Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Preparation of Ellipsoid-Shaped Supraparticles with Modular Compositions and Investigation of Shape-Dependent Cell-Uptake

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Hydrophobic Nanoparticles

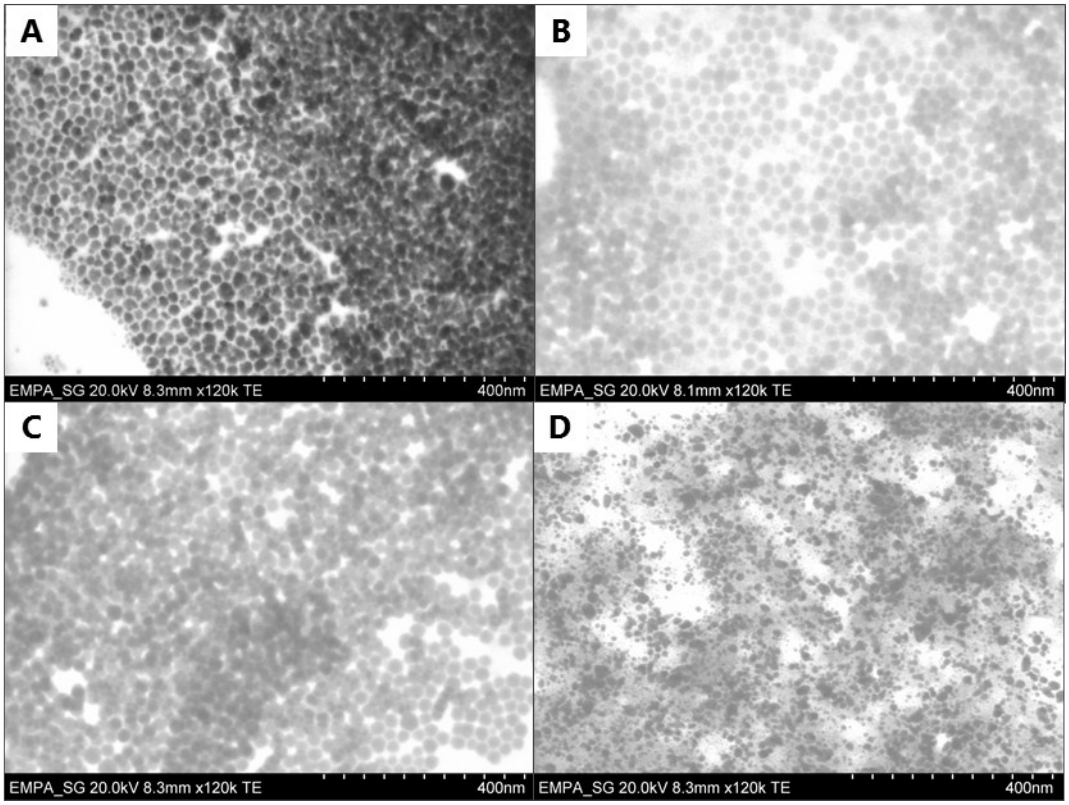

Figure S1. STEM images of the different types of hydrophobic nanoparticles. (A) Magnetite nanoparticles Fe_3O_4NP; (B) anatase nanoparticles TiO_2NP; (C) silica nanoparticles SiO_2NP; (D) silver nanoparticles AgNP.

Absorption and Transmittance Spectra of TiO_2NP and AgNP

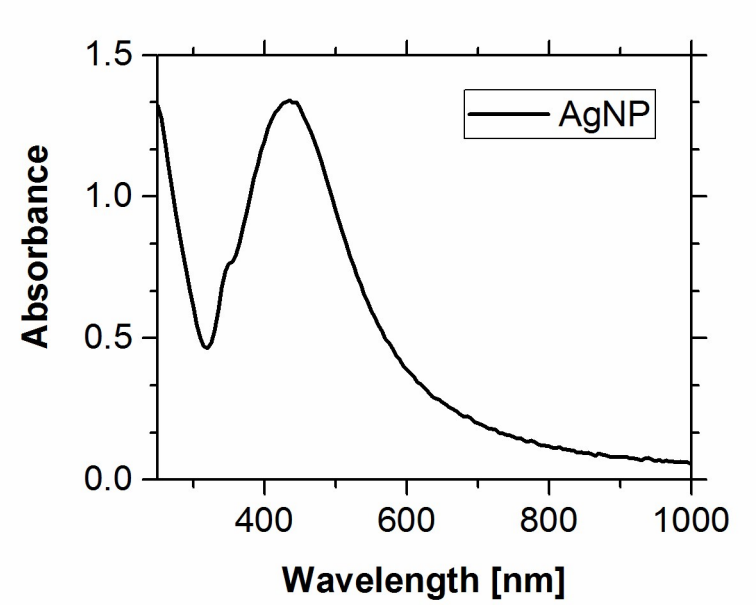

Figure S2. Absorbance of hydrophobic silver nanoparticles AgNP dispersed in chloroform.

