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Supplementary Materials for

Shine and darkle the blood vessels: Multiparameter hypersensitive MR angiography for diagnosis of panvascular diseases

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Text

Synthesis and characterization of PEGylated $NaGdF_4$ nanoparticles

The biocompatible $NaGdF_4$ nanoparticles with appropriate physical parameters were synthesized. In detail, the oleate-capped $NaGdF_4$ nanoparticles with an ultrasmall diameter of 3.53 ± 0.49 nm were firstly prepared by high temperature thermal decomposition, as shown in the transmission electron microscopy (TEM) image together with corresponding size distribution profile in fig. S1. The selected area electron diffraction measurement was conducted and as shown in fig. S2, $NaGdF_4$ nanoparticles are in hexagonal structure according to the JCPDS cards (No. 27-0699). In addition, the hexagonal phases of $NaGdF_4$ nanoparticles were further investigated using the high-resolution TEM (HRTEM) shown in fig. S1, where the lattice spacing of (101) and (200) planes match well with those of corresponding bulk materials. To endow the nanoparticles with biocompatibility, poly (ethylene glycol) (PEG), most of which received FDA approval and were widely used in clinical practice, was selected to modify the surface of the nanoparticles. The asymmetric poly (ethylene glycol) (PEG) ligands were applied to replace the oleate ligand, ultimately obtaining the biocompatible PEGylated $NaGdF_4$ nanoparticles. As displayed in Fig. 1C and fig. S3, the resultant PEGylated $NaGdF_4$ nanoparticles were successfully synthesized with the diameter of 3.77 ± 0.46 nm, suggesting that the procedure of ligand exchange did not lead to evident size changes of the $NaGdF_4$ nanoparticles. The dispersity of nanoparticles was tested through dynamic light scattering (DLS) analysis. As given in fig. S4, the average hydrodynamic diameter (D_h) of PEGylated $NaGdF_4$ nanoparticles was 15.13 nm with a relatively narrow size distribution, suggesting that the water monodispersity of nanoparticles is satisfactory.

Materials and Methods

Materials

Gadolinium chloride hexahydrate ($GdCl_3 \cdot 6H_2O$), oleic acid (OA), 1-octadecene (ODE), and ammonium fluoride (NH_4F) were purchased from Sigma-Aldrich, cyclohexane was purchased from Damao Chemical Reagent Co., Ltd., and methanol, anhydrous ethanol, tetrahydrofuran (THF) with analytical grade were purchased from Sinopharm Chemical Reagent Beijing, Co., Ltd. Sodium hydroxide (NaOH) was obtained from Beijing Chemical Reagents Co. Ltd. The asymmetric poly (ethylene glycol) (PEG) of molecular weight 2000 was supplied from Beijing Oneder Hightech Co. Ltd. Human umbilical vein endothelial cells (HUVECs) were purchased from American Type Culture Collection (ATCC).

^{68}Ga Labeling of nanoparticles

$^{68}Ge/^{68}Ga$ generator was eluted with 0.05M HCl solution. The pH of the eluted $^{68}GaCl_3$ solution was adjusted at 4.5 by the addition of NaOAc solution. During labeling, 111 MBq of $^{68}GaCl_3$ was added to a clean tube with PEGylated $NaGdF_4$ solution (600 μg of nanoparticles included) and the mixture was heated at 37 °C for 20 min. The radiolabeling efficiency was controlled by thin-layer chromatography on TLC Silica gel (TLC-SG) with a methanol and ammonium acetate solvent (volume ratio of 1:1). Excess $^{68}GaCl_3$ was removed by ultrafiltration with 30 kDa MWCO centrifugal filter (Millipore YM-50). The resultant nanoparticles were configured by PBS with the Gd^{3+} concertation of 3 mg/mL.

Characterizations of the $NaGdF_4$ nanoparticles

Transmission electron microscope (TEM) images of the $NaGdF_4$ nanoparticles were obtained on a JEM-2100 transmission electron microscope at an acceleration voltage of 200 kV. The core sizes of $NaGdF_4$ nanoparticles were measured by averaging at least 100 nanoparticles per sample in corresponding TEM image. Dynamic light scattering (DLS) was carried out at 298 K with Nano ZS (Malvern) equipped with a solid state He-Ne laser (λ = 632.8 nm).

Methods

Cell culture

Human umbilical vein endothelial cells (HUVECs, ATCC CRL 1730) were cultured in Endothelial Cell Medium (ECM) with 5% fetal bovine serum (FBS), 1% endothelial cell growth factor (ECGF), and 1% penicillin/streptomycin solution at 37 °C under 5% CO_2 atmosphere. The cell line was tested negative for mycoplasma contamination by the mycoplasma detection kit (Yeasen Cat. No. 40611).

Cell viability assays

Cell Counting Kit-8 (CCK-8) assay on HUVECs was carried out as follows. HUVECs were seeded into a 96-well cell culture plate with a density of 5,000 cells per well under 100% humidity, and then cultured at 37 °C in an atmosphere containing 5% CO_2 for 24 h. Then, the $NaGdF_4$ nanoparticles with different concentrations were added into the wells and incubated with the cells for 24 h at 37 °C under 5% CO_2. After the supernatant containing the excrescent $NaGdF_4$ nanoparticles agent was decanted, the cells were incubated for another 48 h. After that, 10 μL CCK-8 was added to each well, and incubated for 4 h at 37 °C under 5% CO_2. The optical density of each well at 450 nm was recorded on a microplate reader (Thermo, MULTISKAN GO).

Hemolysis test

Briefly, 2 mL blood sample was mixed with 6 mL of normal saline (NS) to purify through the centrifugation for several times. The resultant red blood cells were diluted in 4 mL of NS and the suspension was then diluted 1: 4 into NS (negative control); water (positive control); $NaGdF_4$ solutions (in NS) at different concentrations. The samples were kept in the dark for 4 h at 37 °C and then centrifuged at 3000 rpm for 5 min. After centrifuging, the supernatant was transferred to a 96-well plate and the absorbance at 541 nm was recorded.

