Biomaterials 35 (2014) 4499–4507

Contents lists available at ScienceDirect

Biomaterials

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

Magnetic and fluorescent graphene for dual modal imaging and single light induced photothermal and photodynamic therapy of cancer cells

Ganesh Gollavelli, Yong-Chien Ling*

Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

ARTICLE INFO

Article history:
Received 23 January 2014
Accepted 7 February 2014
Available online 3 March 2014

Keywords:
Graphene
Fluorescence imaging
Magnetic resonance imaging
Photodynamic therapy
Photothermal therapy

ABSTRACT

Developing a simple and cost-effective strategy to diagnose and treat cancer with single and minimal dosage through noninvasive strategies are highly challenging. To make the theranostic strategy effective, single light induced photothermal and photodynamic reagent with dual modal imaging capability is highly desired. Herein, a simple non-covalent approach was adopted to immobilize hydrophobic silicon napthalocyanine bis (trihexylsilyloxide) ($SiNc_4$) photosensitizer onto water dispersible magnetic and fluorescent graphene (MFG) via π–π stacking to yield MFG–$SiNc_4$ functioned as a theranostic nanocarrier. Taking the advantage of broad near infra-red absorption (600–1200 nm) by graphene, photosensitizer of any wavelength within this range will facilitate the single light induced phototherapy. Phosphorescence spectra, singlet oxygen sensor green (SOSG) experiments, and 1,3-diphenyl isobenzofuran quenching studies confirm the generation of singlet $^1O_2$ upon photoirradiation. Confocal microscopic images reveal successful internalization of MFG–$SiNc_4$ in HeLa cells; whereas T_2-weighted magnetic resonance images of MFG reveal a significant concentration dependent darkening effect. *In vitro* photodynamic/photothermal therapeutic studies on HeLa cells have demonstrated that the killing efficacy of MFG–$SiNc_4$ using a single light source is ~97.9%, presumably owing to the combined effects of generating reactive oxygen species, local heating, and induction of apoptosis. The developed MFG–$SiNc_4$ may thus be utilized as a potential theranostic nanocarrier for dual modal imaging and phototherapy of cancer cells with single light source for time and cost effective treatments with a minimal therapy dose.

1. Introduction

Photothermal (PTT) and photodynamic (PDT) therapies are considered to be emerging non-invasive modalities for the treatment of various cancers. Ideally, PTT reagents absorb the excitation light, leading to hyperthermia (>43 °C) and causing photothermal ablation of cancer cells. PDT involves photosensitizers (PSs) to absorb light and transfers energy to surrounding tissue oxygen. The generation of highly reactive oxygen species (ROS) such as singlet oxygen and free radicals can oxidize cellular and sub-cellular compartments including plasma membrane, mitochondria, lysosomal, and nuclear membrane, finally leading to irreversible damage to tumor cells [1,2]. PDT has shown significant efficacy with PS such as Photofrin, a clinically approved first generation photodrug, which was normally activated at 630 nm to take maximum advantage of light penetration into biological tissues (1–3 nm), even though its absorbance is relatively low at this wavelength [3]. The drawbacks of poor penetration depths, excitation at short wavelengths (630 nm), and variation in chemical structure has opened a door in search of alternative PSs. The second generation PSs such as napthalocyanines are activatable at near infra-red (NIR) region (700–850 nm) with penetration depths of almost double than that of Photofrin [3]. Napthalocyanines are very good light absorbers in the first biological window (650–900 nm), where most of the molecules such as hemoglobin (Hb), oxyhemoglobin (HbO_2), and several biological pigments do not absorb in this region [4]. In this perspective, napthalocyanines could be attractive as NIR excitable PSs (~775 nm), which is highly important for an effective phototherapeutic treatment [5]. The inherent hydrophobic nature of PSs limits their clinical usage. To overcome this barrier, several delivery approaches capable of stabilizing hydrophobic PDT drugs in aqueous solution including liposomes, polymeric micelles, gold, silica nanoparticles (NPs), and carbon dots have been demonstrated [6–11]. Among them Au nanorods (NRs) and NPs [6] as well as Au nanocages [12] have been extensively used to carry PSs, owing to their inherent photothermal capabilities, and thereby producing synergistic PTT and PDT (PTT/PDT) effects. In order to observe the

* Corresponding author.
E-mail address: ycling@mx.nthu.edu.tw (Y.-C. Ling).

