SUPPORTING INFORMATION

Controlled Drug Delivery for Cancer Cells Treatment via Magnetic Doxorubicin Imprinted Silica Nanoparticles

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1. Materials

Ethanol (99.8%), methanol (99,9%), acetic acid (99,8%), 3-Aminopropyl)triethoxysilane (APTES) (99%), Tetraethyl orthosilicat (TEOS) (99%), hydrochloric acid (HCl) (37%), nitric acid (HNO_3) (52%), iron (III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$, 99%), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$,97%), acetone (Ac), diethyl ether (Et_2O) were supplied from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 98%) ammonia (NH_4OH) (20%) were purchased from Acros Organics (Halluin, France). MOPS (dry powder) and doxorubicin hydrochloride (DOX) were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany) and used for in vitro and intracellular drug release studies. Human prostate cancer PC-3 cells (CRL-1435) were supplied from American Type Culture Collection (ATCC) (Manassas USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany), while fetal bovine serum (FBS) penicillin and the Alamar Blue assay were purchased from Thermo Ficher Scientific (Carlsbad, CA, USA). All materials were used as received without any purification. Water was distilled and deionized.

2. Instrumentation

Transmission Electron Microscopy (TEM) and High resolution Transmission Electron Microscopy (HRTEM). Nanoparticles were observed using a Jeol-100 CX TEM. A droplet of diluted nanoparticles suspension in water was deposited on a carbon coated copper grid and the excess was drained using filter paper. Size analysis was achieved on TEM images using ImageJ software.

Dynamic light scattering (DLS). Hydrodynamic diameter (dh) measurements were recorded using a (Malvern Instruments Nanosizer).

Fourier Transform Infra-Red (FT-IR.) FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer on pressed KBr pellets. Spectra were obtained at regular time intervals in the MIR region of 4000 – 400 cm–1 at a resolution of 4 cm–1 and analyzed using OPUS software.

Hyperthermia experiments. Hyperthermia experiments for nanoparticles in suspension were conducted on a magneTherm apparatus (magneTherm AC system, Nanotherics Corp., Newcastle under Lyme, UK) at 470kHz and 18 mT. The sample was thermalized at 37 °C before the application of the alternative magnetic field. The temperature was probed using a fluorooptic fiber thermometer.

Ultraviolet-visible spectrophotometry (UV-vis). Absorbance measurements were done with an Avantes UV-visible spectrophotometer, with 100 µm optical fibers. UV/VIS measurements were configured with a range from 200 to 1100 nm. A combined deuterium-halogen light source was used.

Iron Titration. The total iron concentration (M) was determined by atomic absorption spectroscopy (AAS) with a Perkin-Elmer Analyst 100 apparatus after degradation of Fe2O3 NPs in boiling HCl (35%).

3. Experimental

a) *Synthesis of functionalized magnetic nanoparticles*

Maghemite nanoparticles were synthetized as previously described by Massart, using a co-precipitation method. Briefly, 180 g of ferrous chloride (VWR) and 1.59 mol of ferric chloride (VWR) were dissolved in 6% hydrochloric acid (Merck). 1 L of ammonia at 22.5% (Carlo Erba) was added to the medium, under vigorous magnetic stirring at room temperature. Reaction was able to perform for

30 minutes. Then, the as-obtained magnetite was oxidized using 323 g of ferric nitrate (VWR). The suspension was heated at 100°C under magnetic stirring for 30 minutes. Maghemite nanoparticles were then washed three times with acetone (VWR) and two times with diethyl ether (VWR), before being dispersed in water. To stabilize MNPs, the particles were pre-coated with a citrate anion by adding trisodium citrate (a concentration of 25 g/L) to the colloidal solution of MNPs. The specific loss power reached with this magnetic core equalled 116 $W.g^{-1}$ of iron, when measured at 470 kHz and 18 mT.

