

Supporting information

Materials and Methods

Chemicals and Materials: Nanopure water (18.2 MΩ; Millipore Co., USA) was used in all experiments and to prepare all buffers. N-cetyltrimethylammonium bromide (CTAB) and urea were obtained from Alfa Aesar. Ln(NO₃)₃ (Ln = Gd, Eu) was purchased from Aladdin. Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS) and Lipofectamine 2000 were purchased from Invitrogen. **λ-EcoT14 I digest DNA Marker, *Not* I and *Bgl* II endonucleases were purchased from Takara.**

Measurements and Characterizations: A field emission scanning electron microscope (FESEM, S4800, Hitachi) equipped with an energy-dispersive X-ray (EDS) spectrum was applied to determine the morphology and composition of the as-prepared samples. TEM was performed on a JEOL 1011 transmission electron microscope at an accelerating voltage of 200 kV. The crystalline structures of the as prepared samples were evaluated by X-ray diffraction (XRD) analysis on a Rigaku-Dmax 2500 diffractometer by using CuKα radiation (λ=0.15405 nm). N₂ adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The photoluminescence (PL) measurements were performed on a Hitachi F-4500 spectrophotometer equipped with a 150 W xenon lamp as the excitation source. Fluorescence measurements were carried out on Jasco-FP-6500 spectro fluorometer (Jasco International Co. LTD. Tokyo, Japan). UV-vis absorption spectra were recorded using a Varian Cary300 spectrophotometer equipped with a 1-cm path length quartz cell.

Synthesis of GdPO₄:Eu HMNs: The monodisperse GdPO₄:Eu HMNs were prepared according to our previous report.¹

Plasmid DNA Preparation: The plasmid pEGFP-N2 (4.7 kb) containing enhanced green fluorescent protein (EGFP) encoding DNA under control of the cytomegalovirus (CMV) promoter was propagated in *E. coli* and purified using the Plasmid Giga Kit (Qiagen, Valencia, CA). Purified pEGFP-N2, with A₂₆₀/A₂₈₀ purity between 1.8 and 1.9, was dissolved in TE buffer at 1 mg/mL and stored at -20 °C.

Plasmid DNA Loading Experiment: Plasmid DNA loading onto GdPO₄:Eu HMNs was done by mixing 10 μL DNA (0.2 mg/mL) with 500 μL GdPO₄:Eu hollow spheres (40 μg/mL) in different pH of PBS buffer (pH= 5.0, 7.0, 8.0) for 3 h. The supernatant was collected by centrifugation at 12000 rpm for 10 min. The supernatant and stock solution was analyzed by UV-vis spectrophotometry (Cary 300, Varian Inc., Palo Alto, CA) at 260 nm, respectively.

Gel Retardation Assay and Plasmid DNA Protection: The agarose gel was prepared by dissolving 1% (w/v) agarose in TAE buffer containing ethidium bromide. Complexes for this assay were prepared at nanoparticle : DNA ratios (w/w) of 0.5 : 1, 0.6 : 1, 0.8 : 1, 1 : 1, 2 : 1, 5 : 1, 10 : 1. To study the protective effect, double digestion experiment was carried out. Naked plasmid DNA and nanoparticles-DNA vehicles were incubated with *Not* I and *Bgl* II restriction endonucleases for 90 min at 37 °C. Then those samples were loaded on a 1% agarose gel containing 0.01% ethidium

bromide and run at 100 V for 30 min.

Cell Culture: The A549 cells were grown at 37 °C in an atmosphere of 5% (v/v) CO₂ in air, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS. The media was changed every three days, and the cells were digested by trypsin and resuspended in fresh complete medium before plating.

Cytotoxicity Assays: MTT assays were used to probe cellular viability. A549 cells were seeded at a density of 5000 cells/well (100 µL total volume/well) in 96-well assay plates. After 24 h incubation, the as-prepared GdPO₄:Eu HMNs, at the indicated concentrations, were added for further incubation of 48 h. To determine toxicity, 10 µL of MTT solution (BBI) was added to each well of the microtiter plate and the plate was incubated in the CO₂ incubator for an additional 4 h. Then the cells were lysed by the addition of 100 µL of DMSO. Absorbance values of formazan were determined with Bio-Rad model-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). Six replicates were done for each treatment group.

