Multifunctional nanocarriers of Fe3O4@PLA-PEG/Curcumin for MRI, magnetic hyperthermia and drug delivery

**ELECTRONIC SUPPLEMENTARY INFORMATION**

1. **EXPERIMENTS**

**MRI measurement details**

Dilution sets of 0-90 µg/ml (elemental iron) of Fe3O4@PLA-PEG and Fe3O4@PLA-PEG/Cur formulations were prepared with a 15 µg/ml step size and placed in 2-ml vials. Dilution was performed after 2 min stirring by vortex of the 3 mg/ml stock solutions.

Six 2-ml vials containing the diluted solutions were arranged in a circular pattern around a blank vial of distilled water for each of the two IONP formulations. R1 was measured with a variable repetition time (TR) spin-echo sequence and R2 was measured with a multi-echo spin-echo sequence with TR fixed. ParaVison 5.1 software was used to draw regions of interest (ROIs) and calculate relaxation rate values. Decay curves were fit with a mono-exponential decay equation to calculate T2, and a mono-exponential growth saturation recovery equation to calculate T1, , where A is the absolute bias and I is the signal intensity.

T2 of the samples was measured using the Bruker Multi-Slice Multi-Echo (MSME) pulse sequence with the following parameters: Effective (time to echo) TE: 4.25 ms, with 30 echoes ranging 4.25-127.5 ms, TR = 2500 ms, 40 x 40 mm2 x-y field of view (FOV), 30 mm slice thickness, 200 × 200 matrix size providing 200 µm2 x-y resolution, number of averages = 2, total acquisition time = 11 min 55 s.

T1 of the samples was measured using a Rapid Acquisition with Refocused Echoes variable TR (RARE VTR) pulse sequence with the following parameters: TE = 4.25 ms, Rare Factor: 1, 8 TRs ranging from 100 – 2500 ms, 40 x 40 mm2 x-y field of view (FOV), 30 mm slice thickness, 200 × 200 matrix size providing 200 µm2 x-y resolution, number of averages =1, total acquisition time = 27 min 15 s. For DI-H20 T1 & T2, two separate scans were run with effective TE = 40 ms for T2 and TRs ranging from 100-3500 for T1.

1. **RESULTS**

**2.1. Supporting figure legends:**

**Figure S1.** Schematic illustration of the fabrication route of NPs (a) and Curcumin release from Fe3O4@PLA-PEG/Cur under AMF irradiation (b)

**Figure S2**. XRD patterns of Fe3O4, Fe3O4@PLA-PEG, and Fe3O4@PLA-PEG/Cur.

**Figure S3**. Size distribution (n = 100 NPs) determined from TEM images (a) and FeSEM images (b) of Fe3O4, Fe3O4/@PLA-PEG and Fe3O4@PLA-PEG/Cur

**Figure S4.** Hydrodynamic diameters of: Fe3O4 (a), Fe3O4@PLA-PEG (b), and Fe3O4@PLA-PEG/Cur (c).

**Figure S5**. Magnetic hysteresis loops measured at 300 K of the NPs (a); Normalized by the mass of the magnetic core determined from TGA curves (b). The fittings of the normalized loop using Equation (4) (c).

**Figure S6.** Heating curves of 3 mg mL-1 solution of Fe3O4@PLA-PEG/Cur nanocarrier (or 1.63 mgFe mL-1) under AF field of f = 178 kHz of various amplitudes in the range from 3600 to 5040 A/m, interrupting at T= 37 oC (a) and 45 oC (b).

**Figure S7.** Dependence of SAR on H2

**Figure S8.** UV-VIS spectra (a, c) and curcumin release amount (b, d) versus used field amplitude observed at 37 oC (a, b) and 45 oC (c, d).

**Figure S9.** Magnetic inductive heating of Fe3O4@PLA-PEG and Fe3O4@PLA-PEG/Cur nanoparticles in AMF of HAC = 5040 A/m and f = 178 kHz.

**Figure S10.** Graphs showing the re-increase of tumor after the treatment: Group 1: non-injected and non-irradiated mice as a control; group 2: non-injected mice with AMF irradiation; group 3: mice injected with Fe3O4@PLA-PEG and without AMF irradiation; group 4: mice injected with Fe3O4@PLA-PEG and subjected to AMF.