The hemolysis rate was calculated by the following equation:

$$\text{Hemolysis rate} = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}} \times 100\%$$

where D_t, D_{nc}, and D_{pc} were the absorbance of the tested sample, the negative control, and the positive control, respectively.

Establishment of rodent models

Rat model of right middle cerebral artery occlusion (rMCAO)

The Sprague-Dawley (SD) rats (~300 g) were randomly selected to be in abrosia for 12 h besides water. Abdominal anesthesia was conducted with 1% pentobarbital. During surgery, the rectal

temperature was monitored and kept at 37 ± 0.5 °C by using a heating blanket. A midline incision between the sternum and chin was operated to expose the carotid arteries and an MCAO suture (total length of ~45 mm and diameter of 0.38 ± 0.02 mm) with a slightly enlarged silicon-coated tip was inserted via external carotid artery into internal carotid artery and pushed ≈18-20 mm from the carotid bifurcation for building the rMCAO model. After that the skin of rat was sutured, the body temperature was maintained at 37 ± 0.5 °C using a heating pad until the rat woke up. The rat was subjected to MR scanning after 24 h.

Rat model of CRF

The SD rats (~220-250 g) were administrated with nature saline suspension of adenine with the dose of 200 mg/kg every day through gastric gavage. The body weights were recorded every 7 days and the blood biochemical renal function indicators to evaluate the degree of renal impairment. The CRF rat models would be established after ~30 days of adenine administration.

Blood half-life measurement

$NaGdF_4$ nanoparticles or Gd-DTPA contrast agents with 0.1 mmol/kg Gd were intravenously injected into two groups of 6-week-old female BALB/c mice (n = 3), respectively. The PEGylated $NaGdF_4$ nanoparticles. Blood samples were obtained and weighted at 30 s, 1 min, 5 min, 15 min, 30 min, 2 h, 4 h, 8 h, 12 h, 24 h and 48 h post-injection. The Gd content in the blood was determined through ICP-MS (Thermo, ICAP-Qc) after acid digestion.

Histology study after MRI

The tissues of brain were harvested after MRI experiments of AD mice and rMCAO rats, and were fixed in the paraformaldehyde. After being embedded into paraffin, the fixed tissues were sliced. Then the resultant slices were stained with H&E, and subjected to microscopy study for the histological analysis. For AD mice, the brain slice next to H&E slice were further stained with Thioflavin S, Congo red, Masson and Nissl through immunohistochemical method, for judging the blood hypoperfusion of AD. For rMCAO rats, the brain slice next to H&E slice were further stained with Nissl, Tunel and Masson through immunohistochemical method, for judging the arterial occlusion, collaterals, and ischemic penumbra.

Biosafety evaluation of $NaGdF_4$ nanoparticles in healthy mice

The randomly chosen female BALB/c mice (6-week-old) (n = 4) was intravenously injected $NaGdF_4$ nanoparticles with 0.1 mmol/kg Gd and the other four healthy mice were the control group. The body weights of mice were recoded every day. At 14 d post-injection, the mice were sacrificed, and the major organs including heart, liver, spleen, lung and kidney were extracted and subjected to H&E staining and CPN III staining. A healthy mouse was set as the control. The blood collected from each mouse was further used for blood routine and blood chemical examinations.

Biosafety evaluation of $NaGdF_4$ nanoparticles in healthy and CRF rats

15 CRF rats were randomly divided into 3 groups (n = 5). $NaGdF_4$ and Gd-DTPA with the dose of 0.1 mmol Gd per kg body weight were intravenously injected into the two groups of rats, respectively and the third group of rats were set as the control with no injection. Another 10 healthy rats (220 - 250 g) were randomly divided into 2 groups (n = 5). $NaGdF_4$ at the dose of 0.1 mmol Gd per kg body weight was intravenously injected into the one group. The body weights of rats were recoded every day. The body weights of rats were recoded every day. The

blood samples were collected at pre- and 7, 14, 21, 28 d post-injection of contrast agents and subjected for renal and liver function related-blood indexes examinations. After 28 d injection, the rats were sacrificed, and the kidneys and livers of them were extracted, photographed, fixed with paraformaldehyde, cut into slices, and subjected to the H&E. In addition, the main organs including lung, spleen and heart from the representative rats in each group and skin tissues were extracted fixed with paraformaldehyde, cut into slices, and subjected to the H&E.

Biosafety evaluation of $NaGdF_4$ nanoparticles in swine

Following the MRI, $NaGdF_4$ with the dose of 0.1 mmol Gd per kg body weight were intravenously injected into the young swine to be continually cultivated. In addition, another sexually matured swine (four-month male) was adopted with no injection. The rectal temperatures and body weights of the swine were recoded every day. The blood samples were collected from the front cavity vein of swine at 14 d post-injection of $NaGdF_4$ and subjected for blood routine and blood chemical examinations. After two-week injection, swines were sacrificed, and the major organs including heart, liver, spleen, lung, kidney, brain, and bladder were extracted, fixed with paraformaldehyde, cut into slices, and subjected to the H&E and CPN III staining.

Main parameters of MRI studies

The main parameters of MRI studies in the current work were set as follows:

1.5 T MRI:

The detailed parameters for T_1-weighted imaging were set as follows: echo time (TE) = 12.58 ms; repetition time (TR) = 400 ms; number of excitations (NEX) = 6; Echo train length (ETL) = 10, Matrix (MTX) = 360 × 360. The detailed parameters for T_2-weighted imaging were set as follows: TE = 28 ms, TR = 1000 ms, NEX = 6, ETL = 10, MTX = 360 × 360.

T_1 relaxation time was obtained through 2D-Fast Gradient Recalled Echo sequence. The detailed parameters: TE = 8 ms, TR = 300, 500, 700, 900, 1100, 1300, 1500 ms, NEX = 1, ETL = 1, MTX = 128 × 128. T_2 relaxation time was obtained through 2D-Fast Gradient Recalled Echo sequence. The detailed parameters: TE = 8, 12, 16, 20, 24, 28 ms, TR =1000 ms, NEX = 1, ETL = 1, MTX = 128 × 128.

