Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Potential application of functional porous TiO_2 nanoparticles in light-controlled drug release and targeted drug delivery

Tianyi Wang [a,†], Haitao Jiang [b,†], Long Wan [b], Qinfu Zhao [b], Tongying Jiang [b], Bing Wang [a,*], Siling Wang [b,*]

[a] College of Life Science and Health, Northeastern University, 89 Wenhuadong Road, Shenhe District, Shenyang, Liaoning Province 110015, People's Republic of China
[b] Department of Pharmaceutics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang, Liaoning Province 110016, People's Republic of China

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 24 October 2014
Accepted 5 November 2014
Available online xxxx

Keywords:
Porous TiO_2
Nanoparticle
Surface functionalization
Controllable release
Targeting

ABSTRACT

Novel multifunctional porous titanium dioxide (TiO_2) nanoparticles modified with polyethylenimine (PEI) were developed to explore the feasibility of exploiting the photocatalytic property of titanium dioxide to achieve ultraviolet (UV) light triggered drug release. Additionally, in order to further realize targeting delivery, folic acid, which chemically conjugated to the surface of the functionalized multifunctional porous TiO_2 nanoparticles through amide linkage with free amine groups of PEI, was used as a cancer-targeting agent to effectively promote cancer-cell-specific uptake through receptor-mediated endocytosis. And a typical poorly water-soluble anti-cancer drug, paclitaxel, was encapsulated in multifunctional porous TiO_2 nanoparticles. The PEI on the surface of multifunctional porous TiO_2 nanoparticles could effectively block the channel to prevent premature drug release, thus providing enough circulation time to target cancer cells. Following UV light radiation, PEI molecules on the surface were cut off by the free radicals ($OH^{\cdot}$ and O_2-) that TiO_2 produced, and then the drug loaded in the carrier was released rapidly into the cytoplasm. Importantly, the amount of drug released from multifunctional porous TiO_2 nanoparticles can be regulated by the UV-light radiation time to further control the anti-cancer effect. This multifunctional porous TiO_2 nanoparticle exhibits a combination of stimuli-triggered drug release and cancer cell targeting. The authors believe that the present study will provide important information for the use of porous TiO_2 nanomaterials in light-controlled drug release and targeted therapy.

1. Introduction

Chemotherapy is now an essential component of cancer therapies used to treat most cancers [1,2]. Although various strategies for drug delivery have been developed in recent years, in efforts to kill cancer cells effectively, many routinely used chemotherapeutic agents are not able to specifically target cancer cells, and their release is poorly controlled, often resulting in serious undesirable side effects [1,3,4]. Therefore, the ability to achieve specific drug accumulation at tumor sites is still a great challenge for successful cancer chemotherapy [5–7].

In order to improve chemotherapy, great efforts have been devoted to the development of tumor-targeted nanocarriers for the controlled delivery of anti-cancer drugs. To obtain a tumor-targeted nanocarrier, a promising strategy is to modify the carrier with targeting ligands, such as peptides [8], antibodies [9] and small molecules (e.g. folate (FA)) [10], which can selectively recognize and bind to the surface receptors over-expressed on cancer cells and stimulate cancer-cell-specific uptake. Moreover, in order to further reduce systemic toxicity and undesired side effects, keeping the loaded drugs sealed during circulation in the bloodstream and rapidly releasing them on reaching and accumulating in tumor tissues is also very important [11,12].

Therefore, an ideal anti-cancer drug delivery system should be a combination of cancer cell targeting and stimuli-triggered drug release. Porous titanium dioxide (TiO_2) offers the potential to achieve this challenge, owing to its interesting properties, such as a high photocatalytic activity and a functional surface.

