Published in final edited form as:
Exp Eye Res. 2013 November ; 116: . doi:10.1016/j.exer.2013.09.001.

Ocular silicon distribution and clearance following intravitreal injection of porous silicon microparticles

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

Lingyun Cheng: cheng@eyecenter.ucsd.edu

[a]Jacobs Retina Center at Shiley Eye Center at University of California, San Diego, USA

[b]Department of Chemistry and Biochemistry, University of California, San Diego, USA

Abstract

Porous silicon (pSi) microparticles have been investigated for intravitreal drug delivery and demonstrated good biocompatibility. With the appropriate surface chemistry, pSi can reside in vitreous for months or longer. However, ocular distribution and clearance pathway of its degradation product, silicic acid, are not well understood. In the current study, rabbit ocular tissue was collected at different time point following fresh pSi (day 1, 5, 9, 16, and 21) or oxidized pSi (day 3, 7, 14, 21, and 35) intravitreal injection. In addition, dual-probe simultaneous microdialysis of aqueous and vitreous humor was performed following a bolus intravitreal injection of 0.25 mL silicic acid (150 μg/mL) and six consecutive microdialysates were collected every 20 min. Silicon was quantified from the samples using inductively coupled plasma-optical emission spectroscopy. The study showed that following the intravitreal injection of oxidized pSi, free silicon was consistently higher in the aqueous than in the retina (8.1 ± 6.5 vs. 3.4 ± 3.9 μg/mL, $p = 0.0031$). The area under the concentration-time curve (AUC) of the retina was only about 24% that of the aqueous. The mean residence time was 16 days for aqueous, 13 days for vitreous, 6 days for retina, and 18 days for plasma. Similarly, following intravitreal fresh pSi, free silicon was also found higher in aqueous than in retina (7 ± 4.7 vs. 3.4 ± 4.1 μg/mL, $p = 0.014$). The AUC for the retina was about 50% of the AUC for the aqueous. The microdialysis revealed the terminal half-life of free silicon in the aqueous was 30 min and 92 min in the vitreous; the AUC for aqueous accounted for 38% of the AUC for vitreous. Our studies indicate that aqueous humor is a significant pathway for silicon egress from the eye following intravitreal injection of pSi crystals.

Keywords

intravitreal porous silicon; ocular silicon clearance; ocular drug delivery; rabbit eye

1. Introduction

Retinal diseases are often chronic and refractory to treatment. Two unique challenges in developing drugs against these diseases derive from the inherent barriers that prevent drugs in the blood from reaching the retina and from the short vitreous half-life of drugs

*Corresponding author. Department of Ophthalmology, Jacobs Retina Center at Shiley Eye Center, University of California, San Diego, 9415 Campus Point Drive, La Jolla, CA 92093-0946, USA. Tel.: +1 858 534 3780; fax: +1 858 534 7985.
[1]Both authors contributed equally to this work.

Financial disclosure: A. Nieto, None; H. Hou, None; M.J. Sailor, Spinnaker Biosciences (I); W.R. Freeman, Spinnaker Biosciences (C); L. Cheng, Spinnaker Biosciences (C).

administered via intravitreal injection. This makes necessary frequent drug administration if directly injected into the eye, entailing the risk of infection and other complications. A major focus in our research is to devise strategies for bypassing this barrier and developing ocular drug delivery systems able to provide sustained drug levels to the retina (Cheng et al., 2004; Chhablani et al., 2013).