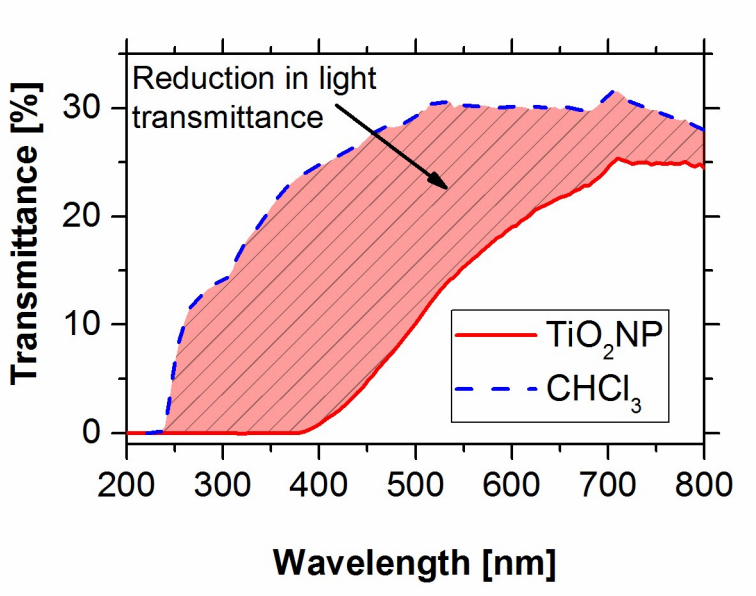

Figure S3. Transmittance spectrum of hydrophobic anatase nanoparticles TiO_2NP measured in chloroform. The area inbetween the two curves for pure chloroform and the nanoparticles dispersion indiated the reduction in light transmission by TiO_2NP.

Parameter: PVA Concentration

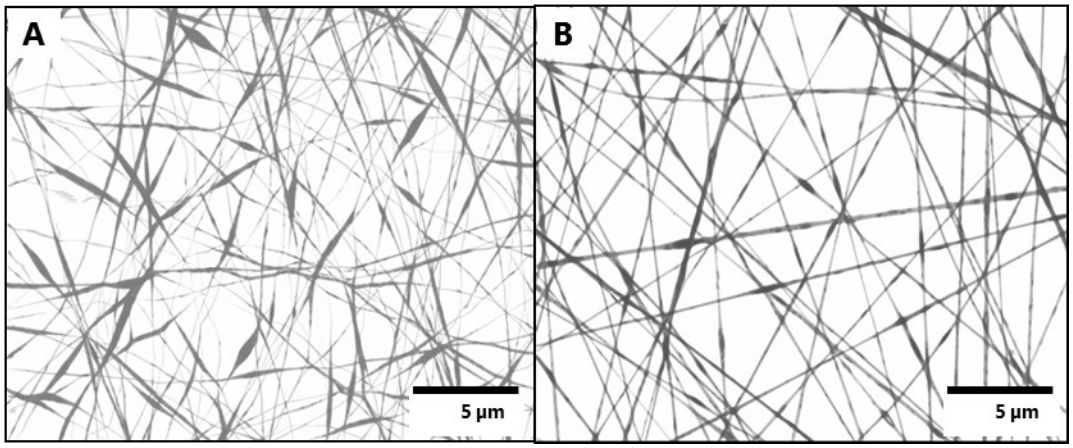

Figure S4. Influence of PVA concentration on electrospun fibers. Both STEM images show Fibers containing ESP of Fe3O4 that were electrospun at (A) 9 wt.% PVA or (B) 10 wt.% PVA.

