Electronic Supporting Information (ESI)

Two-photon Graphene Quantum Dots Modified Gd₂O₃

Nanocomposites for Dual-Mode MRI Contrast Agent and

Cell Labelling Agent

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Fig. S1 Fluorescence excitation and emission spectrum of GQD (insert: digital photos of GQD in water under sunlight and UV lamp).



Fig. S2 TEM results of GQD after ultrasonication in water/DMF solution, evapration at 70 $^{\circ}$ C for 30 min, and centrifuge at 5000 rmp/min.

To synthesis of Gd_2O_3/GQD nanocomposites, Gd_2O_3 in THF solution was added in GQD water solution, after ultrasonication process, evaporation process, centrifugation process and dialysis process, the final product was obtained.

<u>1. Remove un-reacted Gd₂O₃ nanoparticles</u>

The GQD coating integrated Gd_2O_3/GQD nanocomposites with fluorescence property. To eliminate the influence of fluorescence from un-reacted GQD, we use excess Gd_2O_3 nanoparticles in the reaction process. After sonication process and THF evaporation process, there may still have excess and unreacted Gd_2O_3 nanoparticles. Un-reacted Gd_2O_3 nanoparticles are not stable in water solution due to leak of surface modification. Here, we use centrifuge machine to remove these particles under high rotational speed (5000 rmp/min).

2. Remove un-reacted GQD

After remove the un-reacted Gd_2O_3 nanoparticles, the as prepared solution was dialyzed using dialysis bag (100 kDa dialysis membrane) for 24 hours to remove GQD residual. The final obtained solution was stored in glass bottles named as Gd_2O_3/GQD solution.

3. GQD do not aggregate without Gd₂O₃ nanoparticles

To insure all the obtained nanocomposites have Gd₂O₃ nanoparticles inside, we operate a control experiment using the same synthesis method as Gd₂O₃/GQD only exclude Gd₂O₃ nanoparticles. THF solution was added to a certain amount of GQD water solution. The mixture was homogenized for 15 minutes and subsequently stirred at 70 oC for 30 minutes to allow THF evaporation. After centrifugation with the speed of 5000 rmp/min, the obtained clear solution was used to prepare TEM testing sample. The TEM Image was shown Fig.S2. As can be seen in the figure, no aggregation observed in the image which indicating GQD will not aggregate without hydrophobic nanoparticles. This indicates hydrophobic nanoparticles were indispensable in forming nanocomposites.



Fig. S3 VSM results of (a) Gd_2O_3 nanoparticles and (b) Gd_2O_3/GQD solution.



Fig. S4 Thermal gravimetric analysis curves of Gd_2O_3 nanoparticles, GQD and $Gd_2O_3/GQD.$

The thermal gravimetric analysis (TGA) curve of Gd_2O_3 nanoparticles, GQD and Gd_2O_3/GQD were plotted in Fig. S4. Due to the existence of organic surfactant, Gd_2O_3 nanoparticles have only 61.2% of weight when temperature rised up to 700 °C. When temperature increased from 100 °C to 700 °C, GQD sample exhibited 100% weight loss. The weight residual percentage of Gd_2O_3/GQD was 34.8%, indicating 34.8% Gd_2O_3 nanoparticles (did not contain surfactant) in obtained Gd_2O_3/GQD nanocomposites.



Fig. S5 TEM images of (a) Gd₂O₃/GQD, and (b) Gd₂O₃/GQD-L.



Fig. S6 (a) T_1 relaxation rate of Gd₂O₃/GQD-L, and (b) T_2 relaxation rate of Gd₂O₃/GQD-L.

To study the size dependence of MRI relaxivity, another GQD coated sample named Gd_2O_3/GQD -L was prepared. The TEM images and MR relaxivity results were plotted in Fig. R8 and Fig. R9. TEM image showed the size of Gd_2O_3/GQD -L was 100 nm. MRI relaxivity study indicated that smaller Gd_2O_3/GQD nanocomposites had high r₁ relaxivity. One possible explanation for this phenomenon was the smaller nanocomposites have large surface to volume ratio and more surface Gd ions, which enhance synergistic effect of Gd ions and accelerate r₁ relaxation process.³⁻⁵ Additional, larger nanocomposites have more nanoparticles inside; the nanocomposite interior was dominated by hydrophobic Gd_2O_3 nanoparticles. The possible explanation for the smaller r₁ values in large nanocomposites may due to the diminishing water accessibility in nanocomposites.