b) Synthesis of functionalized magnetic nanoparticles

In the first step, 1,5 mL of 1.78M aqueous solution of citrate-Fe_2O_3 NPs, was added to 12.5 mL of water. Then 25 mL of ethanol, 1,5 mL of TEOS and 6 mL of NH_3 (30%) were added to the mixture, followed by vigorous mechanical stirring for 2 hours. Addition of NH_3 initiates the hydrolysis of TEOS in silanols, implied in the polycondensation process, leading to the formation of a first crosslinked silica shell at the nanoparticles' surface. In the next step, 0.4 mL of TEOS, 0.05 mL of aminopropyltriethoxysilane (APTES) and 0.5 mL of doxorubicin hydrochloride ([DOX] = 0.01 $mol.L^{-1}$), were injected into the solution to synthesize a second silica shell, with DOX confined in, thanks to hydrogen bonds with silanol and amino groups, leading to the formation of DOX-loaded-MIP MNPs.. We decided to add in this solution 0.5 mL of DOX from an initial concentration of DOX at 0.01mol/L. We first prepared a solution of DOX with 5,4 mg of DOX in 1 mL of water to obtain a solution with a DOX concentration of 0.01mol/L . Then we took 0,5 mL for the MIP synthesis. Hence we have in solution n = 5mmol of DOX.

We used 9,3 mmol of APTES/TEOS. As one molecule of DOX can do several bondings with the inorganic monomers, we can used less molecules than the functional monomers. At the end after the synthesis, the solution is still colored meaning that DOX is in excess.

c) DOX release experiments

In vitro DOX release was analyzed in different conditions to assess the influence of the temperature and the AMF. We monitored DOX release in medium at pH 7.4 (0.1 M MOPS solution). For all experiments, DOX-loaded-MIP MNPs (2 mL, [Fe] = 50 $mmol.L^{-1}$) were placed in an Eppendorf tube and heated at 37 °C (human body temperature) during 30min. Each 5 min, the supernatant was collected by magnetic separation (2 min) and the amount of DOX released was quantified by UV–VIS spectroscopy.

For the drug release at different temperature, the eppendorf was heated at the desired temperature during 30 min and then the supernatant was collected by magnetic separation (2 min) and the amount of DOX released was quantified by UV–VIS spectroscopy.

d) Cell experiments

Human prostate cancer PC-3 cells (ATCC® CRL-1435™) were grown in adhesion in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich) supplemented with 1 % penicillin and 10 % foetal bovine serum (FBS). They were kept at 37 °C in humidified atmosphere at 5 % CO2 until confluence.

PC-3 cancer cells were incubated at 37 ºC with an aqueous dispersion of DOX-loaded-MIP MNPs or NIP MNPs at fixed iron concentration ([Fe] = 2 $mmol.L^{-1}$, [DOX] = 5 $\mu mol.L^{-1}$ and [Fe] = 5 $mmol.L^{-1}$, [DOX] = 12.5 $\mu mol.L^{-1}$) for 2 hours. After incubation, cells were washed twice with culture medium, and further

incubated for 2 hours (chase period) in complete medium (DMEM supplemented with 10 % FBS) before treatments.

For AMF application, cells were first seeded in1 cm diameter culture well and cultured until confluency. Then, after the incubation and chase period, the cells were submitted for 2 hours to an alternative magnetic field (AMF) produced by a generator device (NanoScale Biomagnetics) with a frequency of 470 kHz and an amplitude of 18 mT. Temperature was probed with a fluoroptic fiber thermometer and recorded every 1 s.

Cell viability after application of different treatments (in presence of free DOX, with and without AMF) was evaluated in the colorimetric Alamar Blue assay. Labelled and treated cells were incubated with 10 % Alamar Blue in DMEM for 2 hours. The Alamar Blue reagent was then transferred to a 96 well plate for analysis with a microplate reader (BMG FluoStar Galaxy) at an excitation wavelength of 550 nm with fluorescence detection at 590 nm. Viability was determined by comparison with control cells.

e) Supplementary figures

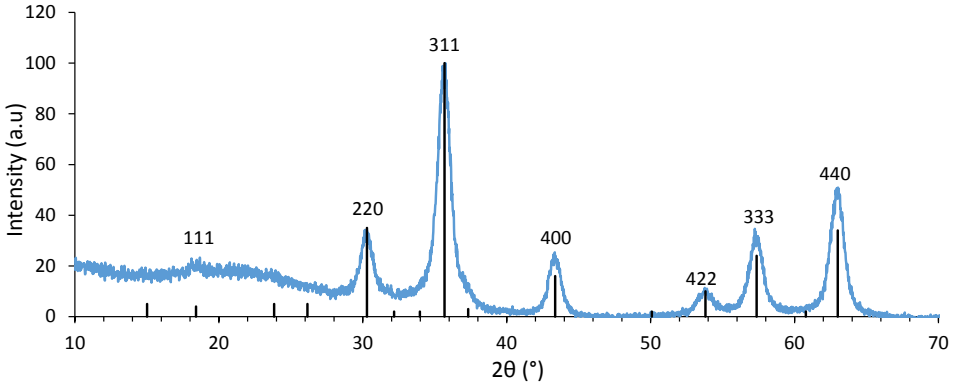

Figure S1. XRD data for bare magnetic nanoparticles. Black lines: theoretical data for maghemite nanoparticles (JCPDS file 39-1346).