Parameter: Solvent Type

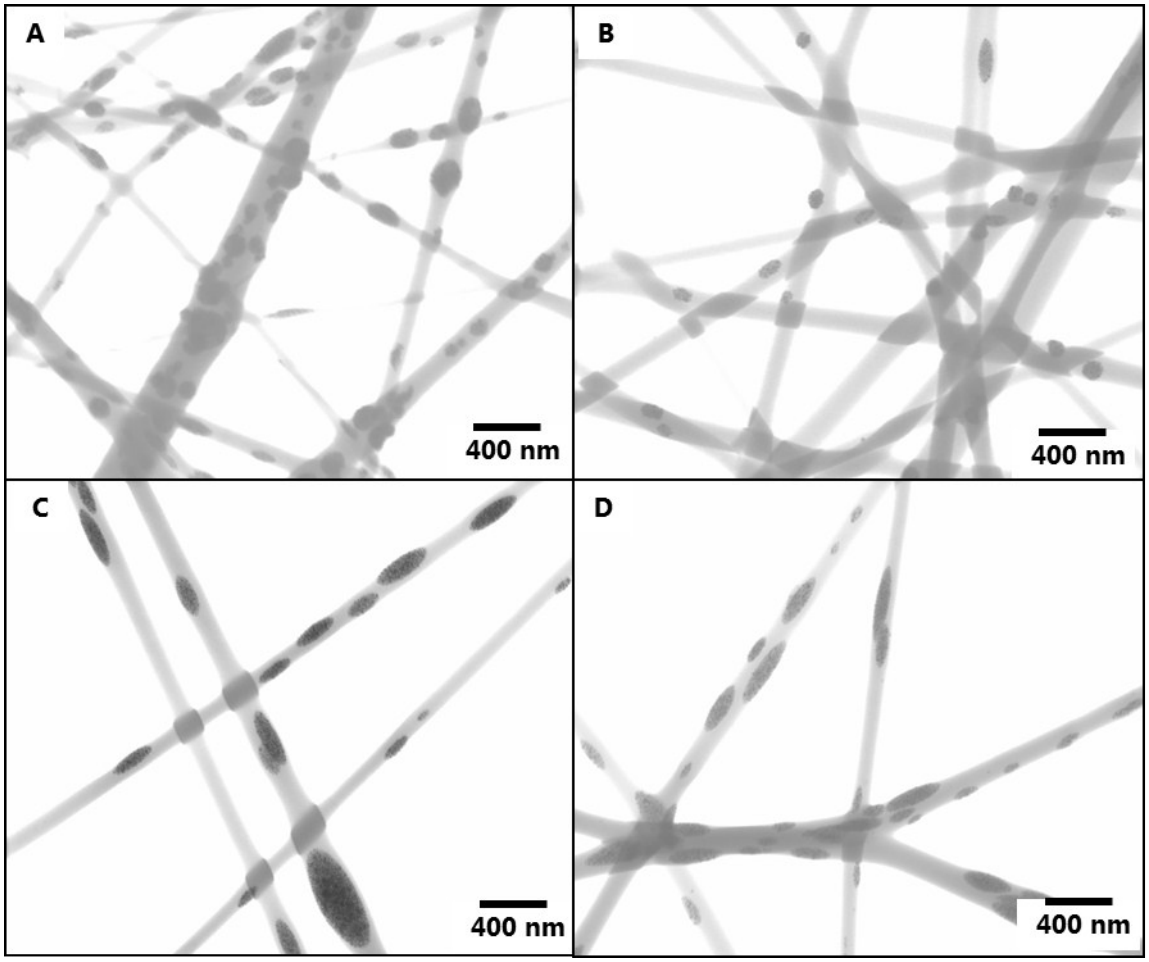

Figure S5. Influence of solvent on the formation of ESPs of Fe_3O_4. (A-D) The STEM images show electrospun fibers containing ESPs from droplets of (A) pure toluene, (B) pure chloroform, (C) pure n-octane, and (D) a mixture of toluene with n-octane 2:1.

Parameter: Relative Humidity

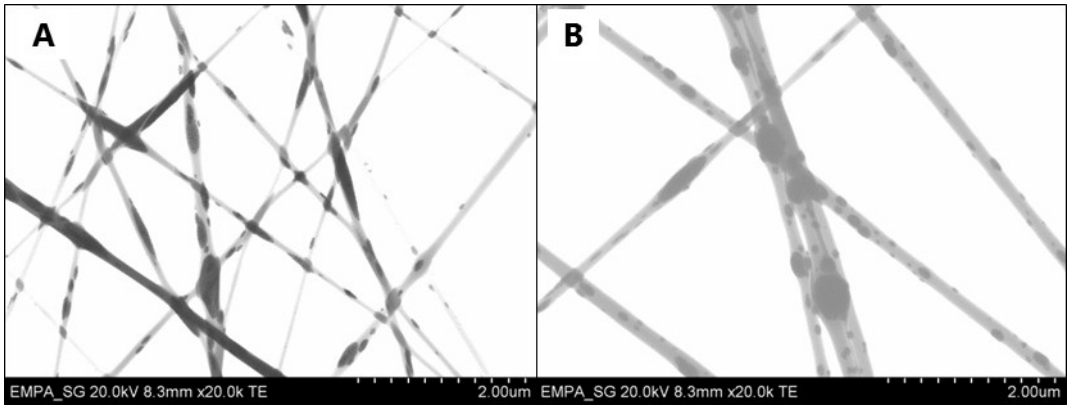

Figure S6. STEM images of PVA fibers containing ESPs of Fe3O4 that were electrospun at (A) 60% relative humidity or (B) 30% relative humidity.