7.0 T MRI:

The detailed parameters for Chest and abdominal cavity 3D MR angiography of mice with mouse body coil were set as follows: TE = 1.400 ms, and TR = 11 ms, FOV = 30 mm × 25 mm × 25 mm, MTX = 180 × 150 × 80, slice thickness = 25 mm.

The detailed parameters for 2D T_1 MRI and SWI of liver and kidney of mice with mouse body coil were set as follows: TE = 7.1 ms, and TR = 455 ms, FOV = 35 mm × 35 mm, MTX = 200 × 200, slice thickness = 1 mm.

The detailed parameters for 3D MR angiography of AD mice with head coil were set as follows: TE = 1.49 ms, and TR = 11.451 ms, FOV = 25 mm × 25 mm × 25 mm, MTX = 200 × 200× 80, slice thickness = 25 mm.

The detailed parameters for 2D SWI of AD mice with head coil were set as follows: TE = 7 ms, and TR = 400 ms, FOV = 25 mm × 25 mm, MTX = 180 × 180, slice thickness = 1 mm.

The detailed parameters for 2D SWI of AD mice with surface coil were set as follows: TE = 8.071 ms, and TR = 400 ms, FOV = 20 mm × 18 mm, MTX = 174 × 157, slice thickness = 0.5 mm.

The detailed parameters for 2D TOF angiography of stroke rat with rat surface coil were set as follows: TE = 1.929 ms, and TR = 10 ms, FOV = 40 mm × 40 mm, MTX = 320 × 320, slice thickness = 0.5 mm.

The detailed parameters for 3D MR angiography of stroke rat with rat surface coil were set as follows: TE = 1.72 ms, and TR = 11.167 ms, FOV = 40 mm × 40 mm × 40 mm, MTX = 320 × 320× 80, slice thickness =40 mm.

The detailed parameters for 2D DWI and SWI of stroke rat with rat surface coil were set as follows: TE = 6.764 ms, and TR = 339 ms, FOV = 35 mm × 35 mm, MTX = 384 × 384, slice thickness = 1 mm.

3.0 T MRI:

The detailed parameters for 3D-TRICKS were set as follows: TE = Minimum, and TR = 3.3 ms, FOV = 320 mm × 288 mm, MTX = 256 × 224, slice thickness = 1.6 mm.

The detailed parameters for 3D BRAVO were set as follows: TE = 3 ms, and TR = 8 ms, FOV = 240 mm × 216 mm, MTX = 256 × 256, slice thickness = 1.0 mm.

The detailed parameters for SWAN were set as follows: TE=Minimum，TR=out of phase，FOV=240 × 192 mm，MTX=288 × 352，slice thickness =2.0 mm.

Supplementary Figures

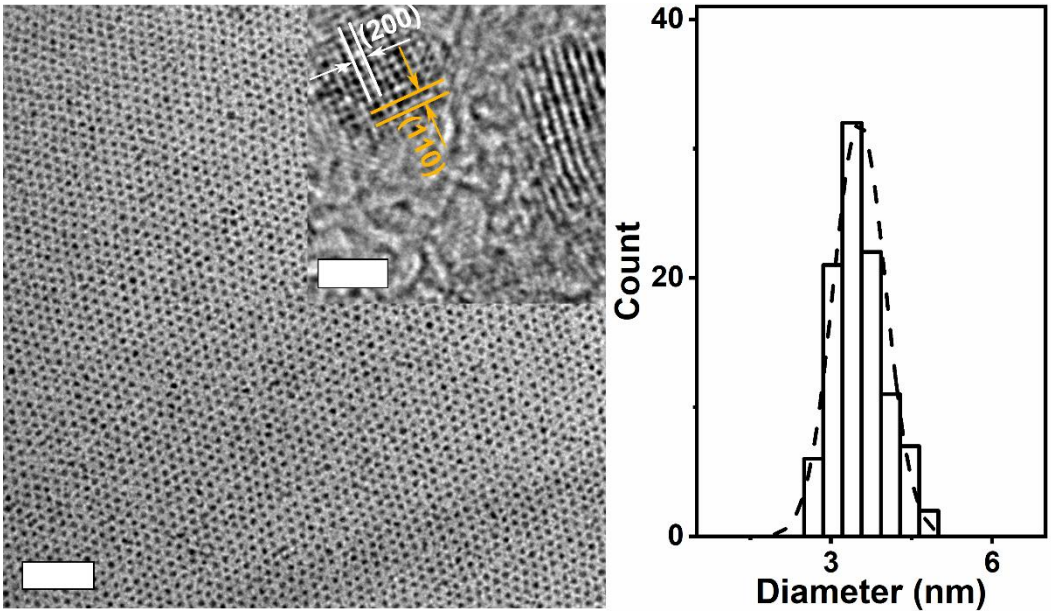

Fig. S1.
TEM images of oleate-capped $NaGdF_4$ nanoparticles together with the histogram profile (scale bar: 50 nm). Inset: the HRTEM image of oleate-capped $NaGdF_4$ nanoparticles (scale bar: 2 nm).

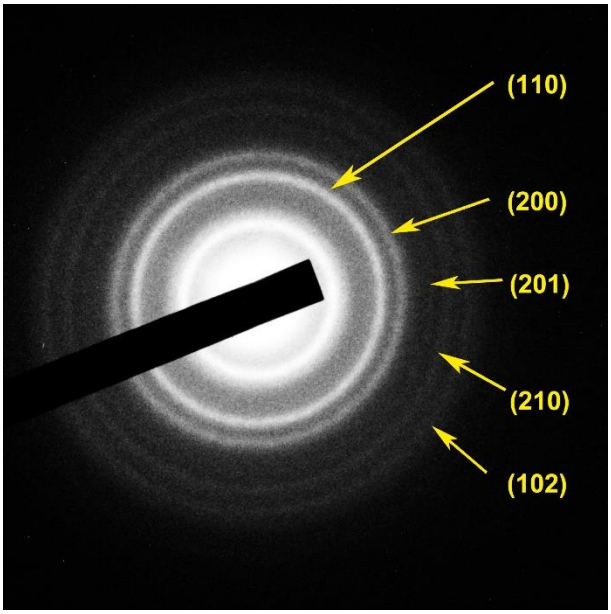

Fig. S2.
Selected area electron diffraction patterns of oleate-capped $NaGdF_4$ nanoparticles.