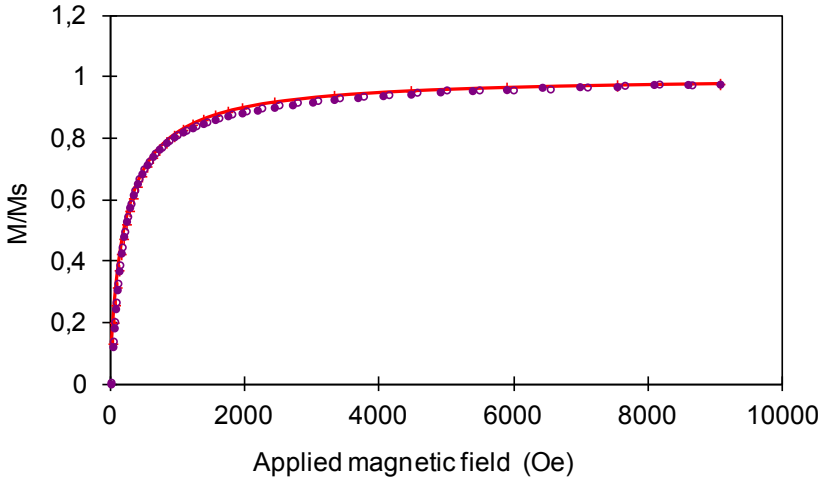

Figure S2. Magnetization curves obtained by vibrating sample magnetometer at room temperature of γ-Fe_2O_3 nanoparticles.

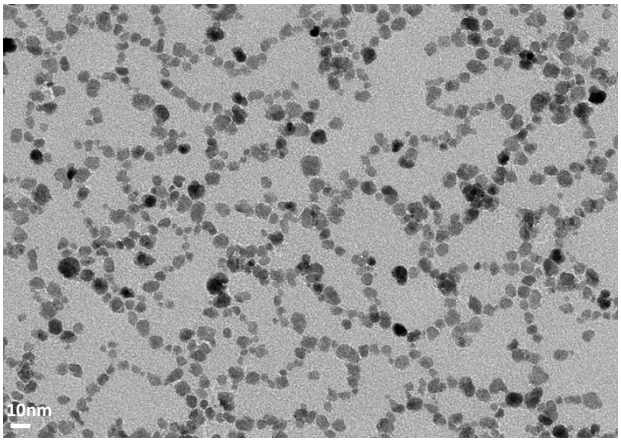

Figure S3. TEM pictures of the size sorted γ-Fe_2O_3 nanoparticles modified with citrate molecules.

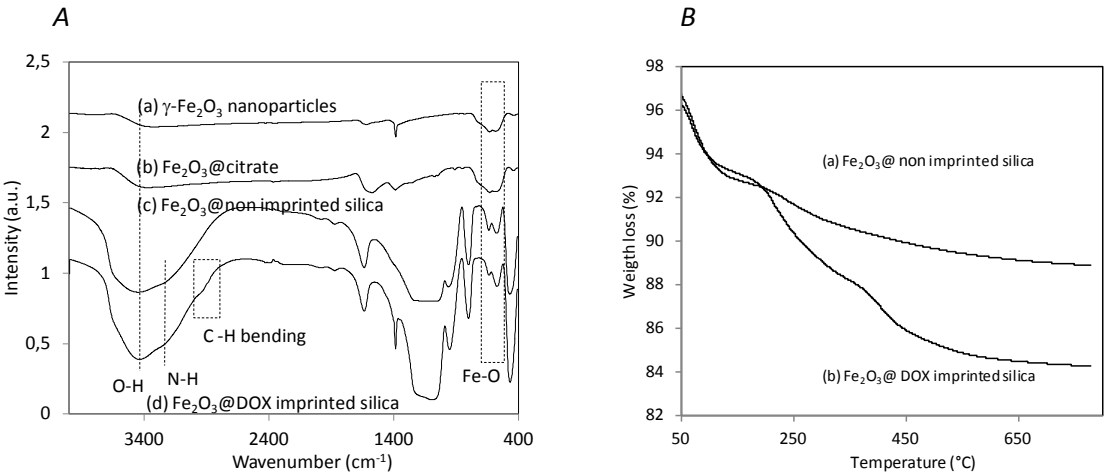

Figure S4. A) IR spectra of a) γ-Fe_2O_3 b) Fe_2O_3@citrate, c) Fe_2O_3@non imprinted silica and d) Fe_2O_3@DOX imprinted silica nanoparticles. B) TGA of a) Fe_2O_3@non imprinted silica and b) Fe_2O_3@DOX imprinted silica nanoparticles.