TiO_2, a typical n-type semiconductor material, has been widely used in many fields, such as photocatalysis [13], energy storage and conversion [14], sunscreening [15] and sensor research [16], and especially in medical fields, as anti-cancer agents [17], implants [18] and substrates for stem cell expansion, owing to its cheapness, chemical stability, innocuity and excellent biocompatibility [19]. Among these, much attention has been drawn in recent years towards the photocatalytic degradation of organic compounds

* Corresponding authors. Tel./fax: +86 24 85820828 (B. Wang). Tel./fax: +86 24 23986348 (S. Wang).
E-mail addresses: wangbing@mail.neu.edu.cn (B. Wang), silingwang@syphu.edu.cn (S. Wang).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2014.11.010
1742-7061/

[20–23]. On ultraviolet (UV) light excitation, the energy absorbed from UV becomes higher than the band gap of TiO_2, and the valence electrons will be excited to the conduction band, creating an electron (e−) and hole (h^+) pair and further generating active free radicals ($OH^·$ and O_2-) which effectively decompose the organic compounds on the surface of the TiO_2 particle [24–26], providing a theoretical foundation for light-triggered drug release. It is worth mentioning that the TiO_2 itself was reported to have a good anti-cancer effect, owing to the production of active free radicals under UV irradiation [17].

Therefore, the present authors have innovatively prepared hybrid polyethylenimine (PEI)-modified porous TiO_2 nanoparticles (MTNP). PEI (a hydrophilic dendritic macromolecule) coated on the surface of MTNP could block the release of drugs as a result of the formation of a hydrophilic layer. Following UV radiation, the PEI molecules could be destroyed by free radicals ($OH^·$ and O_2-) [23,24], and then allow drug release. In addition, FA was covalently linked to the PEI-MTNP. Since folate receptor (FR) is over-expressed on cancer cells, the conjugation (FA with FR) effectively facilitates cellular uptake of the FA functionalized carrier [1,10,29].

Herein, MTNP was developed using non-ionic surfactant F127 as a structure-directing agent. To achieve the targeting and light-controlled release goals, the targeting group (FA) was covalently linked to the bridge molecule (PEI) [30], and then the surface of the MTNP was modified by functional groups (FA-PEI) through the electrostatic adsorption effect. Finally, the anti-cancer drug paclitaxel (PTX) was loaded into the pores of the functionalized carrier by a solvent deposition method [27,28]. The loaded anti-cancer drug was not released during circulation in the bloodstream and normal tissues because of blocking by PEI. Multifunctional MTNP could be efficiently trapped by cancer cells through receptor-mediated endocytosis. And then under UV excitation, the PEI coated on the surface of the carrier could be destroyed, and the anti-cancer drug was rapidly released from the pores. Meanwhile, the TiO_2 carrier itself could also have an anti-cancer effect. The potential of the multifunctional MTNP in tumor targeting and controllable drug release is systematically investigated in the present research.

2. Materials and methods

2.1. Materials

Titanium (IV) isopropoxide (TIP) (95%) was obtained from Alfa Aesar (Lancs, UK). Pluronic block co-polymer F127 was a gift from BASF (Ludwigshafen, Germany). Thiazolyl blue tetrazolium bromide (MTT), Hoechst 33258, streptomycin and penicillin were purchased from Sigma–Aldrich (Santa Clara, USA). Fluorescein isothiocyanate isomer (FITC), PEI and FA were purchased from Aladdin (Shanghai, PRC). KB (human nasopharyngeal carcinoma cell) cell and A549 (human lung carcinoma cell line) cell were purchased from Jia He Biotechnology Co., Ltd. (Shanghai, PRC). PTX was kindly donated by Funing Pharmaceutical Co., Ltd (Shenyang, PRC). The water used for the experiment was Milli-Q (18.2 MΩ). Absolute ethanol (>99.7%) was purchased from Tianjin Bodi Chemical Holding Co., Ltd (Tianjin, PRC). All other solvents were chromatographic grade.

