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Engineering porous silicon nanostructures as tunable carriers for mitoxantrone dihydrochloride

Adi Tzur-Balter [a], Ariel Gilert [b], Naama Massad-Ivanir [b], Ester Segal [b,c,*]

[a] The Interdepartmental Program of Biotechnology, Technion – Israel Institute of Technology, Haifa 32000, Israel
[b] Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel
[c] The Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa 32000, Israel

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ABSTRACT

Nanostructured porous silicon (PSi) thin films, fabricated by the electrochemical anodization of single crystalline Si wafers, are studied as delivery systems for the anticancer drug mitoxantrone dihydrochloride (MTX). The surface chemistry of the PSi carriers was tailored by surface alkylation using thermal hydrosilylation of 1-dodecene and undecylenic acid, followed by physical adsorption or covalent attachment of MTX to the Si scaffold. The nanostructure and the physiochemical properties of the different carriers were characterized by attenuated total reflectance Fourier transform infrared spectroscopy, nitrogen adsorption–desorption and contact angle measurements, demonstrating that surface alkylation results in a pronounced effect on the hydrophobicity/hydrophilicity of the scaffolds and a volumetric gain in pore wall, which in turn results in a decrease in pore diameter (>23%) and available porous volume (>40%). The effect of these key parameters on MTX loading efficacy, release profile, Si scaffold erosion kinetics and in vitro cytotoxicity on human breast carcinoma (MDA-MB-231) cells was studied and compared to the behavior of neat PSi carriers. We show that the chemically modified PSi carriers exhibit sustained release for several days to weeks with minimal to no burst effect, while for the native PSi MTX release was completed within 5 h with a substantial burst release of ~40%. Moreover, our in vitro cytotoxicity experiments have clearly demonstrated that the MTX released from all PSi carriers maintained its cytotoxic effect towards MDA-MB-231 cells, in comparison to the low toxicity of the PSi carriers.

1. Introduction

Nanostructured porous Si (PSi) has emerged over the past several years as a promising and versatile material for biomedical applications [1]. Various PSi-based degradable platforms are under development for applications such as optical biosensing [2–6], biomolecular screening [7], tissue engineering [8–10] and drug delivery [11–21]. The potential impact of PSi on future healthcare is evident by the current assessment of various PSi devices for medical applications in clinical trials [22,23].

Porous Si is typically fabricated by an anodic electrochemical etching of single-crystalline Si wafers in aqueous hydrofluoric acid electrolytes. This process produces a porous layer whose thickness, porosity and average pore size are easily controlled [11,18]. PSi is characterized by several particularly appealing tunable properties predestining it for design of drug delivery platforms: (i) high surface area (up to 800 $m^2 g^{-1}$) that can be functionalized by a wide range of chemical and biological species using a repertoire of reactions, including silanization, hydrosilylation and electrografting; (ii) photonic [24] and photoluminescence [15,25,26] properties for self-reporting drug delivery (e.g. eye implants [27]); (iii) cost-effective and rapid fabrication techniques with the ability to further process into thin membranes and micro- or nanoparticles; and (iv) biocompatibility and most importantly the ability to degrade completely in physiological fluids into non-toxic orthosilicic acid ($Si(OH)_4$), which is the natural form of Si found in the body [28,29]. For in vivo use, PSi behavior may be tuned from bio-inert to bioactive and to biodegradable by varying the pore morphology (porosity and pore size) and surface chemistry. These parameters also affect the loading and release behavior of payloads, e.g. drugs, biomolecules and nanoparticles from PSi hosts, as recently reviewed by Salonen et al. [18] and Anglin et al. [11].

Many studies have shown the loading and in vitro release of different therapeutic and diagnostic agents from PSi (or oxidized PSi) materials, including small drug molecules, such as dexamethasone [30], ibuprofen [19] and the anticancer drugs cisplatin and

* Corresponding author at: Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 4 8295071; fax: +972 4 8293399.
E-mail address: esegal@tx.technion.ac.il (E. Segal).

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doxorubicin [13,14,16,31]. Other payloads include peptides, proteins [32] and nanoparticles [21], with recent studies demonstrating in vivo release [33]. The two prevailing approaches to load molecular payloads into PSi hosts are: (i) adsorption of the drug molecules via specific or nonspecific interactions; and (ii) covalent attachment of the drug molecules to the Si scaffold commonly via hydrosilylation reaction [11,18]. The later method provides a convenient means to trap payloads fairly irreversibly due to the stability of the Si–C bond, formed during hydrosilylation [34]. The payload is only released when the covalent bonds are broken or the supporting Si matrix is degraded [11]. Moreover, these chemical modifications of PSi provide a versatile route for tailoring the dissolution rate of the Si scaffold in aqueous media, and thus dictating the release rates of the drug.