Cell Transfections: A549 cells were seeded at 125000 cells per well in 24-well plates 24 h prior to transfection. Nanoparticle:DNA complexes prepared at 10:1 wt:wt ratios were added to 1 mL of serum free medium to give a final DNA concentration of 2 µg DNA mL⁻¹ in each well and gently rocked to mix. The medium was changed to new medium supplemented with 10% FBS after 6 h and the cells were incubated for another 72 h before viewing using an Olympus BX-51 optical system microscopy equipped with a CCD. **Fluorescence was detected using band-pass excitation filter (330–385 nm) and high pass emission filter (420 nm) and 400 nm dichromatic mirror.** Transfections using the commercial agents, Lipofectamine 2000 were performed following the manufacturer's protocol.

Magnetic Resonance Imaging: 50 µL of different concentrations of samples dissolved in PBS was taken in a 96 well plate and imaged using 1.5 T human clinical scanner. **Cell phantom T1-weighted MR images were done. A549 cells were trypsinized and an aliquot of cell suspension was added to each culture flask to obtain a cell density of 8.0×10^6 cells per flask. The flasks were cultured in DMEM containing 10 % (v/v) FBS at 37 °C in an atmosphere of 5% (v/v) CO₂ in air for 24 h. The media was then removed and replaced with 4 mL of fresh media containing 40 µg/mL GdPO₄:Eu HMNs. The flasks were incubated for 4 h and then washed with PBS for 5 times. Finally, the cells were trypsinized, resuspended in 1 mL PBS, and centrifuged at 3,000 rpm for 15 min to obtain cell pellets.**

Statistical Analysis: All data were expressed in this article as mean result ± standard deviation (SD). All figures shown in this article were obtained from three independent experiments with similar results. The statistical analysis was performed by using Origin 8.5 software.

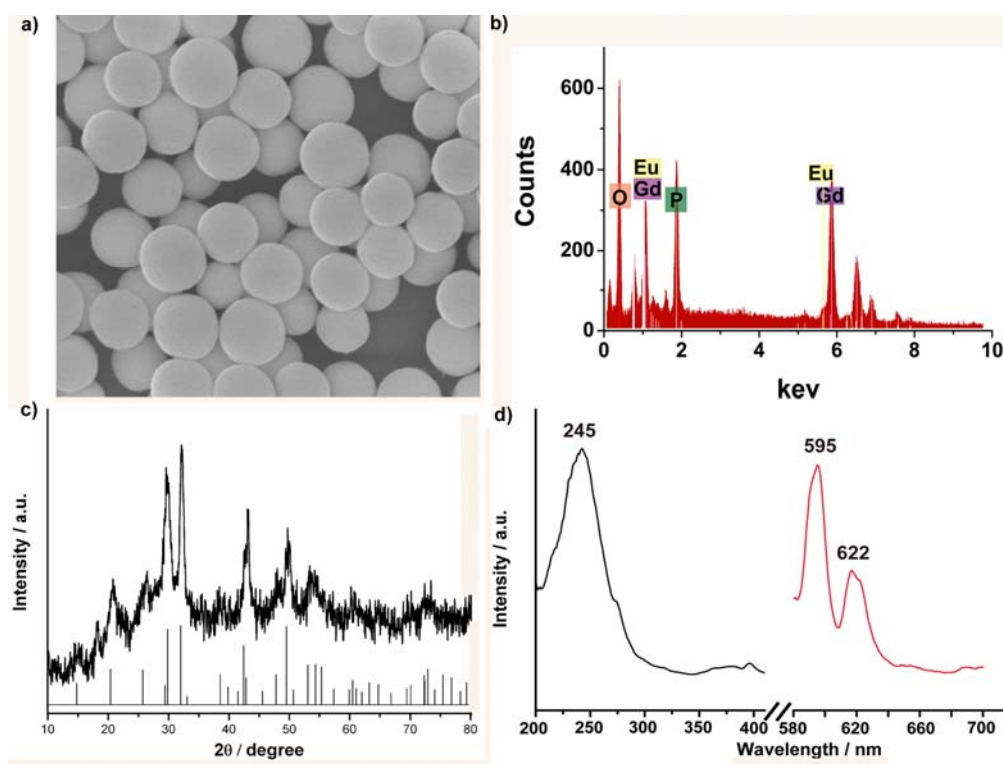


Fig. S1 a) SEM images of the $\text{Gd}(\text{OH})\text{CO}_3:\text{Eu}$ colloidal spheres; b) EDX spectrum of $\text{GdPO}_4:\text{Eu}$ HMNs; c) XRD patterns of $\text{GdPO}_4:\text{Eu}$ HMNs; d) PL excitation (black line) and emission (red line) spectra.