http://dx.doi.org/10.1016/j.biomaterials.2014.02.011
0142-9612/

synergistic therapeutic effects, two different light sources have to be adopted to excite PTT carrier and PS [13,14]. To this end graphene oxide (GO), folic acid-GO, and PEG functionalized GO loaded with hypocrellin A [15] and Chlorin e6 [16,17] were developed via π–π stacking to perform PDT. The PEG functionalized GO–Ce6 composite was excited using 660 and 808 nm lasers to observe the synergistic PTT/PDT therapeutic effects [16]. The improved outcome achieved by multi-laser treatments is very expensive and also prolongs the therapeutic time. Therefore, designing a photo-therapeutic strategy excitable at a single wavelength is highly desired. To perform simultaneous PTT/PDT therapy using a single light source, following alternatives can be considered, (1) Use of two photon femtosecond laser systems to generate heat and singlet oxygen, (2) Design a PTT reagent in accordance with the excitation wavelength of PS, and (3) Selection of an appropriate PS to fit the absorption of PTT reagent. To this end, alternative 1 was successfully demonstrated by Gao et al., in which hypocrellin-loaded gold nanocages were used for PTT/PDT by two photon femtosecond laser excitation, in which simultaneous excitation of nanomaterial (NM) and PS is possible [12]. Alternative 2 was effectively achieved by Wang et al., in which the localized surface plasmon resonance band of gold nanostars were tuned to exactly match with the excitation wavelength of PS (Chlorin e6) [18]. However, the implementation of alternative 3 using a PTT reagent suitable for various PSs to achieve single light induced PTT/PDT is yet to come.

Graphene is an emerging member in carbon family, owing to its extraordinary thermal and optoelectronic properties [19,20]. Its two-dimensional structure, high surface area, easy surface functionality [21], biocompatibility [22–24], high thermal stability, lower cost than Au NRs and carbon nanotubes (CNTs), and enhanced NIR absorption capability in first and second biological window (650–950 nm and 1000–1350 nm) make them as an ideal theranostic platforms for future nanomedicine [25,26]. Owing to its enhanced NIR absorption (~650–1300 nm), graphene could be a versatile platform to immobilize PSs with different excitation wavelengths, thereby achieving improved synergistic PTT/PDT effects. This type of strategy is advantageous by minimizing therapy time and avoiding the utilization of two laser systems. However, poor water dispersibility of graphene severely limits its biological applications. To overcome this problem, graphene was functionalized with various hydrophilic groups to enhance its water dispersibility [27–30]. However, it is necessary to have a balance between sp^2 and sp^3 structure of GO to impart water dispersibility as well as to hold hydrophobic drugs. Apart from simple non-invasive therapeutic strategy, it is also highly desired to possess multi-diagnostic capabilities, such as, fluorescence, magnetic resonance imaging (MRI), computed tomography, and positron emission tomography, which could offer cross validation of the results obtained from different methods, and also provide accurate and realiable disease detection ability to facilitate imaging-guided therapy [31]. This kind of theranostic nanoplatform will minimize the therapy time, cost, and toxicity associated by using different nanomaterials separately [32–35]. Developing cost effective theranostic NMs to treat cancer with minimal doses by means of non-invasive techniques is highly challenging.

Here we report water dispersible magnetic and fluorescent graphene (MFG) functionalized with $SiNc_4$ for dual modal imaging and single light induced PTT/PDT. We believe that MFG–$SiNc_4$ would be advantageous in terms of simple preparation and cost-effectiveness by using graphene.

2. Methods and materials

2.1. Synthesis of graphene oxide (GO)

The GO was synthesized following modified Hummers process with slight modifications, by using pristine graphite flakes as starting material. The graphite flakes (1 g) were added to 98% H_2SO_4 (23 mL) and stirred for ~8 h. $KMnO_4$ (3 g) was slowly added to the above mixture, while maintaining the temperature <20 °C. The reaction mixture was stirred at ~40 °C for ~30 min and then ~65–80 °C for ~45 min. An aliquot (46 mL) of DI water was added and the mixture was heated ~98–105 °C for ~30 min. Finally, the reaction was terminated by the addition of distilled water (140 mL) and 30% H_2O_2 solution (10 mL). The final reaction mixture was subjected to wash with HCl (5%) and distilled water as well as repeated centrifugation yield GO [36,37].

