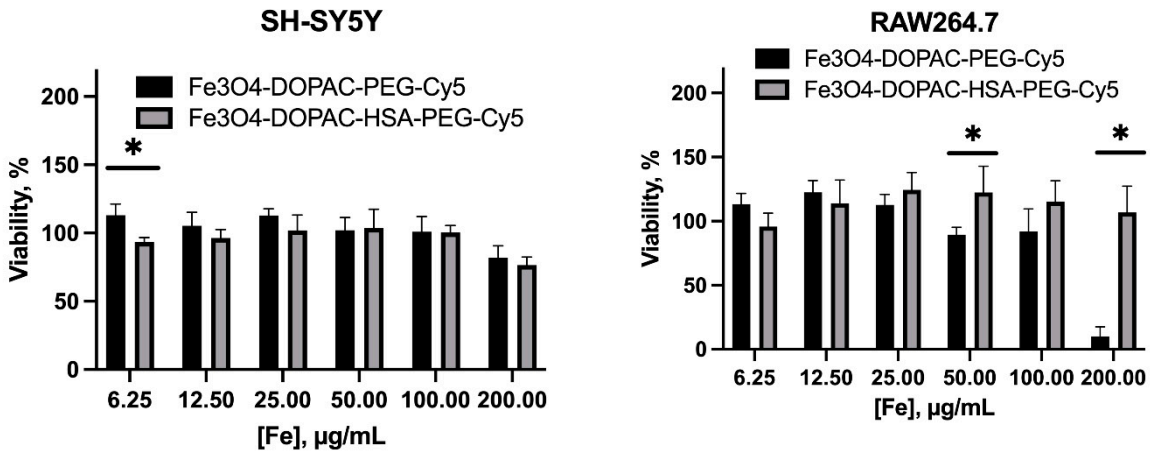

Figure S3. Cytotoxicity assays of RAW264.7 and SH-SY5Y cells, which were incubated with Fe_3O_4-DOPAC-PEG-Cy5 and Fe_3O_4-DOPAC-HSA-PEG-Cy5 MNPs for 48 h. *—*t*-test, significantly different ($P < 0.05$).

Prussian Blue staining.

SH-SY5Y and RAW264.7 cells were seeded confocal petri dish 35 mm (3 × 105 cells/well) and incubated for 24 hours without treatment at 37 C and 5 % CO2. Then the growth medium was replaced and supplemented with MNPs MNPs@DOPAC@PEG@Cy5 or MNPs@DOPAC@HSA@PEG@Cy5 with iron concentration [Fe] = 100 µg/mL and incubated for 2 h. Then cells were washed twice by DPBS and fixed by 4 % paraformaldehyde for 15 min at 4 C. Then, cells were washed by PBS and stained by iron stain kit (HT20-1KT, Sigma) following the manufacturing protocol. The images were obtained using a Nikon Ti2 microscope with 60X 1,25 objective lenses.