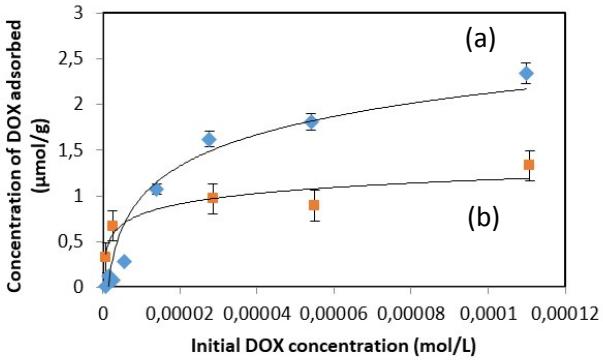

Figure S5. Adsorption isotherm of a) Fe_2O_3@non imprinted silica (in orange) and b) Fe_2O_3@DOX imprinted silica nanoparticles (in blue) toward Doxorubicin.

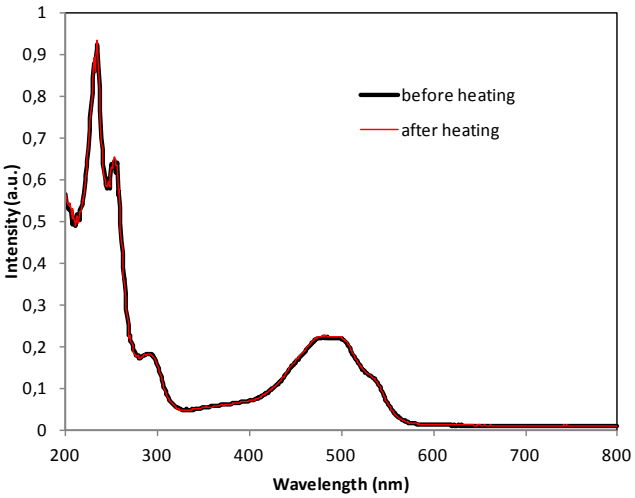

Figure S6. UV-Vis spectra of Doxorubicin before and after heating.

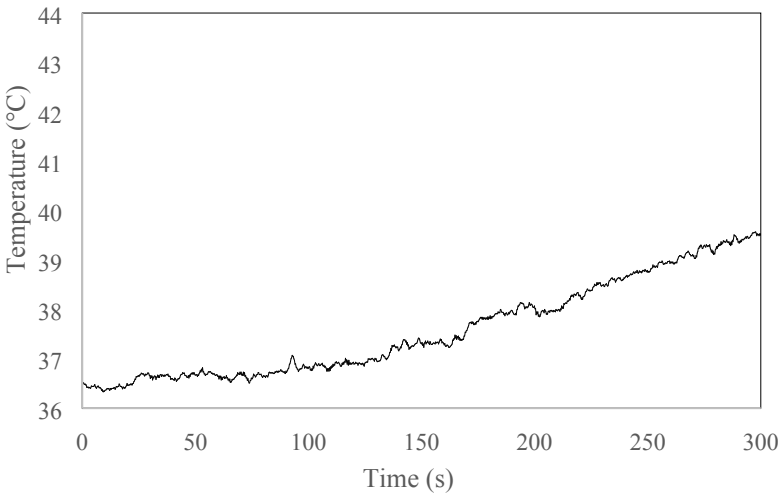

Figure S7. Variation of temperature during AMF treatment in MOPS.

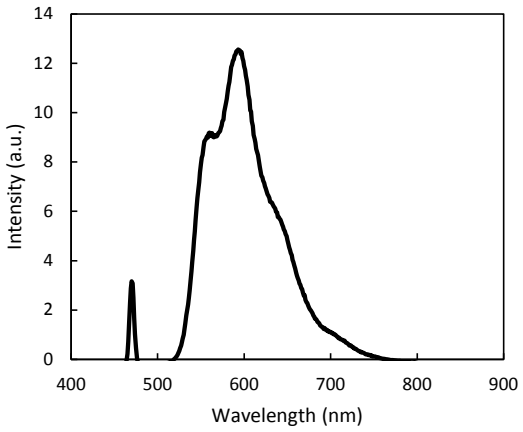

Figure S8. Fluorescent spectra of DOX-loaded-MIP MNPs.

