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Dual-drug delivery by porous silicon nanoparticles for improved cellular uptake, sustained release, and combination therapy

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ABSTRACT

Dual-drug delivery of antiangiogenic drug and chemotherapeutic drug can enhance the therapeutic effect for cancer therapy. Conjugation of methotrexate (MTX) to PSi nanoparticles (MTX–PSi) with positively charged surfaces can improve the cellular uptake of MTX and inhibit the proliferation of cancer cells. Herein, MTX–PSi conjugates sustained the release of MTX up to 96 h, and the released fragments including MTX were confirmed by mass spectrometry. The intracellular distribution of the MTX–PSi nanoparticles was confirmed by transmission electronic microscopy. Compared to pure MTX, the MTX–PSi achieved similar inhibition of cell proliferation in folate receptor (FR) over-expressing U87 MG cancer cells, and a higher effect in low FR-expressing EA.hy926 cells. Nuclear fragmentation analysis demonstrated programed cell apoptosis of the MTX–PSi in the high/low FR-expressing cancer cells, whereas PSi alone at the same dose had a minor effect on cell apoptosis. Finally, the porous structure of MTX–PSi enabled a successful concomitant loading of another anti-angiogenic hydrophobic drug, sorafenib, and considerably enhanced the dissolution rate of sorafenib. Overall, the MTX–PSi nanoparticle system can be used as a platform for combination chemotherapy by enhancing the dissolution rate of the hydrophobic drug and sustaining the release of the conjugated chemotherapeutic drug.

1. Introduction

Nanomedicines have been investigated for cancer therapy for several decades, with several products in clinical trials or in the market [1]. Safe and efficient delivery of poorly-water soluble and/or low permeable chemotherapeutic drugs is still one of the main tasks to be overcome by cancer medicines. Porous silicon (PSi) nanomaterials possess high biocompatibility and have a number of unique properties that render them as potential drug delivery carriers in biomedical applications [2–12], such as increasing the dissolution rate of poorly water-soluble drugs [13], high drug loading capacity [14], and tunable surface structure for tailoring the biological activities by different surface chemistries [8,15–17]. For example, the carboxylic acid- and amine-terminated surfaces of PSi can be used for further chemical functionalization [15,18,19].

Chemical conjugation of anticancer drugs to nanoparticles has been one of the approaches to overcome the drug solubility/permeability obstacles for the delivery of chemotherapeutics [20,21]. Methotrexate (MTX) is a folic acid analog anticancer drug [22]. MTX is able to inhibit the activity of dihydrofolate reductase (DHFR) enzyme in the cytosol of the cells, which results in the suppression of purine and pyrimidine precursor synthesis and consequently DNA biosynthesis, leading to cell apoptosis [23]. MTX has specific affinity to the folate receptor (FR) and good cellular uptake by certain cells due to the FR-mediated cellular uptake pathway [24]. Over-expression of the FR has been found in many human malignancies, especially when associated with aggressively growing tumors [25]. The cancer cells highly expressing the FR include ovarian, endometrial, colorectal, breast, lung, renal cell carcinoma, brain metastases derived from epithelial cancer, and neuroendocrine carcinoma cells [25,26]. However, MTX has low cellular uptake in receptor deficient cells [20].

As with many other chemotherapeutics, drug resistance has been an obstacle faced by patients when treated with MTX [22].

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One mechanism of the MTX resistance is to decrease the receptor expression and thus reduce the receptor-mediated cell uptake [27]. Cationic nanoparticles can be non-specifically up-taken in the cells through endocytosis by interacting with the negatively charged cell membrane. By chemically conjugating MTX to the cationic carriers, the cellular uptake can be increased [28–30]. Conjugation of MTX to chitosan [28], dendrimeric polyamidoamine [29,31,32], poly-L-lysine [20,30,33], targeting peptide cyclo(1,12) PenPRGGSVLVTGC [34], gelatin [35], human serum albumin [36], magnetic nanoparticles [37], quantum dots [38], multi-walled carbon nanotubes [39], and porous silica [40] have been reported to enhance the cell uptake and/or to overcome drug resistance. The conjugation was achieved by a covalent linkage formed between the carboxylic acid group of the MTX and the amine groups of the conjugated carriers.

The 3-aminopropyltriethoxysilane (APTES) surface functionalized thermally carbonized PSi particles have amine-terminated groups on the surface of the PSi [19]. The conjugation of MTX to these biodegradable PSi nanoparticles with cationic charge can aid in improving the MTX cell uptake and inhibit the proliferation of cancer cells. The porous structure of MTX-conjugated PSi nanoparticles can be further used to load another hydrophobic drug for combination therapy. For example, the anti-angiogenic sorafenib is a multi-kinase inhibitor by targeting RAf kinase, platelet-derived growth factor, vascular endothelial growth factor (VEGF) receptor 2&3 kinases and c-Kit receptor [41]. Clinical studies have reported a high interindividual variability in the pharmacokinetics, clinical efficacy and adverse effects of sorafenib treatment [42]. Side effects and variation in pharmacokinetics caused by low solubility increase the risks associated with the clinical applications of sorafenib [43,44]. By enhancing the aqueous solubility of sorafenib, controllable plasma concentrations and more precise control on the effects of sorafenib can be attained, with reduced clinical risks to patients.

