Journal of Controlled Release 178 (2014) 46–54

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Tunable sustained intravitreal drug delivery system for daunorubicin using oxidized porous silicon ☆,☆☆

Huiyuan Hou [a,b,1], Alejandra Nieto [a,b,1], Feiyan Ma [a], William R. Freeman [a], Michael J. Sailor [b], Lingyun Cheng [a,*]

[a] Department of Ophthalmology, Jacobs Retina Center at Shiley Eye Center, University of California San Diego, La Jolla, USA
[b] Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, USA

ARTICLE INFO

Article history:
Received 4 October 2013
Accepted 4 January 2014
Available online 11 January 2014

Chemical compounds studied in this article:
Silicon
Silicon dioxide
Daunorubicin hydrochloride
Hydrofluoric acid
Ethanol
3-Aminopropyltriethoxysilane
Succinic anhydride
N,N-dimethylformamide
N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
Nitrogen

Keywords:
Porous silicon
Controlled drug release
Intravitreal drug delivery
Daunorubicin
Rabbit eye

ABSTRACT

Daunorubicin (DNR) is an effective inhibitor of an array of proteins involved in neovascularization, including VEGF and PDGF. These growth factors are directly related to retina scar formation in many devastating retinal diseases. Due to the short vitreous half-life and narrow therapeutic window, ocular application of DNR is limited. It has been shown that a porous silicon (pSi) based delivery system can extend DNR vitreous residence from a few days to 3 months. In this study we investigated the feasibility of altering the pore size of the silicon particles to regulate the payload release. Modulation of the etching parameters allowed control of the nano-pore size from 15 nm to 95 nm. *In vitro* studies showed that degradation of $pSiO_2$ increased with increasing pore size and the degradation of $pSiO_2$ was approximately constant for a given particle type. The degradation of $pSiO_2$ with 43 nm pores was significantly greater than the other two particles with smaller pores, judged by observed and normalized mean Si concentration of the dissolution samples (44.2 ± 8.9 *vs* 25.7 ± 5.6 or 21.2 ± 4.2 μg/mL, $p < 0.0001$). *In vitro* dynamic DNR release revealed that $pSiO_2$–CO_2H:DNR (porous silicon dioxide with covalent loading of daunorubicin) with large pores (43 nm) yielded a significantly higher DNR level than particles with 15 or 26 nm pores (13.5 ± 6.9 ng/mL *vs.* 2.3 ± 1.6 ng/mL and 1.1 ± 0.9 ng/mL, $p < 0.0001$). After two months of *in vitro* dynamic release, 54% of the $pSiO_2$–CO_2H:DNR particles still remained in the dissolution chamber by weight. *In vivo* drug release study demonstrated that free DNR in the vitreous at post-injection day 14 was 66.52 ng/mL for 95 nm pore size $pSiO_2$–CO_2H:DNR, 10.76 ng/mL for 43 nm $pSiO_2$–CO_2H:DNR, and only 1.05 ng/mL for 15 nm $pSiO_2$–CO_2H:DNR. Pore expansion from 15 nm to 95 nm led to a 63 fold increase of DNR release ($p < 0.0001$) and a direct correlation between the pore size and the drug levels in the living eye vitreous was confirmed. The present study demonstrates the feasibility of regulating DNR release from $pSiO_2$ covalently loaded with DNR by engineering the nano-pore size of pSi.

1. Introduction

Proliferative vitreoretinopathy (PVR) is the major vision threatening complication for rhegmatogenous retinal detachment. Proliferation of endogenous retinal cells, such as retinal pigment epithelium (RPE) and glial cells, as well as visiting immune cells at the vitreoretinal interface leads to the formation of vitreoretinal membranes which cause tractional retinal detachment and vision loss [1]. Inhibition of proliferation of these cells by chemotherapeutic agents has been the primary target of PVR prevention [2,3]. Daunorubicin (DNR) is one of the potential therapeutic agents for unwanted ocular proliferation. It has been shown to be effective for treatment of PVR on animal models and in clinical studies [4–7]. However, the short intravitreal half-life and narrow therapeutic window of DNR [8], which imply frequent intravitreal injections over time to obtain sustained treatment, hinder its further clinical application. An optimal ocular drug delivery system, which could provide a sustained and long-lasting presence of DNR at the disease site, would be an ideal solution. For this purpose we have proposed porous silicon (pSi) as a biodegradable carrier for intravitreal drug delivery [9–11]. The nanostructure of pSi provides reservoirs which host therapeutics and provide sustained drug release after a single intravitreal injection. We have demonstrated that intravitreal pSi injection is safe in rabbit eyes [9]. It degrades to completely soluble and excretable orthosilicates [12]. DNR can be covalently loaded into pSi for sustained intravitreal drug delivery as the carrier degrades [10]. We hypothesize that the rate of drug release, as well as the ocular therapeutic duration may be controllable by altering the nano-pore size of the pSi. Previous work has shown that the rate of degradation of pSi in aqueous media can be dependent on pore size

