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Original article

PEGylated WS_2 nanosheets for X-ray computed tomography imaging and photothermal therapy

Xiao-Zhen Cui [a], Zhi-Guo Zhou [a,*], Yan Yang [a], Jie Wei [a], Jun Wang [a], Ming-Wei Wang [b,c,d,**], Hong Yang [a], Ying-Jian Zhang [b,c,d], Shi-Ping Yang [a]

[a] The Education Ministry Key Lab of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials, and Shanghai Municipal Education Committee Key Laboratory of Molecular Imaging Probes and Sensors, Shanghai Normal University, Shanghai 200234, China
[b] Department of Nuclear Medicine, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[c] Center for Biomedical Imaging, Fudan Univerisity, Shanghai 200032, China
[d] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China

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ABSTRACT

WS_2 nanosheets were prepared by the solvent-thermal method in the presence of *n*-butyl lithium, then the exfoliation under the condition of ultrasound. The formed WS_2 nanosheets were conjugated with thiol-modified polyethylene glycol (PEG-SH) to improve the biocompatibility. The nanosheets (WS_2-PEG) were able to inhibit the growth of a model HeLa cancer cell line *in vitro* due to the high photothermal conversion efficiency of ~35% irradiated by an 808 nm laser (1 W/cm^2). As a proof of concept, WS_2-PEG nanosheets with the better X-ray attenuation property than the clinical computed tomography (CT) contrast agent (Iohexol) could be performed for CT imaging of the lymph vessel.

1. Introduction

In recent years, two-dimensional (2D) nanomaterials have attracted great research efforts because of their fascinating electronic and optical properties. Graphene with excellent electronic, optical, thermal, and mechanical properties has been investigated for a long time [1–3]. Beyond graphene, its analogs of layered transition metal dichalcogenides (MX_2) such as MoS_2 [4], $MoSe_2$ [5], WS_2 [6], and WSe_2 [7] have become a hot research field. Although all of these materials combine each layer with the strong covalent bond, they have weak van der Waals forces between different MX_2 sheets. Therefore, it provides a possibility to prepare the nanosheets by overcoming the van der Waals forces between sheets. With regard to their applications, MX_2 nanosheets have been already extensively investigated in the fields of integrated circuits [8], scanning probe microscopy [9,10], catalysts [11,12], field-effect transistors [13,14], and thermoelectric devices [15,16]. Very few reports have been reported for the theranostic application.

Photothermal therapy (PTT), which depends on a photosensitizer to absorb the near-infrared (NIR) light and generate heat from the optical energy, leads to the thermal ablation of cancer cells. During the past decade, a number of nanomaterials with a relatively high photothermal conversion efficiency have been widely explored by many research groups for PTT ablation of cancer cells, such as metal nanomaterials (Au, Pd) [17–19], carbon nanomaterials (carbon nanotube and graphene) [20,21], metal sulfide and oxide nanomaterials [22,23] as well as organic nanoparticles [24]. Recently, we have developed WO_{3-x} nanorods for X-ray computed tomography (CT) guided photothermal therapy [25], and Fe@Fe_3O_4 nanoparticles for magnetic targeted photothermal therapy [26]. X-ray CT based on different substance with different density is an important molecular imaging technology with the better spatial and density resolution than other imaging modalities. Up to date, the clinical iodine-based CT contrast agents have severe limitations including the relatively short blood circulation time due to the rapid renal clearance, nonspecific biodistribution, renal toxicity, and vascular permeation [27]. Therefore, novel nanoparticulated CT contrast agents have been recently investigated, such as Au [28,29], FePt [30], Bi_2S_3 [31], TaO_x [32], and ytterbium-based nanomaterials [33].

In this paper, we demonstrated the use of WS_2 nanosheets for CT imaging and NIR photothermal ablation of cancer cells. The

* Corresponding author.
** Corresponding author at: Department of Nuclear Medicine, Fudan University Shanghai Cancer Center, Shanghai 200032, China.
E-mail addresses: zgzhou@shnu.edu.cn (Z.-G. Zhou), wang.mingwei88@163.com (M.-W. Wang).

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photothermal therapy effect of PEGylated WS_2 nanosheets was confirmed *in vitro* under the irradiation of an 808 nm laser (1 W/cm^2). Furthermore, the *in vivo* lymph vessel CT imaging was preformed after the injection in the right rear footpad of a mouse.

2. Experimental

2.1. Materials

WS_2 powder was purchased from Alfa Aesar. *N*-Butyl lithium (2.5 mol/L in hexane) was purchased from Energy Chemical. Thiol-modified polyethylene glycol (PEG-SH, MW = 5000 Da) was purchased from Shanghai Yare Biotech. All other reagents were used without further purification. Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm.

