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Multifunctional porous silicon nanoparticles for cancer theranostics

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ABSTRACT

Nanomaterials provide a unique platform for the development of theranostic systems that combine diagnostic imaging modalities with a therapeutic payload in a single probe. In this work, dual-labeled iRGD-modified multifunctional porous silicon nanoparticles (PSi NPs) were prepared from dibenzocyclooctyl (DBCO) modified PSi NPs by strain-promoted azide-alkyne cycloaddition (SPAAC) click chemistry. Hydrophobic antiangiogenic drug, sorafenib, was loaded into the modified PSi NPs to enhance the drug dissolution rate and improve cancer therapy. Radiolabeling of the developed system with ^{111}In enabled the monitoring of the *in vivo* biodistribution of the nanocarrier by single photon emission computed tomography (SPECT) in an ectopic PC3-MM2 mouse xenograft model. Fluorescent labeling with Alexa Fluor 488 was used to determine the long-term biodistribution of the nanocarrier by immunofluorescence at the tissue level *ex vivo*. Modification of the PSi NPs with an iRGD peptide enhanced the tumor uptake of the NPs when administered intravenously. After intratumoral delivery the NPs were retained in the tumor, resulting in efficient tumor growth suppression with particle-loaded sorafenib compared to the free drug. The presented multifunctional PSi NPs highlight the utility of constructing a theranostic nanosystems for simultaneous investigations of the *in vivo* behavior of the nanocarriers and their drug delivery efficiency, facilitating the selection of the most promising materials for further NP development.

1. Introduction

Cancer is the first most common cause of death in Europe [1] and the second leading cause of death in the US [2]. For most of the cancers, earlier detection and treatment provide substantial better survival rate [1,2]. Nanocarrier-based targeted delivery of chemotherapeutic agents to the tumor can increase the local drug concentration in the tumor tissue and lower the systemic exposure, hence reducing the side effects of the drugs [3,4]. On the other hand, accurate diagnostics is a key issue for the early detection and treatment of cancer. The so-called theranostic systems, which combine diagnostic imaging with delivery of therapeutic agents, are a recent advance in the development of nanomedicines [5]. For the development and performance evaluation of these drug delivery systems it is also very important to have the necessary imaging tools to follow the fate of the developed nanocarriers *in vivo* [6].

SPECT (Single photon emission computed tomography) detects the gamma (γ)-rays, i.e., photons resulted from the decay of radionuclides including ^{111}In, ^{99m}Tc, ^{123}I, and ^{131}I, and is in use in clinical applications [7]. Radiolabeling of the nanocarrier-based therapeutic systems with γ-emitting radionuclides can provide a highly sensitive and quantitative evaluation of their *in vivo* biodistribution. X-ray computer tomography (CT) in turn has a high spatial resolution for assessment of anatomic structure, information that is not conferred by the SPECT measurement. Combination

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of SPECT and CT techniques is commonly utilized for clinical and pre-clinical applications, providing a 3-D distribution of the radiotracer with precise anatomical localization [8]. On the other hand, fluorescence dyes are widely used in biomedical research to provide an efficient, convenient and radiation-free modality for preclinical cancer imaging and research.

To meet the theranostic requirements, a single nanocarrier has to include both therapeutic payloads and imaging probes. Nanocarriers such as lipid-based liposomes, polymeric nanoparticles, carbon nanotubes, and inorganic nanosystems (e.g., gold nanoparticles, magnetic nanoparticles, and silicon/silica particles) have been widely investigated for drug delivery applications [5,9–17]. Porous silicon (PSi) displays many advantageous properties that render it as a potential theranostic vector in biomedical applications [18–25], such as high biocompatibility, capability to increase the dissolution rate of poorly water-soluble drugs [26], high drug loading capacity [27], and tunable surface chemistry [16,28,29].

