Talanta 182 (2018) 433–442

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Laser-assisted *in situ* synthesis of graphene-based magnetic-responsive hybrids for multimodal imaging-guided chemo/photothermal synergistic therapy

Mei-Ling Chen[a,**], Zhi-Wen Gao[c], Xue-Min Chen[b,*], Shu-Chao Pang[d], Yi Zhang[a]

[a] College of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
[b] School of Science, Hebei University of Science & Technology, Shijiazhuang 050018, China
[c] School of Materials Science and Engineering, Tianjin University, Tianjin 300193, China
[d] First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China

ARTICLE INFO

Keywords:
Laser ablation
γ-Fe_2O_3
Graphene
MRI
Chemo/photothermal synergistic therapy

ABSTRACT

Magnetic graphene-based hybrids are being increasing recognized as an effective nanotheranostic agent in biomedicine. Conventional technologies for their synthesis have drawbacks not only from a synthetic standpoint, mainly requiring high temperatures and multi-step processes, but also from a biological perspective, chemical precursors or surfactants involved in the chemical process are toxic to cells. Herein, we report a novel approach for one-step fabricating magnetic graphene hybrid nanocomposites based on laser irradiation of an Fe target in GO-PEG aqueous solution at room temperature without using any other chemical reagent. TEM, XPS, FT-IR, XRD, Mossbauer spectrum and VSM observation reveal that γ-Fe_2O_3 nanoparticles were directly grown on the surface of GO-PEG with uniform morphology and superior dispersibility. These GO-PEG-γ-Fe_2O_3 nanocomposites (labeled as GPF) showed low cytotoxicity *in vitro* compared to chemically synthesized nanoparticles since the pulsed-laser-ablation-in-liquid (PLAL) process is free of toxic agents. After tail vein injection of the nanotheranostics, the tumor was clearly mapped by T_2-weighted magnetic resonance of γ-Fe_2O_3, photothermal imaging of graphene and fluorescence imaging of loaded antitumor DOX. Meanwhile the tumor cells both *in vitro* and *in vivo* achieved highly superior inhibition by the synergistic chemo/photothermal therapeutic effect which provided an intense heating effect and enhanced DOX release upon 808 nm NIR light exposure. The results revealed that the magnetic graphene-based hybrids prepared by PLAL is competent for future multi-modal imaging assisted tumor targeted chemo/photothermal synergistic therapy of cancer.

1. Introduction

Nanotheranostics are currently offering various exciting possibilities in personalized 'precision' medicine for cancer through creation of tailor-designed nanomedicine for individualized treatment [1]. Over the past decade, extensive efforts have produced a large arsenal of hybrid nanoplatforms combining both therapeutic and diagnostic functions, such that cancer can be diagnosed for individualized therapy, and therapy can be non-invasively real-time monitored [2]. In particular, magnetic graphene-based hybrids are of increasing interest for chemo/photothermal therapy (PTT) [3], photothermal/photodynamic [4], magnetic resonance imaging [5] and magnetically targeted delivery [6] due to their ultrahigh surface area [7], high charge mobility [8], superparamagnetism [3] and intrinsic NIR region absorption [9]. Various chemical methods [10] have therefore been employed for the synthesis of magnetic graphene-based hybrids. Although these chemical-based strategies have their own virtues, they are limited by the harsh synthesis conditions, time-consuming experimental steps and small scale production, especially the inevitable contamination of the nanoproduct solution originating from additives, precursor reaction or ligand exchange [11], additionally the impurities are usually too stable to be removed and consequently produce cell toxicity [12] and largely restrict their further applications in biomedicine. Therefore, there is urgent need for development of a convenient and scalable approach to prepared biocompatible magnetic graphene-based hybrids by overcoming the drawbacks of conventional chemical methods.

