Journal of Photochemistry & Photobiology, B: Biology 180 (2018) 9–16

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Reduced graphene oxide coated $Cu_{2-x}Se$ nanoparticles for targeted chemo-photothermal therapy

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ARTICLE INFO

Keywords:
$Cu_{2-x}Se$ nanoparticles
Reduced graphene oxide
Doxorubicin
Folic acid
Chemo-photothermal therapy

ABSTRACT

Recently, copper chalcogenide semiconductors have been reported as new near-infrared (NIR) photothermal agents. However, it is difficult to modify them with recognition molecules, and their photothermal conversion efficiencies are relatively low, making it difficult to achieve the targeted photothermal ablation of cancer cells with a high efficiency. In this study, reduced graphene oxide (rGO) was first coated on the surface of $Cu_{2-x}Se$ nanoparticles (NPs) to provide abundant functional groups for the next modification and to increase the photothermal conversion efficiency. Then, doxorubicin (DOX) was loaded and folic acid (FA) molecules were covalently linked onto the surface of $Cu_{2-x}Se$/rGO nanocomposites. The formed DOX@$Cu_{2-x}Se$@rGO-FA nanocomposites were successfully used as chemo-photothermal agents for the targeted killing of cancer cells by utilizing the recognition ability of FA, chemotherapy effect of DOX and photothermal effects of rGO and $Cu_{2-x}Se$ NPs. Under the 980-nm NIR laser irradiation, the nanocomposites showed significantly enhanced chemo-photothermal therapy effect, which can be potentially applied in the nanomedicine field.

1. Introduction

In recent years, photothermal therapy that utilizes photothermal agents to kill cancer cells or tissues, has attracted much interest. First, the electrons of photothermal agents are excited from the ground state to the excited state after absorbing light. Then, the electrons return to the ground state through nonradiative decay and generate heat that is used to destroy local cells or tissues [1]. Conventional photothermal agents include natural chromophores [2,3] and externally added dye molecules [4,5]. However, the low absorption and photobleaching of dye molecules decrease the therapeutic efficacy [1]. To solve these problems, gold nanoparticles (NPs) have been utilized to produce heat to kill cancer cells because of their enhanced visible light absorption cross-sections and higher photostability without photobleaching [6–8]. However, visible light cannot penetrate through skin and tissue [9]. It is essential to use near-infrared (NIR) light to destroy cancer cells because it can easily penetrate tissue, blood, and water [10]. For example, the 980-nm NIR light can penetrate several centimeters in biological tissues [11]. Therefore, gold nanorods [12–14], nanoshells [15–17], nanocages [18–20] and nanostars [21–23] have been used as photothermal agents because they respond strongly to the NIR light. However, these nanomaterials still have some disadvantages. For example, cetyltrimethylammonium bromide (CTAB) on the surface of gold nanorods is highly toxic [24]. The diameters of some nanomaterials are larger than 100 nm, and they can be easily removed by the reticuloendothelial system [25]. In addition, after a long period of laser irradiation, their photostabilities are poor [26].

Recently, copper chalcogenide semiconductors, such as CuS and $Cu_{2-x}Se$ NPs, have been reported as new alternative photothermal agents because of their facile synthesis, good photostability, low cytotoxicity and low production cost [26–32]. Owing to the copper vacancies in the lattice, these semiconductors show strong NIR localized surface plasmon resonance (LSPR) properties [33–36]. Although they are promising photothermal agents, some limitations hinder further applications. First, it is difficult to modify the surface of these semiconductors with recognition molecules, making it difficult to achieve the targeted photothermal ablation of cancer cells; this may cause unavoidable damage to normal cells [14]. Second, the photothermal conversion efficiencies of these semiconductors are relatively low [37], and it is necessary to combine the photothermal therapy with other therapeutic methods. Especially, many studies have combined photothermal therapy with chemotherapy to enhance the therapeutic efficacy because the photothermal effect triggers the release of drug molecules from nanocarriers and thus enhances the chemotherapeutic efficiency

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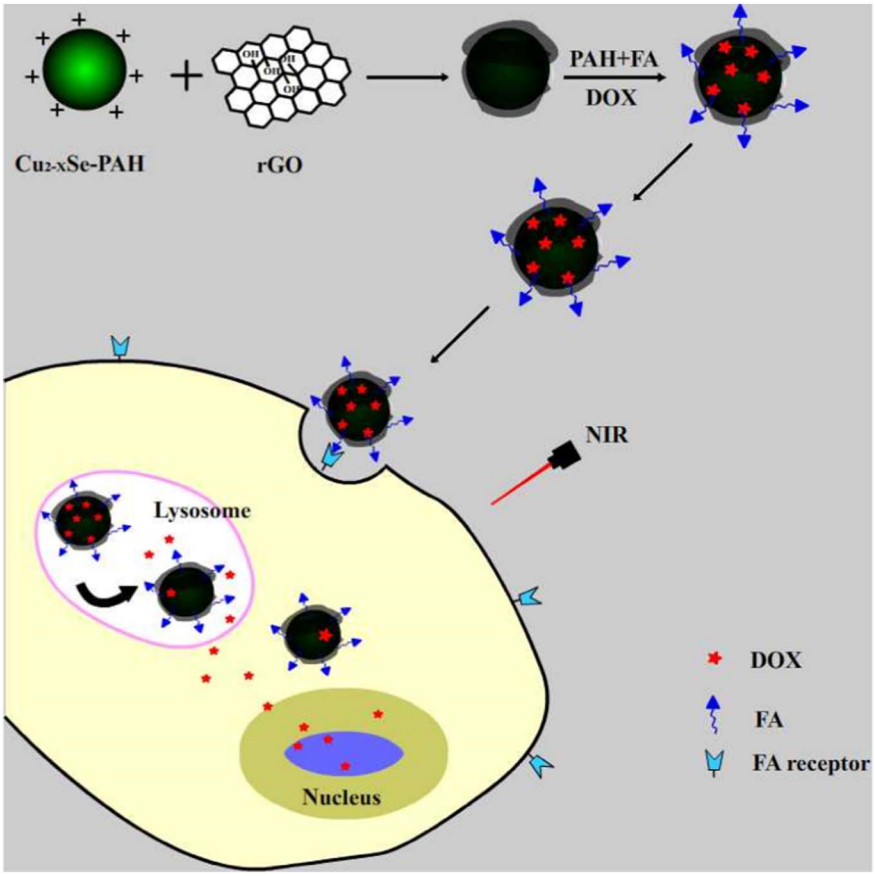
https://doi.org/10.1016/j.jphotobiol.2018.01.020
Received 22 May 2017; Received in revised form 27 December 2017; Accepted 21 January 2018
Available online 20 February 2018
1011-1344/



