Colloids and Surfaces B: Biointerfaces 167 (2018) 299–309

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

An intelligent dual stimuli-responsive photosensitizer delivery system with O_2-supplying for efficient photodynamic therapy

Hongjuan Zhao[a], Li Li[a], Cuixia Zheng[a], Yongwei Hao[a], Mengya Niu[a], Yujie Hu[a], Junbiao Chang[a,b,c], Zhenzhong Zhang[a,b,*], Lei Wang[a,b,*]

[a] Collaborative Innovation Center of New Drug Research and Safety Evaluation, Henan Province, Zhengzhou 450001, PR China
[b] Key Laboratory of Targeting Therapy and Diagnosis for Critical Diseases, Henan Province, Zhengzhou 450001, PR China
[c] School of chemistry and molecular engineering, Zhengzhou University, 100 Kexue Avenue, Zhengzhou, 450001, PR China

ARTICLE INFO

Article history:
Received 26 December 2017
Received in revised form 21 March 2018
Accepted 4 April 2018
Available online 10 April 2018

Keywords:
Photodynamic therapy
Stimuli-responsive
Mesoporous graphitic-phase carbon nitride nanosheets
Hypoxia relieve
Self-supplying

ABSTRACT

The effects of photodynamic therapy (PDT) are limited by the hypoxic tumor microenvironment (TME). In this paper, a new type of biocompatible multifunctional photosensitizer delivery system was fabricated to relieve tumor hypoxia and improve the efficacy of PDT. The photosensitizer hematoporphyrin monomethyl ether (HMME) and catalase (CAT) were encapsulated in the pores of mesoporous graphitic-phase carbon nitride nanosheets (mpg-C_3N_4). Next, hyaluronic (HA) was coated on the surface of the mpg-C_3N_4 via an amide linkage to construct the tumor-targeting HAase/CAT dual activatable and mpg-C_3N_4/HMME response photosensitizer delivery system (HA@mpg-C_3N_4-HMME/CAT). Upon intravenous injection, HA@mpg-C_3N_4-HMME/CAT shows high tumor accumulation owing to the tumor-targeting HA coating. Meanwhile, CAT within mpg-C_3N_4 could trigger decomposition of endogenic TME H_2O_2 to increase oxygen supply in-situ to relieve tumor hypoxia. This effect together with mpg-C_3N_4/HMME dual response is able to dramatically improve PDT efficiency. The hypoxia status of tumors was evaluated *in vivo* to demonstrate the success of the O_2-supplying. And the *in vitro* and *in vivo* results showed the excellent therapeutic effect of the HA@mpg-C_3N_4-HMME/CAT photosensitizer delivery system. O_2-supplying PDT may enable the enhancement of traditional PDT and future PDT design.

1. Introduction

Photodynamic therapy (PDT) involves photosensitizers and light to produce reactive oxygen species (ROS), which damage cells and induce apoptosis [1]. However, the application of PDT is limited by tumor hypoxia ($pO_2 \leq 2.5$ mmHg) due to the oxygen (O_2)-dependent nature of PDT [2]. More serious hypoxia caused by PDT-induced O_2 consumption may lead to irreversible drug resistance and tumor metastasis [3]. Therefore, optimizing the efficacy with O_2-supplying is of great importance for PDT.

Graphitic-phase carbon nitride nanosheets (g-C_3N_4, CNs) are a novel type of nanocarrier that can be used in PDT due to their photosensitivity. Unlike traditional metal oxides, such as TiO_2 and ZnO, which are only activated upon ultraviolet (UV) light irradiation, g-C_3N_4 is a visible light-driven photosensitizer [4,5]. Moreover, the high degree of condensation of the tri-*s*-triazine ring structure in g-C_3N_4 makes it highly photoluminescent and suitable for bioimaging [5,6]. g-C_3N_4 can be synthesized as nanosheets [7,8], quantum dots [9], hollow structures [10], and mesoporous structures [11]. Graphitic-phase mesoporous carbon nitride nanosheets (mpg-C_3N_4, MCNs) possess a uniform mesoporous sheet structure, which not only enables storage of drugs but also enhances ROS generation under visible light illumination due to the higher surface area [4,5,10]. Nevertheless, its low selectivity and tumor targeting function hamper use of mpg-C_3N_4 in PDT [12].