Parameter: Voltage

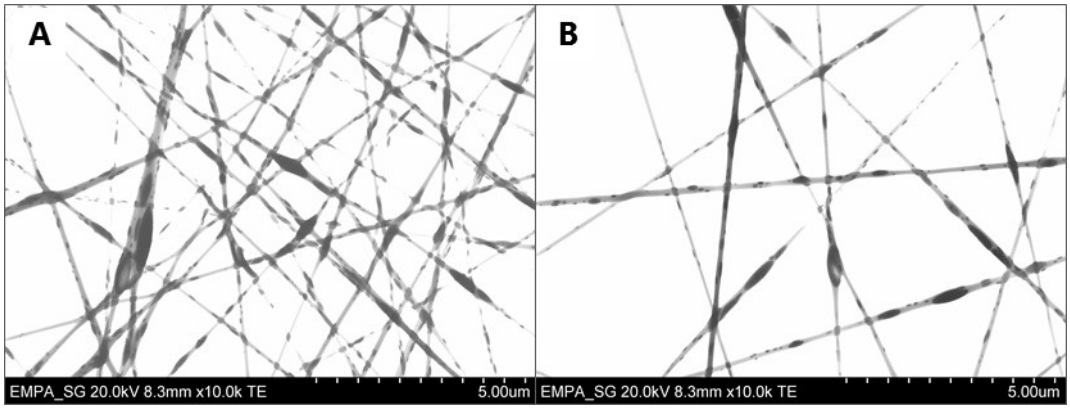

Figure S7. STEM images of PVA fibers containing ESPs of Fe3O4 that were electrospun at (A) 14 kV or (B) 8 kV anodal voltage.

Parameter: Distance

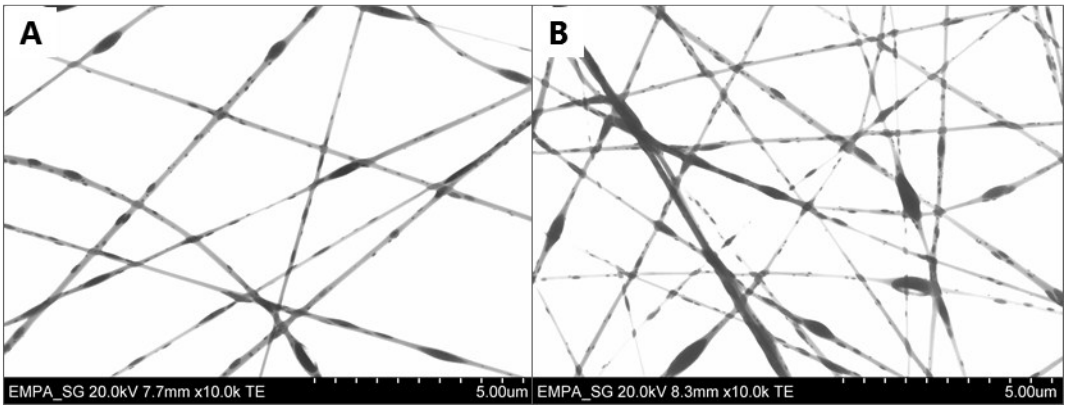

Figure S8. STEM images of PVA fibers containing ESPs of Fe3O4 that were electrospun from a needle tip to collector distance of (A) 25 cm or (B) 10 cm.

Dispersibility of TiO_2NP

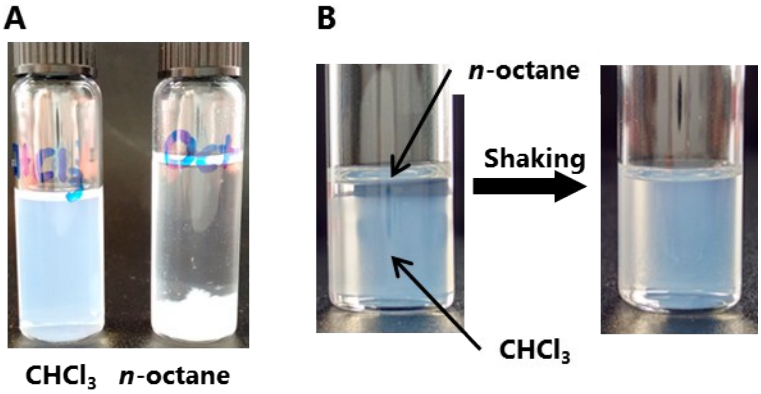

Figure S9. Dispersiblity of TiO_2NP in organic solvents. (A) Dispersiblity in chloroform vs. *n*-octane (equal amount of nanoparticles). (B) Mixing of *n*-octane into a TiO_2NP chloroform dispersion resulting in a clear dispersion for ESP fabrication.

