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Magnetic and fluorescent carbon nanotubes for dual modal imaging and photothermal and chemo-therapy of cancer cells in living mice

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ABSTRACT

Multi-walled carbon nanotubes (MWCNTs) have drawn increasing attention in biomedical fields because of their unique structures and properties, including good photothermal performance, large surface areas, strong near-infrared (NIR) absorbance, and size stability on the nanoscale. However, big challenge for this platform is to achieve fluorescence/magnetic resonance (MR) imaging and photothermal therapy (PTT) therapy in single nanotube. In this work, Multi-walled carbon nanotubes-magnetofluorescent carbon quantum dots/doxorubicin nanocomposites was prepared. The nanocomposite was then used as carriers for targeted drug transport in cancer therapy. These nanocomposites possess high heat-generating ability, pH and NIR responsive drug delivery, and heat-induced high drug release as well. Experiments *in vitro* and *in vivo* show that this platform can deliver anti-cancer drugs to targeted cells, releasing them intracellular upon NIR irradiation, and eliminate tumors effectively through chemo/photothermal synergistic therapeutic effect. Based on the findings of this work, further development of using other CNTs as highly efficient NIR agents can be achieved for vivo tumor imaging and chemo/photothermal synergistic therapeutic.

1. Introduction

Multifunctional nano-theranostics materials that work in the form of combining therapeutic and diagnostic functions into a single hybrid nanomaterial have attracted substantial attentions [1,2]. Carbon nanotubes (CNTs), formed by carbon atoms, have structures of one-dimensional hollow tubular bodies [3]. Since CNTs displayed unique structures and remarkable physical properties, various applications of CNTs have emerged in materials, catalysis and life sciences etc, especially in the matter of tumor therapy [4–6]. As a consequence, they have a dominant position in carrying multiple diagnostics and target deliveries into a specific area of complex biological systems [7]. Furthermore, they are monitored noninvasively with the applying of bio-imaging tools both drugs and their accumulation at the target sites as long as therapeutic and imaging agents are incorporated into CNTs *in vitro* and *in vivo* conditions. Present imaging modalities, including fluorescence, magnetic resonance (MR), photoacoustic, positron emission tomography, and ultrasound images, have significant benefits and limitations [8–10]. Magnetic resonance imaging (MRI) is regarded as one of the most powerful techniques among modern diagnostic medicines as it can penetrate deeply into tissues, providing anatomical details and high quality three-dimensional images of soft tissues in a non-invasive monitoring manner [11]. Fluorescence imaging (FI), in contrast, has the capacity for single-cell sensitivity and subcellular resolution [12]. Apparently, combining the advantages of MRI and FI can bridge gaps in sensitivity and depth between these two modalities, and consequently, leading to an improved reliability in diagnoses [13].

Gadolinium (Gd) has been confirmed to exhibit excellent contrast efficiency because of its unique magnetic property [14]. Many Gd-containing nanoparticles and chelates have been devel-

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oped as effective probes for MR diagnosis. With abilities of inhibiting calcium channels, causing cardiovascular and neurologic toxicity, the free Gd^{3+} is known as highly toxic [15,16]. Therefore, some groups have developed magnetofluorescent carbon quantum dots (CQDs) by doping or conjugating a paramagnetic gadolinium ion for fluorescence and magnetic resonance imaging *in vitro* and *in vivo* [17,18]. However, some of this magnetofluorescent CQDs have limited practical applications, because of low quantum yield and multiple time consuming synthesis steps. There has been a no report of magnetofluorescent CQDs as a nano-theranostic for MR/fluorescence and chemotherapy. It has been shown that doping of hetero elements (including N) during synthesis would significantly improve the fluorescence properties of CQDs [19]. Thus, the purpose of this work is to develop a simple one-pot method to fabricate a magnetofluorescent GdN@CQDs, with excellent magnetic and fluorescence properties, for MR and fluorescence dual-modal imaging [20]. Several researchers have developed magnetofluorescent CNTs by doping or conjugating a paramagnetic carbon quantum dots for fluorescence and magnetic resonance imaging *in vitro* and *in vivo*. However, some of these magnetofluorescent CNTs have the nature of limited practical applications, because of low loading yield and multiple time consuming synthesis steps [21,22]. Therefore, an original strategy to derivatize CNTs with magnetic/fluorescence CQDs is described. With the chemical inertness, the modification of CNTs was typically carried out with noncovalent functionalization. Thus, the purpose of this work aims to develop a magnetofluorescent CNTs, with excellent magnet and fluorescence properties, for MR and fluorescence dual-modal imaging.