* 1. **Supporting tables**

**Table S1:** Size and Zeta potential of the samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Size calculated from XRD patterns (nm)** | **Average size obtained from TEM images (nm)** | **Hydrodynamic diameter**  **(dmean, nm)** | **Zeta potential**  **(mV)** |
| Fe3O4 | 8.5 | 8.5 2.6 | 37 | - 21.2 |
| Fe3O4@PLA-PEG | 10.3 | 9.3 2.8 | 45 | - 40.9 |
| Fe3O4@PLA-PEG/Cur | 11.1 | 12.2 4.3 | 52 | - 32.8 |

**Table S2:** Calculation of SAR ( ) from heating curves

|  |  |  |  |
| --- | --- | --- | --- |
| **H (A/m)** | **dT/dt (oC/s)** | **SAR (W/g)** | **Hf** (**A m−1s−1 )** |
| 3600 | 0.019 | 26.5 | 6.41 108 |
| 3760 | 0.025 | 34.8 | 6.69 108 |
| 4000 | 0.034 | 43.9 | 7.12 108 |
| 4240 | 0.038 | 53.6 | 7.55 108 |
| 4400 | 0.043 | 59.9 | 7.83 108 |
| 5040 | 0.059 | 82.2 | 8.97 108 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S3: Tumor sizes and volumns**   1. **10 day-old tumors** | | | | | | | | | | | | | | | | |
| **Group 1** | | | | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | | **2nd treatment** | | | | **3rd treatment** | | | | |
|  | a | b | V | a | b | | V | a | b | | V | a | b | | V | |
| Head | 1.0 | 1.0 | 0.5 | 1.3 | 1.3 | | 1.1 | 1.4 | 1.4 | | 1.4 | 1.5 | 1.6 | | 1.8 | |
| Back | 1.0 | 1.0 | 0.5 | 1.2 | 1.1 | | 0.8 | 1.3 | 1.1 | | 0.9 | 1.6 | 1.5 | | 1.9 | |
| Tail | 0.8 | 1.1 | 0.4 | 1.2 | 1.4 | | 1.0 | 1.3 | 1.5 | | 1.3 | 1.4 | 1.7 | | 1.7 | |
| Right front leg | 0.8 | 1.1 | 0.4 | 1.1 | 1.3 | | 0.8 | 1.1 | 1.3 | | 0.8 | 1.3 | 1.6 | | 1.4 | |
| Left front leg | 0.8 | 1.0 | 0.3 | 1.2 | 1.3 | | 0.9 | 1.3 | 1.4 | | 1.2 | 1.2 | 1.4 | | 1.0 | |
| Right hind leg | 1.0 | 0.8 | 0.4 | 1.3 | 1.1 | | 0.9 | 1.4 | 1.2 | | 1.2 | 1.5 | 1.3 | | 1.5 | |
| **Mean** |  |  | 0.40 |  |  | | 0.93 |  |  | | 1.12 |  |  | | 1.53 | |
|  |  |  |  |  |  | |  |  |  | |  |  |  | |  | |
| **Group 2** | | | | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | | **2nd treatment** | | | | **3rd treatment** | | | | |
|  | a | b | V | a | | b | V | a | | b | V | a | | b | | V |
| Head | 0.9 | 1.0 | 0.4 | 1.1 | | 1.2 | 0.7 | 1.2 | | 1.3 | 0.9 | 1.3 | | 1.4 | | 1.2 |
| Back | 0.9 | 0.9 | 0.4 | 1.2 | | 1.2 | 0.9 | 1.2 | | 1.2 | 0.9 | 1.3 | | 1.3 | | 1.1 |