2.2. Fabrication of TiO_2 nanoparticles

The TiO_2 nanoparticles were synthesized via a sol–gel route, using F127 as a structure-directing agent and TIP as a TiO_2 source. In detail, 16 g F127 was completely dissolved in 400 ml ethanol, and then 2.4 ml Milli-Q was added to this solution with stirring. Then 7.4 ml TIP was added to the solution at ambient temperature under vigorous stirring. When the clear solution turned into a milky white suspension, the solution was stirred continuously for 50 min, and then the solution was kept at room temperature without stirring to stand for 12 h. Finally, 2 g precursor sample was dispersed in 200 ml absolute ethanol solution, and then the solution was stirred for 2 h at 70 °C. After stirring, the white samples were collected by centrifugation. The samples were extracted three times in this way and then dried at 40 °C overnight. In order to completely remove the surfactant (F127), the sample was extracted again three times in the same way. Finally, the resulting product was dried at 200 °C for a day to remove absolute ethanol completely. The samples obtained were named MTNP.

2.3. Functional modification of MTNP

Before modification of the MTNP, FA was conjugated to PEI at first. The method was as reported by Guo and co-workers [30]: briefly, 1 g FA was dissolved in 50 ml dimethylsufoxide (DMSO) and then 1.1 M excess of *N*-hydroxysuccinimide and *N*, *N*′-dicyclohexylcarbodiimide was added to this solution with stirring. Afterwards, the solution was stirred for 12 h at room temperature. The solution was filtrated to remove the insoluble byproduct, dicyclohexylurea. And then, PEI was dissolved in carboxy-activated FA DMSO solution at a ratio of 1:5 (molar ratio). After stirring for 12 h, the product (FA-PEI) was passed through a Sephadex G-100 column (Pharmacia Biotech) for purification.

MTNP, 80 mg, was suspended in FA-PEI phosphate (PBS) buffer (pH 7.4), and then the solution was stirred for 5 h at room temperature. These modified particles (FA-PEI-MTNP) were then collected by centrifugation (5000 rpm, 5 min) and were dried at room temperature. The FA-PEI-functionalized particles were further labeled with FITC (fluorescein isothiocyanate) by immersing 50 mg TiO_2 particles in carbonate buffer (pH 8.2) and mixing with 500 μl FITC-ethanol solution (1 mg ml^{-1}), followed by stirring this solution for 40 min [30]. After this, the FITC-FA-PEI-functionalized particles were collected by centrifugation (5000 rpm, 5 min). Electrokinetic titrations were measured using a Malvern Zetasizer-3000. The sample solutions were adjusted to different pH values with 0.1 M NaOH and HCl.

2.4. Characterization

Field-emission scanning electron microscopy (SEM), using a SUPRA 35 instrument (ZEISS, GER, operated at 15 kV) and transmission electron microscopy (TEM) (TECNAI G2 20, FEI, USA) were used to examine the morphology of the MTNP obtained. A surface area and pore size analyzer (V-sorb 2800P, Gold App Instruments, CN) was used to measure the pore characteristics of the samples. Samples were degassed at 150 °C for 16 h prior to analysis. The Brunauer–Emmett–Teller (BET) method was used to determine the surface area of the samples. The Barrett–Joyner–Halenda (BJH) method was used to determine the pore size distribution. X-ray diffractometry (XRD; PW3040/60 PANALYII CALB.V NED) was used to determine the crystals of MTNP over the angle range from 5° to 80° (2θ) with a scan rate of 6° min^{-1} and Cu $_{K\text{-}Alpha1\ K\text{-}Alpha2}$ radiation (λ = 1.54 Å, 30 mA, 30 kV).

2.5. Drug loading and release

PTX was loaded into the pores of the MTNP by a solvent deposition method [27,28], involving a combination of soaking equilibrium and solvent evaporation. The details of the drug loading are described in the Supporting Information. PTX released from FA-PEI-PTX-MTNP was determined by the dialysis method. A certain amount of sample was added to 2 ml PBS (pH 7.4) with 0.1% Tween 80, and then this solution was placed in a dialysis

Please cite this article in press as: Wang T et al. Potential application of functional porous TiO_2 nanoparticles in light-controlled drug release and targeted drug delivery. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.11.010