Supporting Information

to

Gd(III)-doped carbon dots as a dual fluorescent-MRI probe

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Materials and methods for characterization

AFM: Atomic Force Microscopy (AFM) images were obtained in tapping mode with a Bruker Multimode Nanoscope 3D using Tap300-G silicon cantilevers with a tip radius <10 nm and ~20-75 N m^{-1} of force constant. For the AFM measurements, isolated Gd-QCDs deposited on a Si-wafer were obtained by a modified technique which combines Langmuir-Schaefer (LS) deposition and self-assembly In a KSV-2000 Langmuir-Blodgett trough, Millipore Q-grade water was used as subphase and a stearic acid solution (0.2 mg mL^{-1})

dissolved in chloroform-methanol (9:1) was spread onto the aqueous subphase. The hydrophobic Si-wafer was then dipped horizontally at a constant surface pressure of 20 mN m^{-1}. After the LS deposition, the surface of the film was rinsed several times with ultrapure water, dried with a flow of N_2 gas and dipped into an aqueous solution of Gd-QCDs (0.5 mg mL^{-1}). Finally, the surface was rinsed copiously with ultrapure water to remove any excess material weakly attached on the surface and dried with N_2 gas.

MRI phantom experiments: MRI phantom measurements were performed on a 1.5 T MRI scanner (GE Signa HORIZON Lx) with acquisition of T1 FSE sequences in coronal plane using the following parameters: TE 15.4, TR 540 ms, FOV 38x36, Matrix 512x224 cm. Gd-QCDs particles as well as the commercial contrast agent Gadovist were diluted in water to the same ionic Gd concentration (0.364 mmol Gd/L) which corresponds to the standard concentration of Gd-based contrast agents used in clinical practice.

Cytotoxicity: In-vitro biocompatibility of QCDs and Gd-QCDs was probed using the MTT assay (MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) against NIH3T3 cells (mouse fibroblast cells). Various concentrations of QCDs and Gd-QCDs were used (0-5000 mg/L). The cells were incubated in the presence of nanoparticles in 96 well plates (P-Lab, Czech Republic) at 37 °C and 5 % CO_2 for 24 h. The absorbances of the resulting solutions were measured using a 96-well microplate reader Synergy HT (BioTek, USA) at 570 nm. The cell viability of the samples was determined as percentage of control cell viability (100× average of test group/average of control group). IC50 indexes (half maximal inhibitory concentration) were statistically determined by 3T3NRU Phototox software (COLIPA, Germany).

Stem cell cultures and labeling: Rat mesenchymal stem cells were isolated from the bone marrow of femurs. The cell suspension was filtered through a 40-μm nylon filter (Falcon) and placed in plastic chambers Cells were grown in Iscove's Modified Dulbecco's Medium

(IMDM; Invitrogen) with 10% fetal bovine serum (FBS) and 2% penicilin/streptomycin (PS) at 37 °C under 5% CO_2. After 24 hours the medium was replaced to remove non-adhered cells. The cells were grown to obtain 80 % of confluence. Cells were incubated with Gd-QCDs in concentration of 25 μg/mL. After 24 hours of incubation the images of labeled cells were obtained by optical and fluorescence microscopy. Visual microscopic detection of emission fluorescence signal of bare Gd-QCDs and labeled stem cells after 24h of incubation with Gd-QCDs was observed by inverted light fluorescence microscope Olympus IX 70 in fluorescence mode using U-MWIBA3 FILTER BLOCK (exc. 460-495, DM 505nm, em. 510-550). All animal experiments were performed in accordance with the Czech Guide for the Care and Use of Laboratory Animals and were approved by the Committee for the Use of Experimental animal at Masaryk University in the Czech Republic.

Supporting figures

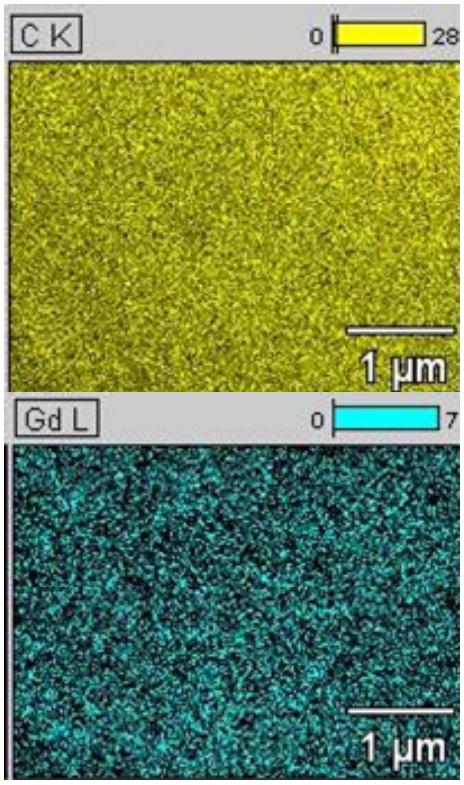

Fig. S1 EDX chemical mapping evidences the homogeneous distribution of C and Gd in the sample.