2.2. Synthesis of magnetic graphene (MG)

The MG was obtained by adding GO (50 mg) and ferrocene (350 mg) into a quartz tube containing ~20 pieces of broken Si wafers, evacuated under vacuum for 30 min, and irradiated inside a focus microwave oven (2.45 GHz; Discover system, CEM corporation, NC, USA) under nitrogen atmosphere (1 atm) for ~1 min at 20 s interval. During irradiation, violent arcing occurred between Si pieces, causing some ferrocene to decompose into Fe, which reacted with graphene to form crude MG. The residual ferrocene was removed by toluene/acetone washings. The crude MG was subsequently collected by an external magnet to yield purified MG, which was dried under vacuum before use [27].

2.3. Synthesis of magnetic and fluorescent graphene (MFG)

The water dispersibility and fluorescence property for MG was introduced by covalent grafting with acrylic acid (AA; Sigma–Aldrich) and fluorescein o-methacrylate (FMA; Sigma–Aldrich) via sonication and microwave irradiation methods. The MG (50 mg) was placed into a bottle containing water (20 mL) and AA monomer (1.5 mL). The benzyl peroxide (BPO, 100 mg; Sigma–Aldrich) in tetrahydrofuran (THF, 1 mL) solution was added to the above mixture and subjected to sonication for 2 min to facilitate the dispersion of MG. The mixture was immediately transferred to a domestic microwave oven (2.45 GHz, 600 W) and subjected to microwave irradiation for 10 s. The microwave energy helps to generate benzoyl radicals and initiates the polymerization process. This process of sequential addition of BPO–sonication–microwave irradiation was repeated twice. The FMA was then added and the process of sequential addition was repeated for another two times. Finally, the surface-functionalized MG was collected and separated from free monomers and unbound polymers by repeated washing with THF, followed by DI water, and centrifuged at 9000 rpm for two times to yield magnetic and fluorescent graphene (MFG) [27].

2.4. Synthesis of MFG–$SiNc_4$

The MFG (1 mg) and silicon 2,3-naphthalocyanine bis (trihexylsilyloxide)($SiNc_4$, 5 mg; Sigma–Aldrich) were mixed in DMSO (1:5 wt%, 10 mL) and vigorously stirred for 24 h. The solution was then centrifuged at 8000 rpm for 20 min several times to remove the excess $SiNc_4$ until the supernatant became colorless. The residue was dried under vacuum for overnight and resuspended in DI water to yield MFG–$SiNc_4$. The amount of $SiNc_4$ functionalized onto MFG was determined by UV–Vis spectrometer at ~775 nm by taking the known $SiNc_4$ concentration (10 μM) as a reference. The calculation means was following the literature [16].

2.5. Phosphorescence experiments

A 3 mL solutions containing $SiNc_4$ (in DMSO) and MFG–$SiNc_4$ (in D_2O) were used to record the $^1O_2$ phosphorescence spectra using a luminescence spectrometer (Edinburgh, FLS 920) equipped with a Xe lamp (450 W) as light source. A LP-850 long pass filter was placed between the sample holder and the detection in order to remove the stray light from the light source. The presented signals were accumulated at an average of 40 scans for better signal-to-noise ratio.

2.6. 1,3-Diphenyl isobenzofuran (DPBF) experiments

DPBF (Sigma–Aldrich) was used as a singlet oxygen trapping reagent in ethanol solution. In the experiment, ethanol solution (2 mL) containing DPBF (0.08 mM) was added to MFG–$SiNc_4$ (0.2 mM) in DI water. A tungsten halogen lamp (300 W) equipped with a band pass filter (750–1380 nm) was used as the light source at an output power of ~300 mW/cm^2. The absorbance of the solution at 410 nm was measured every 2 min for a 12 min period using a UV–Vis spectrophotometer (JASCO V-570), under dark and photo conditions. The decrease of the absorbance caused by photobleaching of DPBF was measured.

2.7. Singlet oxygen sensor green (SOSG) experiment

A 5 μL SOSG solution (0.1 mM; Molecular probes) was added to the solutions of $SiNc_4$ (0.2 μM) in DMSO and MFG–$SiNc_4$ (0.2 μM) in DI water, further irradiated in front of a halogen lamp equipped with a band pass filter of 750–1380 nm for 2 min. The fluorescence intensity of SOSG was monitored at 525 nm with an excitation wavelength of 382 nm. For the singlet oxygen quenching study, NaN_3 (100 mM) and for other ROS ($OH^•$, O_2^-) mannitol (100 mM) was used as a scavenger. The quenching ability was measured in terms of decrease in the fluorescence intensity of SOSG and $^1O_2$ complex formed in-situ by $SiNc_4$/MFG–$SiNc_4$ solutions.