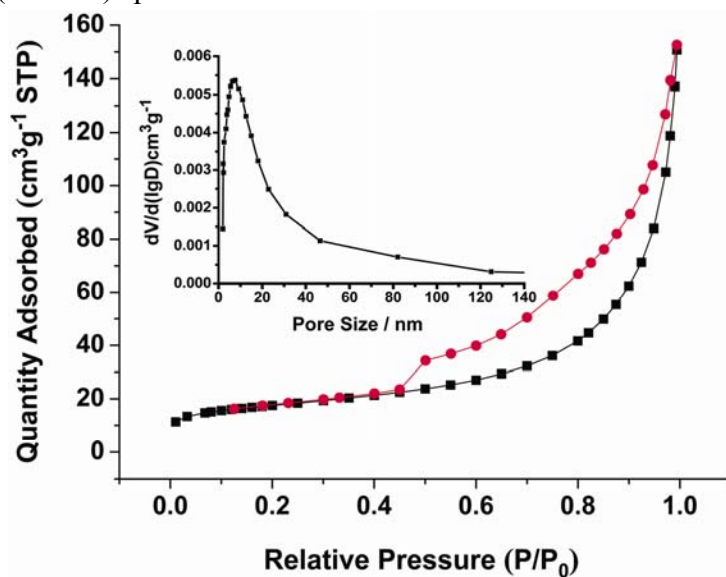


Fig. S2 N_2 adsorption–desorption isotherms and BJH pore-size distribution plots of the samples.

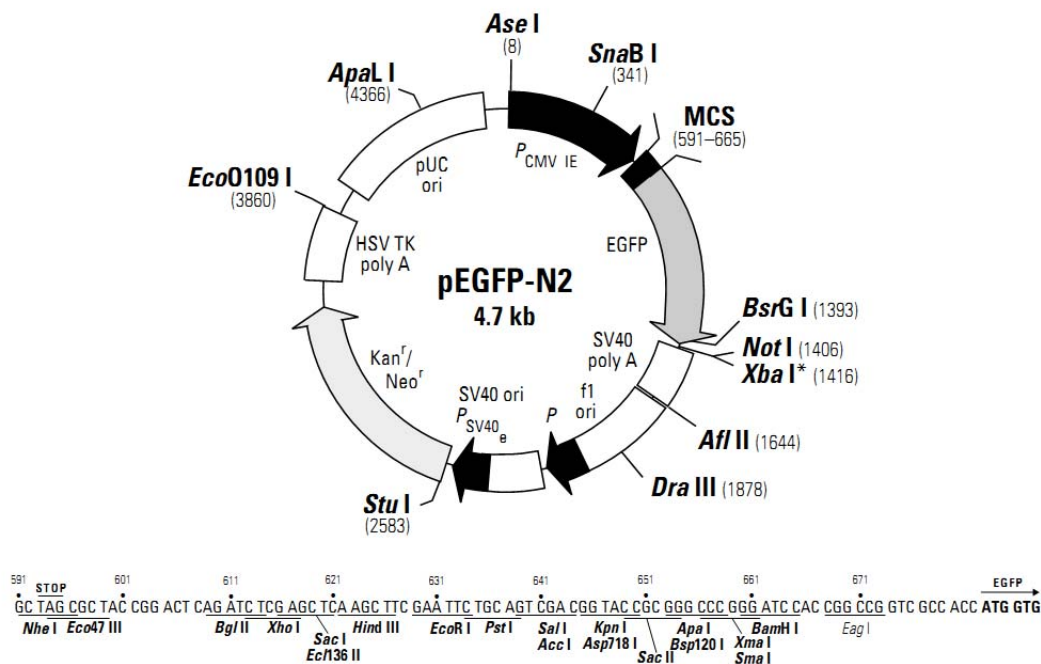


Fig. S3 Restriction map and multiple cloning site (MCS) of pEGFP-N2.