In this work, we tailor the surface properties of nanostructured PSi thin films and demonstrate their applicability as tunable carriers for delivery of mitoxantrone dihydrochloride (MTX), an anthracenedione antitumor drug which has shown significant clinical effectiveness in the treatment of advanced breast and prostate cancers [35,36]. Freshly etched PSi films, fabricated by electrochemical etching of Si wafers, were chemically modified by thermal hydrosilylation of 1-dodecene and undecylenic acid to form dodecyl-terminated and undecanoic acid-terminated PSi carriers, respectively. MTX was loaded within the three types of hosts by physical adsorption or covalent attachment, depending on the surface chemistry of the pore walls. We focus on porous films, still attached to the Si substrate, to allow a careful characterization of the nanostructure and the chemical nature of the carriers (i.e. their porous volume, specific surface area, pore dimensions and hydrophobic/hydrophilic nature). The distinct MTX release profiles exhibited by the different porous films are compared and correlated to the Si scaffold erosion kinetics. The cytotoxic functionality of the released MTX was verified in vitro on human breast carcinoma (MDA-MB-231) cells. We show that loading efficacy and the release behavior are highly affected by the Si surface chemistry and the resulting physiochemical properties of the carrier. Thus, upon proper design nanostructured PSi can be tuned and optimized to release MTX at a rapid or sustained manner. For further clinical applications, the described systems can be applied for carrying different therapeutic cargos and administered both, as an implantable device or processed into injectable particles, for localized cancer therapy. As in most solid cancer cases, e.g. breast cancer, the patient is treated post-operatively (adjuvant therapy) with chemotherapy; an implant that locally elutes the chemotherapeutic agent may potentially improve the antitumor efficacy while minimizing the systemic severe side-effects of these drugs [37].

2. Materials and methods

2.1. Fabrication of porous Si

Mesoporous Si films were fabricated from single-side polished p+⟨100⟩Si wafers (~1 mΩ cm, B-doped, purchased from Siltronix Corp., France) using electrochemical etch process, in a 3:1 (v/v) solution of aqueous HF (48%, Merck, Germany) and ethanol (99.9%, Merck, Germany), at a constant current density of 15 mA cm^{-2} for 225 s. Si wafers with an exposed area of 1.33 cm^2 had a strip of aluminum foil attached to the back side and were mounted in a Teflon etching cell; a platinum spiral coil was used as the counter-electrode. After etching, the surface of the samples was rinsed with ethanol (99.9%, Merck, Germany) several times and dried under a stream of nitrogen gas. These samples are referred to as freshly etched PSi, in this work. (Note: all chemicals were purchased from Sigma–Aldrich unless otherwise mentioned.)

2.2. Hydrosilylation of porous Si

Freshly etched PSi carriers were chemically modified using thermal hydrosilylation of 1-dodecene (⩾95%) or undecylenic acid (⩾95%) inside a customized Schlenk flask to form dodecyl-terminated PSi (d-PSi) or undecanoic acid-terminated PSi (u-PSi), respectively. Briefly, PSi samples were placed under nitrogen in a Schlenk flask containing the deoxygenated neat reagent and allowed to react at 120 °C for 2 h. The resulting samples were thoroughly rinsed with acetone (Frutarom, Israel) and ethanol (99.9%, Merck, Germany) to remove unreacted species from the surface, and then dried under a stream of nitrogen gas.

2.3. Physical characterization of porous Si

2.3.1. Scanning electron microscopy

High-resolution scanning electron microscopy (HR-SEM) micrographs of neat PSi films were obtained using a Carl Zeiss Ultra Plus Field Emission SEM, at an accelerating voltage of 1 keV.

2.3.2. Infrared spectrometry

Surface modification of the PSi is characterized by attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. Spectra were recorded using a Thermo 6700 FTIR instrument equipped with a Smart iTR diamond ATR device.

2.3.3. Advancing and receding contact angle measurements

Contact angle measurements were collected on a minimum of two samples and were measured by a computerized contact angle analyzer (CAM200, KSV), with a reported accuracy of ±0.1°. A micro syringe (Hamilton #81341, 0–1000 μl, Merck Eurolab) was used to create the drop on the surface. Liquid was continuously added to measure the advancing contact angle. To measure the receding contact angle the liquid was continuously removed. The needle was kept in the drop throughout the procedure.

2.3.4. Nitrogen adsorption–desorption measurements

Nitrogen adsorption–desorption isotherms of the different PSi carriers were recorded at 77 K using a Micromeritics ASAP 2010 physisorption instrument. Prior to the adsorption experiment, the samples were degassed in situ at 473 K for 6 h. Nitrogen doses were administered, and the adsorbed amount was registered as a function of the equilibrium pressure. The specific surface area and porous volume values were determined using the Brunauer–Emmett–Teller (BET) model and the pore dimensions were calculated by applying the Barrett–Joyner–Halenda (BJH) method [38,39].

2.4. Loading of MTX within porous Si carriers

2.4.1. Loading MTX into freshly etched PSi and d-PSi carriers

Drug loading was achieved using the impregnation method [40,41]. A volume of 25 μl of MTX dissolved (⩾97%) in dimethyl sulfoxide (DMSO) or methanol (solution concentration 9.66×10^{-3} M) was added to the freshly etched PSi or dodecyl terminated PSi (d-PSi) carriers, respectively, and allowed to infuse into the nanostructure for 2 h. Subsequently, the samples were rinsed with phosphate buffered saline (PBS, pH 7.4) to remove excess free drug followed by overnight drying in a vacuum oven at 30 °C.