Supporting Information

Multifunctional Superparamagnetic Iron Oxide Nanoparticles for Combined Chemotherapy and Hyperthermia Cancer Treatment

Christopher A. Quinto,[a] Priya Mohindra,[a] Sheng Tong,[b] Gang Bao*[a,b]

[a]*Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA*

[b]*Department of Bioengineering, Rice University, Houston, TX 77030, USA*

**Corresponding author. Department of Bioengineering, Rice University, Houston, TX 77030, E-mail address:* gang.bao@rice.edu (G. Bao).

S1. Normalized magnetic field strength profile within inductor coil.

The normalized magnetic field strength profile within a 7.5-turn inductor coil (EasyHeat 2.4kW, Ameritherm, Scottsville, NY) of 3 cm length and 2.54 cm inner diameter was modeled using Matlab using the Biot-Savart law.

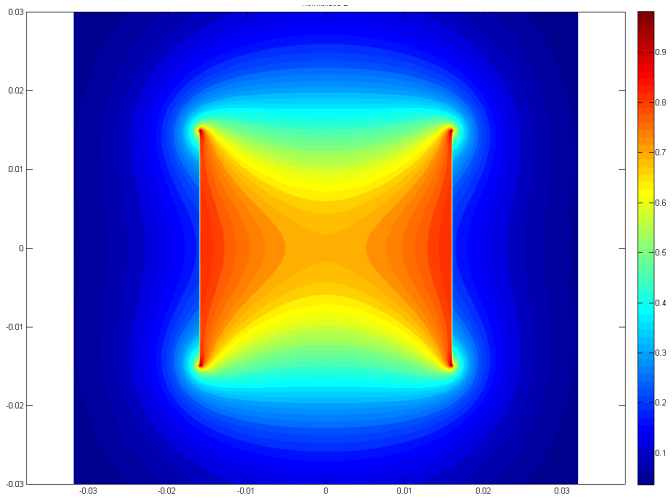

Figure S2. Normalized magnetic field strength profile within inductor coil.

S2. Synthesized SPIO core size distribution.

The sizes of the SPIO core population were measured from the TEM micrograph in Figure 1a using the Image-Pro Plus 6.3 software (Media Cybernetics, Rockville, MD).

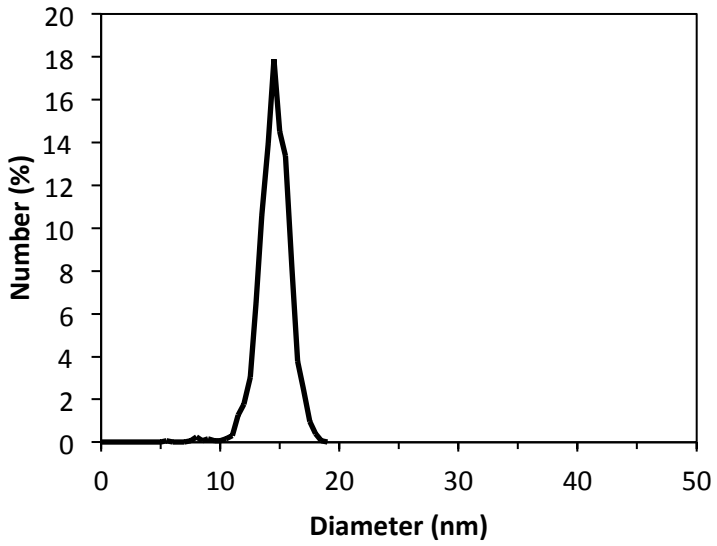

Figure S2. Synthesized SPIO core size distribution.

S3. Temperature increase for specific absorption rate calculation.

Aqueous solutions of SPIOs coated with DSPE-PEG 2000 and 5000 at 0.4 mg/ml Fe were prepared in 1 ml of deionized water. The samples were placed inside a polystyrene-insulated inductor coil (EasyHeat 2.4 kW, Ameritherm, Scottsville, NY). An alternating magnetic field (23.77 kA/m, 355 kHz) was generated within the coil, causing the SPIOs to produce heat. The resulting temperature rise in the ferrofluid was measured with a fiber optic temperature probe (FLUOTEMP, Photon Control Inc., Burnaby BC, Canada) and recorded in real time.

