Acta Biomaterialia 51 (2017) 197–208

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Photothermal and biodegradable polyaniline/porous silicon hybrid nanocomposites as drug carriers for combined chemo-photothermal therapy of cancer

Bing Xia [a,b,*], Bin Wang [b], Jisen Shi [a], Yu Zhang [c], Qi Zhang [b], Zhenyu Chen [a], Jiachen Li [b]

[a] Key Laboratory of Forest Genetics & Biotechnology (Ministry of Education of China), Nanjing Forestry University, Nanjing 210037, PR China
[b] Advanced Analysis & Testing Center, College of Science, Nanjing Forestry University, Nanjing 210037, PR China
[c] State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering & Collaborative Innovation Center for Suzhou Nano Science and Technology, Southeast University, Nanjing 210096, PR China

ARTICLE INFO

Article history:
Received 3 September 2016
Received in revised form 9 December 2016
Accepted 5 January 2017
Available online 6 January 2017

Keywords:
Porous silicon nanoparticles
Polyaniline
Biodegradability
Photothermal effect
Drug delivery
Combination therapy

ABSTRACT

To develop photothermal and biodegradable nanocarriers for combined chemo-photothermal therapy of cancer, polyaniline/porous silicon hybrid nanocomposites had been successfully fabricated via surface initiated polymerization of aniline onto porous silicon nanoparticles in our experiments. As-prepared polyaniline/porous silicon nanocomposites could be well dispersed in aqueous solution without any extra hydrophilic surface coatings, and showed a robust photothermal effect under near-infrared (NIR) laser irradiation. Especially, after an intravenous injection into mice, these biodegradable porous silicon-based nanocomposites as non-toxic agents could be completely cleared in body. Moreover, these polyaniline/porous silicon nanocomposites as drug carriers also exhibited an efficient loading and dual pH/NIR light-triggered release of doxorubicin hydrochloride (DOX, a model anticancer drug). Most importantly, assisted with NIR laser irradiation, polyaniline/PSiNPs nanocomposites with loading DOX showed a remarkable synergistic anticancer effect combining chemotherapy with photothermal therapy, whether *in vitro* or *in vivo*. Therefore, based on biodegradable PSiNPs-based nanocomposites, this combination approach of chemo-photothermal therapy would have enormous potential on clinical cancer treatments in the future.

Statement of Significance

Considering the non-biodegradable nature and potential long-term toxicity concerns of photothermal nanoagents, it is of great interest and importance to develop biodegradable and photothermal nanoparticles with an excellent biocompatibility for their future clinical applications. In our experiments, we fabricated porous silicon-based hybrid nanocomposites via surface initiated polymerization of aniline, which showed an excellent photothermal effect, aqueous dispersibility, biodegradability and biocompatibility. Furthermore, after an efficient loading of DOX molecules, polyaniline/porous silicon nanocomposites exhibited the remarkable synergistic anticancer effect, whether *in vitro* and *in vivo*.

1. Introduction

With the cancer death rate dropping by ~20% since 1991, a great progress on cancer therapy had been made in the past decades [1]. However, ~14.1 million new global cancer cases and ~8.2 million cancer deaths occurred in 2012, thus there is still a great demand for the development of better cancer treatments today [2]. Recently, a novel technology of photothermal therapy based on a large variety of near-infrared (NIR) absorbing inorganic and organic nanomaterials have aroused a tremendously increasing interest, including various gold nanostructures, palladium nanosheets, lanthanide-doped upconversion nanoparticles, copper sulfide nanoparticles, carbon-based nanomaterials, organic nanoagents containing NIR dyes, or conjugated polymeric nanoparticles [3–12]. These above-mentioned photothermal nanomaterials can

* Corresponding author at: Key Laboratory of Forest Genetics & Biotechnology (Ministry of Education of China), Nanjing Forestry University, Nanjing 210037, PR China.
E-mail address: xiabing@njfu.edu.cn (B. Xia).

http://dx.doi.org/10.1016/j.actbio.2017.01.015
1742-7061/

efficiently absorb light, preferably NIR light with superior tissue penetration ability to generate heat, resulting in the death of cancer cells and the subsequent tumor ablation. Except for the thermal ablation of tumor, photothermal therapy is also helpful to trigger and promote other therapeutic approaches (e.g., chemotherapy or radiotherapy) for the synergistic anticancer effect, which is called as "combination therapy" [13–15]. In many pre-clinical animal models, the ideal efficacy of the combination therapy based on inorganic nanomaterials has been reported [3–9], but their potential long-term toxicity concerns hold them back from clinical trials, resulted from their non-biodegradable nature, poor renal clearance, or heavy metal components foreign to the human body [16–18]. As a potential replacement for inorganic nanomaterials, organic nanoagents containing NIR dyes or conjugated polymeric nanoparticles for photothermal therapy have attracted much attention in the past few years, because of their better biocompatibility [10–12]. Especially, conjugated polymeric nanoparticles with extended π-electrons (e.g., polyaniline, polypyrrole, or poly(3,4-ethylenedioxythiophene):poly(4-styrenesulfonate)) exhibited a strong and stable hyperthermia effect under repeated NIR laser irradiation [19–22]. However, some limitations from these conjugated polymeric nanoparticles such as poor water-dispersibility and low loading amount of anticancer drugs prevented them from their further applications on the combination therapy of cancer. Besides, their metabolism, biodegradability, and long-term toxicology profiles *in vivo* still need be explored and improved [11]. To overcome these above-mentioned drawbacks, it is of great interest and importance to develop novel biodegradable and photothermal nanocarriers with better biocompatibility for combination cancer therapy, aiming at next clinical translations [23–26].