Malignant cancer cells generate excess H_2O_2 (50–100 μM) in the tumor microenvironment because of the aberrant metabolism of cancer cells [13,14]. Catalase (CAT) mediates decomposition of H_2O_2 to oxygen and water inside the tumor [15]. CAT, which can convert about 5 million H_2O_2 molecules per minute, has been explored in recent years to overcome tumor relieve [13,16]. Therefore, a CAT-based strategy to relieve tumor hypoxia and concentrate ROS in the tumor area seems feasible [17].

In this study, we developed a HAase/H_2O_2 dual stimulation photosensitizer-loading system to promote photosensitizer release, achieving O_2-enriched PDT to relieve tumor hypoxia and

* Corresponding authors at: Collaborative Innovation Center of New Drug Research and Safety Evaluation, Zhengzhou, Henan Province, 450001, PR China.
E-mail addresses: zhenzhongz@126.com (Z. Zhang), hahalei@139.com (L. Wang).

https://doi.org/10.1016/j.colsurfb.2018.04.011
0927-7765/

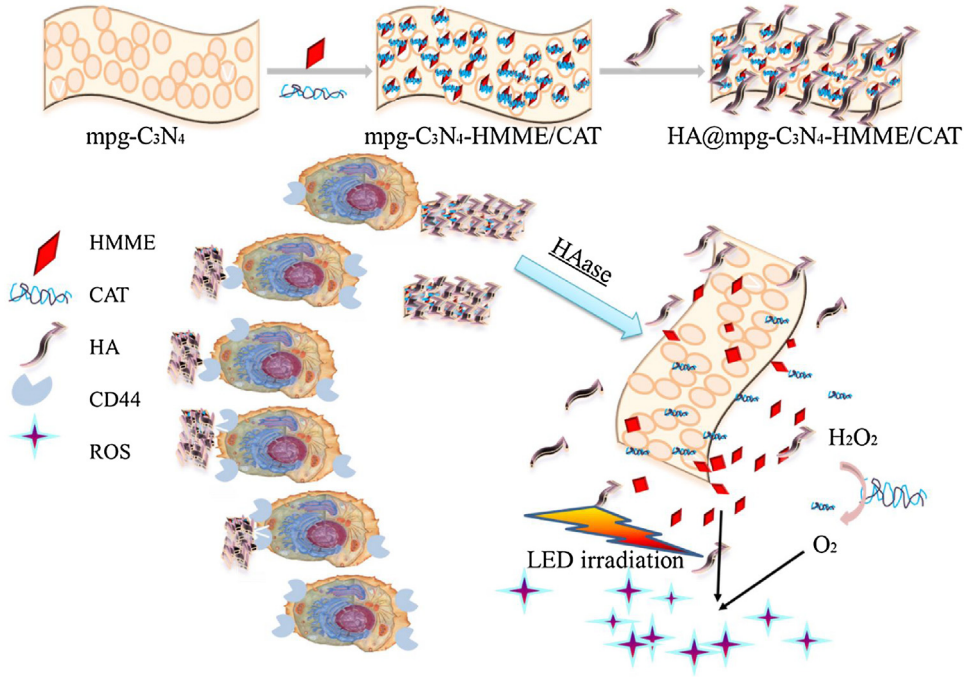

Fig. 1. Scheme illustration for preparation of HA @mpg-C_3N_4-HMME/CAT.