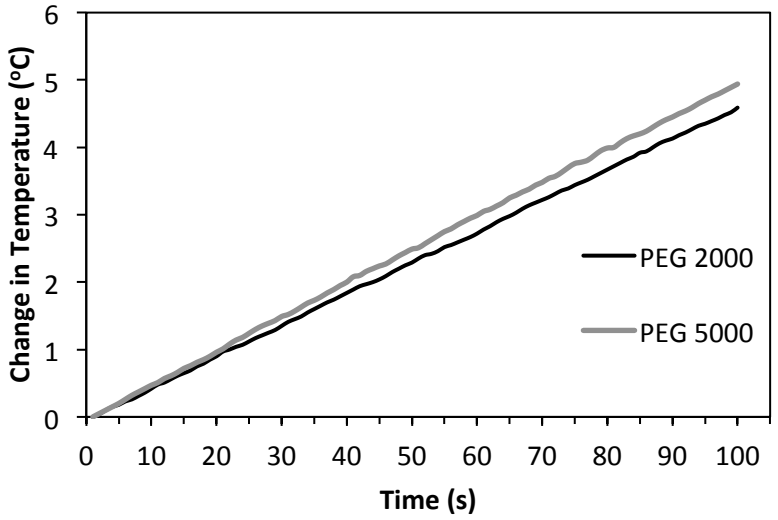

Figure S3. Temperature profile for specific absorption rate calculation. Temperature change of a 1 ml aqueous solution containing SPIOs coated with PEG 2000 and 5000 at 0.4 mg/ml Fe during exposure to an alternating magnetic field of 23.77 kA/m and 355 kHz.

S4. Effect of DOX loading on the zeta potential of coated SPIOs.

The zeta potentials of DSPE-PEG 2000 and 5000 coated SPIOs and DOX-SPIOs were measured using a Malvern Instruments Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom). Non-loaded and DOX-loaded SPIO samples were suspended at 50 μg/ml Fe in phosphate buffered saline (13.7 mM NaCl, 1 mM Na_2HPO_4, 0.27 mM KCl) at pH 4, 7, and 10 in a disposable folded capillary cell.

