ELECTRONIC SUPPLEMENTARY INFORMATION

Tunable Magnetothermal Properties of Cobalt-doped Magnetite-Carboxymethylcellulose Ferrofluids: Smart Nanoplatforms for Potential Magnetic Hyperthermia Applications in Cancer Therapy

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Synthesis of colloidal nanoparticles. Magnetic iron oxide nanoparticles (MIONs) were prepared based on the co-precipitation of Fe(II) and Fe(III) oxide nanoparticles in a water medium in the presence of NH_4OH at 80 ± 2 ˚C under nitrogen atmosphere. In brief, 0.02 M of $FeSO_4$ and 0.04 M of $FeCl_3$ were dissolved in the CMC solution (1.0 % w/v, 200 mL) pre-heated to 40 ± 2 ˚C under vigorous stirring. Then, the mixture was heated up to 80 ± 2 ˚C under a nitrogen atmosphere, and 12 mL of NH_4OH solution (25.0 % v/v) was added to the reaction flask and homogenized under moderate stirring for 20 min. Subsequently, the solution was cooled down to room temperature and dialyzed for 18 and 24 h using cellulose membrane (M_w cut-off of 12 kDa) under moderate stirring at room temperature. After purification, the colloidal solution was stored at 6 ± 2 ˚C for further use. For the synthesis of cobalt-doped nanoparticles (Co-MIONs), a similar procedure was performed except for the amount of Fe(II) precursor used. In that case, Fe(II) species were partially replaced by 3.0% ,

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5.0% or 10.0% (mol/mol) of Co(II) species. As a reference, uncoated magnetite nanoparticles were also prepared using a similar protocol without the addition of CMC in the medium (*i.e.*, without capping ligand).

Experimental Protocol – Mitochondrial activity (MTT) assay. All of the biological tests were conducted according to ISO 10993-5:2009/(R)2014 (Biological evaluation of medical devices: Tests for in vitro cytotoxicity). HEK 293T (passage 11) U87 (passage 44) cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Gibco BRL, USA) with 10% FBS (fetal bovine serum, Gibco BRL, USA), penicillin G sodium (10 units mL^{-1}, Gibco BRL, USA), streptomycin sulfate (10 mg mL^{-1}, Gibco BRL, USA), and amphotericin-b (0.025 mg mL^{-1}, Gibco BRL, USA) in a humidified atmosphere of 5% CO_2 at 37 ˚C.

All of the cells were plated (1×10^5 cells/well) in 96-well plates. Cell populations were synchronized (arrested in the G0 cell phase, quiescence state) by nutrient deprivation for 24 h (DMEM medium without FBS). After that, the volume of media was suctioned and replaced with DMEM medium containing 10% FBS and incubated for 24 h. Then, magnetite and cobalt doped magnetite nanoparticles (MION@CMC, Co3-MION@CMC, Co5-MION@CMC, and Co10-MION@CMC) solutions were added to individual wells at each different final concentration. Control samples were designed as follows: control group (cell culture with DMEM and 10% FBS); positive control (cell culture with DMEM, 10% FBS and 1.0% v/v Triton™ X-100 (Sigma-Aldrich, USA); and negative control (cell culture with DMEM, 10% FBS and chips of sterile polypropylene Eppendorf®, 1 mg mL^{-1}, Eppendorf, Germany). After 24 h, the total volume of the solution of each well was aspirated and replaced with 60 µL of culture media containing serum to each well. The MTT reagent (5 mg mL^{-1}, > 98%, Sigma-Aldrich, USA) was added to each well and incubated for 4 h in an oven at 37 ˚C and an atmosphere of 5% CO_2. Next, 40 µL SDS (sodium dodecyl sulfate, ≥ 99.0 %, Sigma-Aldrich, USA) solution/4 % HCl (37 %, Sigma-Aldrich, USA) was placed in each well and incubated for 16 h in an oven at 37 ˚C in an atmosphere of 5% CO_2. Then, the volume of 100 µL from each well was aspirated and transferred to a similar blank 96-well plate, and the absorbance was measured using iMark™ Microplate Absorbance Reader (Bio-Rad®) with a filter of wavelength at λ=595 nm. The percentage of cell viability was calculated after blank corrections, according to Equation S1, where the values of the control group were set to 100% cell viability.

$$Cell\ viability\ =\ \frac{Absorbance\ of\ sample\ and\ cells}{Absorbance\ of\ control}\ \text{x}\ 100 \qquad \text{(S1)}$$

Statistical significance was tested using one-way ANOVA followed by Bonferroni's method. At α confidence level value $\alpha < 0.05$ was considered statistically significant. The experiments were performed at least in triplicate ($n \geq 3$).