ESP of TiO_2

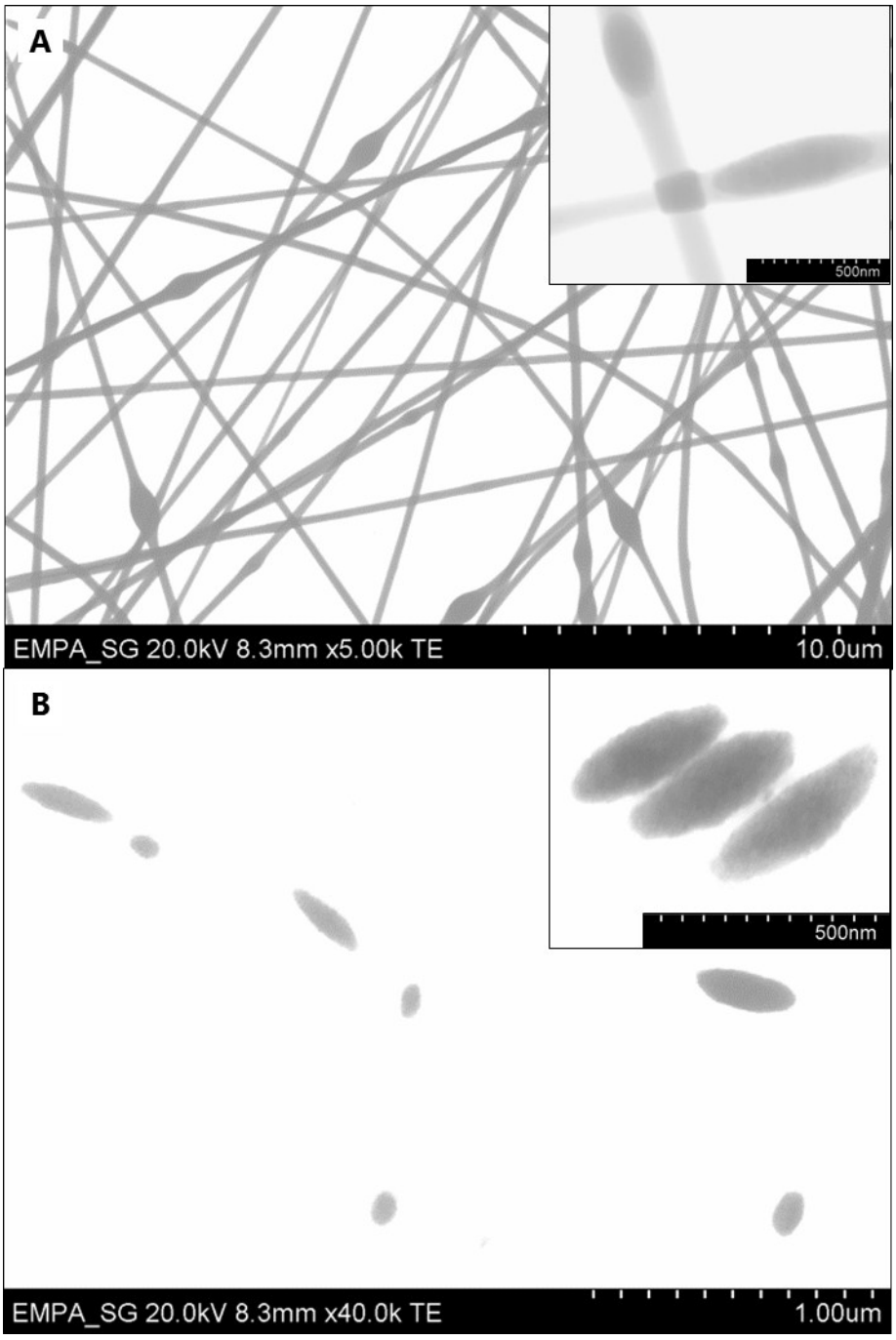

Figure S10. Fabrication of ESP of TiO2. (A) STEM image of electrospun PVA fibers containing ESP of TiO2. (B) STEM image of ESPs of TiO2 from dispersion after dissolution of the PVA fibers.

