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# Glucose functionalized carbon quantum dot containing organic radical for optical/MR dual-modality bioimaging

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ABSTRACT

The organic paramagnetic compounds nitroxides have great potential as magnetic resonance imaging (MRI) contrast agents. Herein, we report the synthesis and characterization of glucose modified carbon quantum dot containing 2,2,6,6-tetramethyl-piperidinooxy (TEMPO) for targeted bimodal MR/optical imaging of tumor cells. CQD-TEMPO-Glu shows the greatest potentials for bioimaging applications in view of low cytotoxicity, good biocompatibility, green fluorescence emission and high T1 relaxivities. The *in vitro* MR and optical imaging results confirm enhanced cellular internalization of CQD-TEMPO-Glu in cancer cells through GLUT mediated endocytosis. These results confirm that CQD-TEMPO-Glu is expected to be widely exploited as dual-modal contrast for cancer imaging.

## 1. Introduction

Hybrid materials and inorganic materials are upcoming materials in the biomedical sciences and engineering fields. The functional hybrid organic/inorganic nanocarriers have been widely explored in biosensors, drug and genetic materials delivery, [1–3] mediated optogenetics [4], near-infrared photodynamic therapy [5] and even the remotely controlled therapeutic delivery [6]. In addition, they are also used in purification and biomineralization of peptides [7,8], development of field emitters [9] and remotely actuated applications [10]. Very recently, the advances of atomic layer deposition technology further expanded their potential application areas [11].

The widely used magnetic resonance (MR) contrast agents enhance MR signal and improve diagnosis in clinical practice. Nowadays, Gadolinium (Gd) based contrast agents (GBCAs) have accounted for essentially all of the MR contrast agents used in clinical enhanced magnetic resonance imaging (MRI) scans [12]. However, it was gradually reported that GBCAs administration may lead to accumulation of toxic free Gd in important tissues, even associate with the development of nephrogenic systemic fibrosis [13–15]. Nitroxide stable free radicals (NSFR)are paramagnetic organic compounds with a single unpaired electron, which can shorten $T_1$ and r2/r1 and provide bright contrast. As early as eighties of last century, NSFR have already been reported could be used as intravenous MR contrast agents in addition to paramagnetic metals [16–19]. A series of related experiments [20–23] *in vitro* and *in vivo* showed that nitroxyl spin labels possessed some favorable features similar to GBCAs, such as electron paramagnetism, fast renal elimination, long half-life and chemical versatility, but metal-free and relatively low toxicity. MR contrast agents containing 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) have been successfully synthesized and evaluated in our previous work [24]. These metal-free organic paramagnetic contrast agents may be a promising choice.

The fluorescent materials with features of low toxicity and high efficiency were often applied in various labeling, imaging and therapeutic studies for gene delivery [2,25], photodynamic therapy [5], targeted photothermal ablation [26,27], bioimaging and biodetection [28,29] and the detection of organophosphorus pesticides [30]. In recent years, the focuses of the development of novel MR contrast agents had not only been the improvement of $^1$H water relaxivities and water dispersibility, but also the achievement of multimodal imaging. Conjugation with conventional fluorescent dye or fluorescein had been used to build the multimodal contrast agents in our previous work [24,31], but we still suffered from their stubborn disadvantages. Very recently, fluorescent carbon quantum dot (CQD) has attracted much our

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attention. Compared with the conventional dyes, the key advantages of non-blinking photoluminescence and excellent photostability make CQD in favor of single-molecule tracking and real-time imaging [32]. Similar to the toxic and poorly soluble semiconductor quantum dots (QDs), non-metallic CQD also have the properties including low cost and easy to synthesis, small size and high biological compatibility, excellent light stability and height adjustable photoluminescence, chemical inertness and easy for functionalization [32–34]. The good fluorescence properties and the robust platform for surface specific modification provided by CQD were much conducive to synthesis of multifunctional agents. We noted that many biological applications of functional CQD including bioimaging, biosensing and drug/gene delivery have been reported [35–37], but used for MR contrast medium developing was relatively less. This time, through a good integration between CQD and NSFR with the potential of chemical bonding [22], we attempted to design CQD modified with TEMPO to act as $T_1$-enhanced, fluorescent contrasted and non-metallic bimodal MR contrast agents.

Choosing the appropriate targeting ligands may contribute to the maximization of molecular probe performance. Glucose transport (GLUT) is a relatively well-studied carrier mediating sugar intake in various normal and tumor cells [38–41]. The classic "Warburg effect" indicated that malignant tumor cells with high metabolism more depend on the aerobic glycolysis, which means more glucose consumption and over-expression of GLUTs compared to normal cells, especially the GLUT-1 [42–46]. Based on the theory, targeting GLUTs were often considered in the developments of the targeted probes and anti-cancer drug [47,48]. Thus, we supposed that the MR contrast agents modified with glucose may be more sensitive and significant detection by the GLUTs over-expressing tumor cells.

In this work, CQD prepared *via* a simple reaction of a microwave-assisted method were further modified with TEMPO and glucose to be the GLUT-targeting MRI/optical imaging bimodal contrast agents (CQD-TEMPO-Glu). The basic physicochemical properties, cytotoxicity and uptake specificity of the agents by GLUTs-overexpressing on human liver cancer $HepG_2$ cells *in vitro* were reported and compared to the normal human liver HL-7702 cells. Furthermore, the glucose-mediated cancer specific uptake efficacy of the CQD-TEMPO-Glu was also evaluated by MRI and optical imaging *in vitro*.

