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Gadolinium-doped carbon dots with high quantum yield as an effective fluorescence and magnetic resonance bimodal imaging probe

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ABSTRACT

It is highly desired to develop the dual-modality fluorescence and magnetic resonance imaging (MRI) probes in medical imaging because it can provide high-resolution macroscopical anatomical information and high-sensitivity microscopical optical signal simultaneously. In this study, harmless gadolinium-doped carbon dots (Gd-CDs) were prepared *via* a convenient one-pot hydrothermal approach for fluorescence/MR bimodal imaging. The derived Gd-CDs exhibit enhanced blue photoluminescence with a quantum yield as high as 69.86% and significantly improved longitudinal relaxivity ($r_1 = 14.33$ mM^{-1} s^{-1}, 0.5 T) in comparison with commercial Magnevist (Gd-DTPA, $r_1 = 4.5$ mM^{-1} s^{-1}, 0.5 T). Here Gd^{3+} ions are simply chelated onto CDs by carboxyl groups. Moreover, unlike the previous reports, Gd^{3+} chelation does not perturb core optical properties of CDs. Excellent water solubility, good cell-membrane permeability and negligible cytotoxicity make Gd-CDs an ideal dual-modal fluorescence/MR imaging nanoprobe, suggesting its potential and significance in practical biological and clinic applications in the future.

1. Introduction

Magnetic resonance imaging (MRI) is regarded as one of most powerful techniques in modern diagnostic medicine because it can penetrate deeply into tissue, providing anatomical details and high quality three-dimensional images of soft tissue in a non-invasive monitoring manner [1,2]. Unfortunately, this technique has just been able to resolve objects larger than a few micrometers in size, thus exhibits much lower sensitivity than radioactive or optical method [3,4]. Fluorescence imaging (FI), in contrast, has the capacity for single-cell sensitivity and subcellular resolution, but possesses poor spatial resolution and tissue penetration, which restricts its application just to surface or near-surface phenomena [5–7]. Apparently, combining the advantages of MRI and FI can bridge gaps in sensitivity and depth between these two modalities, and consequently, leads to an improved reliability in diagnosis [8–10].

Up to now, developing FI/MRI bimodal nanoprobes usually adopt two different strategies. One logical strategy is based on incorporating paramagnetic ions into semiconductor quantum dots (QDs) such as CdSe, CdS and CdTe [10–13]. These nanoprobes show good photostability, high relaxivity and quantum yield (QY), but high toxicity and potential pollution hazard limit their biological and medical applications [10–13]. The other is to coat the magnetic cores and fluorophores with shells such as silica or modifying silica-capped fluorescent cores with paramagnetic ions [14,15]. In general, these probes have lower toxicity compared with those obtained by the above strategy thanks to the encapsulation of silica shell. However, silica encapsulating not only makes the synthesis process more complex and difficult, but also increases the particle size by a value of 4–7 nm [14,15].

Current trend in the development of dual-modal fluorescence/MR imaging probes suggests that an ideal nanoprobe should not only own stronger fluorescent intensity and higher relaxivity, but also should possess low toxicity and good stability in a biological environment. Gadolinium (Gd) has been confirmed to exhibit excellent contrast efficiency because of its unique magnetic property. Many Gd-containing nanoparticles and chelates have been developed as effective probes for MR diagnosis [16–21]. What's

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more, some Gd chelates are commercially available, but they are expensive and their contrast efficiency needs to be further improved. Therefore, one challenge for MRI contrast agents is to design Gd-containing agents with high contrast efficiency, high stability, low-cost, low toxicity, and easy preparation process [22,23]. As a rising star of the carbon family, fluorescent carbon dots (CDs) is becoming a superior framework to construct multi-modal imaging probes, because of their favorable optical properties, good biocompatibility, excellent water solubility, and low toxicity along with green synthesis and the ease of surface functionalization [24,25]. Bourlinos [4] and Ren [26] as well as their co-workers reported the synthesis of Gd-doped CDs for fluorescence/MR imaging by the low temperature pyrolysis of the precursors containing Gd and carbon. The resultant Gd-doped CDs showed good MR response and photoluminescence (PL) properties with longitudinal relaxation rate (r_1) and QY of 5.5–6.4 mM^{-1} s^{-1} and 2.6–8.9%, respectively [4,26]. One-pot approach such as hydrothermal treatment of citrate acid (CA), ethanediamine and $GdCl_3$ at 200 °C [8], or microwave-assisted polyol method using sucrose, concentrated H_2SO_4 and $GdCl_3$ as the starting materials [22] had also been successfully demonstrated to prepare Gd-doped CDs with a largely improved r_1 of 11.356 mM^{-1} s^{-1}, but a low QY of 5.4% was accompanied. Additionally, Gd^{3+} encapsulated carbonaceous dots were also prepared by simple calcination of gadopentetic acid (Gd-DTPA) in the air [27]. Although a relatively high QY of 19.7% was present, the r_1 value of 5.88 mM^{-1} s^{-1} was unsatisfactory [27]. Recently, Shi et al. reported a dual-modal imaging probe based on Gd chelates functionalized CDs *via* a complex three-step approach [28]. r_1 of Gd-doped CDs was sharply improved to 56.72 mM^{-1} s^{-1} due to increasing their surface-chelated Gd^{3+}. However, compared with Chen's work [27], the QY was degenerated to 5.4%, suggesting that an enhancement of MR property is at the expense of sacrificing fluorescent intensity. Also, the involving three-step process is cost- and time-consuming [28]. Therefore, the development of multi-modal imaging nanoprobes based on CDs still remains in the early stage. Considering the diversity in the preparation, structure, surface functionalization, and PL property of CDs, continued efforts should be exerted.