Thermogravimetric Analysis of Nanoparticles

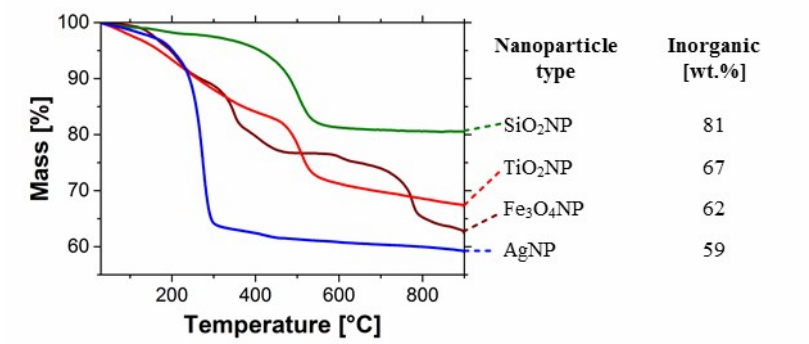

Figure S11. Thermogravimetric Analysis of the used nanoparticles. The inorganic content was determined as the remaining mass after the highest temperature was reached.

Calcination of HESPs

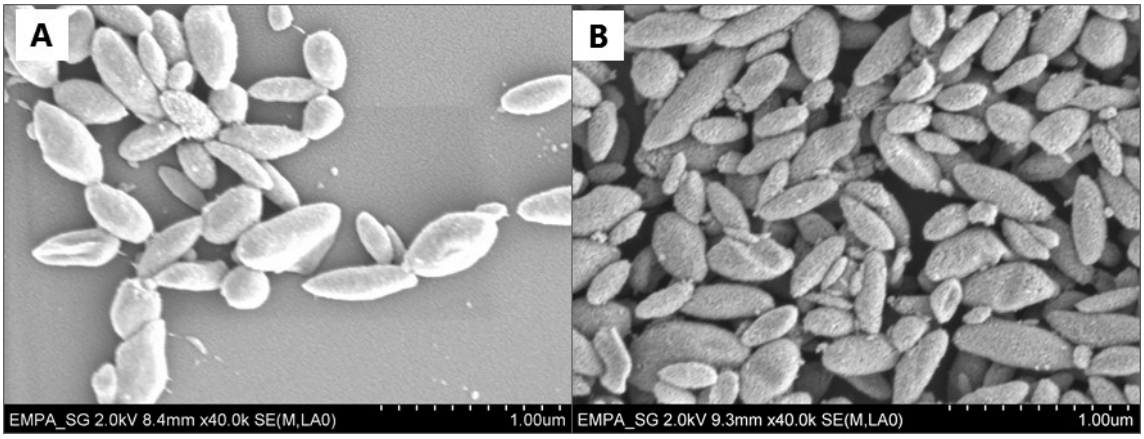

Figure S12. SEM images of HESP of Fe3O4/TiO2 (A) without calcination from direct drop-casting from aqueous dispersion on a silicon wafer or (B) after calcination for 6 h at 600 °C.

Magnetic Orientation

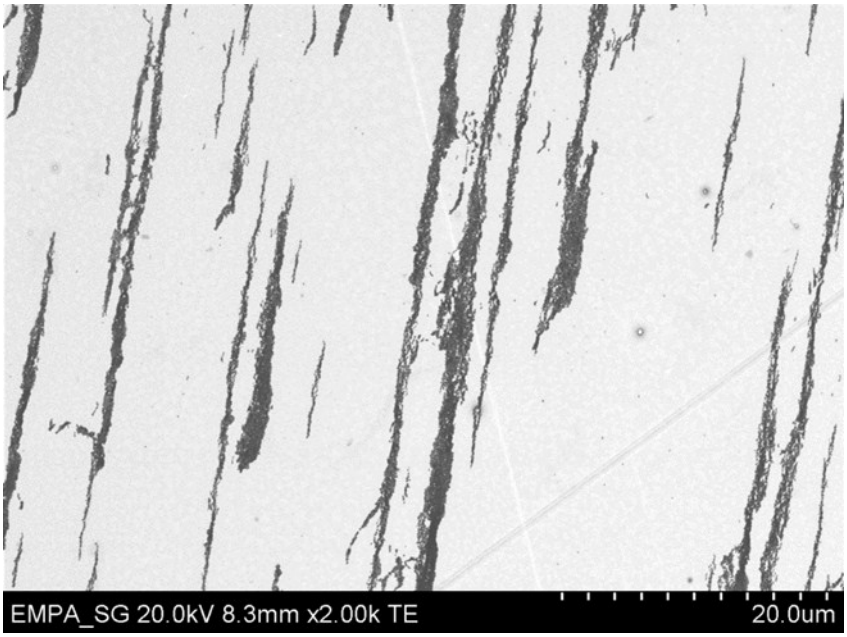

Figure S13. STEM image of ESPs of Fe3O4 that were drop-cast from aqueouos dispersion on a TEM copper grid and dried in 4 cm distance to a strong NdFeB permanent magnet (40 x 40 x20 mm). The water was left to evaporate at room temperature.