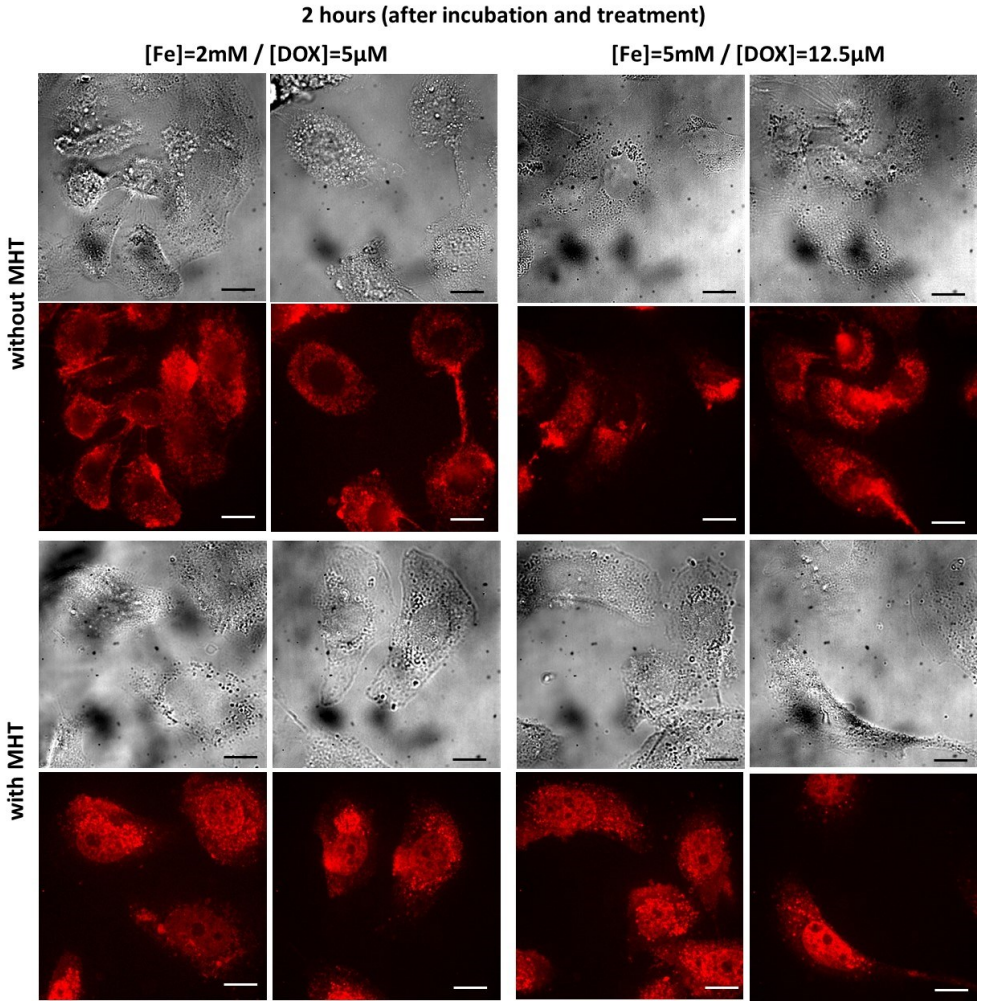

Figure S9. Confocal microscopy images (top: bright field, bottom: DOX fluorescence) of cells incubated with DOX-loaded-MIP MNPs 2 hours after incubation at [DOX] = 5 and 12.5 µmol.L^{-1}. Scale bars: 20 µm.