| Tail | 0.8 | 1.0 | 0.3 | 1.1 | | 1.3 | 0.8 | 1.2 | | 1.4 | 1.0 | 1.3 | | 1.5 | | 1.3 |
| Right front leg | 0.8 | 1.1 | 0.4 | 1.2 | | 1.4 | 1.0 | 1.3 | | 1.5 | 1.3 | 1.3 | | 1.6 | | 1.4 |
| Left front leg | 0.9 | 1.0 | 0.4 | 1.2 | | 1.4 | 1.0 | 1.2 | | 1.4 | 1.0 | 1.4 | | 1.6 | | 1.6 |
| Right hind leg | 0.9 | 0.8 | 0.3 | 1.3 | | 1.2 | 1.0 | 1.3 | | 1.2 | 1.0 | 1.4 | | 1.3 | | 1.3 |
| **Mean** |  |  | 0.36 |  | |  | 0.90 |  | |  | 1.02 |  | |  | | 1.29 |
|  |  |  |  |  | |  |  |  | |  |  |  | |  | |  |
| **Group 3** | | | | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | | **2nd treatment** | | | | **3rd treatment** | | | | |
|  | a | b | V | a | | b | V | a | | b | V | a | | b | | V |
| Head | 0.9 | 0.8 | 0.3 | 1.2 | | 1.1 | 0.8 | 1.2 | | 1.1 | 0.8 | 1.4 | | 1.2 | | 1.2 |
| Back | 0.7 | 0.9 | 0.2 | 1.2 | | 1.3 | 0.9 | 1.2 | | 1.3 | 0.9 | 1.3 | | 1.2 | | 1.0 |
| Tail | 0.8 | 1.1 | 0.4 | 1.0 | | 1.2 | 0.6 | 1.1 | | 1.2 | 0.7 | 1.3 | | 1.4 | | 1.2 |
| Right front leg | 0.8 | 1.0 | 0.3 | 1.1 | | 1.3 | 0.8 | 1.1 | | 1.3 | 0.8 | 1.2 | | 1.5 | | 1.1 |
| Left front leg | 0.8 | 1.0 | 0.3 | 1.0 | | 1.2 | 0.6 | 1.1 | | 1.2 | 0.7 | 1.3 | | 1.4 | | 1.2 |
| Right hind leg | 1.0 | 1.1 | 0.6 | 1.2 | | 1.3 | 0.9 | 1.2 | | 1.3 | 0.9 | 1.3 | | 1.5 | | 1.3 |
| **Mean** |  |  | 0.35 |  | |  | 0.78 |  | |  | 0.82 |  | |  | | 1.15 |
|  |  |  |  |  | |  |  |  | |  |  |  | |  | |  |
| **Group 4** | | | | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | | **2nd treatment** | | | | **3rd treatment** | | | | |
|  | a | b | V | a | | b | V | a | | b | V | a | | b | | V |
| Head | 0.7 | 0.8 | 0.2 | 0.9 | | 1.0 | 0.4 | 1.0 | | 1.1 | 0.6 | 1.0 | | 1.1 | | 0.6 |
| Back | 0.8 | 1.0 | 0.3 | 1.0 | | 1.1 | 0.6 | 1.1 | | 1.0 | 0.6 | 1.2 | | 1.2 | | 0.9 |
| Tail | 1.0 | 1.0 | 0.5 | 1.1 | | 1.0 | 0.6 | 1.2 | | 1.1 | 0.8 | 1.2 | | 1.3 | | 0.9 |
| Right front leg | 0.8 | 1.1 | 0.4 | 0.9 | | 1.0 | 0.4 | 1.1 | | 1.0 | 0.6 | 1.3 | | 1.2 | | 1.0 |
| Left front leg | 0.7 | 0.7 | 0.2 | 0.7 | | 0.9 | 0.2 | 0.9 | | 1.0 | 0.4 | 0.9 | | 1.0 | | 0.4 |
| Right hind leg | 1.0 | 1.2 | 0.6 | 1.1 | | 1.0 | 0.6 | 1.0 | | 1.1 | 0.6 | 1.2 | | 1.3 | | 0.9 |
| **Mean** |  |  | 0.36 |  | |  | 0.47 |  | |  | 0.58 |  | |  | | 0.78 |