☆ Financial support: This study was supported by the National Institutes of Health under grant number NIH EY020617.
☆☆ Disclosure: W.R. Freeman, Spinnaker Biosciences (C); M.J. Sailor, Spinnaker Biosciences (I); L. Cheng, Spinnaker Biosciences (C).
* Corresponding author at: Department of Ophthalmology, Jacobs Retina Center at Shiley Eye Center, University of California San Diego, 9415 Campus Point Drive, La Jolla 92093-0946, USA. Tel.: +1 858 534 3780; fax: +1 858 534 7985.
E-mail address: cheng@eyecenter.ucsd.edu (L. Cheng).
[1] Both authors contributed equally to this work.

0168-3659/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jconrel.2014.01.003

and surface morphology [13]. Most recently, Martinez et al. demonstrated *in vitro* a positive correlation between pore size and degradation rate of oxidized and 3-aminopropyl triethoxysilane functionalized pSi particles in phosphate buffered saline. However, subsequent quantum dot infiltration loading and release showed that the release rate was negatively associated with the pore size of the pSi particles [14]. In the current study, we investigated the influence and capacity of changing pore size of $pSiO_2$ microparticles on the rate of drug release using DNR as a model drug. We are interested in knowing if the relationship between pore size and pSi degradation would translate into a similar relationship between pore size and daunorubicin release if we use a covalent drug loading strategy instead of infiltration loading which released daunorubicin too fast and caused retinal toxicity [10]. We are also interested in knowing the capacity of this tenability in a living eye to gauge the feasibility of this intravitreal drug delivery system to prevent and treat PVR.

2. Material and methods

2.1. Synthesis of pSi microparticles

pSi microparticles were prepared by anodic electrochemical etch of highly doped, (100)-oriented, p-type silicon wafers (Siltronix Inc., Archamps, France, boron-doped, 1.00 ± 0.01 mΩ·cm resistivity), as previously described [10,15]. A silicon wafer with an exposed area of 8.04 cm^2 was contacted on the backside with a strip of aluminum foil and mounted in a Teflon etching cell that was fitted with a platinum counter-electrode. The etching conditions to obtain pSi microparticles with different pore sizes, including current density and etch duration, are summarized in Table 1. Particles A and B were etched in a 3:1 (v/v) solution of 48% aqueous hydrofluoric acid (HF) and absolute ethanol (Fisher-Scientific, Pittsburg, PA). Particles C and D were etched in a 1:1 (v/v) solution of 48% aqueous HF and absolute ethanol. The wafers were etched at a constant current density (mA/cm^2), and the porous layer resulting from every etch was removed from the silicon substrate by electropolishing in a 1:29 solution of 48% aqueous HF and absolute ethanol for120 s (Table 1). The etching and electropolishing procedure was repeated 20 times per wafer. The films were harvested every 4 etches and the resulting porous layers were ultrasonicated in ethanol (FS5 dual action ultrasonic cleaner, Thermo Fisher Scientific, Pittsburg, PA) for 30 min to form the pSi particles. After ultrasonic treatment, the supernatant was removed and the particles were resuspended in ethanol. This procedure was repeated a total of three times until the supernatant was transparent. The pSi particles were isolated, dried at room temperature and stored under vacuum in a desiccator.

