



Functionalized graphene oxide/Fe₃O₄ hybrids for cellular magnetic resonance imaging and fluorescence labeling

Chaohui Zhou^a, Hui Wu^a, Mingliang Wang^{c,*}, Chusen Huang^a, Dapeng Yang^{b,*}, Nengqin Jia^{a,b,**}

^a The Education Ministry Key Laboratory of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials and Shanghai Municipal Education Committee Key Laboratory of Molecular Imaging Probes and Sensors, Department of Chemistry, Shanghai Normal University, Shanghai 200234, China

^b College of Chemical Engineering and Materials Science, Quanzhou Normal University, Quanzhou 362000, Fujian Province, China

^c Department of Radiology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

ARTICLE INFO

Article history:

Received 29 November 2016

Received in revised form 10 April 2017

Accepted 13 April 2017

Available online 23 April 2017

Keywords:

GO/Fe₃O₄ hybrids

Contrast agents

MRI

Fluorescence labeling

ABSTRACT

In this work, we developed a T₂-weighted contrast agent based on graphene oxide (GO)/Fe₃O₄ hybrids for efficient cellular magnetic resonance imaging (MRI). The GO/Fe₃O₄ hybrids were obtained by combining with coprecipitation method and pyrolysis method. The structural, surface and magnetic characteristics of the hybrids were systematically characterized by transmission electron microscopy (TEM), vibrating sample magnetometer (VSM), AFM, Raman, FT-IR and XRD. The GO/Fe₃O₄ hybrids were functionalized by modifying with anionic and cationic polyelectrolyte through layer-by-layer assembling. The fluorescence probe fluorescein isothiocyanate (FITC) was further loaded on the surface of functionalized GO/Fe₃O₄ hybrids to trace the location of GO/Fe₃O₄ hybrids in cells. Functionalized GO/Fe₃O₄ hybrids possess good hydrophilicity, less cytotoxicity, high MRI enhancement with the relaxivity (r₂) of 493 mM⁻¹ s⁻¹ as well as cellular MRI contrast effect. These obtained results indicated that the functionalized GO/Fe₃O₄ hybrids could have great potential to be utilized as cellular MRI contrast agents for tumor early diagnosis and monitoring.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Magnetic resonance imaging (MRI) is widely applied to visualize anatomical structures in biomedical research and clinical medicine for early tumor detection, diagnosis and monitor, which is capable of resolving physiological and anatomical details as its non-invasive nature, real-time monitoring and high spatial resolution [1–3]. In order to obtain excellent diagnosis quality, MRI contrast agents (CAs) were introduced to significantly enhance the imaging contrast between normal and pathological sites [4]. It is well known that paramagnetic Gd³⁺ complexes (e.g. Gd³⁺ chelate, Gd doped nanoparticles (NaYF₄:Gd [5]) or lanthanide oxides (Gd₂O₃) [6,7]) are usually used as T₁ contrast agents. Furthermore, Gd-based nanoparticles with fluorescent lanthanide elements of multifunctional systems have attracted great attention for MRI and fluorescence imaging due to superior optical and magnetic property. Some multifunctional systems have also been fabricated as

mul-bimodal probes such as PEGylated Gd₂O₃:Tb³⁺ nanoparticles [8], Eu_{0.2}Gd_{0.8}PO₄·H₂O NPs [9], Er³⁺/Yb³⁺ and Tm³⁺/Yb³⁺ doped GdF₃ NPs [10] and Gd₂O₂S:Eu³⁺ [11] nanoparticles and so on. Nowadays, the Gd³⁺ chelate-based T₁ MRI CAs are the most extensively used in clinic [12], however, they give rise to renal failure of patients in some case and are restricted by the US Food and Drug Administration (FDA) [13]. Thus, it is highly desired to develop new MRI CAs with high safety and efficacy.