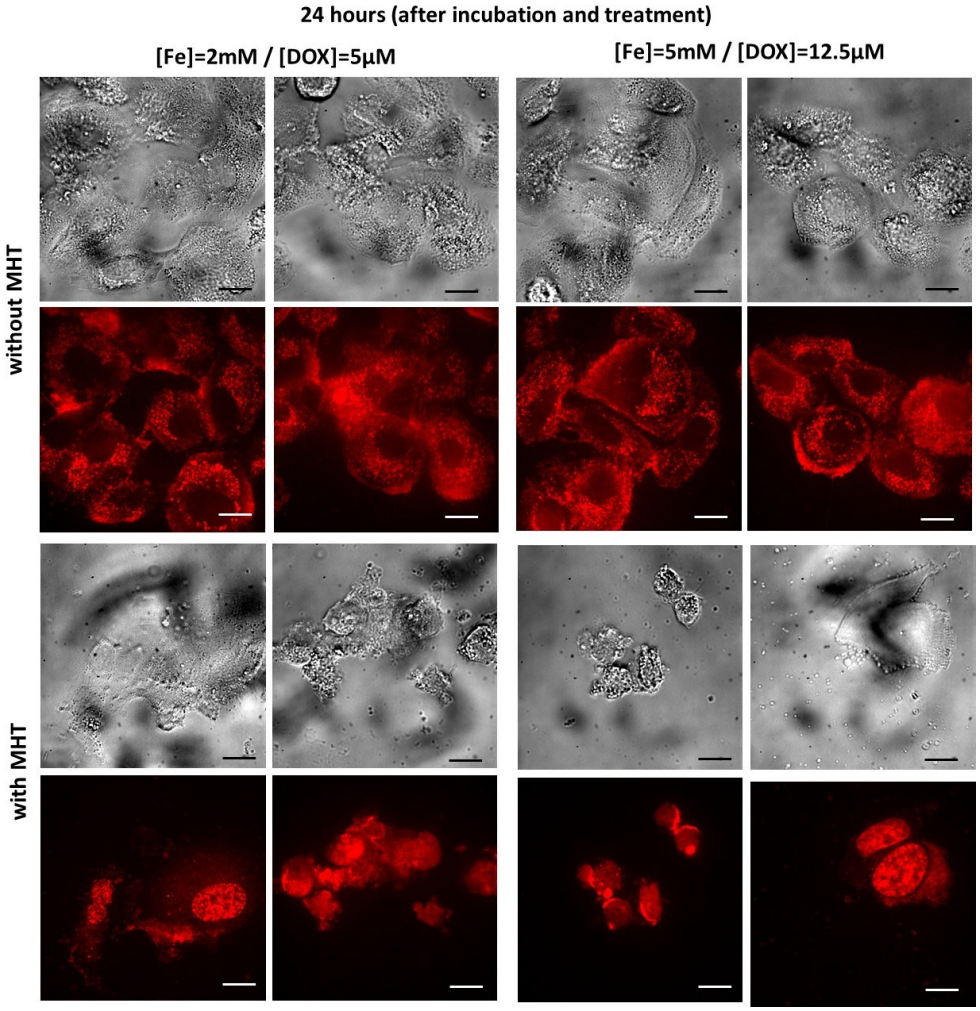

Figure S10. Confocal microscopy images (top: bright field, bottom: DOX fluorescence) of cells incubated with DOX-loaded-MIP MNPs 24 hours after incubation at [DOX] = 5 and 12.5 µmol.L^{-1}. Scale bars: 20 µm.

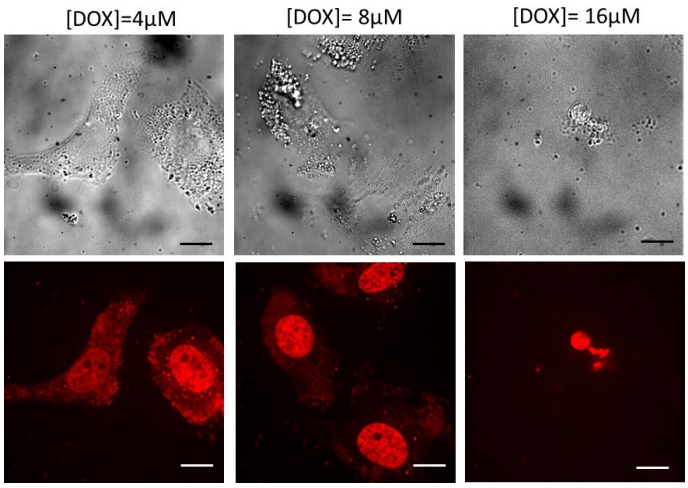

Figure S11. Confocal microscopy images (top: bright field, bottom: DOX fluorescence) of cells incubated with free DOX at different concentrations ([DOX] = 4, 8 and 16 µM) 24 hours after incubation. Scale bars: 20 µm.