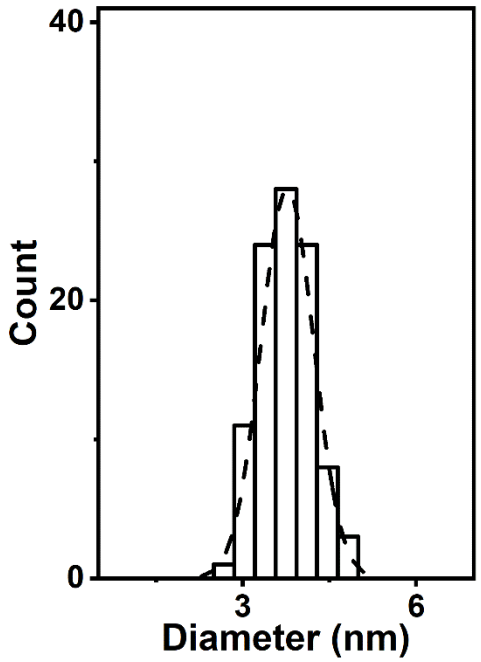

Fig. S3.
The histogram of the PEGylated $NaGdF_4$ nanoparticles size distribution profile summarized by TEM images.

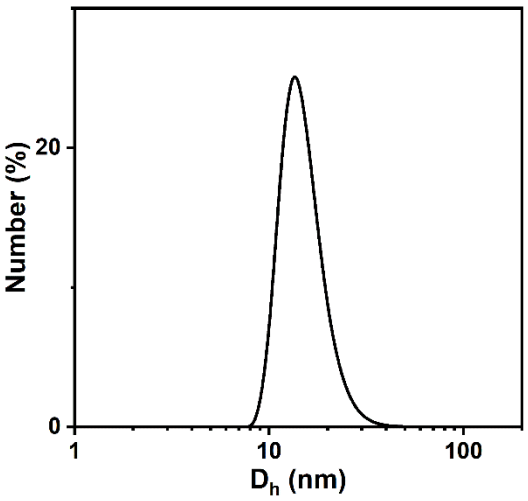

Fig. S4.
The DLS result of the PEGylated $NaGdF_4$ nanoparticles.

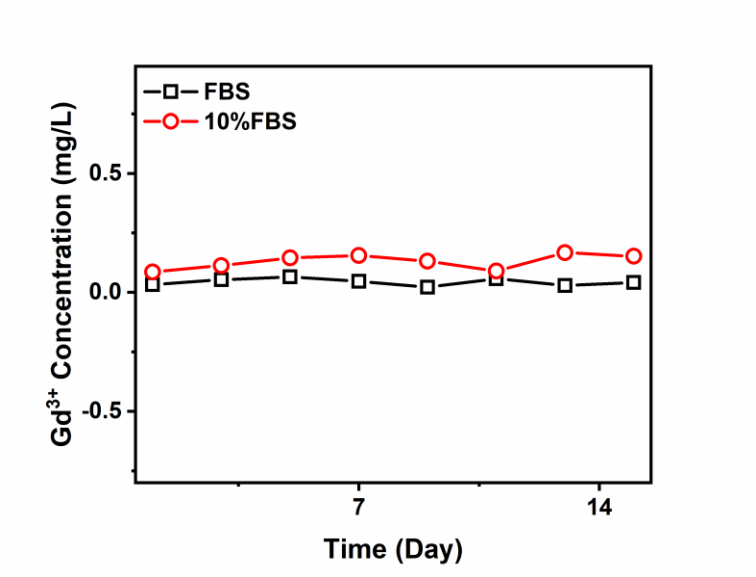

Fig. S5.

The 14-day monitoring of the potentially released Gd^{3+} ions from the $NaGdF_4$ nanocrystal incubated in FBS and 10% FBS solutions.

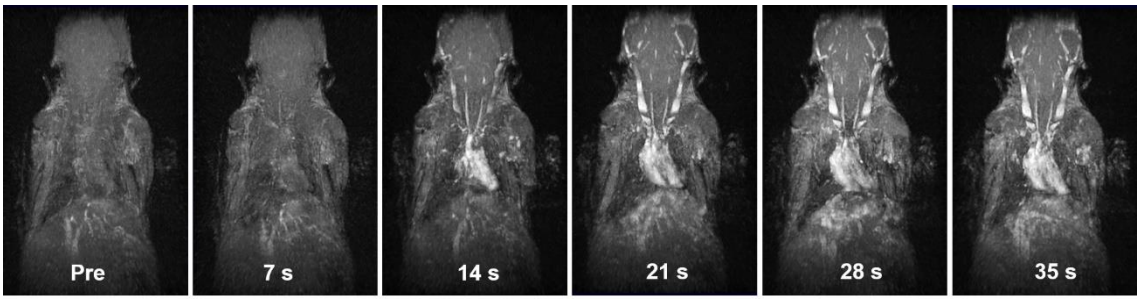

Fig. S6.

Real-time DCE MR angiography obtained pre- and post-injection of $NaGdF_4$ nanoparticles with 0.1 mmol/kg.

