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Engineered gadolinium-doped carbon dots for magnetic resonance imaging-guided radiotherapy of tumors

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ABSTRACT

The effectiveness of radiotherapy can decrease due to inaccurate positioning of machinery and inherent radioresistance of tumors. To address this issue, we present a novel theranostic nanoplatform based on gadolinium-doped carbon dots (Gd-doped CDs) designed specifically for magnetic resonance imaging (MRI)-guided radiotherapy of tumors. The Gd-doped CDs (~18 nm) with dispersibility in water and stable photoluminescence were synthesized via a one-step hydrothermal approach. After tail vein injection of the Gd-doped CDs, they exhibited a relatively long circulation time (~6 h), enabled efficient passive tumor targeting. Gd-doped CDs accumulate in the kidney and could be cleared out of the body from bladder. Importantly, they exhibited favorable biocompatibility with excellent performance in longitudinal relaxivity rate (r_1) of 6.45 $mM^{-1}S^{-1}$ and radiosensitization enhancements. These results show that Gd-doped CDs are excellent T_1 contrast agents and radiosensitizers, possessing great promise for MRI-guided radiotherapy of tumors.

1. Introduction

Radiotherapy is a mainstay of noninvasive cancer treatment without surgical risks and complications and without the systematic toxicity of chemotherapy [1–3]. Clinically, this technique uses high-energy X-rays or γ-rays to generate toxic photoelectrons and radicals to induce apoptosis of cancerous cells or suppress their proliferation. Despite the fact that radiotherapy is presently the most efficient treatment against solid tumors, it has two urgent limitations that need to be addressed in order to improve the efficacy of cancer therapy [4]. Firstly, more precise localization should be achieved using a variety of techniques and methods to obtain high-definition images. Inaccuracy in localization of radiation therapy could lead to damage of the surrounding normal tissue or insufficient radiation to the tumor [5]. Secondly, improving the sensitivity of tumors to radiotherapy is vital for effective eradication of cancerous cells. Radiation may induce systemic acquired resistance within tumor cells in the hypoxic zone of the solid tumors or trigger underlying molecular repair processes that lead to radioresistance [6]. Therefore, the development of new effective theranostic agents for enhancing resolution of medical imaging and tumor radiosensitivity becomes an urgent priority.

Gadolinium (Gd), a lanthanide element, has been widely used in magnetic resonance imaging (MRI) contrast agents and radiosensitization enhancement due to its unique physical and chemical properties [7–10]. In clinical applications, Gd-chelator complexes possess seven unpaired electrons with a large magnetic moment and are most commonly used as T_1 contrast agents for high-resolution imaging [11]. However, Gd ions released from complexes accumulate in the body and cannot be metabolized. Gd ions can cause considerable biological toxicity by inhibiting calcium channels, and can lead to nephrogenic system fibrosis in patients with renal dysfunction [12]. Recently, research has shown that

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encapsulating Gd into nano-carriers can effectively suppress Gd release while maintaining the T_1-shortening capacity [13]. Materials used for this strategy include inorganic nanoparticles [14], liposomes [15], micelles [16], and microcapsules [17]. It is worth mentioning that Gd capsulated into carbonaceous dots by simple calcination remains stable with minimal Gd leakage even in harsh biological conditions [18]. Additionally, the high atomic number of Gd ($Z = 64$) provides dose enhancement during radiation therapy because of the high X-ray photon capture cross-section and Compton scattering effect [19]. Therefore, local deposition of Gd-based radiosensitizers on the tumor site could enhance the effects of X-rays, necessitating a decreased radiation dose and resulting in fewer side effects for normal tissues. Several studies have shown that Gd-based nanoparticles are not only useful as T_1 positive contrast agents for MRI, but also as effective radiosensitizers for tumor treatment [20]. Especially, Motexafin gadolinium (MGd) as a chemotherapeutic drug could deplete the available pool of DNA repair substrates, making them unavailable for repairing the oxidative damage on DNA strands induced by radiation [21]. Despite these advantages, pharmacokinetic limitations of Gd-based compounds include short circulation half-lives and nonspecific distribution, which are the main causes of tumor radiotherapy failure.