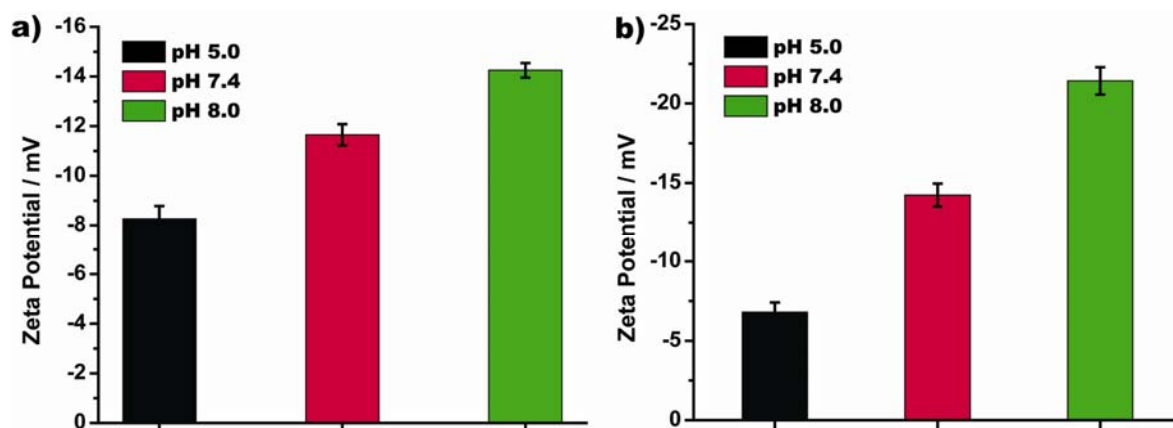


Fig. S4 The zeta potential of pEGFP-N2 (a) and GdPO₄:Eu HMNs at various pH.

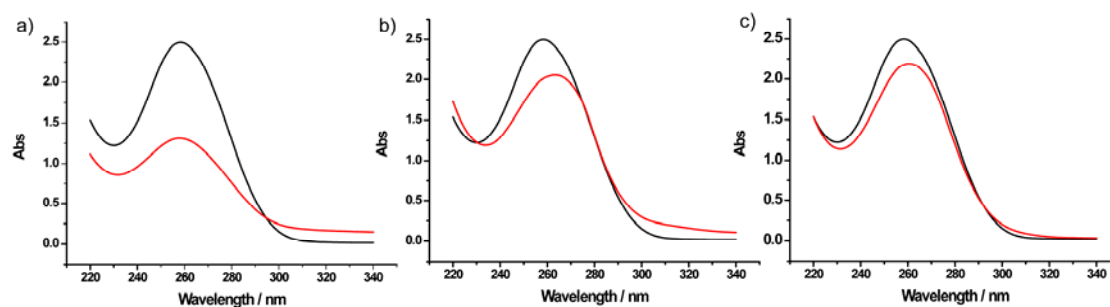


Fig. S5 Quantification of plasmid DNA loading onto GdPO₄:Eu HMNs at different pH: a) pH = 5.0; b) pH = 7.4; c) pH = 8.0. The black line represent the stock solution

of DNA and the red line represent the supernatant of DNA after loading onto GdPO₄:Eu HMNs.