2.2. Characterization

The phase and crystallography of the products were characterized with a Rigaku DMAX 2000 diffractometer equipped with Cu/Kα radiation at a scanning rate of 4°/min in the 2θ range of 10–80° (λ = 0.15405 nm, 40 kV, 40 mA). Transmission electron microscopy (TEM) images were obtained on a JEOL JEM-2010 transmission electron microscope operating at 200 kV. The concentration of tungsten was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Vistampxicp Varian, America). The absorption spectra and zeta potential were obtained on the spectrometer of Beckman Coulter DU730 Life Science and Malvern Zetasizer Nano ZS90, respectively. An 808 nm laser was bought from Shanghai Xilong Optoelectronics Technology Co., Ltd. The temperature was monitored by a thermal imaging system (FLIR A300, USA). The optical absorption of formazan at 490 nm was measured on an enzyme-linked immunosorbent assay reader (Multiskan MK3, USA). CT information was performed on a GE Light Speed VCT 64-detector CT (GE Amersham Healthcare System, Milwaukee, WI). Cell morphology was observed on an inverted optical microscope (Olympus, IX71, Japan) with a magnification of 200×. Laser scanning confocal microscopic imaging was performed on a Leica TCS SP5-II inverted microscope (Germany). The *in vivo* CT imaging was obtained on a Siemens Biograph mCT scanner.

2.3. Synthesis of WS_2 nanosheets

WS_2 powder (0.4 g) was reacted with *n*-butyl lithium in hexane (2.5 mol/L, 5 mL) in a Teflon-lined autoclave (10 mL) at 120 °C for 7 h. After the autoclave was cooled to room temperature, the upper solution was removed. The obtained precipitation was washed with dry hexane to remove the remaining *n*-butyl lithium twice. For the exfoliation, 100 mL water was added under the condition of ultrasound with the power of 500 W for 4 h. The formed opaque suspension was centrifuged at 10,000 rpm for 5 min to remove the unexfoliated WS_2. The final product was collected by centrifugation with 12,000 rpm for 8 min, purified with ethanol for three times, and dialyzed against water using dialysis bag of MW 3500 for 4 days.

2.4. Synthesis of PEG-conjugated nanosheets (WS_2-PEG)

PEG-SH (25 mg) was dissolved in the opaque suspension of WS_2 nanosheets (15 mL, 10 mg/mL). The mixture solution was stirred at room temperature for 24 h, then separated by centrifugation with 17,000 rpm for 15 min and washed with ethanol for three times.

2.5. 2.5Photothermal experiments of WS_2-PEG nanosheets

An aqueous suspension containing WS_2-PEG nanosheets with different concentrations (0, 10, 25, 50, 75 and 100 μg/mL, respectively) was put in a quartz cuvette with an optical path length of 1 cm. The cuvette was illuminated by an 808 nm laser with a power density of 1 W/cm^2 for 600 s. The diameter of the laser spot was 1 cm. The temperature was monitored by a thermal imaging system.

2.6. Photothermal conversion efficiency

The photothermal conversion efficiency (η) was calculated using the following equation [34]:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{808}})}$$

where h is heat transfer coefficient, S is the surface area of the container, T_{max} is the equilibrium temperature, T_{surr} is the beginning temperature. Q_{dis} is the loss of heat which is absorbed by the quartz sample cell. I is the power of laser. A_{808} is the absorbance of the aqueous solution of WS_2-PEG nanosheets at 808 nm.

2.7. CT Imaging and HU measurements

WS_2-PEG nanosheets or the clinically used CT contrast agent (Iohexol) with different concentrations were prepared in 2 mL Eppendorf tubes and placed in a self-designed scanning holder. The imaging parameters were shown as follows: slice thickness, 0.625 mm; pitch, 0.984:1; voltage, 80 kV; current, 500 mA; field of view, 512 × 512; gantry rotation time, 0.4 s; table speed, 40 mm/rotation; view, 84 × 84.

2.8. Photothermal ablation of HeLa cells in vitro

PBS (500 μL) or the solution of WS_2-PEG nanosheets with different concentrations (0, 10, 25, 50, 75 and 100 μg/mL, respectively) were added to a 12-well cell culture plate containing HeLa cells. Then, HeLa cells were incubated for 4 h at 37 °C. The adherent cell solution was exposed to an 808 nm laser for 10 min (0.4 W/cm^2). After the laser irradiation, HeLa cells were cultured for another 1 h for MTT assay. All measurements were done in triplicate.

2.9. Trypan blue staining

PBS (100 μL) or the solution of WS_2-PEG nanosheets (75 μg/mL in PBS buffer solution with 10% FBS) were added to a 96-well cell culture plate containing HeLa cells, then HeLa cells were incubated for 4 h. The adherent cell solution was exposed to an 808 nm laser for 10 min (0.4 W/cm^2), then HeLa cells were cultured for another 1 h. After that, HeLa cells were stained with 0.4% trypan blue solution for 3 min. After removal of the medium, the adherent cells were washed with PBS for three times. Cell morphology of the adherent cells in PBS (100 μL) was observed. Cells stained by trypan blue were counted as dead cells. Each experiment was carried out in triplicate.

2.10. Laser scanning confocal microscopy

For Calcein-AM/PI (3,8-diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide) assay, 500 μL PBS or the solution of WS_2-PEG nanosheets (75 μg/mL in PBS