improve PDT effect. HMME and CAT were wrapped in the pores of mpg-C_3N_4, and HA was coated onto the surface to facilitate photosensitizer delivery to tumors (HA@mpg-C_3N_4-HMME/CAT). HMME was used due to its high quantum yield of ROS in the therapeutic excitation window (350–550 nm). CAT encapsulated in mpg-C_3N_4 with largely retained enzyme activity and increased stability protect from protease is able to degrade endogenous H_2O_2 inside tumor to enhance *in situ* O_2 concentration. HA targets tumors via CD44 receptors, which are overexpressed on tumor cells [18,19]. It also enhances the intracellular uptake of MCNs and improves the therapeutic efficacy. After being taken up by cancer cells, HA@mpg-C_3N_4-HMME/CAT intracellular HAase degraded the HA coating, resulting in release of HMME and CAT. Intracellular H_2O_2 penetrated the pores and was degraded by CAT to generate O_2; this also promoted release of HMME due to efflux of O_2. Finally, HMME and mpg-C_3N_4 generated ROS in the presence of O_2 to kill cancer cells under light-emitting diode (LED) light irradiation. The mechanism of the dual stimulation (H_2O_2-activatable and hyaluronidase-stimulation), dual response (photosensitizer mpg-C_3N_4 and HMME), and O_2-supplying photosensitizer-delivery system (HA@mpg-C_3N_4-HMME/CAT) is shown in Fig. 1.

2. Experimental section

2.1. Materials and reagents

Hematoporphyrin monomethyl ether (HMME, purity >98%) was sourced from Beijing Yi-He Biotech Co. Ltd (Beijing, China). All solvents and reagents were analytical grade. Sodium hyaluronate (purity >98%, MW = 7.7 kDa) was bought from Bloomage Freda Biopharm Co. Ltd (Jinan, Shandong). The dialysis bags (molecular weight cutoff = 8–14 kDa) were got from Spectrum Laboratories (Rancho Dominguez, CA, USA). Cyanamide and 12 nm SiO_2 particles (Ludox HS40), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), N-Hydroxysuccinimide (NHS) and dimethyl sulfoxide (DMSO) and all the other reagents were obtained from Sigma-Aldrich (St Louis, MO, USA).

2.2. Preparation of bulk g-C_3N_4 and mpg-C_3N_4

The bulk g-C_3N_4 was prepared by heating 2 g of cyanamide ($CN-NH_2$) in a covered crucible, which was heated in a furnace up to 550 °C and held for 4 h. The mpg-C_3N_4 was synthesized using the hard template method [11,20]. 4.0 g of cyanamide was dissolved in 16.0 g of dispersed solution with 40% of 12 nm SiO_2 particles in water. The mixture was vigorous stirring at 65 °C in an oil bath to remove water. The resultant white powder was transferred into a covered crucible, and then heated it at a rate of 3 °C/min to reach a temperature of 550 °C and maintained this temperature for an additional 4 h. The brown-yellow product was treated with 400 mL ammonium bifluoride (NH_4HF_2, 4 M) for 2 days to remove the silica template. The powders were then centrifuged and washed with distilled water for several times and with ethanol twice, and dried at 70 °C under vacuum overnight [11,21].

2.3. Preparation of mpg-C_3N_4-HMME and mpg-C_3N_4-HMME/CAT

HMME and mpg-C_3N_4 (mass ratio = 3/1) were dissolved in the ethanol-water solution (volume ratio = 1/1) in an ultrasonic bath for about 2 h. After evaporation, the product mpg-C_3N_4-HMME was dispersed in the deionized-water [22].

For preparation of mpg-C_3N_4-HMME/CAT, HMME ethanol solution and CAT phosphate buffer solution were added in to mpg-C_3N_4 solution and ultrasonic for 2 h (HMME: CAT: mpg-C_3N_4 mass ratio = 3/1/1). After evaporation the ethanol and water were removed, and the product was dispersed in deionized-water for further use.

2.4. Construction of the HA coating

HA (MW = 7.7 kDa) was dissolved in 20 mL H_2O, then EDC·HCl (250 mg) and NHS (150 mg) were added, followed by stirring for 30 min. mpg-C_3N_4, mpg-C_3N_4-HMME and mpg-C_3N_4-HMME/CAT were added to the activated HA solution drop by drop, and reacted at room temperature for 2 h, respectively. After that, the products were separated by centrifugation for 15 min at 12,000 rpm. Finally,