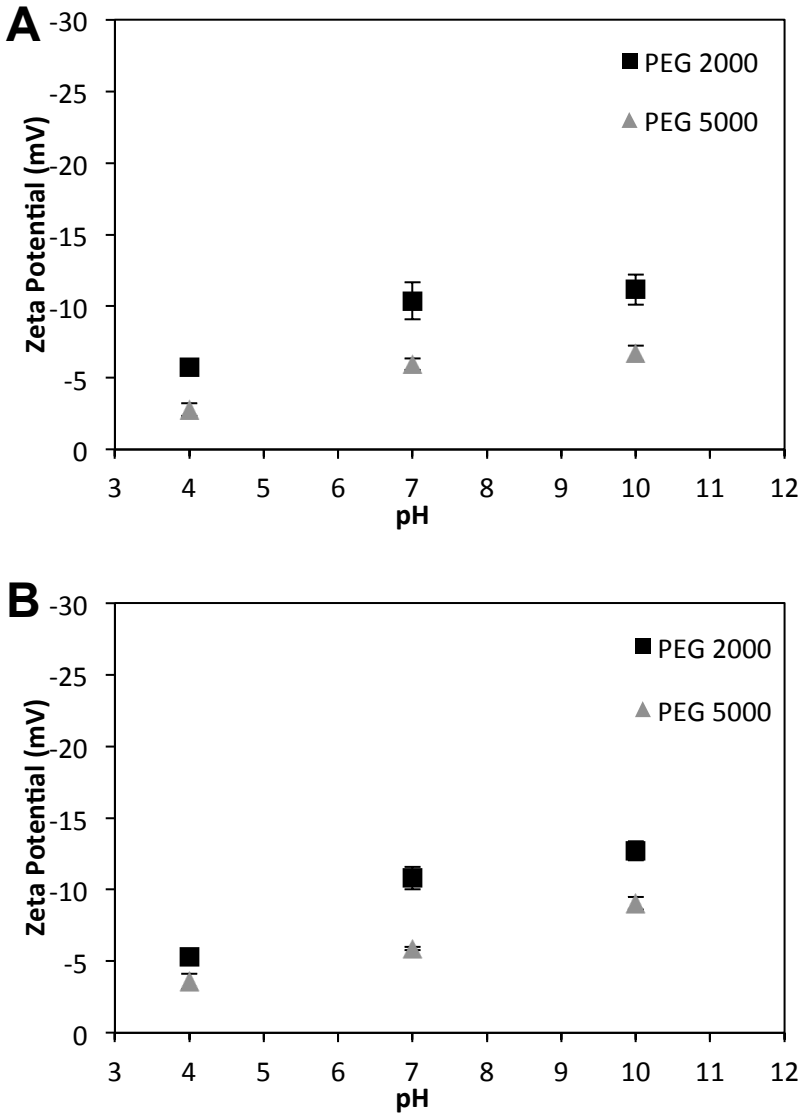

Figure S4. Effect of PEG length and DOX loading on the zeta potential of coated SPIOs. Zeta potential of (A) SPIOs without and (B) with Doxorubicin loading. Error bars represent standard error.

S5. Combined effect of hyperthermia and DOX.

HeLa cells were seeded into a 96 well plate at 5000 cells per well. After an overnight incubation, free DOX was added to the wells at a range of concentrations up to 10 μg/ml DOX. The plate was then placed in a water bath to maintain a temperature of 43°C for 1 hour. Following the hyperthermia, the plate was returned to a 37°C incubator. Cell viability was measured 48 hours post treatment using an MTT assay.

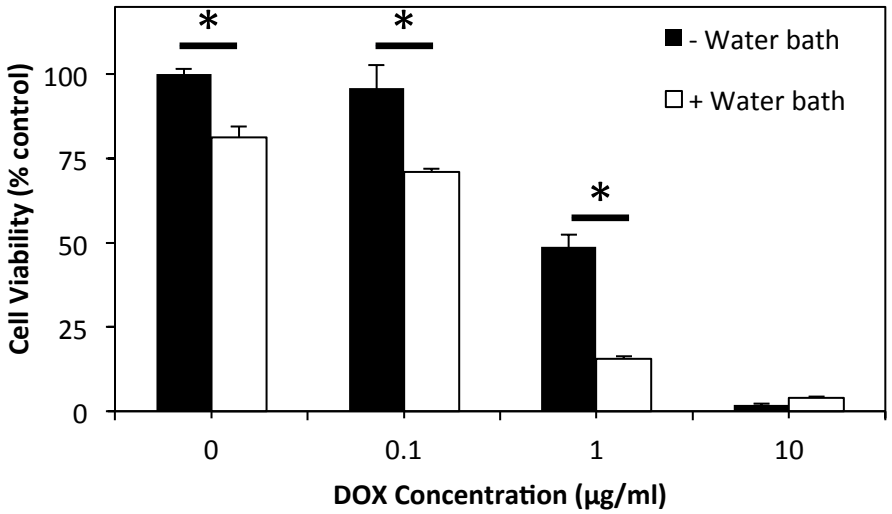

Figure S5. Combined effect of hyperthermia and DOX. HeLa cell viability 24 hours post treatment with a range of free DOX concentrations with or without 43°C water bath heating for 60 minutes. * = significant difference (Tukey test $p<0.01$). Error bars represent standard error.

S6. Cell morphology following DOX/hyperthermia treatment.