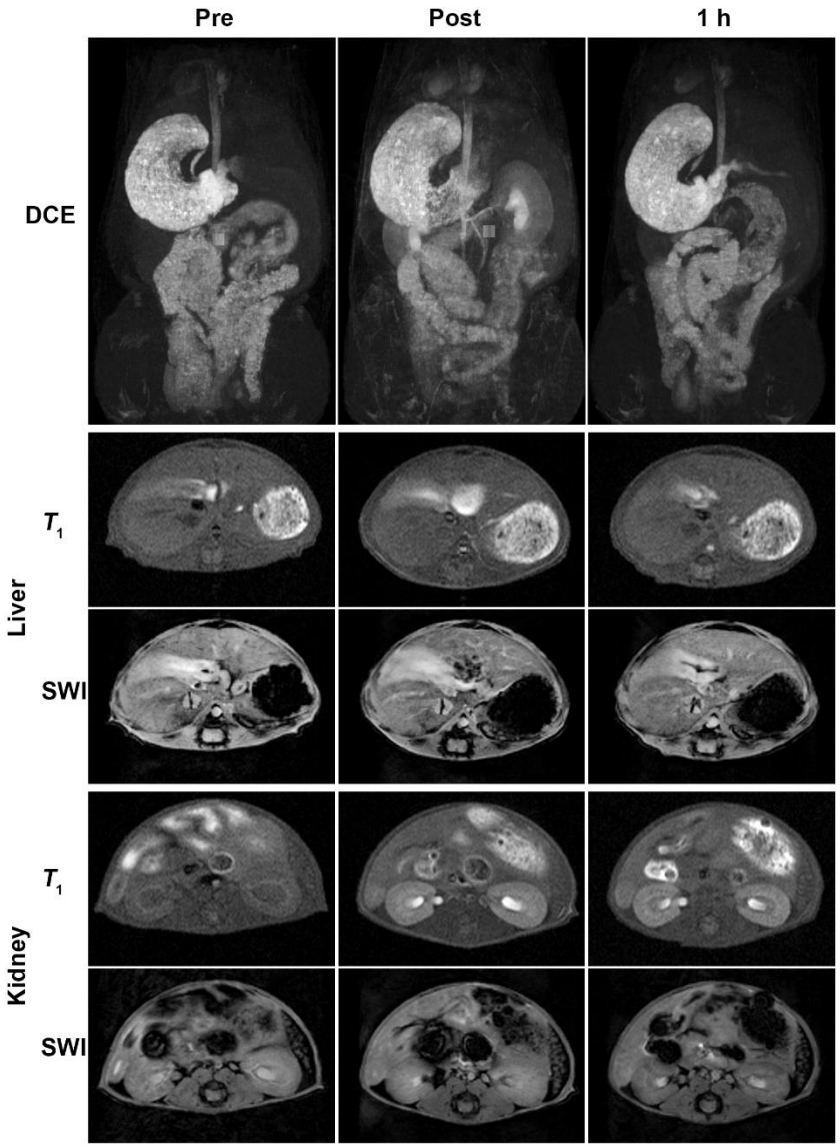

Fig. S7.

3D DCE-MRI, T_1 MRI and SWI of liver and kidney of mice acquired before and at different time points after injection of Gd-DTPA.

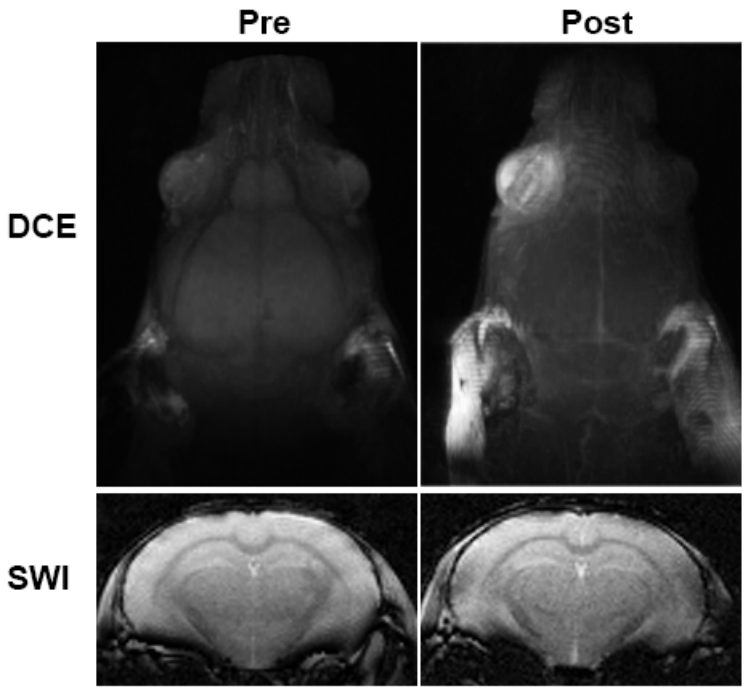

Fig. S8.
3D DCE-MRI and SWI of the mouse brain acquired before and after injection of Gd-DTPA.