Carbon quantum dots (CDs), a new formulation of carbon nanomaterial, have drawn tremendous attention due to their remarkable optical properties, chemical inertness, and biocompatibility [22–24]. Taking advantage of these properties, CDs can be used in a wide range of biomedical applications such as bioimaging [25], bio-labeling [26], bio-sensing [27], drug delivery [28], gene transfection [29] and photodynamic therapy [30]. Especially, Ge and co-workers reported a novel red-emissive CDs with strong photoacoustic response and high photothermal conversion efficiency ($\eta \approx 38.5\%$), which enable the C-dots to act as multifunctional theranostics for simultaneous diagnosis and therapy of cancer [31]. Our previous work demonstrated that doping CDs with heteroatoms effectively tunes their intrinsic properties and introduces beneficial features [32–35]. Based on the results of previous studies, we prepared Gd-doped CDs by simple hydrothermal carbonization of gadopentetic acid (Gd-DTPA) as the gadolinium source material and glycine as the passivation agent. By virtue of the inertness of carbon cages, Gd-doped CDs could minimize Gd leakage even under harsh biological conditions. These specifically designed multifunctional theranostic nanoparticles have great potential as MRI contrast agents with efficient metabolic pathways *in vivo* and higher longitudinal relaxation efficiency. More importantly, Gd-doped CDs as enhancers of radiosensitivity highlight a novel avenue for overcoming resistance of solid tumors to radiotherapy.

2. Materials and methods

2.1. Materials

Gadopentetic acid (Gd-DTPA) was purchased from Bayer Pharma AG (Germany). Quinine sulfate (98%, suitable for fluorescence), glycine, and glucose were acquired from Sigma (New York, USA). Ammonia water, NaH_2PO_4, Na_2HPO_4, and H_2SO_4 were obtained from Guangfu Fine Chemical (Tianjin, China). Propidium Iodide (PI), fetal bovine serum (FBS), Dulbecco's Minimum Essential Medium (DMEM) medium were purchased from Invitrogen China limited (China, Shanghai). The Cell Counting Kit-8 (CCK-8) was bought from Dojindo (Kumamoto, Japan). All other chemicals were purchased from Aladdin Chemical Reagent (China) unless otherwise noted.

2.2. Synthesis of Gd-doped CDs

First, 1 g glycine was diluted in 20 mL water, then was mixed with varying amounts of Gd-DTPA (0–2 g) under vigorous stirring to form a transparent homogeneous solution. This solution was transferred into a 50 mL Teflon-lined stainless steel autoclave and heated at 180 °C for different time periods (1–6 h). After cooling to room temperature, the reaction mixture was centrifuged at 5000 rpm for 15 min to remove the black precipitates. The brown-yellow supernatant was transferred into a dialysis membrane (MWCO of 1000) and was dialyzed against ultra-pure water for 4 days to remove residues. The dialysis solution was collected and freeze-dried using a vacuum freeze dryer. The Gd-doped CD powders thus obtained were saved for further characterization.

2.3. Instrumentation and characterizations

The morphologies of the Gd-doped CDs were examined by high-resolution transmission electron microscopy (HRTEM) on a JEM-2100 microscope (Jeol, Japan) under an accelerating voltage of 200 kV. UV-Vis absorption spectra were recorded using a UV-2450 UV-Vis Spectrophotometer (Shimadzu, Japan). Photoluminescence (PL) emission measurements were made using a Cary Eclipse Fluorometer (Varian, America). The chemical structures of Gd-doped CDs were analyzed using a Fourier Transform Infrared (FT-IR) spectrometer (Nicolet Nexus 470, America). The elemental composition of Gd-doped CDs was determined by XPS measurements in a MK II photoelectron spectrometer, using Al-Ka (1486.6 eV) as the X-ray source. The crystal structure of the Gd-doped CDs was characterized via X-Ray diffraction (XRD) patterns on a Rigaku-D/MAX2500 diffractometer (Rigaku, Japan) equipped with Cu Kα ($\lambda = 0.15405$ nm) radiation at a scanning speed of 4°/min in the range from 5°to 80°.

2.4. Measurement of fluorescence quantum yields

The quantum yield (Φ) of the prepared carbon nanodots was determined by a comparative method [36]. Quinine sulfate (reported quantum yield: 54%) was dissolved in 0.1 M $H_2SO_4$to create a standard solution [refractive index (η) = 1.33]. Aqueous solutions containing varying concentrations of Gd-doped CDs were prepared by dissolving them in distilled water ($\eta = 1.33$). Absorbance of the solutions at the excitation wavelength was measured using a UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the samples were recorded using a FLS920 fluorimeter at an excitation wavelength of 360 nm. The samples were then analyzed using the PL spectrometer and the PL emission intensity of the excitation wavelength at which the CDs and the reference showed the same UV absorbance. The quantum yield was calculated using the following equation:

$$\Phi_X = \Phi_{st}\left(\frac{Grad_X}{Grad_{st}}\right)\left(\frac{\eta_X^2}{\eta_{st}^2}\right)$$

The ST and X denote the standard group and test group, respectively. Φ is the fluorescence quantum yield. Grad is the gradient from the plot of integrated fluorescence intensity *vs.* absorbance, and η is the refractive index of the solvent. To minimize reabsorption, absorbance in the 10 mm fluorescence cuvette was not allowed to exceed 0.1 at the excitation wavelength.

2.5. Hemolysis assay

Hemolysis assay was carried out according to the procedure reported in the literature with slight modification. In brief, fresh