Experimental Protocol –Magnetic Hyperthermia *in vitro* cellular. All of the cells were plated (1×10^5 cells/well) in 24-well plates. Cell populations were synchronized in serum-free media for 24 h. After that, the media volume was suctioned and replaced with Leibowitz medium (L-15, Gibco BRL, USA) containing 10 % FBS for 24 h. Then, the solutions of MION@CMC and Co10-MION@CMC were added to individual wells at a final concentration of 15 μg mL^{-1}. The samples were designed and prepared as follows: control groups (U87: cell culture with L-15 and 10 % FBS, and U87-MHT: cell culture with L-15 and 10 % FBS exposed to an alternating magnetic field, AMF); U87-MION@CMC and U87-Co10-MION@CMC groups (cell culture with L-15 and 10% FBS incubated with nanoparticles); U87-MION@CMC -MHT and U87-Co10-MION@CMC-MHT groups (cell culture with L-15 and 10% FBS incubated with nanoparticles and exposed to an AMF for 60 min). After 3 h of incubation in an atmosphere without CO_2 at 37 ˚C, U87, U87-MION@CMC, and U87-Co10-MION@CMC groups were washed with PBS, trypsinized (250 μL of 0.2 % trypsin, 7 min), centrifuged (1400 rpm for 5 min) and resuspended in 600 μL of L-15 supplemented with 10% FBS. The characterization of cancer cell death was performed using mitochondrial activity based on the *in vitro* MTT bioassay. Therefore, 400 μL of the MTT reagent (5 mg mL^{-1}) was added to each well with the cells and incubated for 2.5 h at 37 ˚C. Formazan crystals were dissolved using 400 μL isopropanol solution/4 % HCl. Then, 100 μL of the solution was removed from each well and transferred to a 96-well plate, and the absorbance was measured (iMark™ Microplate Absorbance Reader, Bio-Rad®, with a wavelength filter at λ = 595 nm). The experiments were performed in 10 replicates (n = 10), and the cell viability response was expressed according to Equation S1.

For U87-MHT, U87-MION@CMC -MHT, and U87-Co10-MION@CMC-MHT groups, after 3 h of incubation in an atmosphere without CO_2 at 37 ˚C, these systems

were exposed to an alternating magnetic field (AMF, H = 19.9 kA m^{-1}, frequency = 112.6 kHz) for 60 minutes. After the exposure to the AMF, the cells were treated as previously described.

FIGURE

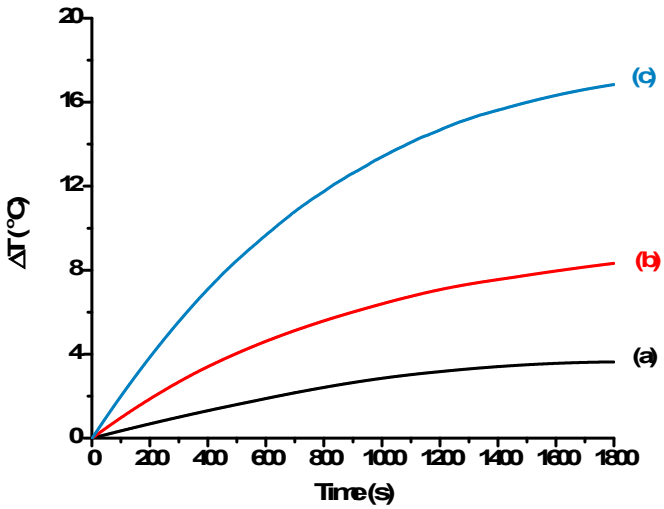

Fig. S1 Variation in the "net" temperature versus the time for MION@CMC with concentrations of (a) 2.5 mg/mL, (b) 5.0 mg/mL, and (c) 20.0 mg/mL.