The majority of iron-based magnetic nanomaterials have been developed to T₂ MRI CAs, which is considered safer. For example, iron oxide nanomaterials (Fe₃O₄, γ-Fe₂O₃, α-Fe₂O₃) [14–16], iron alloy-based nanomaterials (FeCo, CoFe₂O₄ [17], NiFe₂O₄ [18], MnFe₂O₄ [19], FePt [20] and others). Furthermore, iron-based magnetic nanomaterials multifunctional systems have been extensively explored as mul-bimodal probes. Examples of such systems include magneto-fluorescent imaging agents [21–23], T₁–T₂ imaging agents [18], magneto-motive ultrasound imaging agents [24] and so on. Super-paramagnetic iron oxide (Fe₃O₄) NPs have been commonly used for T₂-weighted CAs and could cause decrease in regional signal leading to darker images as its shortening spin–spin proton transverse relaxation times (T₂) [25,26]. But Fe₃O₄ NPs tend to aggregate and often form precipitation in practical application, restricting their application both in vitro and vivo [27]. To address this issue, it is often desirable to modify Fe₃O₄ NPs with suitable functionalities or fabricate magnetic hybrids.

* Corresponding authors.

** Correspondence to: N. Jia, The Education Ministry Key Laboratory of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials and Shanghai Municipal Education Committee Key Laboratory of Molecular Imaging Probes and Sensors, Department of Chemistry, Shanghai Normal University, Shanghai 200234, China.

E-mail addresses: wang.mingliang@zs-hospital.sh.cn (M. Wang), yangdp@qztc.edu.cn (D. Yang), nqjia@shnu.edu.cn (N. Jia).

Graphene is a novel one-atom-thick two-dimensional structure carbon materials, which has attracted a great deal of interest in material science because of their unique electronic, thermal, mechanical, and structural properties [28,29]. Graphene and graphene derivatives have been explored applications in the nanomedicine field, such as molecular imaging, drug delivery, cancer therapies, and biosensing [30–32]. Graphene oxide (GO), one of the most extensively used graphene derivatives in the biomedical field owing to its excellent water solubility and low toxicity [33,34]. In addition, graphene oxide (GO) can confer enhancements in mass transport and higher effective surface area. The combination of graphene oxide (GO) with nanomaterials is able to offer numerous excellent physicochemical properties and functions for various applications [35]. A versatile nanocomposites based on GO have been built up by loading inorganic nanoparticles for cell imaging and drug delivery. Moreover, it has been reported graphene and graphene derivatives could not have obvious toxicity in vitro and in vivo experiments [36]. Several groups reported that the formation of aggregates of Fe_3O_4 nanoparticles could improve MRI contrast [35], but the uncontrolled aggregation of Fe_3O_4 nanoparticles can cause precipitation of the NPs and limited the application of Fe_3O_4 nanoparticles in vitro and vivo [37]. Therefore, the GO was employed to serve as a platform due to their highly effective surface area and controlled formation of MNP aggregates on the GO sheets, which could enhance MRI contrast of Fe_3O_4 nanoparticles [37]. Recently, the hybrids of Fe_3O_4 NPs and GO have attracted substantial attention due to both the superior properties of GO and magnetite nanoparticles for potential use as the contrast agent for MRI [38,39]. There are quite a few reports on synthetic methods of GO/ Fe_3O_4 hybrids including in situ reduction of acetylacetone iron, chemical precipitation method, ion exchange and subsequent calcinations method, chemical deposition method and so on [40–48], but those methods still own some drawbacks such as uncontrolled loading amount of Fe_3O_4 , easily leaching out from the GO in the process of application and having low saturation magnetization strength [48,49]. Therefore, it is believed that GO/ Fe_3O_4 hybrids synthesized by suitable method may have great potential for applications in MRI.

In this work, we constructed a potentially excellent contrast agent for cellular MR Imaging and fluorescence labeling. GO/ Fe_3O_4 hybrids were synthesized by combining with co-precipitation method and pyrolysis method, followed by modification of anionic and cationic polyelectrolytes via layer-by-layer assembling to obtain functionalized GO/ Fe_3O_4 hybrids. Further, FITC as a fluorescence labeling was grafted to the surface of functionalized GO/ Fe_3O_4 hybrids for monitoring their cellular internalization process. The MTT assay, prussian blue staining and fluorescence imaging experiments showed that the functionalized hybrids possess low toxicity, favorable water solubility and biocompatibility. Finally, magnetic resonance imaging study showed that the functionalized hybrids can be used as potential magnetic resonance imaging contrast agents for tumor early diagnosis and monitoring because of their excellent magnetic resonance imaging effect.