Surface modification is necessary to introduce fluorophores, chelators for radiolabeling, and hydrophilic targeting moieties to modulate the properties of PSi for cancer drug delivery [16,20,29,30]. Intravenously administrated nanocarriers can be delivered to the tumor site by the enhanced permeability and retention (EPR) effect or by receptor-mediated active targeting after conjugation of targeting ligands [31]. In addition, the surface biofunctionalization of NPs will modulate the tissue distribution and elimination profiles of the nanocarriers [32,33]. Strain-promoted azide-alkyne cycloaddition (SPAAC) click reaction is an approach for the surface modification of nanomaterials for biomedical applications [34–36]. SPAAC provides several advantages, such as high selectivity, efficiency, and mild/aqueous solution reaction conditions needed [37]. iRGD is a 9-amino acid cyclic variant of RGD (Arg-Gly-Asp) peptide, identified by phage display technology as a tumor targeting and tissue penetrating peptide [38] that has been used to target nanoparticles to cancer therapy applications [39].

Intratumoral administration can provide 100% delivery of the anticancer agent to the injection site. However, conventional small drug molecules can be rapidly pumped out from the tumor into the bloodstream after intratumoral injection, due to the high pressure environment in the tumor or the proteins that exclude drugs from tumor cells and tissues [40,41]. For example, polymeric nanocarriers and cationic liposomes have been shown to increase the tumor retention of loaded drugs after intratumoral administration [40,42].

Here, dual-modality nanotheranostic PSi NPs we prepared by conjugating 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA), Alexa Fluor 488 and the peptide iRGD on the surface of the particles using the SPAAC reaction, followed by radiolabeling of the NPs with ^{111}In for monitoring the biodistribution of the administered NPs using SPECT/CT imaging. Alexa Fluor 488 was covalently conjugated to the nanocarrier for long-term biodistribution studies. In this work, we hypothesize that we can improve the efficacy of the anticancer therapy of the anti-angiogenic drug sorafenib by using PSi-based nanotheranostic systems. By iRGD-modification of the PSi NPs, we aimed for enhancing the nanoparticle tumor accumulation and retention when administered intravenously and intratumorly, respectively.

2. Materials and methods

2.1. Materials and cell culture

Dibenzocyclooctyne-PEG4-amine (DBCO-PEG4-amine) was purchased from Click Chemistry Tools (Scottsdale, USA). Azidoalanine-modified iRGD was purchased from GenicBio (China). 1,4,7,10-Tetraazacyclododecane-1,4,7-tris acetic acid-10-(azidopropyl-ethylacetamide) (Azido-mono-amide-DOTA) was purchased from Macrocyclics Inc (USA). Sorafenib was obtained from LC laboratories® (USA). ^{111}In labeling was carried out with no-carrier-added $^{111}InCl_3$ in 0.04 M HCl (370 MBq/mL, Mallinckrodt Medical B.V., The Netherlands). All the other chemicals were obtained from Sigma–Aldrich (USA) and used as received, unless otherwise stated. Dulbecco's phosphate buffer saline (10× PBS), Hank's balanced salt solution (10× HBSS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin (2.5%), sodium pyruvate, nonessential amino acids (100 × NEAA), L-glutamine (100×), penicillin-streptomycin (100× PEST) were all purchased from HyClone (USA).

The highly metastatic prostate cancer cell PC3-MM2 was cultured in DMEM supplemented with 10% FBS, 1% NEAA, 1% L-glutamine, and 1% PEST (100 IU/mL) in 75 cm^2 flasks incubated at 37 °C in a humidified atmosphere (95%) and 5% CO_2.

2.2. Multifunctional PSi NPs preparation

Undecylenic acid-modified thermally hydrocarbonized PSi (UnTHCPSi) NPs were prepared by electrochemical etching and wet-milling, as described elsewhere [43]. DBCO-PEG4-amine and Alexa Fluor 488 (Life Technologies, USA) were conjugated to UnTHCPSi NPs via N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) coupling reaction. Briefly, 1 mg of UnTHCPSi NPs was mixed with 3 mg of NHS and 2 μL of EDC in 2 mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 5.5) buffer for 30 min. After that, 1 mg of DBCO-PEG4-amine and 10 μg of Alexa Fluor 488 were added to the reaction mixture, and the pH was adjusted to 7.8 with 1 M NaOH. After 1 h, the NPs were harvested by centrifugation (Sorvall RC 5B plus, Thermo Fisher Scientific, USA) at 10,000 *g* for 3 min and washed three times with 1 mL of dimethylformamide (DMF), water and ethanol (99.6%, Altia Corporation, Finland) to obtain Alexa Fluor 488-labeled and DBCO-modified PSi NPs (UnTHCPSi-Alexa488-DBCO).

