Biomaterials 175 (2018) 123–133

Contents lists available at ScienceDirect

Biomaterials

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

A multifunctional nanotheranostic for the intelligent MRI diagnosis and synergistic treatment of hypoxic tumor

Ruixue Song [a], Meng Zhang [b], Yanyan Liu [a], Zhaowen Cui [b], Hua Zhang [c], Zhongmin Tang [b], Xiaoyan Chen [a], Haihong Wu [a, ***], Zhenwei Yao [c, **], Mingyuan He [a], Wenbo Bu [a, b, *]

[a] Shanghai Key Laboratory of Green Chemistry and Chemical Processes, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200062, PR China
[b] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China
[c] Department of Radiology, Huashan Hospital, Fudan University, Shanghai 200040, PR China

ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form
20 April 2018
Accepted 13 May 2018

Keywords:
Hypoxia tumor
Nanotheranostics
Chemotherapy
Magnetic resonance imaging
Nanotechnology

ABSTRACT

Hypoxia, as an inevitable characteristic of solid tumors, has been regarded as a noticeably causative factor to therapeutic resistance and metastatic variants. Exploring novel theranostics to realize the accurate diagnosis of hypoxia and the simultaneous implementation of effective therapy is a promising prospect for the successful treatment of tumors. In the present study, we designed and synthesized a multifunctional rattle-structured nanotheranostic, with the inner core coated by hollow mesoporous silica for chemical drug Doxorubicin (DOX) storage and hypoxia-sensitive MnO_2 enrichment. In various acidic micro-environments caused by hypoxia, MnO_2 nanosheets could be degraded into manganese ions (Mn^{2+}), which were chelated by the modified Tetraxetanum (DOTA) ligands for real-time T_1-magnetic resonance imaging (T_1-MRI), with on-demand DOX release to realize both normoxia and hypoxia-sensitive chemotherapy by overcoming hypoxia. Nanotheranostics integrating hypoxia-driven T_1-MRI with synergetic chemotherapy have tremendous potential in the intelligent diagnosis, personalized treatment and excellent prognosis of solid tumors in the future.

1. Introduction

Hypoxia, associated with the vulnerable invasion, metastasis and resistance to conventional therapies, is a distinguishing feature of solid tumors, which leads to the poor prognosis of cancer patients [1,2]. The exploration of novel theranostics against hypoxic tumors has attracted increasing attention in biotechnology research as well as clinical studies. However, it is difficult to obtain an accurate diagnosis and effective treatment of hypoxia *in vivo*, let alone achieve both using a single system. For hypoxia detection, several existing techniques, such as the O_2 needle electrode method [3] and immunostaining [4], could not meet the requirements of "real-time" and "accuracy" in the clinic. We previously reported an ultrasensitive O_2 nanoprobe for the detection of hypoxia *in vivo*, but this fluorescence imaging technique [5] suffered from the limited tissue-penetration depth of light [6]. Among modern medical imaging instruments, the recently developed positron emission tomography (PET) [7,8] could quantitatively offer accurate 3D assessment of hypoxia relying on the radiotracer probes [9], but the accompanying ionizing radiation is an unwanted and inconvenient factor for patients. In contrast, magnetic resonance imaging (MRI) [10,11], with the unique advantages of high spatial resolution, no penetration depth limit, no ionizing radiation, etc. [12], can safely provide information for on-site and real-time pathological conditions, representing a competitive candidate in hypoxic tumor imaging. Moreover, using functionalized nanoparticles as contrast agents (CAs) [13–15] that can amplify the magnetic resonance signals and respond to specific tumor microenvironments [16], such as subacidity [17,18] and reducibility [19], sensitive hypoxia detection and simultaneous therapy would be more feasible with an integrated nanoplatform.