2. Materials and methods

2.1. Materials

Natural flake graphite used to prepare GO was purchased from Qingdao Dingding Graphite Products Factory. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS), polyethylenimine (PEI) (25 K), polystyrene sulfonate sodium salt (PSS) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich and used as received. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, diethylene glycol (DEG), diethanolamine (DEA), sodium hydroxide, and ethanol were obtained from Sinopharm Chemical Reagent Co. Ltd. and used without further purification. All other reagents used were available commercially and were of the high purity grade.

2.2. Synthesis of GO/ Fe_3O_4 hybrids

The GO was prepared by modified Hummer's method from purified natural graphite powder [50,51]. The GO/ Fe_3O_4 hybrids were synthesized via combining with co-precipitation method and pyrolysis method with some modification following those reported literatures [52–54]. In a typical experimental procedure, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (120 mg), and $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ (450 mg) were dissolved in 10 mL of hot DEG at 90 °C in an oil bath, after 30 min of stirring, 2.5 mL of DEA was added. Meanwhile, the mixture of NaOH (6 mmol) and 5 mL of hot DEG were also introduced and kept stirring for 10 min again. Afterwards, the mixed solution of GO (15 mg) and 10 mL of DEG was homogenized under vigorous stirring. Finally, the mixture was brought to a 50 mL Teflon-lined autoclave, and the sealed autoclave was heated to 180 °C for 8 h. The products were collected and separated by centrifugation, washed with ethanol and water for three times, and then dried under vacuum.

2.3. Preparation of water-dispersed polymer-functionalized GO/ Fe_3O_4 hybrids

In order to endow the GO/ Fe_3O_4 hybrids disperse in water with excellent solution stability, good biocompatible and further functionalization, the GO/ Fe_3O_4 hybrids were grafted with functionalized polymer by layer-by-layer assembling method [55]. Briefly, 3 mg of GO/ Fe_3O_4 were added into a 20 mL 1 wt% aqueous solution of an anionic polyelectrolyte PSS (polystyrene sulfonate sodium salt), the mixture solution was homogenized under vigorously stirring at 65 °C for 12 h. The large aggregates or contaminants were removed by filtering and excess of PSS was separated by a magnet. Then, the PSS wrapped GO/ Fe_3O_4 were re-dispersed in pure water with brief sonication. We can make sure that the polymer were successful modified to the surface of GO/ Fe_3O_4 hybrids by measuring of surface potential of GO/ Fe_3O_4 hybrids. The GO/ Fe_3O_4 @PSS hybrids were further dispersed into a 20 mL solution containing 1 wt% of the cationic polyelectrolyte (PEI) and kept stirring for another 3 h. Finally, GO/ Fe_3O_4 @PSS@PEI was obtained by centrifuging, fully washing with three times. And the particles were re-dispersed in pure water for further applications.

2.4. Fluorescence labeling of functionalized GO/ Fe_3O_4 hybrids with FITC

FITC as fluorescence probe was further conjugated to the surface of functionalized GO/ Fe_3O_4 by $-\text{N}=\text{C}=\text{S}$ to the amine groups of PEI coupling reaction to investigate the cellular uptake behavior of the hybrids [56]. Typically, 3 mg/mL functionalized GO/ Fe_3O_4 hybrids dispersed in ethanol was firstly mixed with 1 mg/mL FITC solution, triethylamine was then added to the mixture for adjusting pH to slightly alkaline, and continuously shocked in the dark for 12 h. Finally, the mixture was centrifuged, washed three times with ethanol to remove excess FITC, and the FITC labeled-GO/ Fe_3O_4 hybrids were re-dispersed in PBS solution.

2.5. Characterization

The Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet Avatar 370 FT-IR spectrophotometer using KBr pellets. X-ray diffraction (XRD) patterns were determined by a Rigaku DMAX 2000 diffractometer equipped with Cu/K α radiation ($\lambda = 0.15405$ nm) (40 kV, 40 mA). The ultraviolet-visible (UV-vis) absorption spectra were obtained with a UV-7502PC spectrophotometer. The morphology and composition of the hybrids were characterized by transmission electron microscopy (TEM) equipped with JEOL model JEM2100 high-resolution transmission electron microscope (HR-TEM) instrument. AFM images were obtained using a Multi-Mode V AFM (Veeco). Zeta potentials and size of the sample were measured by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS90). MTT assay could be determined by

Download English Version:

<https://daneshyari.com/en/article/5434477>

Download Persian Version:

<https://daneshyari.com/article/5434477>

[Daneshyari.com](https://daneshyari.com)