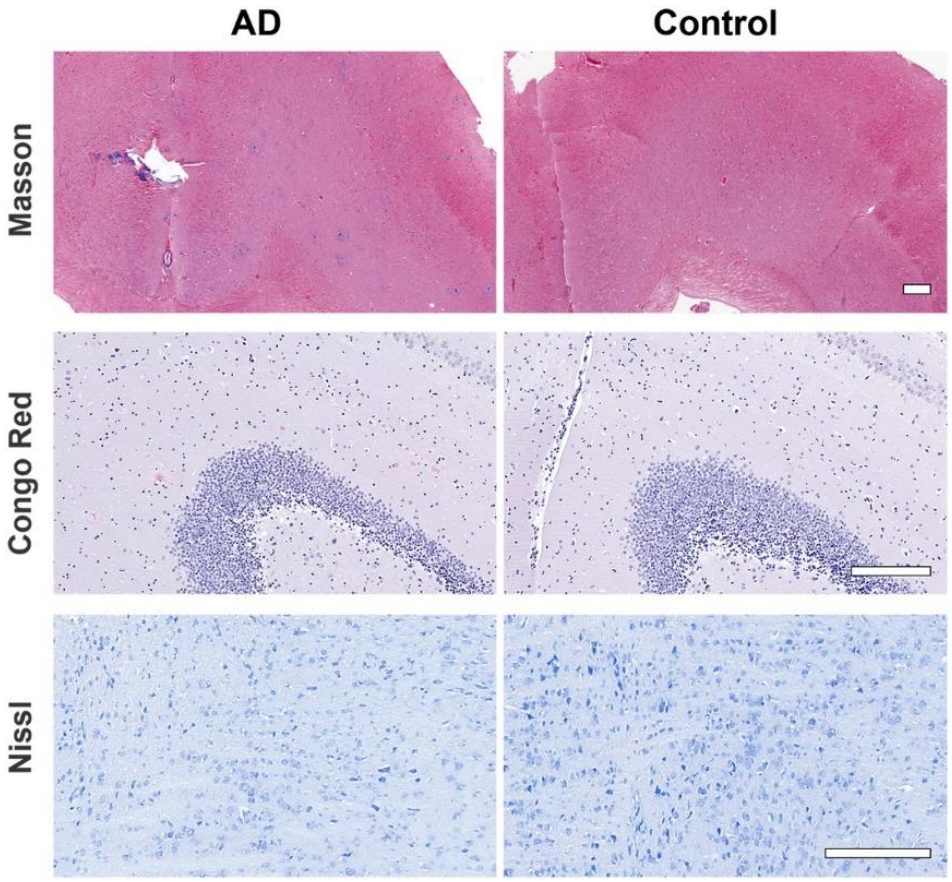

Fig. S9.

The enlarged Masson, Congo Red and Nissl staining images of the healthy and AD mouse brain. The embedded scale bar corresponded to 200 μm.

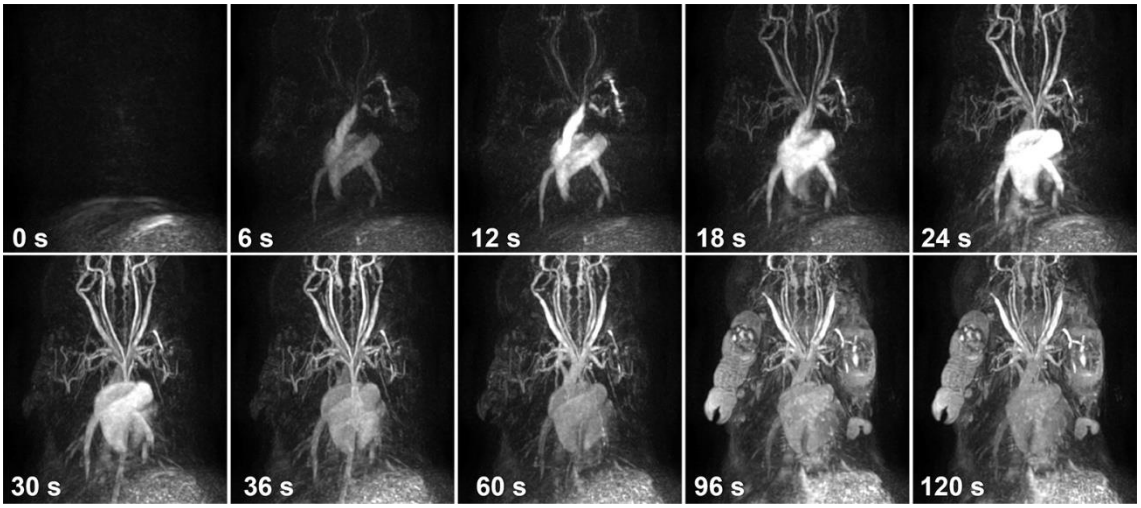

Fig. S10.
3D-TRICKS of swine during the first 120 s before and after injection of $NaGdF_4$ nanoparticles.

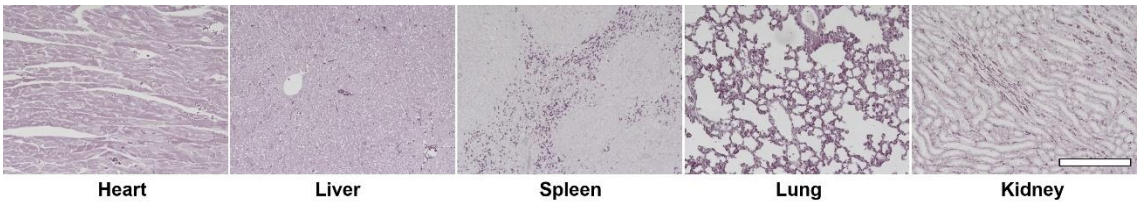

Fig. S11.
CPNIII staining of tissue slices of major organs from mice treated with $NaGdF_4$ nanoparticles. The embedded scale bar corresponded to 200 μm.

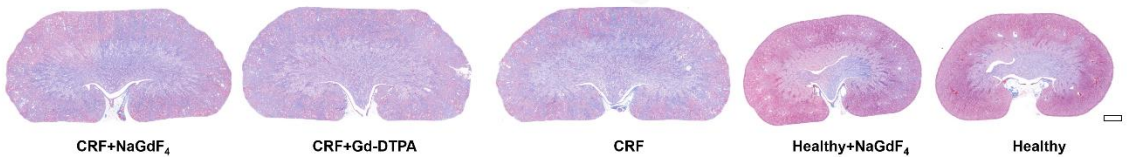

Fig. S12.
Masson staining of kidney of healthy mice and CRF treated with $NaGdF_4$ nanoparticles or not at 28 d post-injection. The embedded scale bar corresponded to 2 mm.

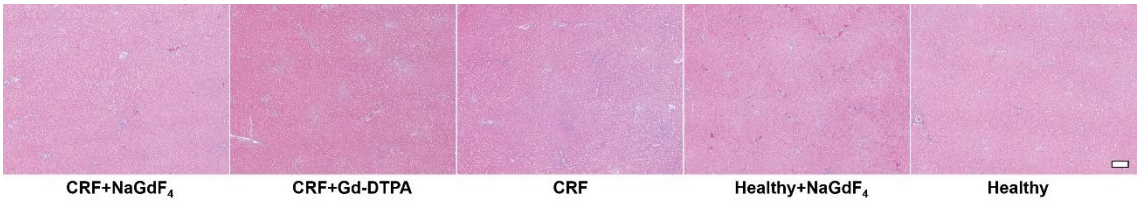

Fig. S13.

Masson staining of liver of healthy mice and CRF treated with $NaGdF_4$ nanoparticles or not at 28 d post-injection. The embedded scale bar corresponded to 200 μm.