Mesoporous silicon microparticles have been proposed as a drug delivery vehicle to achieve a slow drug release and a long-lasting therapeutic effect. (Anglin et al., 2008; Salonen et al., 2008), (Kashanian et al., 2010) In general, porous silicon has demonstrated good biocompatibility. (Bimbo et al., 2010) However, biocompatibility is dependent on location and tissue types (Leoni et al., 2002; Park et al., 2009) as well as surface chemistry of the porous silicon particles. (Low et al., 2006) The eye is a unique organ with clear media that offers a direct view of compounds or delivery systems injected into the vitreous. The eye is especially suitable for a porous silicon drug delivery system because the direct imaging of porous silicon particles may enable non-invasive monitoring of payload release from the nanoscale pores. (Wu et al., 2011) We previously showed that intravitreally injected porous silicon particles were not toxic in rabbit eyes, showing good biocompatibility and slow elimination from the body. (Cheng et al., 2008) Elimination of micron-size particles of porous silicon from the eye necessarily involves degradation of the material into water-soluble products. The degradation of porous silicon *in vivo* involves two main processes: oxidation of the elemental silicon component and dissolution of the silicon oxide thus formed. The soluble silicon-containing species are various protonated forms of the orthosilicate ion, SiO_4^{4-}, and its oligomers. The form of orthosilicate that predominates at neutral pH is silicic acid $Si(OH)_4$, which is naturally found in numerous tissues. Silicon is considered an essential trace element and in aqueous environment at neutral pH, $Si(OH)_4$ is excreted from the body through the urine (Mertz, 1981). For the purpose of ocular drug delivery, a significant quantity of porous silicon particles needs to be placed in the vitreous. It is therefore important to assess the fate of the silicic acid degradation product of the particles. The ocular distribution and clearance pathway of silicic acid has not yet been documented. This issue is not only of academic interest, but it is also a practical issue to better understand this ocular drug delivery system and possible safety issues associated with higher doses and longer-term exposure of ocular tissues involved in the clearance pathway of silicic acid. Theoretically, porous silicon particles degrade in the vitreous and the final product, silicic acid, may be removed from the eye through the retina to the choroid then to systemic circulation or through the aqueous humor to Schlemm's canal and then to systemic circulation. The surface chemistry plays an important role in the rate at which particles degrade; as-prepared porous silicon degrades faster than material whose surface has been modified by oxidization or hydrosilylation (Cheng et al., 2008). The purpose of the present study was to determine the ocular elimination kinetics and pathways of silicon clearance from the eye for two important formulations of porous silicon microparticles: as-prepared particles (pSi) which consist of Si–Si and Si–H bonds, and thermally oxidized porous silicon particles ($pSiO_2$) which contain Si–O–Si bonds as the primary structural motif. The correlation of pSi degradation with drug release will depend on individual drug loaded such as daunorubicin shown previously by us (Wu et al., 2011). However, the silicon clearance pathway is independent to payload and is the objective of the current study.

2. Materials and methods

Porous silicon (pSi) microparticle preparation

Porous Si (pSi) microparticles were prepared by anodic electrochemical etch of highly doped, (100)-oriented, p-type silicon wafers (boron-doped, 1.10 mΩ·cm resistivity; obtained from Siltronix Inc., Archamps, France), in an electrolyte consisting of a 3:1 (v:v) solution of

48% aqueous hydrofluoric acid (HF) and ethanol (Fisher-Scientific, Pittsburg, PA). A Si wafer with an exposed area of 8.04 cm^2 was contacted on the back side with a strip of aluminum foil and mounted in a Teflon etching cell that was fitted with a platinum counter electrode. The wafer was etched using a current density waveform (J) previously described: (Wu et al., 2011) $J = A_0 + A \cdot \cos(kt + a)$, where A_0 is current density offset (mA/cm^2), A is current density amplitude (mA/cm^2), k is frequency (s^{-1}), t is time (s), and a is phase shift (s^{-1}). The values used for A_0, A, and a were 90.2 mA/cm^2,12.4 mA/cm^2, and 0, respectively. The current density waveform generates a porosity modulation in the porous silicon layer that acts as a 1-dimensional photonic crystal. (Vincent, 1994), (Thonissen and Berger, 1997) The photonic crystal displays a sharp peak in the optical reflectance spectrum whose wavelength is directly proportional to k. The value of k was adjusted to yield a reflectance peak whose maximum occurred in the spectrum at ~600 nm; a typical value for k was 2.25. The waveform was etched into the silicon wafer for a total of 400 s. The resulting porous layer was then removed from the silicon substrate by replacing the electrolyte with a 1:29 (v:v) solution of 48% aqueous hydrofluoric acid and ethanol and then applying an anodic pulse (6.2 mA/cm^2) for 120 s. Each wafer was subjected to four such etching protocols, and the porous layers were collected and ultrasonicated in ethanol for 30 min in an FS5 dual action ultrasonic cleaner (Thermo Fisher Scientific, Pittsburg, PA), then rinsed with ethanol 3 times.

Porous silicon oxide ($pSiO_2$) microparticle preparation

The $pSiO_2$ formulation was prepared from pSi by air oxidation in a ceramic boat inside a muffle furnace (Thermo Fisher Scientific, Pittsburg, PA). The temperature in the furnace was increased from room temperature to 800 °C at a heating rate of 10 °C min^{-1} and then held in air for 1 h. The furnace was allowed to cool to room temperature for an additional 3 h prior to removal of the samples. The oxidation reaction was accompanied by a color change of the particles from brown to transparent, corresponding to conversion of Si to SiO_2 in the porous matrix. However, the particle size and shape were not affected by the oxidation procedure. For sustained ocular drug delivery purposes, we have found that oxidized porous silicon shows a longer vitreous half-life (Cheng et al., 2008) and the surface is more amenable to covalent functionalization for subsequent drug grafting (Chhablani et al., 2013).