2.2. DNR loading into porous silica ($pSiO_2$) microparticles

DNR was loaded into particles A, C and D by covalent attachment by creating a chemical bond between the drug and functional groups placed on the particle surface, as previously described [10]. Briefly, pSi particles were placed in a ceramic boat, heated from room temperature to 800 °C inside a muffle furnace (Thermo Fisher Scientific, Pittsburg, PA), and maintained at 800 °C for 1 h in order to fully oxidize pSi to $pSiO_2$. The samples were removed from the chamber after the furnace had cooled to room temperature. The resulting $pSiO_2$ particles were then treated with an aqueous HCl solution (2% concentrated HCl by volume) for 1 h, rinsed three times with water and dried. The particles were then vortexed in a 1% 3-aminopropyltrimethoxysilane (Sigma-Aldrich) in ethanol solution for 1 h, rinsed with ethanol, and dried, resulting in alkylamine-modified particles. The amine-functionalized porous SiO_2 particles were reacted with 0.1 M succinic anhydride (99%, Sigma-Aldrich) in N,N-dimethylformamide (DMF, Sigma-Aldrich) for 16 h and rinsed with water to obtain a carboxylic acid functional surface ($pSiO_2$–CO_2H). The carboxylic acid group resulted from the ring opening of succinic anhydride through reaction with the amine group on the surface of the particles [10,16]. The surface carboxyl species were then activated by treatment with an aqueous solution containing 68 mM N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich; 8.45 mg) and 6.5 mM N-hydroxysulfosuccinimide (Sulfo-NHS, Pierce Biotechnology Inc., Rockford, IL; 0.92 mg). The coupling reagents were added to a dispersion of $pSiO_2$–CO_2H particles in aqueous phosphate buffered saline (PBS, pH 7.4, Fisher Scientific) containing 10% dimethyl sulfoxide by volume (DMSO, Alfa Aesar, Ward Hill, MA). DNR was then coupled to the activated surface by addition of an aqueous solution (200 μL) containing 1 mg/mL daunorubicin hydrochloride (Tocris Biosciences, Bristol, UK) to the particle mixture, as previously described [10]. After the loading procedure, the particles were pelletized by centrifugation and carefully rinsed with ethanol five times until the washing solution was close to transparent in order to remove unloaded drug and any excess cross linkers. At this point, the particles were observed to have changed from an initial white translucent appearance to the deep orange color of DNR. Average particle size and pore size of the $pSiO_2$ particles were determined from scanning electron microscope (SEM) plan-view images from randomly selected particles ($n > 5$) using a Phillips XL30 field emission electron microscope operating at an accelerating voltage of 5 kV (FEI Phillips, Hillsboro, OR) [10,17]. The particle thickness and open porosity were calculated by optical measurements of the reflectivity spectrum as a function of liquid infiltration using the spectroscopy liquid infiltration method (SLIM) [18]. The textural properties of the $pSiO_2$ particles were analyzed by nitrogen sorption at −196 °C on an ASAP 2020 porosimetry apparatus (Micromeritics, Norcross, GA). Prior to the sorption experiment, approximately 50 mg of the porous silicon sample was outgassed overnight at 105 °C. The specific surface area (m^2/g) and pore volume (cm^3/g) of the particles were calculated from the N_2 adsorption/desorption isotherms by using the BET (Brunauer–Emmett–Teller) and BJH (Barrett–Joyner–Halenda) methods, respectively [19–21].

The presence of the functional linker on the $pSiO_2$–CO_2H surface as well as the successful covalent attachment of DNR to the microparticles was confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy using a Nicolet 6700 FT-IR spectrometer with Smart-ATR attachment (Nicolet Instruments Inc., Madison, WI). Drug loading efficiency of each type of particle ($pSiO_2$–CO_2H:DNR) was analyzed by thermogravimetry (TGA). The DNR-loaded samples (~2 mg) were placed in a 90 μL alumina sample cup. Samples were heated at a constant rate of 10 °C/min up to 800 °C in nitrogen atmosphere with a purge rate of 10 mL/min using a Q600 simultaneous TGA/DSC apparatus (TA Instruments, New Castle, DE). Weight percent loading efficiency of DNR in the samples was determined by analyzing TGA curves of $pSiO_2$–CO_2H ($pSiO_2$ with carboxylic acid functional surface) as well as $pSiO_2$–CO_2H:DNR ($pSiO_2$ containing DNR covalently attached to the pore walls), as shown previously [10,11].

Table 1
Parameters of pSi microparticles synthesis.

Particle	Etch current density (mA/cm^2)	Etching solution (48% HF: Ethanol)	Etch duration (s)	Lift-off current density (mA/cm^2)	Lift-off duration (s)
A	90.2	3:1	300	6.2	120
B	300	3:1	120	6.2	120
C	60	1:1	480	6.2	120
D	70	1:1	400	6.2	120

HF: hydrofluoric acid.
s: seconds.

2.3. In vitro degradation test of empty $pSiO_2$ particles with different pore sizes

The effect of the different textural characteristics of the $pSiO_2$ particles on *in vitro* degradation behavior was examined by immersing particles A,