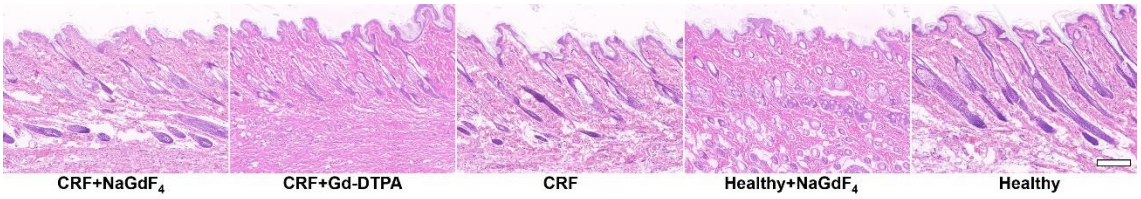

Fig. S14.

H&E staining of skin tissues from the representative rats in each group. The embedded scale bar corresponds to 200 μm.

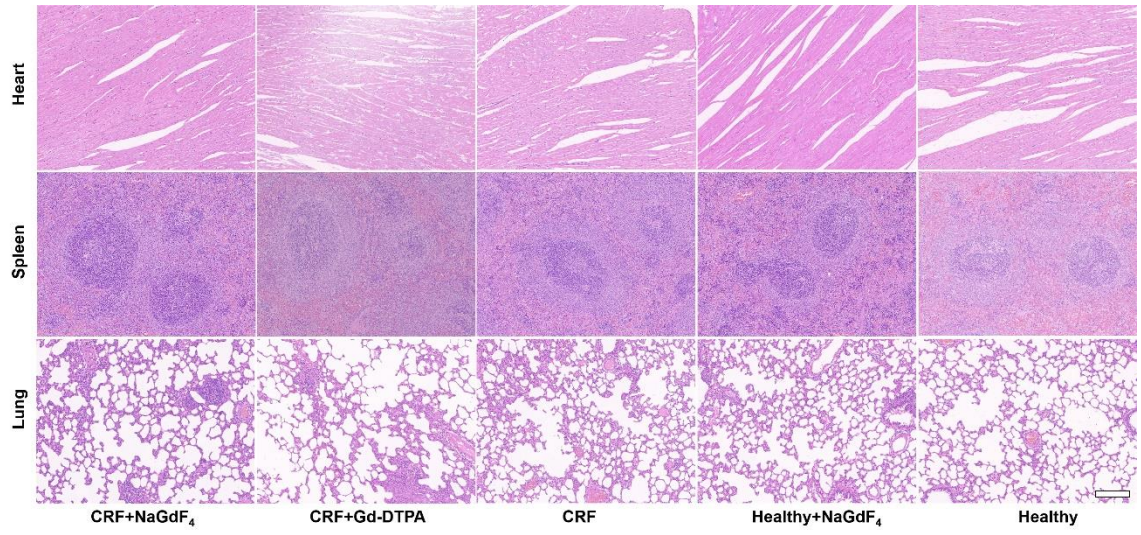

Fig. S15.

H&E staining of lung, spleen and heart from the representative rats in each group. The embedded scale bar corresponds to 200 μm.

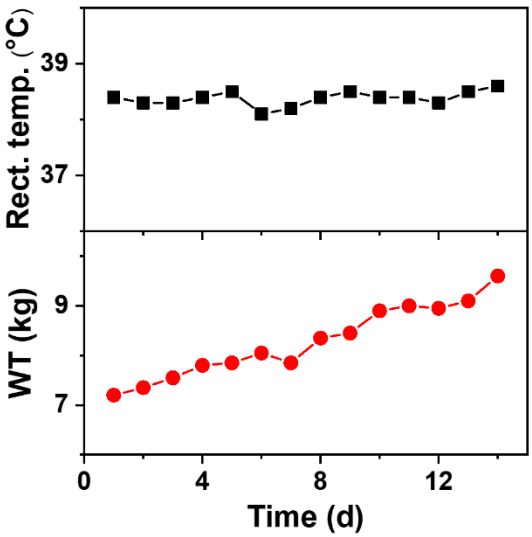

Fig. S16.

Fluctuations in the rectal temperature and the increase of body weight of young swine after $NaGdF_4$ nanoparticles administration.

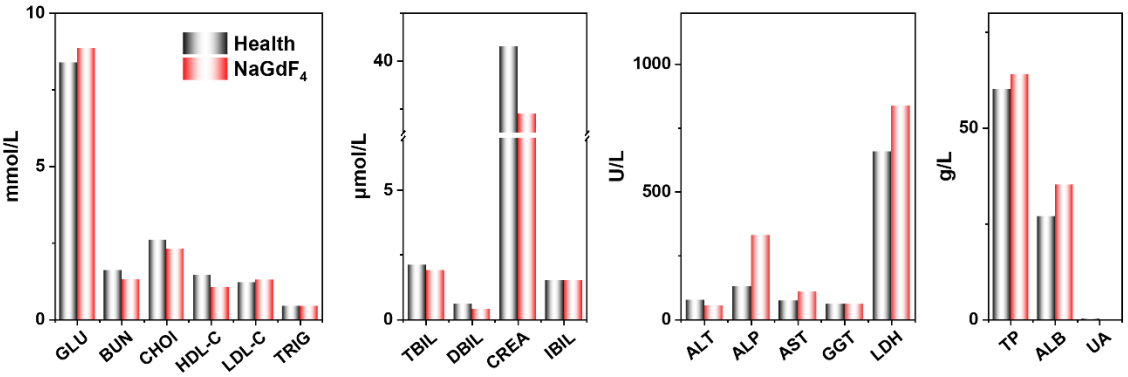

Fig. S17.

Blood biochemical test results of young swine treated with $NaGdF_4$ nanoparticles contrast agents.