There is an increasing interest in using photothermal therapy (PTT) induced by near-infrared (NIR) laser as a highly effective alternate to conventional cancer treatment approaches [23,24]. Imaging-guided photothermal therapy (PTT), a novel adjuvant therapeutic method of precision therapy, could be used to eliminate remaining cancer cells completely with precise guidance in the future [25,26]. Owing to the unique physical and chemical properties of carbon nanotubes (CNTs), CNTs have generated substantial interest in nanomedicine for applications in thermal therapy. CNTs can absorb NIR laser light to produce a local high temperature, which may kill cancer cells [27–29]. CNTs have emerged as promising candidates for highly efficient delivery of drugs and biomolecules due to their unique structure and properties [30]. CNTs can be conjugated with anti-cancer drugs by covalent and noncovalent interaction methods. Inspired by the properties of CNTs, we pay increasing attention to the combination of PTT and chemotherapy in cancer treatments. Although previous studies have demonstrated that PTT is efficient in cancer therapy with the help of the above CNTs, single PTT cannot always eradicate cancers completely, leading to cancer recurrence [31,32]. However, study shows that heat derived from CNTs have efficiency to enhance the effect of chemotherapy. The combination of photothermal therapy (PTT) and chemotherapy, termed chemo-thermotherapy, can achieve enhanced anti-cancer efficacy via synergistic effects.

EGFR antibody is an emerging class of targeting ligands which also serve as biological drugs that can be used to treat various diseases [33]. Comparing to other targeting agents, EGFR antibody possess distinctive advantages: low synthesis cost, low-immunogenicity, small size that makes it easy to penetrate into solid tumors, and high affinity comparable to monoclonal antibodies for binding almost any molecules [34]. As escort molecules, EGFR antibody is able to deliver drugs or nanoparticles encapsulating drugs, target cells via high-affinity and specific binding [35].

Here we report the magnetofluorescent MWCNTs as a nano-theranostic for MR/fluorescence imaging and chemo/PTT therapy. The GdN@CQDs-MWCNTs/DOX-EGFR possess, we revealed, strong heat-generating ability. Drug delivery experiment reveals that the GdN@CQDs-MWCNTs/DOX-EGFR exhibit both pH and heat responsive drug release behaviors. Furthermore, experiments *in vitro* and *in vivo* demonstrate the excellent cancer ablation ability of GdN@CQDs-MWCNTs/DOX-EGFR. The strategy of material synthesis combining PTT and chemotherapy is illustrated in Fig. 7A. Our research demonstrates the as-synthesized GP-GdN@CQDs-MWCNTs/DOX-EGFR are promising cancer ablation nanoplatforms for combining PTT and chemotherapy.

2. Materials and methods

2.1. Materials and chemicals

MWCNTs (i.d./o.d. = 2–4 nm/10–20 nm) were purchased from Shenzhen Nanotech Port Co. Ltd. Citric acid ($C_6H_8O_7$, CA), gadolinium chloride ($GdCl_3$) 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). All chemicals were analytical grade and used as received without further purification. Distilled water was used throughout the whole experiment. Hydroxy-2,5-dioxopyrrolidine-3-sul fonicacid sodium salt (Sulfo-NHS, 97%) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%) were acquired from Aladdin. Chemical Co. (Shanghai, China). Fetal bovine serum (FBS) and DMEM was obtained from SunShine Biotechnology Co., Ltd. (Nanjing, China). The A549, H522, MDA-MB-231, and MCF-10A were purchased from the Cell Bank of Culture Collection of Chinese Academy of Sciences (Shanghai, China). All other reagents and solvents were of analytical grade and used as received.

2.2. Characterization

The photoluminescent (PL) spectra were recorded using molecular fluorescence spectrometer (Cary Eclipse, varian, USA). The infrared spectrum was performed on a (FT-IR) Nexus 670 FTIR type (Nicolet). The X-ray diffraction (XRD) analysis was performed using a D/Max 2500V/PC diffractometer (Rigaku Corporation, Japan). The thermogravimetric (TG) measurements were performed on a Perkin-Elmer TG 7 instrument. UV–Vis spectroscopy measurements were performed on a Cary 5000 UV–Vis–NIR spectrometer (Varian). The surface composition and element analysis of the samples were recorded using X-ray photoelectron spectroscopy (XPS, EscaLab-250, Thermo, USA). The hydrodynamic size and zeta-potential were measured on a Malvern ZEN 3600 Zetasizer (Malvern Instruments, UK). The transmission electron microscopy (TEM) images were acquired on a JEM-2100F transmission electron microscope. MR images were acquired by a 7 T BioSpec 70/30 experimental scanner (70/30 Bruker BioSpin; Ettlingen, Germany). The fluorescent images of cells were acquired by confocal laser scanning microscope (TI-E-A1R, Nikon, Japan). Details of methods used for material characterization are described in the "Experimental Section" (Supporting Information).

2.3. Synthesis of GdN@CQDs

GdN@CQDs were synthesized according to previous a CQDs method with fine modification. Briefly, 0.5 g of CA, 0.3 g of polyglutamic acid, and 0.1 g of GdCl3 were dispersed into 10 mL of double distilled water (DDW) in sequence under vigorous stirring. Subsequently, the above mixture solution was added into a 25 mL Teflonlined stainless steel autoclave and heated to 200 °C for 6 h. The dark brown products were obtained after cooling to room temperature. The large and agglomerated nanoparticles were removed by centrifuging at 12,000 rpm for 10 min. The supernatant