As a multifunctional nanoplatform, porous silicon nanoparticles (PSiNPs) have showed an excellent performance on cancer diagnosis and therapy [27–31]. PSiNPs have a versatile loading capability of various exogenous organic molecules, biomacromolecules, or even nanoparticles etc., attributed to their tunable pore size and porosity, large surface area, and tailored surface functionalization [32–34]. Compared with other inorganic nanomaterials, PSiNPs with an excellent biocompatibility and biodegradability have greater potential on their future clinical translation. They can be efficiently excreted from the body through the urine and alleviate the long-term reticuloendothelial systems accumulation concerns, via self-degrading into non-toxic orthosilicic acid ($Si(OH)_4$) *in vivo* [30,35–43]. Recently, to develop novel drug delivery systems with an ideal biodegradability and photothermal effect, we adopted PSiNPs as nanocarriers to co-load NIR dyes and anticancer drugs via electrostatics interactions [44]. However, we found that NIR dyes molecules were easily released from PSiNPs and fast decomposed in the aqueous solution under NIR laser irradiation, limiting their future applications *in vivo*. Herein, to fabricate biodegradable nanocarriers with a strong and stable photothermal effect, polyaniline was chosen to be covalently grafted onto PSiNPs via surface initiated polymerization (shown in Fig. 1a), due to their robust photothermal effect [19,45–48], and excellent biocompatibility [49]. In order to analyze chemical components, size, morphology, and photothermal effect of as-prepared polyaniline/PSiNPs hybrid nanocomposites (PANi-PSiNPs), multi-characterizations including X-ray photoelectron spectra (XPS), transmission Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), transmission electron microscope (TEM), and thermal imaging had been performed in our experiments. Furthermore, their loading and dual pH/NIR light-triggered release of anticancer drugs was also assessed in different biological environments. Finally, their biocompatibility, biodegradability, and performances on chemo-photothermal therapy were systemically explored, whether *in vitro* or *in vivo*.

2. Materials and methods

2.1. Preparation of PSiNPs-based nanocomposites

Single side polished, (100) oriented, and boron-doped p^+-type silicon wafers with 8–10 Ω cm resistivity (Hefei Kejing Materials Technology Co. Ltd., China.) were boiled in 3:1 (v/v) concentrated H_2SO_4/30% H_2O_2 for 30 min and rinsed copiously with deionized (DI) water (⩾18 MΩ cm resistivity, Millipore). The porous silicon samples were prepared by electrochemically etching in a 40% HF/ethanol electrolyte (1:1, v/v) at the current intensity of 100 mA/cm^2 for 15 min. As-prepared porous silicon samples were sonicated in *N*-methyl-2-pyrrolidinone (NMP) solution containing 4-vinylaniline (3%, v/v) (Sigma-Aldrich Chemicals, USA), and then incubated under microwave heating at 100 °C for 1 h. Using 30 min centrifugation at 1.2×10^5 rpm, the PSiNPs samples were washed by NMP to prepare vinylaniline-terminated PSiNPs (VANi-PSiNPs, shown in Fig. 1a). The oxidative polymerization grafting of aniline (Sigma-Aldrich Chemicals, USA) onto VANi-PSiNPs was carried out in 1 mol/L aqueous solution of HCl, containing 1 mg/mL VANi-PSiNPs, 10 mmol/L aniline, and the corresponding amount of $(NH_4)_2S_2O_8$ oxidant with a molar ratio of 1:1 (monomer/oxidant). With stirring in ice-bath, the polymerization reaction was need to proceed for 12 h. The surface-modified PSiNPs was subsequently washed by DI water, NMP, and ethanol to prepare polyaniline-terminated PSiNPs (PANi-PSiNPs, shown in Fig. 1a). In our experiments, others chemicals were bought from Sinopharm Chemical Reagent Co. Ltd. in China.

2.2. Characterization of PSiNPs-based nanocomposites

Size and zeta potential of PSiNPs-based nanocomposites were analyzed by Zetasizer Nano ZS dynamic-light-scattering (DLS) measurements (Malvern Instruments, UK) at 25 °C. To monitor the elemental components and atomic concentrations of PSiNPs-based nanocomposites, XPS were recorded using Kratos AXIS Ultra DLD system (UK) with a monochromatic Al Kα X-ray beam (1486.6 eV) at 150 W in a residual vacuum of $<4 \times 10^{-9}$ Pa. The chemical composition and functional groups of PSiNPs-based nanocomposites were also detected using FTIR (Vertex 80, Bruker, USA). Their morphology was observed by SEM (JEOL JSM-7600F, Japan) with the accelerating voltage of 15 kV and TEM (JEOL JEM-1400, Japan) with the accelerating voltage of 120 kV, respectively.

2.3. Degradation studies in vitro

A serial of bare PSiNPs and PANi-PSiNPs samples with the concentration of 50 μg/mL was incubated in 3 mL PBS buffer (pH = 7.4) at 37 °C. An aliquot (0.45 mL) of the above degradation solutions was taken at different time points, and then ultracentrifugated (1×10^6 rpm, 15 min) to remove non-degraded particles from the degradation solutions. An aliquot (0.4 mL) of the resultant supernatant was diluted with 4.6 mL HNO_3 (2%, v/v), and then measured by inductively coupled plasma optical emission spectrometry (ICP-OES, LEEMAN LABS Prodigy, USA) to analyze silicon elemental amount. Meanwhile, an aliquot (0.05 mL) of the resultant supernatant was also diluted with 0.95 mL DI water, and then subjected to the analysis of silicon elemental amount using a molybdenum blue colorimetric method, which was described in detail elsewhere [50].

2.4. Biodistribution, biodegradation, and toxicity analysis in vivo

All animal work was carried out under protocols approved by Laboratory Animal Center of Simcere Pharmaceutical Group in