Physical characterization of porous microparticles

Average particle size and pore size were determined from plan-view images of randomly selected particles ($n > 10$) using a Phillips XL30 field emission scanning electron microscope operating at an accelerating voltage of 5 kV (FEI Phillips, Hillsboro, OR). Surface chemistry was characterized by Fourier transform infrared (FTIR) spectroscopy using attenuated total reflectance (ATR) mode on a Nicolet 6700 Smart-iTR spectrometer (Thermo Fisher Scientific, Pittsburg, PA). The textural properties of the particles were analyzed by nitrogen adsorption at −196 °C on an ASAP 2020 porosimetry apparatus (Micromeritics, Norcross, GA). Prior to the adsorption experiment, approximately 50 mg of the porous Si sample was outgassed overnight at 105 °C. The specific surface area and pore volume of the particles were calculated from the N_2 adsorption isotherms using the BET (Brunauer-Emmett-Teller) and BJH (Barrett-Joyner-Halenda) methods, respectively (Gregg and Sing, 1982), (Brunauer et al., 1938; M. Kruk and Jaroniec, 1999).

Animal studies

All animal experiments were carried out in adherence to ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

1) Ocular pharmacokinetic tissue sampling: Forty pigmented rabbits were used for the study and only one eye of each rabbit was used for microparticle intravitreal injection. Out of 40

rabbits, 20 were used for the fresh porous silicon (pSi) particle study and the other 20 were used for the oxidized porous silicon ($pSiO_2$) particle study. For the pSi particle study, 1 mg of pSi particles in 100 μL of balanced salt solution (BSS; Thermo Fisher Scientific, Pittsburg, PA) was injected into the right eye of each of the 20 rabbits using a 27 gage needle. At the post-injection time points day 1, 5, 9, 16, and 21, four rabbits were subjected to a comprehensive eye exam, including anterior segment biomicroscopy, posterior indirect ophthalmoscopy and intraocular pressure (IOP) measurements, before the planned sacrifice. Four animals at each time point were used to account for the inherent variation of pSi microparticle suspension concentration injected as well as variation between individual animals. Immediately after sacrifice, the enucleated eye globes were dissected individually into aqueous, vitreous, and retina as described previously (Cheng et al., 2004; Nan et al., 2010). For the $pSiO_2$ particle study, 2 mg of particles in 100 μL of BSS were injected with a 27 gage needle and a longer study course was performed because of the slower dissolution property of $pSiO_2$ microparticles (Cheng et al., 2008). The rabbits injected with the $pSiO_2$ particles were sacrificed at post-injection day 3, 7, 14, 21, and 35, four animals at each time point. Prior to both intravitreal injection and the scheduled sacrifice, 1 mL of blood was sampled for silicon quantitation. The collected eye tissues and plasma were kept at −80 °C until the quantitation of Si (as dissolved silicic acid) was performed by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2) Simultaneous microdialysis of aqueous and vitreous humor: To better understand the distribution and elimination of orthosilicate from the eye, a dual-probe microdialysis was performed as previously described (Chen et al., 2013). For this acute study, one rabbit was used (3.7 kg body weight). The rabbit had a comprehensive eye exam before the microdialysis experiment. After general anesthesia with intramuscular ketamine (35 mg/kg body weight) and xylazine (6.25 mg/kg body weight) and topical anesthesia with proparacaine hydrochloride (Ophthalmic solution USP 0.5% (wt/vol); Bausch & Lomb, Rochester, NY), an anterior chamber microdialysis probe (CMA 30 linear, 4 mm membrane length custom made, 6000 Da molecular weight cut-off; CMA Microdialysis, North Chelmsford, MA) was installed followed by installation of a vitreous microdialysis probe (CMA 20 Elite, 4 mm membrane length, 20,000 Da molecular weight cut-off; CMA Microdialysis, North Chelmsford, MA). Tissue adhesive (Vetbond 1469SB; 3M Corporation, St Paul, MN) was applied around the probe entry point to prevent ocular fluid leaks. Thirty minutes after the installation of the probes, intravitreal injection of 0.250 mL of silicic acid solution (150 μg/mL) was performed to yield final vitreous concentration of 25 μg/mL, assuming rabbit vitreous volume to be 1.5 mL The saturated stock silicic acid solution was prepared by dissolving 5 mg pure silicic acid crystals (Spectrum Laboratory Products, Gardena, CA) in 10 mL 50 mM sodium hydroxide (NaOH; Sigma–Aldrich, St Louis, MO) in phosphate buffered saline solution (PBS; Thermo Fisher Scientific, Pittsburg, PA) and incubated at 37 °C for one week. The solution pH was adjusted to 7 with hydrochloric acid (HCl; Sigma–Aldrich, St Louis, MO) before intravitreal injection of the supernatant. During the microdialysis experiment, both probes were perfused with PBS at 1 μL/min using a microsyringe pump (NE-100; New Era Pump Systems Inc, Farmingdale, NY). The vitreous and aqueous perfusate samples were collected every 20 min for total of 6 collections. During the course of the microdialysis experiment, the rabbit remained on a water blanket (TP650; Gaymar Industries, Orchard Park, NY) to help prevent body heat loss and anesthesia was maintained by boosting every 40 min using one half volume of the first dose. Every other dose was ketamine only starting with the first boost because xylazine stays in the system longer (Veilleux-Lemieux et al., 2012). At the end of the microdialysis procedure, a blood sample was acquired before the animal was sacrificed and the eye globe enucleated. The eye globe was dissected under a surgical microscope to sample aqueous humor, vitreous humor, and retina. These eye tissues were separately stored at −80 °C until silicon was quantitated using ICP-OES.