1. **5 day-old tumors**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group 1** | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | **2nd treatment** | | | **3rd treatment** | | |
|  | a | b | V | a | b | V | a | b | V | a | b | V |
| Head | 0.6 | 0.6 | 0.11 | 0.9 | 1.1 | 0.45 | 1.0 | 1.1 | 0.55 | 1.1 | 1.1 | 0.67 |
| Back | 0.6 | 0.6 | 0.11 | 0.7 | 0.8 | 0.20 | 1.0 | 1.1 | 0.55 | 1.4 | 1.5 | 1.47 |
| Tail | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.17 | 0.9 | 1.0 | 0.41 | 1.0 | 1.2 | 0.60 |
| Right front leg | 0.5 | 0.5 | 0.06 | 0.8 | 0.8 | 0.26 | 0.9 | 1.0 | 0.41 | 0.9 | 0.9 | 0.36 |
| Left front leg | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.17 | 0.9 | 0.9 | 0.36 | 0.9 | 0.9 | 0.36 |
| Right hind leg | 0.5 | 0.5 | 0.06 | 0.8 | 1.0 | 0.32 | 0.9 | 1.0 | 0.41 | 1.0 | 1.3 | 0.65 |
| **Mean** |  |  | 0.08 |  |  | 0.26 |  |  | 0.45 |  |  | 0.69 |
|  | | | | | | | | | | | | | |
| **Group 2** | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | **2nd treatment** | | | **3rd treatment** | | |
|  | a | b | V | a | b | V | a | b | V | a | b | V |
| Head | 0.4 | 0.4 | 0.03 | 0.6 | 0.7 | 0.1 | 0.7 | 0.8 | 0.2 | 1.1 | 1.1 | 0.7 |
| Back | 0.5 | 0.5 | 0.06 | 0.7 | 0.8 | 0.2 | 0.8 | 0.9 | 0.3 | 1.0 | 1.1 | 0.6 |
| Tail | 0.4 | 0.4 | 0.03 | 0.6 | 0.6 | 0.1 | 0.7 | 0.7 | 0.2 | 0.9 | 0.9 | 0.4 |
| Right front leg | 0.4 | 0.4 | 0.03 | 0.5 | 0.6 | 0.1 | 0.7 | 0.7 | 0.2 | 1.2 | 1.2 | 0.9 |
| Left front leg | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.2 | 0.7 | 0.7 | 0.2 | 1.2 | 1.2 | 0.9 |
| Right hind leg | 0.4 | 0.4 | 0.03 | 0.6 | 0.6 | 0.1 | 0.7 | 0.7 | 0.2 | 1.0 | 1.0 | 0.5 |
| **Mean** |  |  | 0.04 |  |  | 0.13 |  |  | 0.20 |  |  | 0.63 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Group 3** | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | **2nd treatment** | | | **3rd treatment** | | |
|  | a | b | V | a | b | V | a | b | V | a | b | V |
| Head | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.17 | 0.7 | 0.8 | 0.20 | 1.0 | 1.2 | 0.60 |
| Back | 0.4 | 0.4 | 0.03 | 0.8 | 0.9 | 0.29 | 0.8 | 1.0 | 0.32 | 0.9 | 1.0 | 0.41 |
| Tail | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.17 | 0.7 | 0.7 | 0.17 | 1.0 | 1.0 | 0.50 |
| Right front leg | 0.5 | 0.6 | 0.08 | 0.8 | 0.8 | 0.26 | 0.9 | 1.0 | 0.41 | 1.1 | 1.2 | 0.73 |
| Left front leg | 0.5 | 0.5 | 0.06 | 0.7 | 0.7 | 0.17 | 0.8 | 1.0 | 0.32 | 1.1 | 1.1 | 0.67 |
| Right hind leg | 0.5 | 0.5 | 0.06 | 0.8 | 0.8 | 0.26 | 0.9 | 0.9 | 0.36 | 1.1 | 1.1 | 0.67 |
| **Mean** |  |  | 0.06 |  |  | 0.22 |  |  | 0.30 |  |  | 0.59 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Group 4** | | | | | | | | | | | | | |
| **Mouse** | **Before treatment** | | | **1st treatment** | | | **2nd treatment** | | | **3rd treatment** | | |
|  | a | b | V | a | b | V | a | b | V | a | b | V |
| Head | 0.5 | 0.5 | 0.06 | 0.7 | 0.8 | 0.20 | 0.8 | 0.8 | 0.26 | 1.0 | 0.8 | 0.40 |
| Back | 0.5 | 0.5 | 0.06 | 0.6 | 0.7 | 0.13 | 0.7 | 0.8 | 0.20 | 0.9 | 0.9 | 0.36 |
| Tail | 0.5 | 0.5 | 0.06 | 0.9 | 0.9 | 0.36 | 0.9 | 0.9 | 0.36 | 0.8 | 0.8 | 0.26 |
| Right front leg | 0.5 | 0.5 | 0.06 | 0.6 | 0.7 | 0.13 | 0.8 | 0.9 | 0.29 | 1.0 | 0.9 | 0.45 |
| Left front leg | 0.5 | 0.5 | 0.06 | 0.6 | 0.9 | 0.16 | 0.7 | 0.9 | 0.22 | 0.8 | 0.9 | 0.29 |
| Right hind leg | 0.4 | 0.4 | 0.03 | 0.7 | 0.7 | 0.17 | 0.7 | 0.8 | 0.20 | 0.9 | 0.8 | 0.32 |
| **Mean** |  |  | 0.06 |  |  | 0.19 |  |  | 0.25 |  |  | 0.35 |

V: tumor volumn (cm3); a: tumor length (cm); b: tumor width (cm)