HeLa cells were grown to 80% confluency in a T-75 cell culture flask. The cells were detached with 0.05% trypsin/EDTA and resuspended in cell culture media with 10 µM HEPES. The cells were counted and $5x10^4$ cells were pipetted into eight different cryovials. The vials were then filled to 1 ml of either media, media with 100 µg/ml Fe DSPE-PEG 2000 SPIOs (without DOX), 100 µg/ml Fe DOX-loaded DSPE-PEG 2000 SPIOs (16.6 µg/ml DOX), or an equivalent free DOX solution. Each of these groups had a +AMF/-AMF sample with the +AMF samples undergoing a 1 hour AMF treatment (23.77 kA/m, 355 kHz) and the –AMF samples kept in a cell culture incubator at 37^{o}C. Two hours after the AMF treatment, the media in each sample was then replaced with fresh media and seeded into an 8-well chamber slide (Nunc Lab-Tek II) at 25,000 cells per well so that the total incubation time with the SPIO/SPIO-DOX/DOX was 3 hours. Phase contrast images of the live HeLa cells were taken 24 hours later using a Zeiss AxioVert S100 fluorescent microscope.

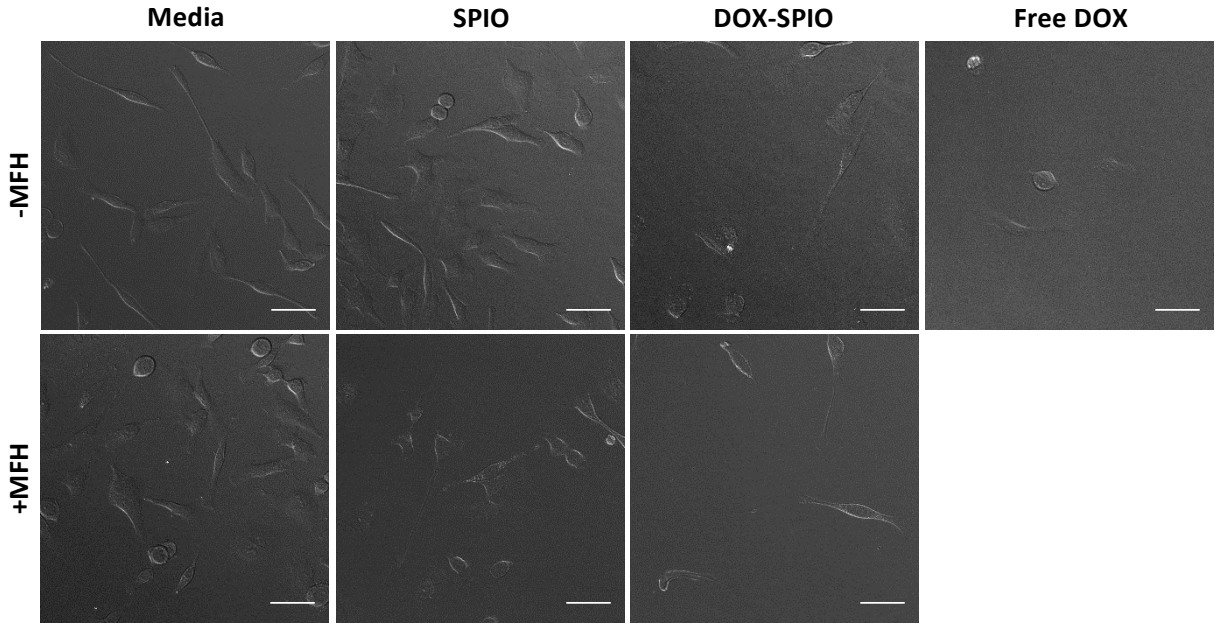

Figure S6. Cell morphology following DOX/hyperthermia treatment. Phase contrast images of HeLa cells 24 hours post treatment with media alone, just SPIOs without DOX), DOX-SPIOs, or free DOX. Each of the indicated groups had samples with or without a 1 hour exposure to an AMF (23.77 kA/m, 355 kHz). (Scale bar = 25 µm).