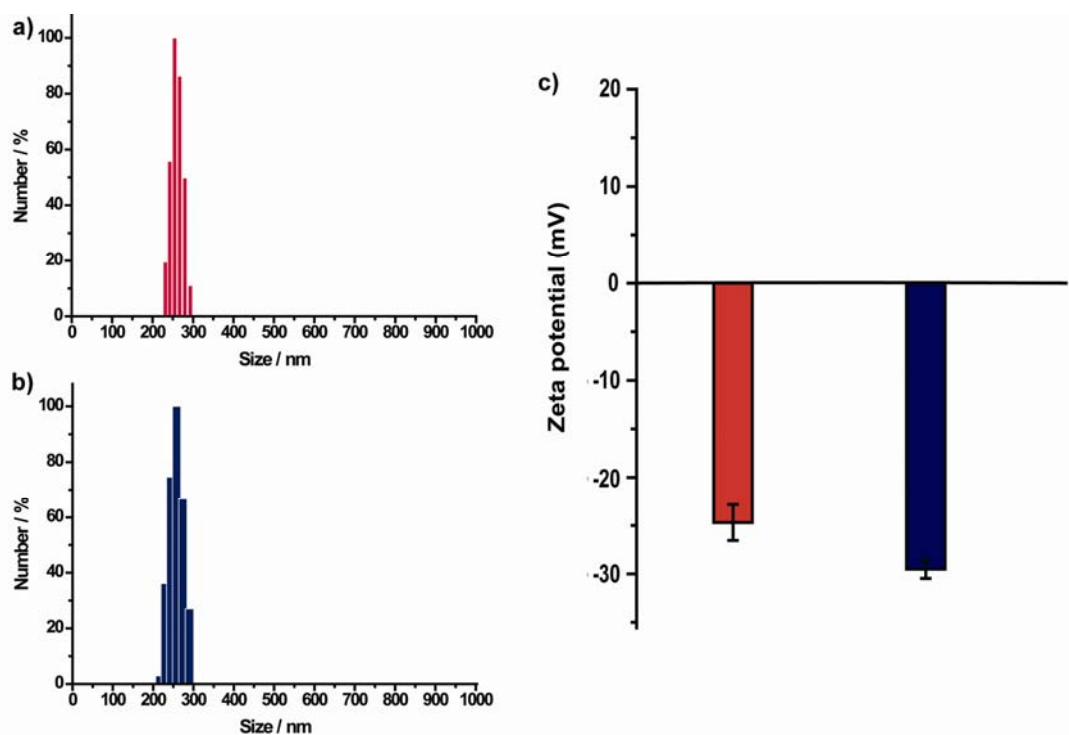


Fig. S6 Hydrodynamic distribution of GdPO₄:Eu HMNs (a); GdPO₄:Eu HMNs-DNA (b); c) Zeta-potential of different samples: GdPO₄:Eu HMNs (left); GdPO₄:Eu HMNs-DNA (right). The hydrodynamic size for them was calculated to be 254.4 and 258.7 nm

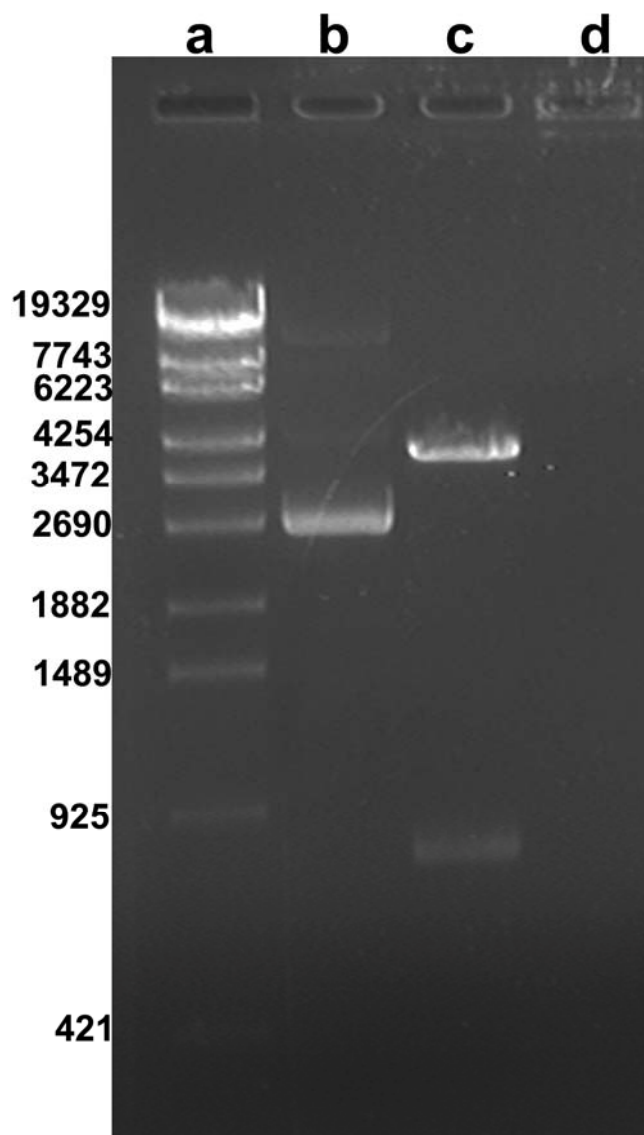


Fig. S7 Restriction endonucleases protection assay. Lane a, λ -EcoT14 I digest DNA Marker; Lane b, naked plasmid DNA; Lane c, plasmid DNA digested with *Not* I and *Bgl* II endonuclease for 90 min; Line d, GdPO₄:Eu HMNs-DNA complex treated with *Not* I and *Bgl* II endonuclease for 90 min.