It is well known that free Gd^{3+} is highly toxic because they inhibit calcium channels and cause cardiovascular and neurologic toxicity [20,29]. Therefore, decreasing total amount of Gd^{3+} in Gd-doped CDs while maintaining the excellent MR and fluorescent properties, would favor their practical applications in bio-imaging. In this study, a dual-modal imaging probe with high fluorescence and improved relaxivity for T_1-weighted MR imaging was prepared and investigated in detail. As shown in Scheme 1, Gd-doped CDs were synthesized *via* a convenient one-step hydrothermal method using citric acid (CA), diethylenetriamine (DETA), and gadolinium chloride ($GdCl_3$) as the starting materials. CA was selected as carbon source because it can provide more carboxyl groups which may chelate gadolinium ions during and after formation of CDs than other nontoxic acid [8,22,29]. Moreover, CA is more easily polymerized in the presence of DETA [30,31]. As-prepared Gd-doped CDs exhibits excellent water solubility and cell-membrane permeability, which provides potentials for dual-modal MR/fluorescence imaging *in vitro* and *in vivo*. Experimental results indicate that the synthesized Gd-CDs in the present work contain lower Gd^{3+} content, while exhibiting good longitudinal relaxivity, significantly improved QY, and negligible cytotoxicity. Most importantly, the doping of paramagnetic Gd^{3+} does not cause the loss of fluorescent intensity but largely enhances the QY of CDs to 69.86%. Gd-doped CDs have also been demonstrated for dual-modal MR/fluorescence imaging of HeLa cells in real-time, suggesting that these nanoprobes have great potential applications in biological and medical fields in the future.

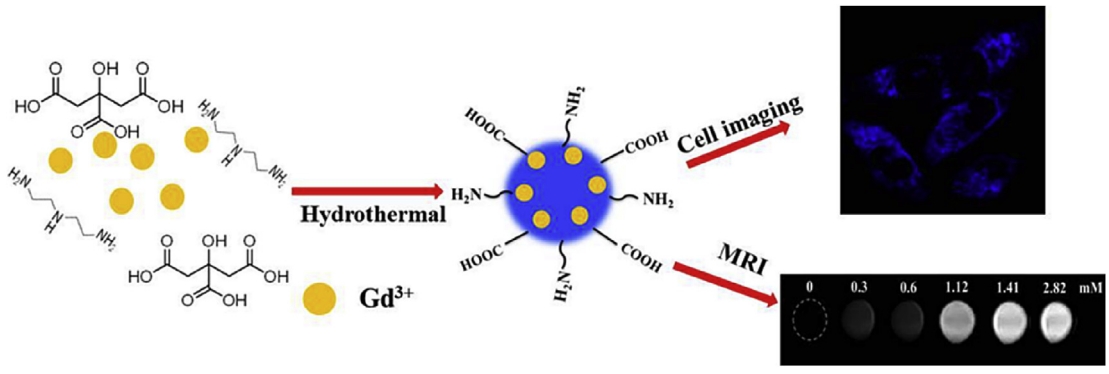
2. Experimental section

2.1. Materials

Citric acid ($C_6H_8O_7$, CA) and diethylenetriamine ($C_4H_{13}N_3$, DETA) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Dialysis membranes of 500–1000 Da (USA, Spectrumlabs) were purchased from Toscience Biotechnology Co, Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8), gadolinium chloride ($GdCl_3$), Xylenol orange (XO) and Eriochrome black T (EBT) were purchased from Sigma-Aldrich Trading Co, Ltd. (Shanghai, China). Fetal bovine serum (FBS), trypsin and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco Life Technologies Co. (Grand Island, USA). Human cervical carcinoma (HeLa cells) was purchased from Shanghai Institute of Cell Biology (Shanghai, China). All chemicals were analytical grade and used as received without further purification. Distilled water was used throughout the whole experiment.

2.2. Synthesis of Gd-CDs

CDs and Gd-CDs were prepared *via* a simple hydrothermal treatment of CA and $GdCl_3$ in the presence of DETA. Briefly, 3 g CA, 0–1.2348 g $GdCl_3$ (0–5 at.%), and 0–1 mL DETA were dispersed into 6 mL distilled water in sequence under vigorous stirring. Subsequently, the above mixture solution was added into a 10 mL Teflon-lined stainless steel autoclave and heated to 150–200 °C for 0.5–5 h. The dark brown products were obtained after cooling to room temperature. The large and agglomerated nanoparticles were removed by centrifugation at 12,000 rpm for 15 min. The resultant



Scheme 1. Schematic illustration for the one-step hydrothermal synthesis of Gd-CDs as a bimodal imaging probe.