* Corresponding author. Shanghai Key Laboratory of Green Chemistry and Chemical Processes, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200062, PR China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: hhwu@chem.ecnu.edu.cn (H. Wu), zwyao@fudan.edu.cn (Z. Yao), wbbu@chem.ecnu.edu.cn (W. Bu).

https://doi.org/10.1016/j.biomaterials.2018.05.018
0142-9612/

The timely and effective treatment and accurate diagnosis of hypoxia are also enormous challenges, as malignant tumors lack sufficient molecular oxygen and thereby become resistant to both chemotherapy and radiotherapy. Recently, we summarized the progress of the diagnosis and treatment of hypoxic tumors and proposed three treatment strategies—"evading hypoxia, overcoming hypoxia, or utilizing the low oxygen concentration"—to reverse therapy resistance [20]. Considerable research efforts have been dedicated to determine the problem by implementing these strategies [21–23], but those strategies were either unintelligent for hypoxia-driven self-adaption treatments or unable to overcome the inherent shortcomings. For example, we previously reported the evasion of hypoxia by the integration of scintillators and semiconductors to achieve synchronous radiotherapy, and depth-insensitive PDT could diminish oxygen dependence of radiotherapy, but neither of them could achieve treatment based on the state of the hypoxic condition [24]. Recently, tirapazamine (TPZ), as a specialized drug for hypoxia tumors, was used with photosensitizers for synergistic chemotherapy and photodynamic therapy by utilizing the characteristics of hypoxia [25]. Although TPZ is efficient for hypoxic cells, it shows negligible lethality to normoxia, which increases the risk of using multiple drugs. Thus, the using of a single drug (such as DOX [26]) would be more efficient to treat both normoxic and hypoxic cells by overcoming the deficiency of oxygen according to the tumor treatment need as well as the amount of the released drug that could be effectively tracked. Therefore, an intelligent nanoplatform that could produce a self-regulating stable oxygen concentration environment based on the degree of hypoxia to recover chemo-sensitivity and simultaneously respond to the tumor micro-environment to realize on-demand stimuli-responsive drug release is urgently needed.

Herein, a multifunctional rattle-structured nanotheranostic was designed and fabricated, with an upconversion nanoparticle (UCNP) as the core, hollow mesoporous silica wrapped on the outside of the UCNP with DOX filled within the cavity, MnO_2 nanosheets [27] modified in mesopores as hypoxia-sensitive agents, and PEG and DOTA ligands grafted onto the outer surface of the nanoparticles (named DOX-UCHSM-DOTA). Because the novel nanotheranostic could sensitively respond to the specific micro-environment of the tumor to degrade MnO_2 nanosheets to manganese ions (Mn^{2+}), concomitantly obtaining the following two goals: 1) the produced Mn^{2+} relying on hypoxic degree could be immediately chelated by DOTA for the real-time T_1-MRI diagnosis of hypoxia; and 2) with the degradation of MnO_2 nanosheets, the nanoplatform could intelligently release DOX and replenish O_2 to realize normoxic and hypoxic sensitivity in chemotherapy with a single drug. This constructed nanotheranostic showed great potential to meet the demands of real-time and non-invasive hypoxic regions diagnosis and on-demand hypoxic sensitivity therapy.

2. Materials and methods

2.1. Materials

Cetyltrimethylammonium chloride solution (CTAC, 25% by wt. in H_2O), 1-Octadecene (90%), Ammonium fluoride (NH_4F), PVP (Mw = 40,000), $TmCl_3 \cdot 6H_2O$, $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, CO-520 (NP-5), Triethanolamine (TEA), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and Doxorubicin Hydrochloride (DOX) were purchased from Sigma-Aldrich. Oleic acid (OA), Tetraethyl orthosilicate (TEOS), Potassium permanganate ($KMnO_4$), Ammonia solution (25–28%), Sodium chloride (NaCl), Ethanol, Methanol, Cyclohexane, $MnSO_4$ and Sodium hydroxide (NaOH) were obtained from Sinopharm Chemical Reagent Co., Ltd. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic Acid (DOTA) was purchased from Alfa Aesar Chemical Co., Ltd. *N*-Hydroxysuccinimide (NHS) and *N*'-(3-Dimethylaminopropyl)-*N*-Ethylcarbodiimide Hydrochloride (EDC) were purchased from Aladdin Industrial Corporation. HS-PEG-NH_2 (2000) and Pimonidazole HCl were purchased from Yare Biotech, Co., Ltd. (Shanghai, China). All reagents were analytical grade and without further purification.