	Core	NP Size (nm)	Coating Materials	Field (T)	r ₁	Ref.
1	Gd ₂ O ₃	3.46	PEG-TETT	7	8.06	1
2	Gd_2O_3	3.46	CTX-PEG-TETT	7	8.41	
3	Gd_2O_3	2.2	polysiloxane	7	8.8	2
4	Gd_2O_3	1.1	polysiloxane	7	9.4	3
5	Gd ₂ O ₃	3~4	DEG	1.5	2.05	4
6	Gd_2O_3	3.92	Fluorescein- PEI	1.5	6.76	5
7	Gd ₂ O ₃	3	PEG	1.5	9.4	6
8	Gd_2O_3	6.4	GQD	7	15.9	This work

Table S1 MR relaxivity summary of various previously reported Gd2O3contrast agents

Table S2 Cellular cytotoxicity of Gd₂O₃ based contrast agents

Gd Source	Incubate Time (h)	Cell	Gd Concentratio n	Cell Viability	Ref. No
Gd-DTPA	24	S18	[Gd] = 10 μM	> 90%	7
CTX-PEG- Gd ₂ O ₃	24	C6 glioma	[Gd] =5 mg/L	< 80%	8
Gd₂O₃/Gluc uronic acid	24	DU145	[Gd] =5 mg/L	> 90%	9
Gd_2O_3	24	S18	[Gd] = 10 μM	≈ 90%	10
Oleic acid- Gd ₂ O ₃	24	HDF	[Gd] = 50 μM	> 70%	11
Gd₂O₃/GQD	24	MCF-7	[Gd] = 40 μM or [Gd] =6.3 mg/L	> 90%	This work

From literature, a simple pH-dependent iron dissolution study on hydrophobic oleic acid-coated nanoparticles encapsulated with

amphiphilic polymeric coating, leaching of metal ions at pH range of 6– 10 (liberated from the magnetic nanocomposites after 7 days incubation at 37° C) were rather negligible.¹² This suggested that at neutral pH range, metal ions were unlikely to leach from magnetic nanocomposites that contained hydrophobic oleic acid capped nanoparticles. In terms of the chemical structure, GQD contained numerous oxygen-containing functional groups, inclusive of carboxylic acid. These carboxylic acid functional groups normally only will be protonated (forming –COOH) at low pH environment (acidic), resulting in a neutral charge. At normal/neutral pH condition, the carboxylic acid functional groups will be deprotonated into negatively charged –COO⁻ groups which are able to interact favorably with various metal ions.^{13,14} Both these reasons resulted in the low toxicity of Gd₂O₃/GQD nanocomposites.



Fig. S7 (a) Fluorescence emission spectra of GQD and Gd_2O_3/GQD solution under excitation of 365 nm, (b) UV-vis absorption spectrum of GQD and Gd_2O_3/GQD solution, (c) emission spectra of Gd_2O_3/GQD solution under excitation of 365nm (one-photon) and 750 nm (two-photon), (d) fluorescence mechanism of Gd_2O_3/GQD , and (e) schematic diagram of Gd_2O_3/GQD nanocomposites.

The fluorescence emission spectra of GQD and Gd_2O_3/GQD solution were shown in Fig. S7a. The emission peak showed no shift after GQD coating on Gd_2O_3 nanoparticles. The emission spectrum of GQD and Gd_2O_3/GQD showed no significant difference. In Gd_2O_3/GQD nanocomposites, Gd_2O_3 nanoparticles were coated with several layers of GQD, which was calculated by comparing the shell thickness and GQD size. Due to multi-layered coating structure, the fluorescence of Gd_2O_3/GQD originated from outer GQD shell. The emission property of GQD was influenced by size and surface chemistry of the GQD. The coating process did not change the size and surface chemistry of the outer shell GQDs. This indicated that the surface coating process did not change the fluorescence property of GQD. UV-vis absorption spectrum of GQD and Gd₂O₃/GQD aqueous solutions (in Fig. S7b) showed weak shoulder at 320 nm. Gd₂O₃ nanoparticles were encapsulated in the center of the obtained nanoclusters and formed a core-shell structure.

Fig S7c exhibited the emission spectrum of Gd₂O₃/GQD sample irritated under 365 nm (one-photon) and 750 nm (two-photon), respectively. The maximum emission wavelengths of both spectrums were located at 515 nm. Two-photon fluorescence spectrum showed a narrower bandwidth than that of one-photon fluorescence spectrum. Fluorescence mechanism was illustrated in Fig. S7d. Similar to the chemical structure of polyaromatic compounds, the fluorescence in GQD may originate from the large π conjugated system.¹⁵ Furthermore, the lone pair electrons from the strong electron donating group dimethylamido which is doped to the aromatic ring of N-GQD, can be excited to the aromatic rings to form the p- π conjugation, further enlarging the π -conjugated system.¹⁶ The fluorescence spectrum can be considered as a transition from lowest unoccupied molecular orbital (LUMO) to the highest occupied molecular orbital (HOMO). During the thermal cutting stage, dimethlamine was conjugated with GQD. The strong orbital interaction between dimethlamido and π -conjugation system in GQD elevated a HOMO to a higher energy orbit HOMO*. Formation of HOMO* reduced the band gap and induced a red shift of fluorescence spectrum. Meanwhile, the large π -conjugation system in GQD and strong electron donating effect of dimethlamido can facilitate the charge transfer and enhance the two-photon absorption. Thus, GQD could be excited by both 365 nm light for one-photon fluorescence emission and 750 nm light for twophoton fluorescence emission.

Fig. S7e showed the schematic structure of Gd₂O₃/GQD nanocomposites. For the simple demonstration, the figure exhibited a two-layer GQD coated Gd_2O_3/GQD nanostructure.

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