Scheme 1. Illustration of synthesis of DOX@$Cu_{2-x}Se$@rGO-FA composites and combined chemotherapy and photothermal therapy for cancer cells.

in a synergistic manner [38–40].

Herein, a new $Cu_{2-x}Se$ NP based chemo-photothermal therapy platform was designed. As shown in Scheme 1, the surfaces of $Cu_{2-x}Se$ NPs were first modified with positively charged poly(allylamine) hydrochloride (PAH). Then, the surface of $Cu_{2-x}Se$ NPs was covered with negatively charged reduced graphene oxide (rGO), an exciting photothermal agent [41], through electrostatic interaction. Next, doxorubicin (DOX), a typical chemotherapy agent, was attached onto the surface of $Cu_{2-x}Se$/rGO nanocomposites through π–π stacking and electrostatic interaction between DOX and rGO. Another layer of PAH was coated on the surface of rGO, and then folic acid (FA), recognition molecules for targeting specific cells with FA receptors, was covalently linked onto the surface of $Cu_{2-x}Se$/rGO nanocomposites through amide reaction between the carboxyl groups of FA and the amine groups of PAH. The obtained DOX@$Cu_{2-x}Se$@rGO-FA nanocomposites can be used as chemo-photothermal agents for the targeted killing of cancer cells by utilizing the recognition ability of FA, chemotherapy effect of DOX and photothermal effect of rGO and $Cu_{2-x}Se$ NPs. After entering the cancer cells mediated by FA, DOX is released from the surface of nanocomposites due to the low pH in cellular endosomes and enters the nucleus of the cells to kill them [42]. At the same time, both the $Cu_{2-x}Se$ NPs and rGO generate heat under NIR irradiation for the targeted ablation of cancer cells. Therefore, the cancer cells are killed by both chemotherapy and photothermal therapy effects.

2. Materials and Methods

2.1. Materials

Copper sulfate ($CuSO_4·5H_2O$, 99%) was purchased from Sinopharm Chemical Reagent (Shanghai, China). Selenium dioxide (SeO_2, 99.9%) and PAH (MW 70 kDa) were obtained from Aladdin Chemistry (Shanghai, China). Polystyrene sulfonate (PSS, MW 70 kDa) was purchased from Alfa Aesar (USA). Vitamin C (Vc) was obtained from Sigma-Aldrich (USA). DOX·HCl (98%) was purchased from Dalian Meloney Biotechnology (Dalian, China). GO was purchased from Nanjing XFNano Material Tech. 5-(and-6)-carboxyfluorescein diacetate succinimidyl esters (CFSE) were obtained from Dojindo Laboratories. All other reagents were of analytical grade. 18.2 MΩ Millipore water was used in all the experiments.

2.2. Synthesis of PSS-stabilized $Cu_{2-x}Se$ NPs (PSS-$Cu_{2-x}Se$ NPs)

PSS-$Cu_{2-x}Se$ NPs were synthesized following our previously developed method with slight modification [35]. Briefly, 1.0 mL 5 mg/mL PSS and 8.1 mL water were added to a round-bottom flask, and then 0.1 mL 0.2 mol/L SeO_2 and 0.2 mL 0.4 mol/L Vc were added under vigorous stirring. After 10 min, a mixture of 0.1 mL 0.4 mol/L $CuSO_4$ and 0.5 mL 0.4 mol/L Vc were added and reacted at 30 °C for 30 min. Then the mixture was heated up to 45 °C until the color of the solution changed to green in 6 h, indicating that PSS-$Cu_{2-x}Se$ NPs were obtained. To remove the free small molecules, the as-prepared product was purified via dialysis (molecular weight cut-off: 10 kDa) for one day with six changes of distilled water. Finally, the product in an aqueous solution was stored in a 4 °C refrigerator.

2.3. Synthesis of $Cu_{2-x}Se$@rGO Nanocomposites

First, rGO was prepared following our previously published method [42]. Then, negatively charged PSS on the surface of $Cu_{2-x}Se$ NPs was reacted with positively charged PAH through electrostatic interaction. $Cu_{2-x}Se$@rGO nanocomposites were prepared through electrostatic interaction between the negatively charged rGO and the positively charged PAH on the surface of $Cu_{2-x}Se$ NPs. The detailed experimental