Electronic Supporting Information

Ultra-sensitive diagnosis of orthotopic patient derived hepatocellular carcinoma by Fe@graphene nanoparticles in MRI

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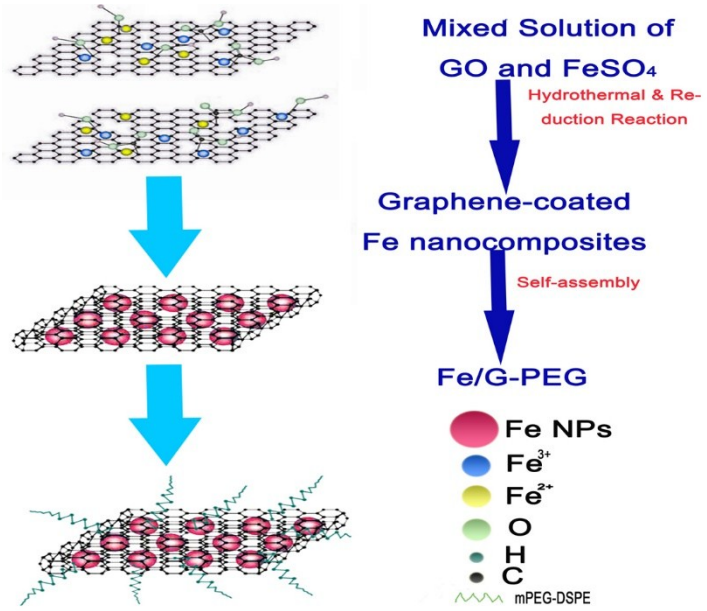
Materials. Graphite (>99.99%), Iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were purchased from Aladdin (China). 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol) (mPEG-DSPE Mw=5,000 Da) was purchased from Jenkem Technology Co., Ltd. All the chemicals were of analytical grade and used as received without further purification. HCC-LM3 human hepato carcinoma cells (HCC-LM3) were obtained from Fudan University (Shanghai, China). The human liver tumor tissue was from the department of oncology in Drum Tower Hospital, Nanjing University (China). Male BALB/c mice (6-8 weeks, 20-22g) were purchased from Animal Central Laboratory of Nanjing Medical University (Nanjing, China). The nude mice (4-6 weeks, male) were purchased from Comparative Medicine Centre of Yangzhou University.

Synthesis of Fe/G-PEG. The Fe/G NPs were synthesized via the hydrothermal and reduction reactions according to our previous report[30]. The detailed process was schematically illustrated in Scheme 1. After strong oxidation, the negative charged Graphene Oxide (GO) was prepared from high-purity graphite by Modified Hummer's method[31]. Then iron ions were absorbed onto GO sheets via electrostatic interaction after the addition of ferrous sulfate solution to the GO solution. Finally Fe/G NPs were formed under the hydrothermal condition and a reduction by hydrogen. The Fe/G-PEG NPs were synthesized by intermolecular forces between mPEG-DSPE and Graphene. Briefly, 60mg Fe/G NPs were suspended in 15 mL tetrahydrofuran (THF). After 5 minutes of ultrasonication, 35 mL of PBS suspension containing 25 mg mPEG-DSPE was added into aforementioned Fe/G solution. The mixture was allowed to react for 12 hours at room temperature under the mechanical stirring. The obtained Fe/G-PEG NPs were harvested by three cycles of magnetic separation and washing process with PBS to remove excess reactant. The purified Fe/G-PEG NPs were re-suspended in PBS and stored at 4 ^{o}C for further usage.



Scheme s1 Schematic illustration for the synthetic process of Fe/G-PEG NPs.

Characterization. Morphology of the as-synthesized Fe/G-PEG NPs was investigated by transmission electron microscopy (TEM, JEOL TEM-100) and high resolution TEM (JEOL, TEM-2100). The X-ray diffraction (XRD) pattern was recorded in the 2θ range of 20-60° on a Hitachi X-ray diffractometer using Cu κ1 radiation (λ=1.54056 Å) at 40 kV and 200 mA. Surface element composition was investigated by X-ray photoelectron spectroscope (XPS, ThermoFisher K-Alfa) with a focused monochromatic Al X-ray (1486.6ev) source. Fourier transform infrared spectroscopy (FT-IR) was performed on a Bruker IFS 66V vacuum-type spectrometer. Lyophilized powder was mixed with KBr (5mg: 1g, W/W) and then pressed to a plate before the FT-IR measurement.