Analysis of Cell Viability (MTS Assay): To assess cell viability 1.5 x 10^4 A549 cells per well were seeded in 200 µL complete cell culture medium in a 96-well cell culture plate and grown overnight under standard growth conditions. Thereafter cells were treated for 24 h with 200 µL standard growth medium per well containing increasing concentrations of the supraparticles or the positive control agent $CdSO_4$. CellTiter 96® AQueous One Solution (Promega) containing MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H) as the water-soluble tetrazolium compound was used according to the manufacturer's protocol.

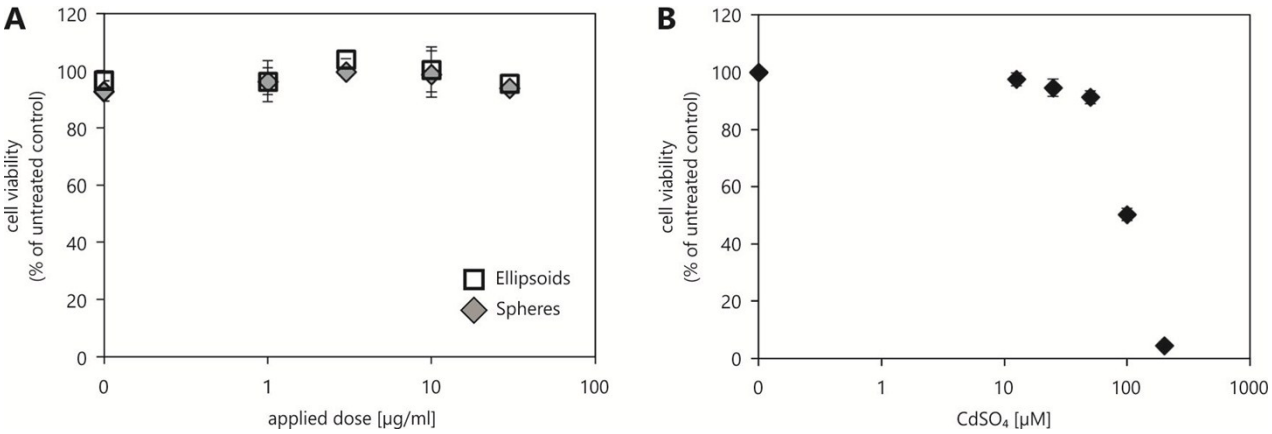

Figure S14. Cell viability analysis using the MTS assay. (A) Both supraparticle types do not influence cell viability at concentrations up to 30 µg/mL and over an incubation period of 24 h. (B) In contrast the assay internal positive control ($CdSO_4$) reduces cell viability in a dose-dependent manner.

Flow cytometry analysis of supraparticle uptake: Untreated A549 cells served as the negative control sample. Gates were adjusted to place 97-99% of all cells in the lower right quadrant (+-). Cells in this area exhibit average granularity values. An increase in cell granularity, due to uptake of nanoparticles, results in an increase in side-scatter (SS) values. Consequently such cells appear in the upper right quadrant (++) of the dotplot. The number of cells appearing in the upper right quadrant can thus be quantified as a measure of particle uptake efficacy.