Pulsed laser ablation in liquids (PLAL) is a simple and effective method to produce additive-free nanoparticles without the necessity for

* Corresponding author.
** Corresponding author.
E-mail addresses: custard.chen@tjutcm.edu.cn (M.-L. Chen), chxm0058@163.com (X.-M. Chen).

https://doi.org/10.1016/j.talanta.2018.02.030
Received 27 January 2018; Received in revised form 6 February 2018; Accepted 7 February 2018
Available online 08 February 2018
0039-9140/

any ligands or post synthesis cleaning [13]. Hereby the nanoparticles with clean surfaces get initiated by laser irradiation of a solid target in a liquid medium under ambient conditions. In particular, liquid media is recognized as an important parameter in the synthesis of nanoparticles by PLAL because it not only has the advantage of confining the resulting nanomaterials, but also allows efficient *in situ* conjugation in a single-step process during pulsed laser ablation in liquid without using any matrix binder or stabilizers [14]. More importantly, the conjugated agents may be selected primarily on the basis of the intended application of the nanoparticles. For example, various agent types (organic and inorganic) have already been utilized to fabricated composites by PLAL, such as polymer [15], biomolecule [16], graphene [17], carbon nanotube [18] and TiO_2 [19] for heterogeneous catalysis, energy and biological application. Moreover, the PLAL-synthesized colloidal nanoparticles without the shielding and pH effect by ligands are easier to be adsorbed by supports and more prone to reach the dispersed adsorbent surface efficiently compared with the chem-nanoparticles [17]. Thus, PLAL brought new opportunities to build theranostic nanoplatforms owing to its green and convenient assemble technique. In this sense, *in situ* assembly of nanoparticles onto a graphene-based support material by PLAL is expected to be an efficient and convenient method for fabrication of heterogeneous theranostic nanoplatform. However, to the best of our knowledge, there is no report about construction of colloidal nanoparticle-graphene theranostic nanoplatforms by PLAL.

In this work, we present a novel green, scalable and one step approach for synthesis of magnetic nanoparticle-GO hybrid nanocomposites by using nanosecond laser irradiation an Fe target immersed in GO-PEG aqueous solution as shown in Scheme 1. GO-PEG in the solution induce steric adsorption and *in situ* size quenching effects, resulting in the PLAL-synthesized γ-Fe_2O_3 nanoparticles tightly bounded to the GO-PEG sheets. The obtained GO-PEG-γ-Fe_2O_3 nanocomposites named as GPF showed lower cell toxicity compared to the chem-GO-PEG-γ-Fe_2O_3. Finally, we evaluated the multimodal imaging and chemo/photothermal synergistic antitumor performance of GPF used as a multifunctional theranostic nanoplatform.

2. Experimental

2.1. Chemicals

All chemical were of analytical grade and used without further purification. PEG modified graphite oxide (GO-PEG) was obtained from XFNANO in China. Fe target with a purity of 99.99% was from General research institute for nonferrous materials, China. Doxorubicin hydrochloride (DOX) was purchased from Shanghai Sangon Biotech Co. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay kit was purchased from Nanjing KeyGEN Biotech Co., China. DMEM (high glucose) cell culture medium, trypsin and antibiotic-antimycotic were purchased from Gibco Invitrogen. The other chemicals, *e.g.*, $FeCl_3{\cdot}6H_2O$, and $FeCl_2{\cdot}4H_2O$, HNO_3 and aqueous ammonia (25%) were obtained from National Medicines Corporation Ltd, China. Deionized (DI) water of 18 MΩ·cm was used throughout the experments.