***In vitro* MRI study.** Different concentrations of Fe/G-PEG NPs and Fe_3O_4 NPs (synthesized *via* coprecipitation method[1] which is the most common method for ultrasmall size Fe_3O_4 NPs.) were diluted in PBS and then mixed with thick yogurt in the Eppendorf tubes. T2 weighted MR study of samples was conducted a clinical 3T whole-body MR scanner (Siemens Skyra, Erlangen, Germany) by using sense-8-head coil. A TurboRARE sequence (field of view = 3.00 × 3.00 cm, repetition time = 1848.7ms, echoes = 1, slice thickness = 1 mm, number of averages = 4, matrix size = 512×512 and total scan time = 3 min 56s 636ms) was used for T2 weighted imaging.

***In vitro* cytotoxicity assay (MTT assay).** The cytotoxicity of Fe/G-PEG NPs was detected *via* a MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Briefly, HCC-LM3 cells were suspended in 100 μL Iscoves modified Dulbecco's medium, seeded in a 96-well plate at a density of 10^4 cells/well. After 24 hours, different concentrations of Fe/G-PEG NPs were added into each of the wells. After incubation for another 24 hours, the medium was replaced with 95 μL of fresh medium plus 10 μL of MTT (5 mg/mL). Then the cells were incubated at 37°C for another 4 hours in the dark. Next, the MTT medium solution of each well was removed, and 100 μL of DMSO (dimethyl sulfoxide) was added into each well. The absorption of DMSO solution at 490 nm was measured using iMark Enzyme mark instrument (BIO-RAD Inc., USA).

***In vivo* MRI imaging.** *In vivo* liver MRI was carried out in a 7.0 T small animal MR Scanner (Bruker PharmaScan, Germany). A gradient-echo T2*-weighted FLASH sequence was performed in healthy BALB/c mice and PDX bearing nude mice respectively. Healthy BALB/c underwent different treatments prior to imaging. Two groups of mice were tested 24 hours and 6 hours post the intravenous injection of Fe/G-PEG NPs at a dose of 0.5mg/kg weight while the other was tested with no injection as control. Three group nude mice were transplanted the human liver tumor tissue block (All experiments are ethical and passed by the relevant departments, and all the protocols for animal test were reviewed and approved by committee on animals in Nanjing University (China) and carried on according to guidelines given by National Institute of Animal Care). These tumor bearing mice received the same treatment as the healthy BALB/c mice did before the *in vivo* MRI. The parameters of the T_2* weighted imaging were as follows: field of view = 3.19×3.19 cm, repetition time = 298.5ms, echoes = 1, slice thickness = 1 mm, number of averages = 3, matrix size = 512×512, flip angle = 30°, and total scan time = 4.34 min.

Fe bio-distribution in mice. Healthy BALB/c mice were intravenously injected with Fe/G-PEG at a dosage of 0.5 mg/kg and sacrificed at 1 h, 3 h, 6 h, 12 h and 24 h post-injection (n = 3 at each time point), respectively. Subsequently, the heart, liver, spleen, lung, kidney and brain, were excised and collected. The organs were weighted and decomposed in heat nitric acid to colorless solution. After being evaporated to dryness, they were dissolved in 3.0 mL diluted nitric acid solution (1%). Then the Fe concentration in the solution was measured by inductively coupled plasma-mass spectrometry (ICP-MS, Perkine Elmer Corporation, USA). The data were normalized to the tissue weight and expressed as percentage of injection dose/g organ at each test

point.

Fe bio-distribution in liver. The PDX bearing nude mice were intravenously injected with Fe/G-PEG NPs at a dosage of 0.5 mg/kg and sacrificed 6 hours post the injection. Subsequently, liver was excised and collected. It was fixed with 4% formalin, embedded in paraffin and sectioned into slices with 40 μm thickness. Sections from the liver were stained with hematoxylin and eosin (H&E) and Prussian blue respectively. Afterwards, they were observed under a light microscope at 40×, 100×, 200×, 400× magnification respectively by an experienced physician, and representative images were provided.