After the *in vivo* microdialysis experiment, the probes were placed in deionized water (WFI-quality, cell culture grade, Cellgro, Manassas, VA) for continued flushing overnight to clean the silicic acid from the system before performing a test to determine the recovery rate of silicon for the probes. For this purpose, each probe was soaked in a vial of 25 (μg/mL silicic acid solution and the perfusate was collected every 20 min for 6 consecutive times.

Silicon quantification

To determine the silicon content as dissolved silicon (silicic acid) in the aqueous and vitreous, the collected fluid samples were weighed and centrifuged at 11,000 rpm for 10 min. Aqueous fluid supernatant (0.1 mL) was diluted with 2% (v/v) nitric acid aqueous solution to make a final volume of 3 mL (HNO_3; EMD, Darmstad, Germany). Vitreous fluid supernatant (0.07 mL) was diluted with 2% (v/v) HNO_3 aqueous solution to make a final volume of 3 mL (Park et al., 2009), (Dyck et al., 2000; Hauptkorn et al., 2001; Wills et al., 2008) To determine the silicon content in the retina tissue, the wet samples were weighed and digested with hydrogen peroxide 30% (w/w) (0.05 mL) (H_2O_2; Sigma–Aldrich, St. Louis, MO) and concentrated $HNO_3 \sim 15.7$ M (0.250 mL) for 2 days. To determine the silicon concentration in plasma, 0.150 mL of sample was digested with a solution consisting of 0.025 mL of 30% (by mass) aqueous H_2O_2 and 0.125 mL of concentrated HNO_3 for 2 days. Mechanical homogenization of the tissue samples was performed with a pellet pestle (Sigma–Aldrich, St. Louis, MO) applied for 10 s after addition of the digesting reagents, resulting in a homogeneous slurry. The digested tissues were centrifuged at 11,000 rpm for 10 min and the supernatant (0.1 mL) was diluted with 2% (v/v) HNO_3 aqueous solution to make a final volume of 3 mL This open vessel digestion procedure with HNO_3–H_2O_2 was designed to ensure that as much of the analyte that is available for recovery is rendered soluble and relatively stable in aqueous acidic medium. Silicon calibration standard solutions were prepared from a stock solution containing 1000 μg/mL Si (Trace Cert Silicon standard for ICP in nitric acid; Fluka, Milwaukee, WI). External calibration standard solutions were prepared in 2% (v/v) HNO_3 solution in water (WFI-quality, cell culture grade; Cellgro, Manassas, VA) by serial dilution to reach final concentrations 8, 4, 2,1, 0.5, 0.1 and 0.01 μg/mL Calibration standards and samples were prepared in polystyrene tubes. Normal rabbit aqueous, vitreous, retina and plasma from rabbits that had not received any injection of pSi or $pSiO_2$ particles (InVision BioResources, Seattle, WA) were treated following the same procedure as the samples and used as controls to determine the baseline levels of silicon.

Soluble silicon, in the form of protonated orthosilicate species, was detected by inductively coupled plasma-optical emission spectroscopy (ICP-OES) in an argon plasma spectrometer (Optima 3000 DV; Perkin–Elmer, Norwalk, CT) equipped with a standard torch, Scott-type spray chamber, GemTip cross-flow nebulizer and an AS-90 autosampler (Perkin–Elmer, Norwalk, CT). The ICP-OES instrument parameters are summarized in Table 1.

Data analysis

Pharmacokinetic (PK) parameters of silicon content in ocular tissues and plasma were calculated using non-compartmental methods and sparse sampling setting within the Phoenix® WinNonlin® software (version 6.3; Pharsight Corp, Mountain View, CA). The area under the curve from time zero to the last measurable concentration (AUC_{0-t}) was calculated. A terminal rate constant of elimination was calculated using a minimum of three measurable concentrations and a terminal elimination half-life was calculated using 0.693/*k*el. The pharmacokinetic parameters of silicon content from the microdialysis of vitreous and aqueous humor were calculated using the PK software module using a one-compartment model and IV-Bolus input setting. For comparison of silicon concentrations detected among the ocular tissues, the data from all the time points of the same tissue were pooled and

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.