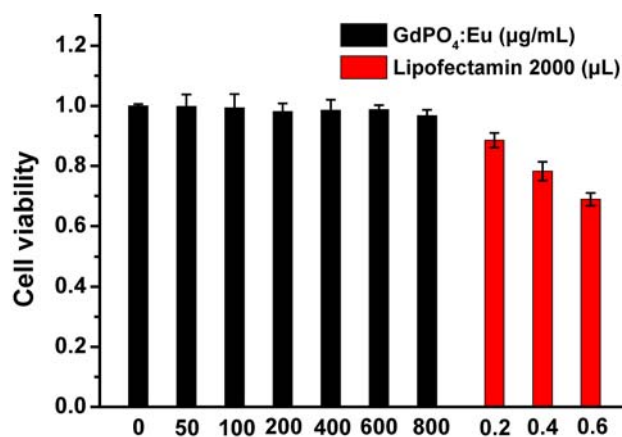


Fig. S8 Concentration-dependent cell survival data of A549 cells treated with $\text{GdPO}_4\text{:Eu}$ particles and Lipofectamin 2000.

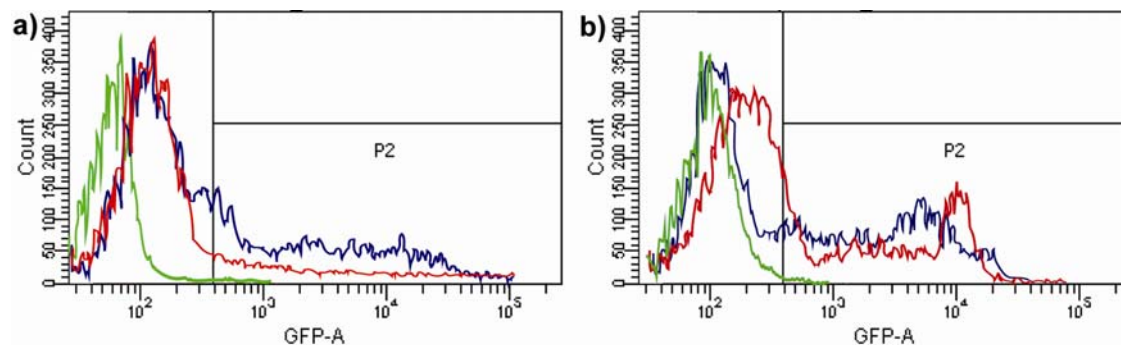


Fig. S9 FACS analysis showed the profiles of A549 cells transfected with Lipofectamine 2000-DNA (blue line) and $\text{GdPO}_4\text{:Eu}$ HMNs-DNA complexes (red line) for 48 h (a) and 72 h (b), respectively. The green line was the control.

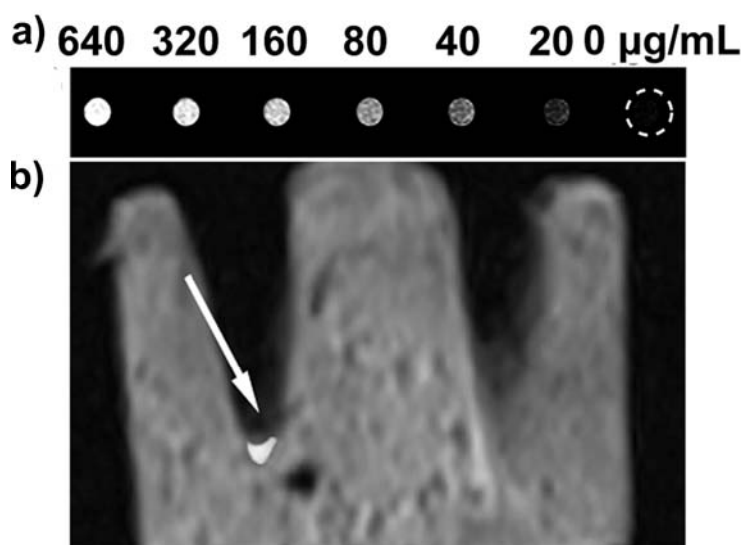


Fig. S10 Phantom NMR images of $\text{GdPO}_4\text{:Eu}$ HMNs dissolved in PBS solution showing T_1 -weighted bright contrast (a): T_1 -weighted images of A549 cell pellets incubated with (left) and without (right) $\text{GdPO}_4\text{:Eu}$ (b)

Reference

1. Z. Li, Z. Liu, M. Yin, X. Yang, J. Ren and X. Qu, *Adv. Healthcare Mater.*, 2013, DOI: 10.1002/adhm.201200364.