## 2. Experimental

### 2.1. Materials

All reagents and solvents were purchased from commercial sources. Solvents were dried according to standard procedures. Deionized water (DI water) was used to prepare all the aqueous solutions. Citric acid, thiourea, 4-amine-2,2,6,6-tetramethyl-piperidinooxy (4-amine-TEMPO), D-Glucosamine hydrochloride (GAH), triethylamine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS) and *N*,*N*-dimethylformamide (DMF) were purchased from Adamas (China). Methyl thiazolyl tetrazolium (MTT), dimethyl sulfoxide (DMSO), paraformaldehyde and 4′,6-diamidino-2-phenylindole (DAPI) were acquired from Sigma-Aldrich (USA).

### 2.2. Characterization

The size and morphology of the synthesized NPs were observed by a HITACHI H-7650 transmission electron microscope (TEM) operating at 200 kV. The mean size distribution of the particles was measured using a Malvern Zeta-size 3000HS. IR spectra were measured using a Shimadzu FTIR-8100 spectrophotometer. Absorbance detections were carried out on Shimadzu UV-2450 spectrophotometer. Fluorescence spectra were obtained using a Hitachi F-4500 spectrofluorometer with a xenon lamp and 1.0 cm quartz cells.

### 2.3. Synthesis of CQD-TEMPO-Glu

The carboxyl-terminated CQD were synthesized according to methods described in the literature with some modifications [49]. In brief, thiourea (0.03 g, 0.5 mmol) and citric acid (0.96 g, 5 mmol) were dissolved in distilled water (10 mL) and stirred well. The mixture was microwave-assisted reacted at 720 W for 5 min. After precipitation with MeOH and centrifugal separation, the carboxyl-terminated CQD were obtained by vacuum drying. Finally, CQD were dispersed in purified water at a concentration of 1.0 mg/mL for subsequent experiments.

The carboxyl-terminated CQD (200 mg), then EDC·HCl (80 mg) and Sulfo-NHS (80 mg) were added to 10 mL DI water and stirred for 1 h. The 4-amine-TEMPO (60 mg) in DMSO (5 mL) was added in the above carboxyl-terminated CQD solvent and stirred for 48 h at room temperature. The pure product was obtained by dialysis against DI water for 48 h using a tubular membrane of cellulose (MWCO 1500 Da). The residual suspension was finally freeze-dried to give CQD-TEMPO.

CQD-TEMPO (100 mg) were dissolved in 5 mL DI water, EDC·HCl (30 mg) and Sulfo-NHS (30 mg) were added and stirred for 1 h. Then GAH (10 mg) and triethylamine (50 μL) were added in the solvent and reacted for 48 h at room temperature. The pure product was obtained by dialysis against DI water for 48 h using a tubular membrane of cellulose (MWCO 1500 Da). The residual suspension was finally freeze-dried to give CQD-TEMPO-Glu.

### 2.4. T1 values measurement and T1 relaxation rate calculation

CQD-TEMPO-Glu was prepared at the concentration of 0, 1.25, 2.5, 5, 10 mg/mL in 5 mL Eppendorf tubes respectively. The T1 of these solutions were determined by Philips Achieva 3.0T MR scanner using a T1 mapping sequence. The scan parameters of the sequence were as follows: TR:3000 ms,TE:10 ms,IR:50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000 ms; slices thickness: 4 mm; gap: 0.5 mm; field of view: 52.491; NSA: 2. Images of the phantoms were analyzed by defining regions of interest (ROI) in each test tube. Relaxivity (r1) was calculated *via* curve-fitting of T1 ($s^{-1}$) *vs* the CQD-TEMPO-Glu concentration (mg/mL).

### 2.5. Cell culture and in vitro studies

#### 2.5.1. Cell culture

All the cell culture supplies were purchased from Corning (USA) and Life Technologies (USA). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (USA). Human liver cancer HepG2 cell lines and normal human liver HL-7702 cell lines were gifted from the Research Center of Clinical Medicine in Nanfang Hospital (Guangzhou, China). HepG2 cell lines were cultured in DMEM, supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 U/mL), and HL-7702 cell lines were supplemented in the same medium besides with 20% FBS. All the cells were incubated at 37 °C with 5% $CO_2$ in atmosphere.

The solutions of CQD-TEMPO-Glu and CQD-TEMPO were dissolved in DI water and further diluted with culture medium to be an appropriate concentration using in the following cellular experiments.

#### 2.5.2. MTT cytotoxicity assay

HepG2 cells and HL-7702 cells were seeded in a 96-well plate at 6500 cells/well and co-incubated until the cell confluence reached 80%. Subsequently, the old culture medium was replaced by 200 μL medium containing CQD-TEMPO-Glu at different concentrations (1.25, 2.5, 10, 20 mg/mL) and the cells were kept incubation for 24 h or 48 h. Six replicate wells were used for one concentration. After incubation, the medium was removed and 200 μL medium containing MTT (5 mg/mL) was added into and incubated for another 4 h. The formazan crystals in each well was dissolved by 150 μL DMSO and completely dissolved *via* being shaken on tablet oscillator for 15 min, then the