Next, DOTA and iRGD were sequentially conjugated to UnTHCPSi-Alexa488-DBCO by the SPAAC reaction. First, the azido-mono-amide-DOTA (10 μg in DMF) was added to 1 mg of UnTHCPSi-Alexa488-DBCO (in Milli-Q water) for reaction at 37 °C for 30 min. The unreacted azido-mono-amide-DOTA was removed by washing with DMF, ethanol and Milli-Q water to obtain DOTA-labeled PSi NPs (UnTHCPSi-Alexa488-DBCO-DOTA). After that, 1 mg of azidoalanine-iRGD was added to the vial containing UnTHCPSi-Alexa488-DBCO-DOTA NPs. After the reaction at 37 °C for 1 h, the NPs were collected from the reaction mixture by centrifugation and washed with 50% (v/v) ethanol–water, water and ethanol to obtain iRGD-modified multifunctional PSi NPs (UnTHCPSi-Alexa488-DBCO-DOTA-iRGD).

For simplicity, below we have designated the prepared UnTHCPSi-Alexa488-DBCO-DOTA and UnTHCPSi-Alexa488-DBCO-DOTA-iRGD NPs as PSi and PSi-iRGD, respectively, unless otherwise stated.

2.3. Physicochemical characterization of the NPs

The porosity, pore volume and the specific surface area of the UnTHCPSi NPs were determined by N_2 sorption at −196 °C using TriStar 3000 (Micromeritics Inc., USA), as described in Supporting information. The qualitative analysis of the surface modification of the NPs was performed by Fourier transform infrared spectroscopy (FTIR) with a Vertex 70 spectrometer (Bruker Optics, USA) using a horizontal attenuated total reflectance (ATR) accessory (MIRacle, PIKE Technologies, USA). The spectra were recorded between 4000 and 650 cm^{-1} with a 4 cm^{-1} resolution. Hydrodynamic diameter (*Z*-average) and zeta (ζ)-potential measurements of the NPs were carried out using a Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C.

2.4. Cell viability studies

The cytotoxicity of the multifunctional NPs was evaluated by ATP-based cell viability kit, as described elsewhere [29]. Briefly, PC3-MM2 cells were seeded in 96-well plates at the density of 1.5 × 10^4 cells/well and allowed to attach overnight. Then, the cell culture medium was replaced by 100 μL of medium (with or without 10% FBS) containing different concentrations of SF-loaded NPs (25, 50, 100, and 250 μg/mL). After 24 h incubation, the number of living cells was determined by the ATP-based cell viability kit (CellTiter-Glo® luminescent cell viability assay kit, Promega, USA), according to the manufacture's protocol. Each experiment was performed at least in triplicate. PC3-MM2 cells cultured in growth medium without NPs or with 1% of Triton X-100 served as positive and negative controls, respectively. The luminescence was measured with a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA). The *in vitro* anti-proliferation effect of the SF-loaded PSi and PSi-iRGD NPs against the cancer cells growth was also evaluated using the same cell viability assay and conditions, as described above.

2.5. Drug loading and release

The poorly water-soluble antiangiogenic drug, sorafenib (SF), was loaded to the NPs for chemotherapy applications. The PSi and PSi-iRGD NPs were immersed in 50 mg/mL of SF dissolved in DMF solution with a ratio of 1 mg of NPs to 10 mg of drug in solution for drug loading studies. The loading degrees were determined by immersing 200 μg of the drug-loaded NPs to 1 mL of acetonitrile/water mixture (42:58, v/v) under vigorous stirring for 1 h and the supernatant was analyzed by high-performance liquid chromatography (HPLC) to determine the amount of SF loaded in the NPs. The dissolution profiles of SF from the PSi and PSi-iRGD NPs or free SF were performed at 37 ± 1 °C in sink conditions using a shaking-flask method with a shaking speed rate of 100 rpm. More detailed information on the assays is presented in Supporting Information.