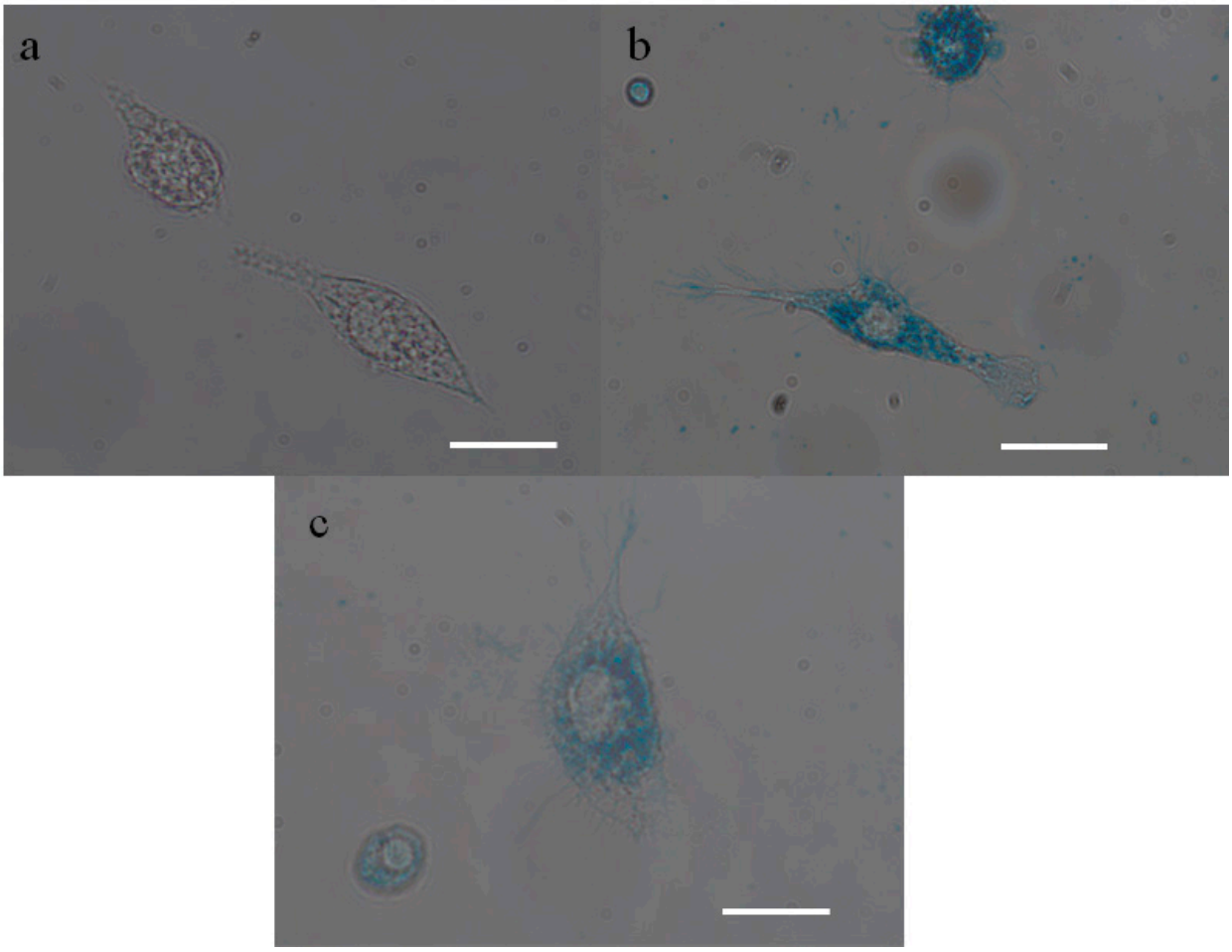

Figure S4. Prussian Blue staining of RAW264.7 cells. a – RAW264.7 cells non treated with MNPS, b - RAW264.7 cells treated with Fe3O4-DOPAC-HSA-PEG-Cy5, c - RAW264.7 cells treated with Fe3O4-DOPAC-PEG-Cy5. Blue color corresponds to signal MNPs. Scale bar - 10 um.

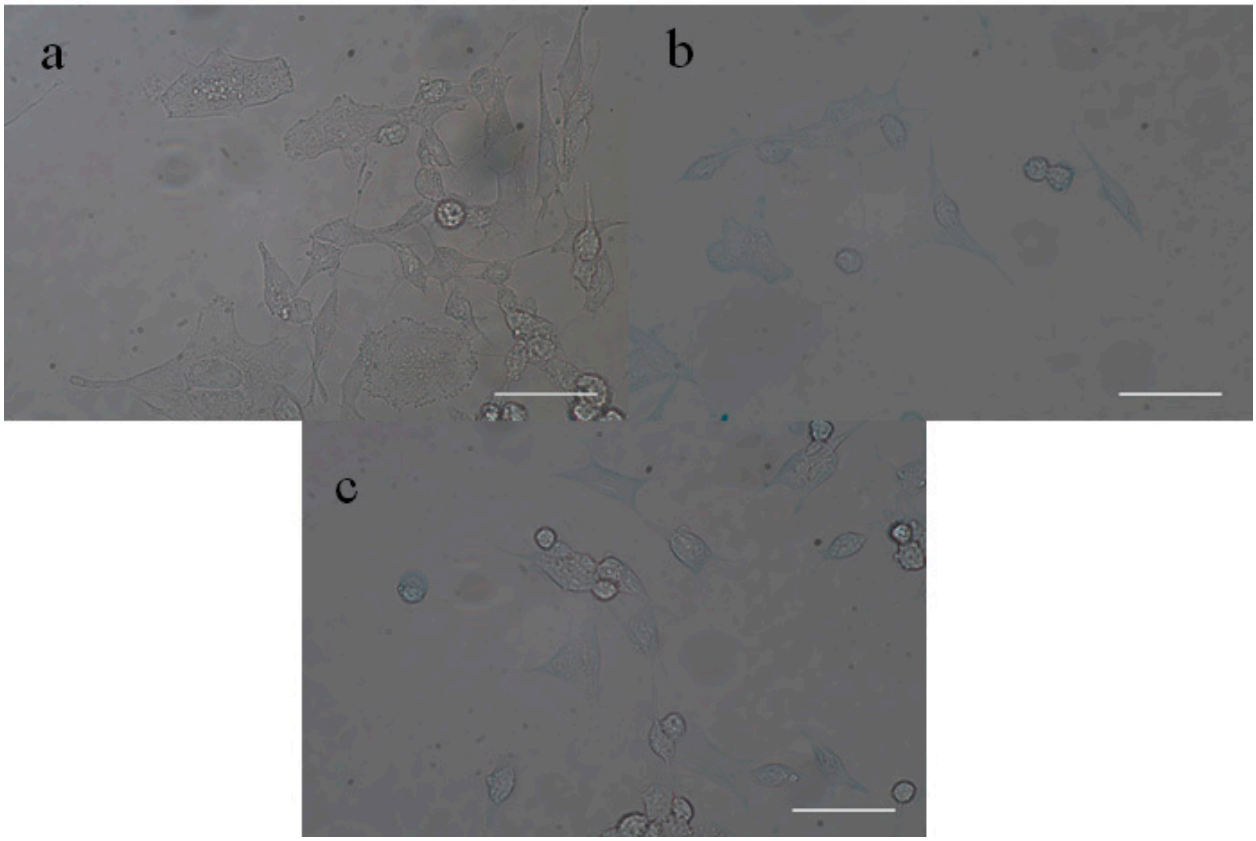

Figure S5. Prussian Blue staining of SH-SY5Y cells. a – SH-SY5Y cells non treated with MNPS, b - SH-SY5Y cells treated with Fe3O4-DOPAC-HSA-PEG-Cy5, c - SH-SY5Y cells treated with Fe3O4-DOPAC-PEG-Cy5. Blue color corresponds to signal MNPs. Scale bar - 100 um.