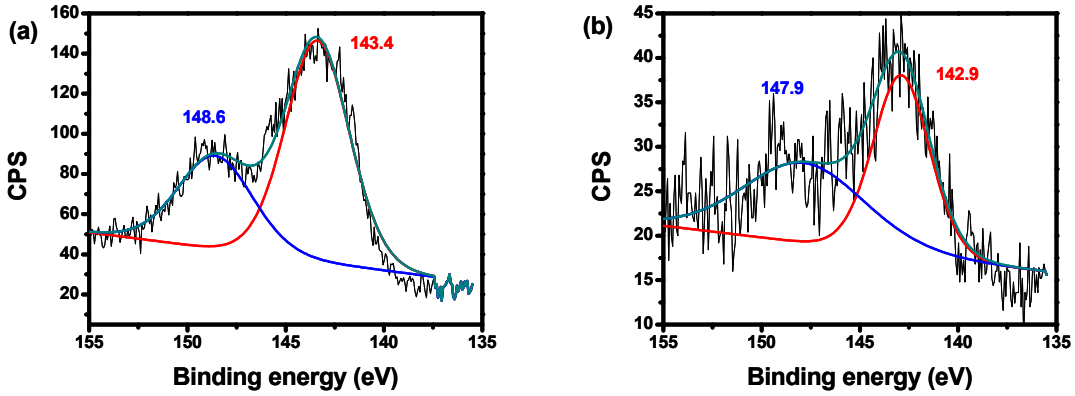

Fig. S2 Gd 4d spectrum of (a) gadopentetic acid and (b) Gd-QCDs.

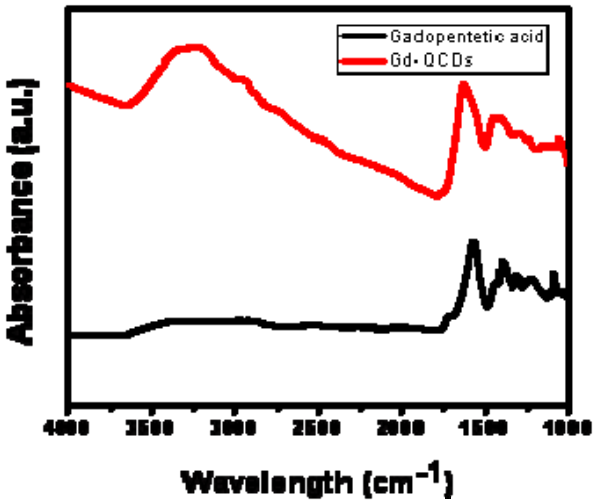

Fig. S3 FT-IR spectra of gadopentetic acid and Gd-QCDs in KBr.

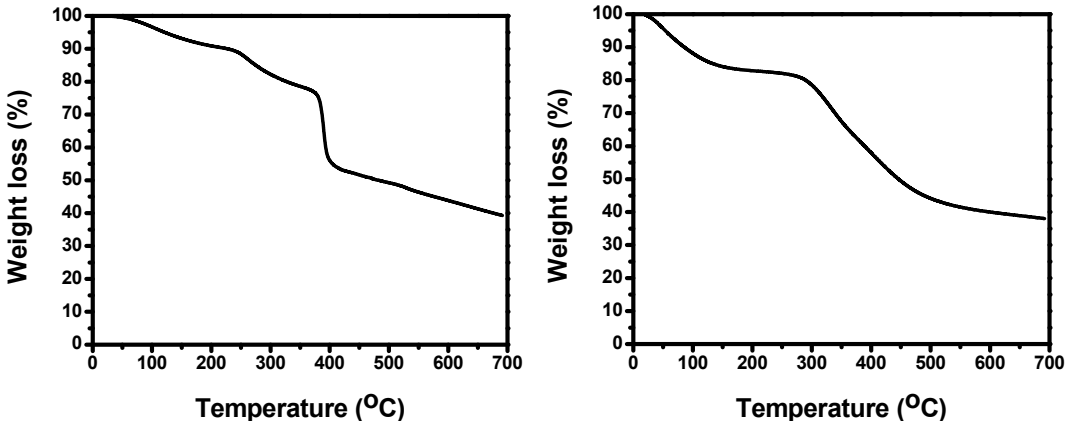

Fig. S4 TGA traces in air of gadopentetic acid (left) and Gd-QCDs (right).