In this work, the chemotherapeutical anticancer drug MTX was chemically conjugated to the amine-terminated thermally carbonized PSi to form MTX–PSi nanoparticles for enhancing the cell uptake and sustaining the release of MTX. Simultaneously, the hydrophobic anti-angiogenic sorafenib was loaded to the MTX–PSi to enhance the dissolution rate of this drug for combination therapy. The propose of the dual-drug delivery system developed in this work was to: (1) enhance the dissolution rate of the poor water soluble anti-angiogenic drug, sorafenib, which can inhibit the kinase of the neovascular cells on the cell membrane to slow down the tumor growth; and (2) to enhance the cellular uptake and sustain the release of the chemotherapeutical drug MTX to kill tumor cells. The conjugation of MTX, sorafenib loading to MTX–PSi nanoparticles, the dissolution profiles of MTX and sorafenib, as well as the anti-proliferation and cell apoptosis of MTX–PSi were evaluated in this study.

2. Materials and methods

2.1. Materials and cell culturing

MTX was purchased from TCI (Chuo-ku, Japan). Sorafenib was obtained from LC Laboratories (Woburn, USA). All other chemicals and solvents purchased were of analytical grade from Sigma–Aldrich (USA) and used as received. Hypoxanthine–aminopterin–thymidine (50× HAT) was purchased from Gibco® (Carlsbad, USA). Dulbecco's phosphate buffered saline (10× PBS) and 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×) were all purchased from HyClone (Waltham, USA). CellTiter-Glo® luminescent cell viability assay kit was purchased from Promega (Madison, USA). MTX was dissolved in dimethylsulfoxide (DMSO, 2 mM) as stock solution for further tests.

Endothelial EA.hy926 (ATCC, USA) cells were incubated in DMEM with high glucose and 2% HAT, while brain cancer cells U87 MG (ATCC, USA) were cultured with DMEM with low glucose. Both cell lines were cultured in 75 cm^2 flasks for further experiments at 37 °C with humidified atmosphere containing 5% CO_2, and the DMEM was supplemented by 10% FBS, 1% sodium pyruvate, 1% NEAA, 1% L-glutamine, and 1% penicillin–streptomycin (100 IU/mL).

2.2. Preparation of PSi nanoparticles

Amine-functionalized thermally carbonized PSi nanoparticles were prepared by adapting the procedure as previously reported elsewhere [19]. Briefly, multilayer PSi films were produced by electrochemically etching monocrystalline *p*+-type Si⟨100⟩ wafers of 0.01–0.02 Ω cm resistivity in a 1:1 (v/v) aqueous hydrofluoric acid (HF, 40%) – ethanol electrolyte as described previously [4]. The free standing films were thermally carbonized with acetylene at 500 °C and 820 °C [13], after which the obtained films were immersed into HF to generate surface silanol termination for APTES attachment, following the previously described process using a 10 v-% APTES-toluene solution [19]. The size reduction of the PSi multilayer films to nanoparticles was performed by wet-milling using a 5 v-% APTES-toluene solution as the milling liquid. After milling, the excess silane was removed by replacing the liquid and re-dispersing the nanoparticles to fresh toluene and ethanol at least 3 times using centrifugation. The final size selection of the nanoparticles was done by centrifugation.

2.3. Conjugation of MTX to PSi nanoparticles

MTX was conjugated to the amine-terminated PSi nanoparticles using N-hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) coupling reaction. Briefly, 2.2 mg (5 μmol) of MTX was dissolved in 1 mL DMSO with 10 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 5.5), 1.7 mg (15 μmol) of NHS and 13 μL (60 μmol of EDC) were added to the solution. The mixture was stirred for 30 min at room temperature to activate the carboxylic acid group of MTX. After that, 1 mg of the amine-terminated PSi nanoparticles in ethanol was added to the reaction mixture, and the pH was adjusted to 7.5 by 1 M NaOH. After 45 min reaction, the PSi nanoparticles were collected from the reaction mixture by centrifugation (Sorvall RC 5B plus, thermo Fisher Scientific, USA) at 10,000 rcf for 3 min and washed with 1 mL of DMSO, water and ethanol 3 times to obtain the MTX conjugated PSi nanoparticles (MTX–PSi), which were then re-suspended in ethanol for further use.

2.4. Physicochemical characterization of PSi nanoparticles

The physical parameters of the PSi nanoparticles were determined by nitrogen sorption at −196 °C using TriStar 3000 (Micromeritics Inc., USA). The specific surface area of the PSi nanoparticles was calculated using the Brunauer–Emmett–Teller theory. The total pore volume was obtained as the total adsorbed amount at a relative pressure $p/p_0 = 0.97$. The average pore diameter was calculated from the obtained surface area and pore volume by assuming the pores as cylindrical.

The qualitative analysis of the MTX–PSi nanoparticles were performed by Fourier transform infrared spectroscopy (FTIR) with a Vertex 70 FTIR spectrometer (Bruker Optics, USA) using a

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