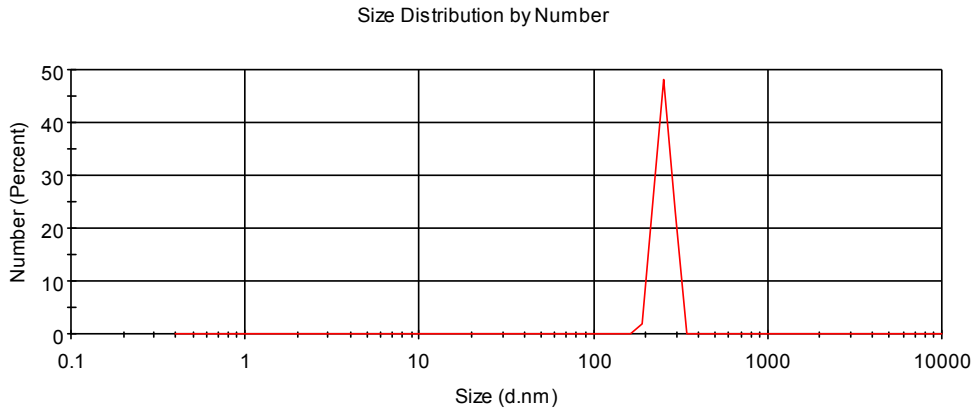


Figure s1. The hydrodynamic size of original Fe/G-PEG NPs

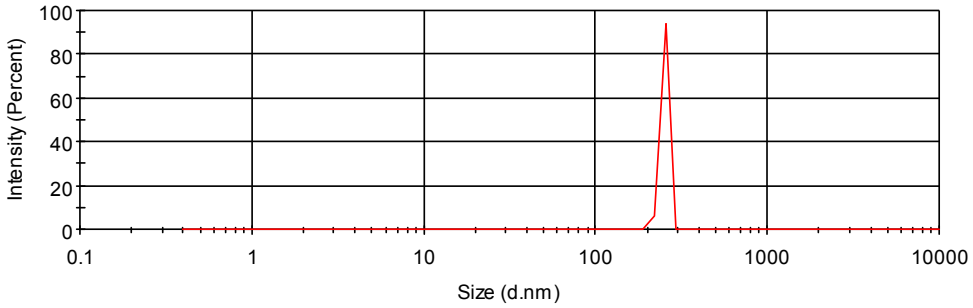


Figure s2. The hydrodynamic size of Fe/G-PEG NPs incubated in the solution after two months