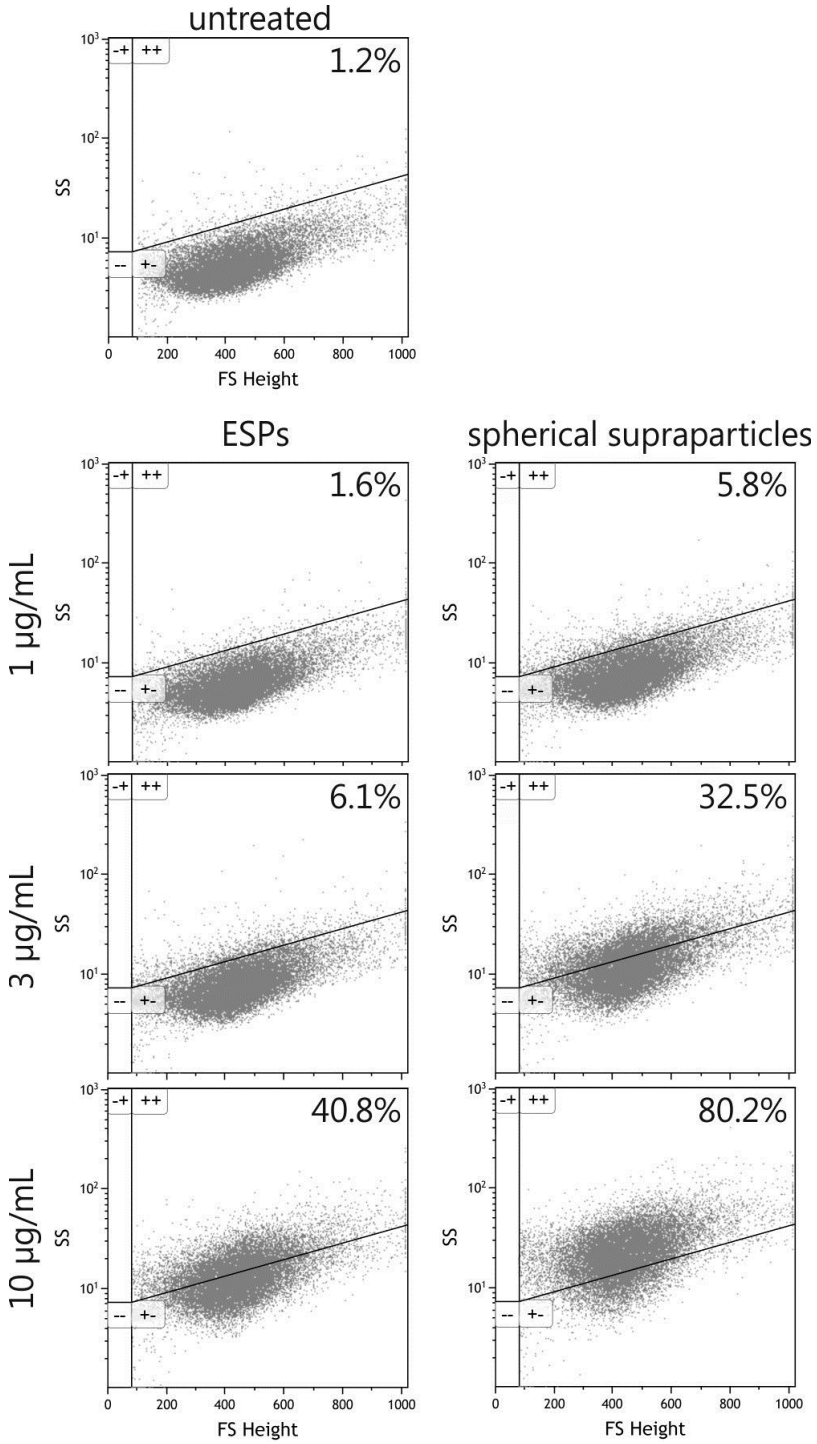

Figure S15. Representative example of flow cytometry results. A549 cells were treated with increasing concentrations of the respective supraparticles for 24 h and cell numbers in the upper right quadrant (++) were quantified as a measure of particle uptake. Cell numbers are given in % of total cell count. 20000 cells per sample were analyzed.

Quantification of total iron content in treated A549 cells by ICP-OES: For ICP-OES measurements A549 cells were treated for 24 h with 10 µg/mL of ESPs or spherical supraparticles of Fe_3O_4 or remained untreated. Cells were harvested with Trypsin/EDTA and pelleted by centrifugation (200g, 5 min). Cell pellets were resuspended in complete cell culture medium and counted using a Neubauer counting chamber. Equal amounts of cells for each sample were pelleted again (200g, 5 min), resuspended in 0.1 mL nitric acid (65%; p.A.), transferred into a Teflon pressure vessel and treated with micro waves at 500 W for 5 min (MLS, 1200 mega, Leutkirch, Germany). The resulting solution was diluted with ddH_2O to a final volume of 5 mL and analyzed for iron content by atomic emission spectrometry (ICP-OES Optima 300 from Perkin Elmer, Schwerzenbach, Switzerland) with external calibration. Total iron content in each sample was finally normalized to applied cell number.

Table S1. Iron content in A549 cells.

treatment	**total iron content [µg]**	**cell number [x10^6]**	**normalized iron content [µg/ 10^6 cells]**
untreated	0.087	1.2	0.07
ESPs	6.37	1.2	5.31
spherical supraparticles	15.5	1.4	11.1