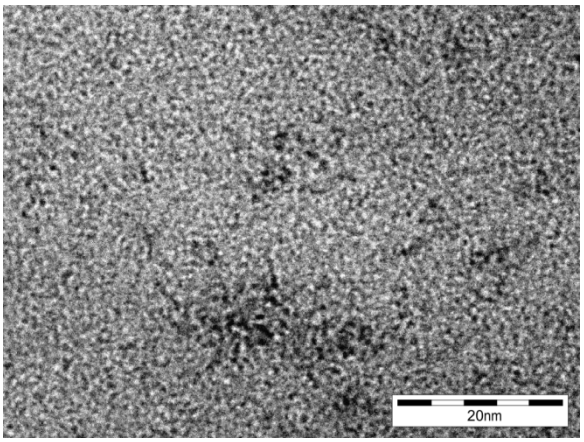

Fig. S5 TEM image of ultrafine nanoparticles 3 nm in size (dark spots).

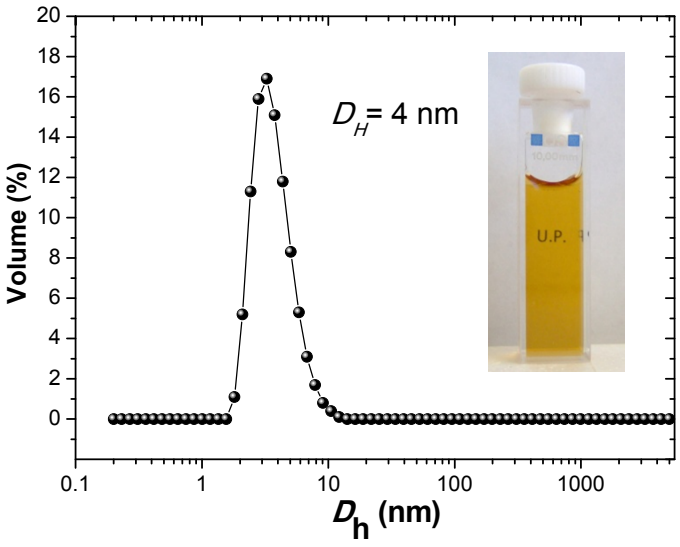

Fig. S6 Distribution plot of the hydrodynamic diameter of the Gd-QCDs aqueous dispersion (0.25 mg mL^{-1}).

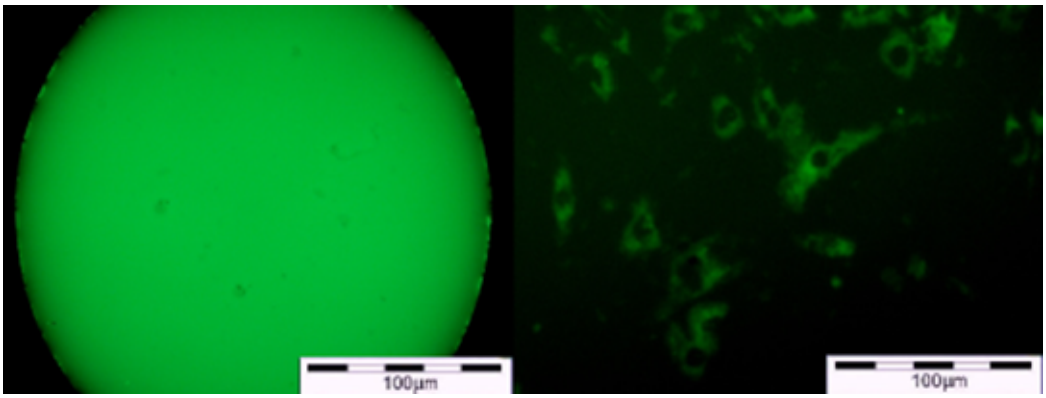

Fig. S7 Optical fluorescence microscopy images of Gd-QCDs: a) a drop of Gd-QCDs aqueous dispersion, b) rat mesenchymal stem cells incubated with Gd-QCDs (25 μg/mL) for 24 hours.