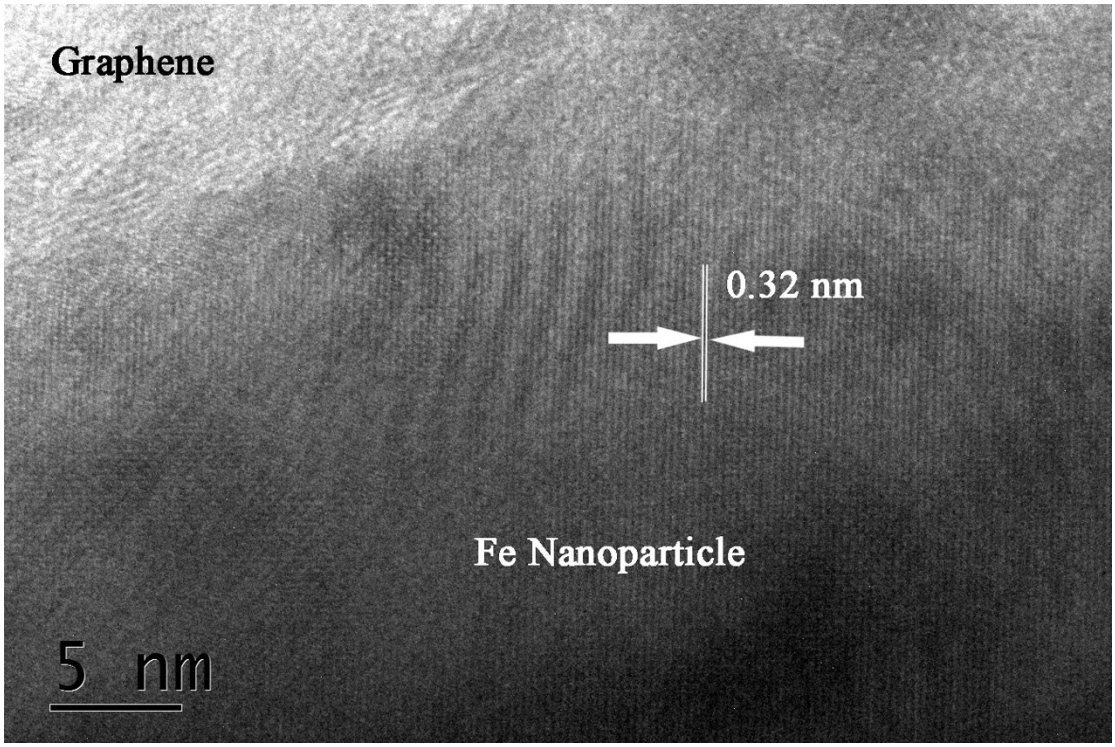


Figure s3. High resolution TEM image of Fe/G-PEG NPs with highlighted lattice spacing.

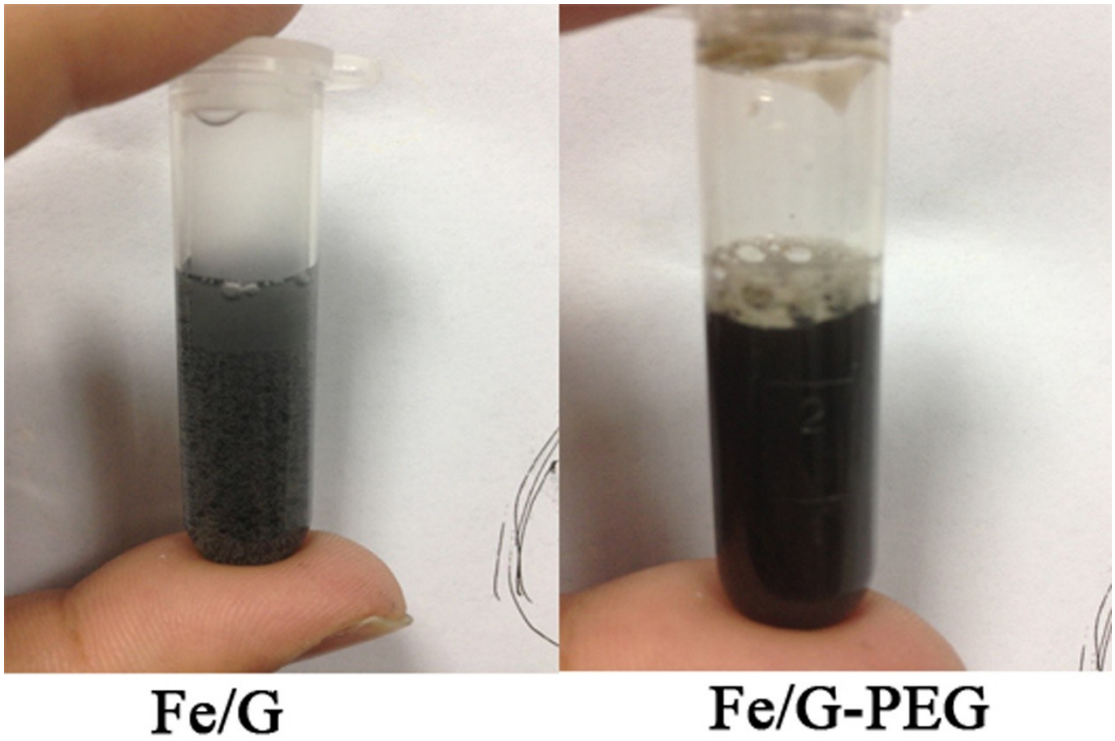


Figure s4. The dispersion of Fe/G NPs and Fe/G-PEG NPs in water solution post 3 minutes and 3 days of rest respectively.

References

1 P. F. Su, G. Chen and J. Zhao, *Chemical Journal of Chinese Universities* 2011, **7**, 1473-1477.