2.2. Synthesis of $NaYF_4$:Yb,Tm nanoparticles

The monodisperse $NaYF_4$:20%Yb,0.5%Tm nanocrystals prepared by thermal decomposition method [28]. $YCl_3 \cdot 6H_2O$ (482.3 mg, 1.59 mmol), $YbCl_3 \cdot 6H_2O$ (155.0 mg, 0.4 mmol), and $TmCl_3 \cdot 6H_2O$ (3.83 mg, 0.01 mmol) which were dispersed in deionized water were added to 100 mL flask containing 15 mL oleic acid and 30 mL 1-Octadecene. The mixture was stirred well at room temperature for one hour. Then water was removed from the mixture by slowly heating to 120 °C under the protection of an argon atmosphere, and it was further got rid of by keeping the temperature at 156 °C for an hour in an argon atmosphere. The system was a homogeneous transparent yellow solution and then cooled down to room temperature with the flowing of argon. 10 mL methanol solution containing NaOH (200 mg, 5 mmol) and NH_4F (296.3 mg, 8 mmol) was then added to the system, and the mixture was stirred for about two hours at room temperature. After getting rid of the methanol, the solution was slowly heated to 280 °C and maintained the temperature at 280–290 °C for 1.5 h. The products were precipitated by adding 20 mL ethanol and collected by centrifugation at 13,000 rpm for 15 min. After washing several times with cyclohexane and ethanol, the nanoparticles were dispersed in 20 mL cyclohexane (100 mM).

2.3. Synthesis of $NaYF_4$:Yb,Tm@$NaYF_4$ nanoparticles

For the synthesis of $NaYF_4$:Yb,Tm@$NaYF_4$ nanocrystal, about 1.0 mmol $NaYF_4$: Yb, Tm was firstly prepared using the similar procedures as mentioned above. 800 μmol $YCl_3 \cdot 6H_2O$ in water solution was added in to a 100 mL flask containing 15 mL oleic acid and 30 mL 1-octadecene. Then the mixture was stirred well and got rid of water. Then 10 mL methanol solution of NH_4F (1 mmol) and NaOH (1.685 mmol) were added and the solution was stirred evenly. Next removing methanol form the system, the solution was heated to 270–280 °C and kept temperature for 1.5 h. The same washing steps were followed and sample was re-dispersed in 20 mL cyclohexane.

2.4. Synthesis of $NaYF_4$:Yb,Tm@NaYF4@$dSiO_2$

Using the anti-microemulsion method, as-prepared $NaYF_4$:Yb,Tm@$NaYF_4$ nanoparticles evenly were wrapped a layer of dense silicon oxide on the surface. The mixture that composed of igepal CO-520 (NP-5, 2 mL) and cyclohexane (40 mL) was stirred slowly for one hour. After that, as-prepared $NaYF_4$:Yb,Tm@$NaYF_4$ hexane solution (2 mL) was added into the mixture and stirred for three hours. Then ammonium hydroxide (280 μL, 30%) was added dropwise into the solution and the mixture was stirred for another two hours. The solution containing tetraethyl orthosilicate (TEOS, 4 mL) and cyclohexane (16 mL) was added into the system for 1 h by using a syringe pump which was used to control the adding rate as 0.1 mL/h. After that, the system continued to stir for 24 h. Then, methanol was added into the system to terminate the reaction and collected by centrifugation at 13,000 rpm for 15 min. After washed several times with ethanol to remove excess NP-5, the nanoparticles were dispersed in 10 mL deionized water.