2.2. Instrumentation

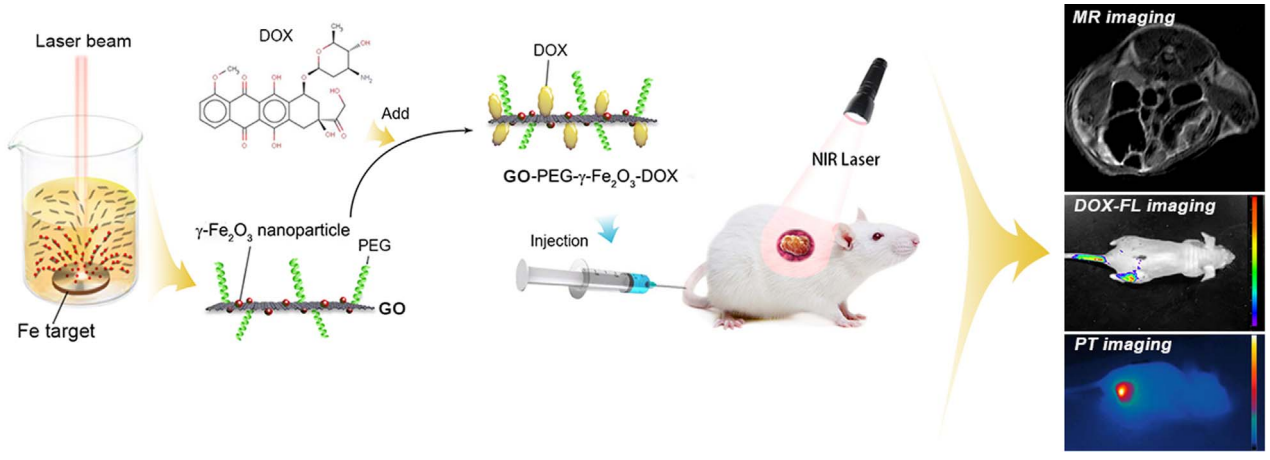
The morphology was observed by a JEOL-2100F transmission electron microscope (TEM, JEOL, Japan) equipped with a field-emission gun, and the composition was measured by the EDS attached to the TEM. The phase structures were investigated by using a Bruker D8 advance XRD. XPS analysis was performed on an ESCALAB 250 surface analysis system (Thermo Electron, USA). Room temperature ^{57}Fe Mössbauer spectra are measured using a FH-1918 Mössbauer spectrometer (Beijing Nuclear Instrument Factory, China), with a room temperature palladium matrix cobalt-57 source and was calibrated with a natural α-iron foil. The spectra were fitted with symmetric quadrupole doublets using a standard least squares fit procedure. ATR-FT-IR spectra were obtained using a Nicolet 6700 Fourier transform infrared spectrometer (Thermo Fisher, USA) within 4000–400 cm^{-1}. Dynamic light scattering (DLS) and zeta potential analysis were performed with a Zetasizer Nano ZS90 at 25 °C (Malvern, England). The hysteresis loops were obtained with a vibrating sample magnetometer (VSM, Lakeshore 7300, USA). Absorption spectra were recorded on a U-4100 UV–Vis spectrophotometer (Hitachi, Japan). Cell viability was assessed by MTT assay with multidetection microplate reader (μQuant, Bio-tek) at 570 nm. Cell fluorescence images were recorded on an inverted fluorescent microscope (Leica, green excitation). The determination of Fe in GPF was conducted by using an Agilent 7500a inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technolog ies Inc.).

2.3. Preparation of GPF

An Fe target (5 mm thick) was ablated with a nanosecond pulsed Nd: YAG laser device with a wavelength of 1064 nm in 10 mL aqueous GO-PEG dispersions with the concentration of 10 μg/mL which was continuously stirred. The laser energy, pulse width and frequency used in the experiments were 200 mJ/pulse, 7 ns and 15 Hz, respectively, and the irradiation time was kept at 25 min. During laser irradiating, the liquid environment was maintained at ambient temperature and pressure. 0.11 mmol/L (C_{Fe}) was derived in the GPF through ICP-MS analysis.

2.4. Preparation of GPF by chemical method (Chem-GPF)

Chem-GPF was obtained by oxidation of GO-PEG-Fe_3O_4 composite,



Scheme 1. Schematic illustration of GPF-DOX for multimodal imaging guided chemo/photothermal synergistic therapy.