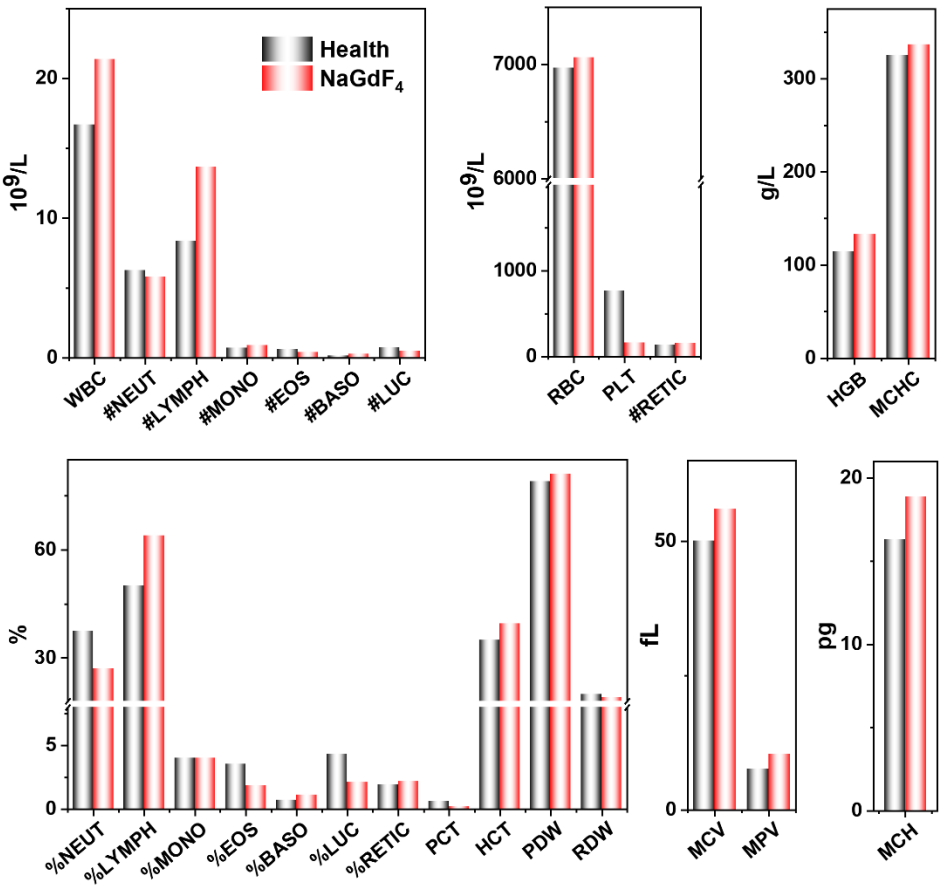

Fig. S18.

Routine blood test results of young swine treated with $NaGdF_4$ nanoparticles.

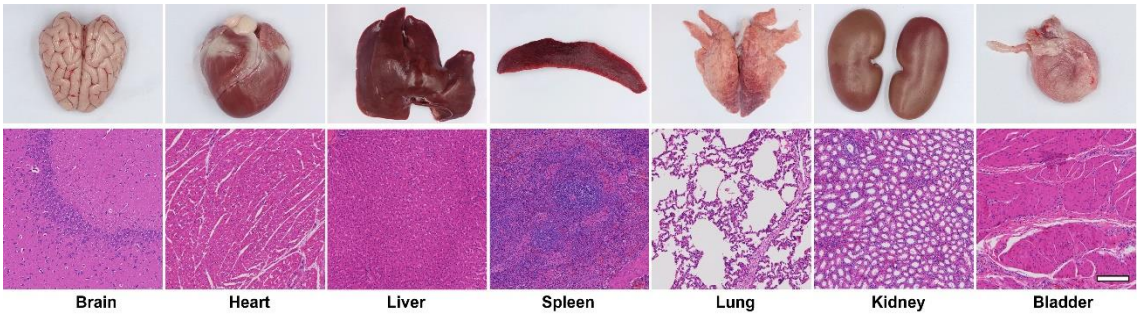

Fig. S19.
H&E staining of tissue slices from major organs of young swine treated with $NaGdF_4$ nanoparticles at 14 d post-injection. The embedded scale bar corresponded to 200 μm.

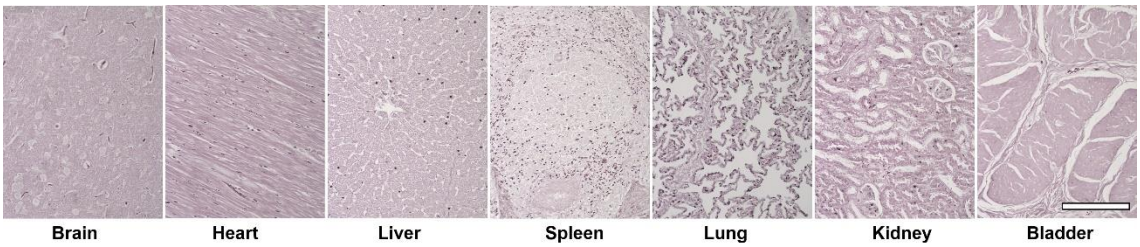

Fig. S20.
CPNIII staining of tissue slices of major organs from swine treated with $NaGdF_4$ nanoparticles. The embedded scale bar corresponded to 200 μm.

Movie S1.
3D MRI vascular structures of the health mouse from heart to kidney after intravenous injection of $NaGdF_4$.

Movie S2.
3D MRI vascular structures of the health mouse from kidney to lower limbs after intravenous injection of $NaGdF_4$.

Movie S3.
3D DCE MR angiography of the brain of the AD mouse after intravenous injection of $NaGdF_4$.

Movie S4.
3D DCE MR angiography of the brain of the health mouse after intravenous injection of $NaGdF_4$.

Movie S5.
Axial TOF angiography show of the rat head.

Movie S6.

3D DCE MR angiography of the stroke rat head before intravenous injection of $NaGdF_4$.

Movie S7.

3D DCE MR angiography of the stroke rat head after intravenous injection of $NaGdF_4$.

Movie S8.

The spatial evolution of blood vessels of swine derived from 3D BRAVO imaging (slice by slice) after intravenous injection of $NaGdF_4$.

Movie S9.

The spatial evolution of blood vessels of swine derived from 3D BRAVO imaging (slice by slice) after intravenous injection of Gd-DTPA.