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UNIVERSITY OF CALIFORNIA, SAN DIEGO

**CHEMICALLY MODIFIED AND NANOSTRUCTURED POROUS SILICON AS A
DRUG DELIVERY MATERIAL AND DEVICE**

A Dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Chemistry

by

Emily Jessica Anglin

Committee in charge:

Professor Michael J. Sailor, Chair
Professor Seth M. Cohen
Professor Sadik C. Esener
Professor David N. Hendrickson
Professor Alexander Hoffmann

2007

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The dissertation of Emily Jessica Anglin is approved, and it is acceptable in quality and form for publication on microfilm:

Chair

University of California, San Diego

2007

DEDICATION

To my family and friends

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Anglin, E.J., Cunin, F., Devoisselle, J-M., Tourne-Peteilh ,C., Cheng, L. Freeman, W.R., Sailor, M.J., Sustained release of a monoclonal antibody from electrochemically fabricated porous silica. (Manuscript in preparation)

Cheng, L., Anglin, E.J., Cunin, F., Kim, D., Sailor, M.J., Falkenstein, I., Tammewar, A., Freeman, W.R., Intravitreal properties of porous silicon photonic crystals: a potential self-reporting intraocular drug delivery vehicle. (Manuscript submitted).

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3. Anglin, E.J., Cunin, F., Devoisselle, J-M., Tourne-Peteilh ,C., Cheng, L. Freeman, W.R., Sailor, M.J., Sustained release of a monoclonal antibody from electrochemically fabricated porous silica. (Manuscript in preparation)
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5. Cunin, F., Milhiet, P-M., Anglin, E., Sailor, M.J., Espenel, C., Le Grimellec, C., Brunel, D., Devoisselle, J-M., Continuous planar phospholipid bilayer supported on porous silicon thin film reflector. *Ultramicroscopy* (2007) doi:10.1016/j.ultramic.2007.02.041 (Manuscript in press)
6. Freeman, W.R., Sailor, M.J., Cheng, L., Cunin, F., Anglin, E.J., Li, Y.Y., Porous photonic crystals for drug delivery to the eye. *PCT. Int. Appl.* (2006), 26 pp.
7. Anglin, E.J., Schwartz, M.P., Ng, V.P., Perelman, L.A., Sailor, M.J., Engineering the chemistry and nanostructure of porous silicon Fabry-Perot films for loading and release of a steroid. *Langmuir* (2004), 20(25), 11264-11269.
8. Harris, J.D., Anglin, E.J., Hepp, A.F., Bailey, S.G., Sheiman, D.A., Castro, S.L., Space environmental testing of dye-sensitized solar cells. *Proceedings of the 6th European Space Power Conference*, Porto, Portugal, (2002), 629-632.
9. Bailey, S.G., Harris, J.D., Hepp, A.F., Anglin, E.J., Raffaele, R.P., Clark, H.R., Gardner, S.T.P., Sun, S.S., Thin-film organic based solar cells for space power. *Proceedings of the 37th Intersociety Energy Conversion Engineering Conference*, Washington, D.C. (2002), Paper # 20154.

ABSTRACT OF THE DISSERTATION

CHEMICALLY MODIFIED AND NANOSTRUCTURED POROUS SILICON AS A DRUG DELIVERY MATERIAL AND DEVICE

by

Emily Jessica Anglin

Doctor of Philosophy in Chemistry

University of California, San Diego, 2007

Professor Michael J. Sailor, Chair

This thesis describes the fabrication, chemical modification, drug release, and toxicity studies of nanostructured porous silicon for the purposes of developing a smart drug delivery device.

The first chapter is an introductory chapter, presenting the chemical and physical properties of porous silicon, the concepts and issues of current drug delivery devices and materials, and how porous silicon can address the issues regarding localized and controlled drug therapies.

The second chapter discusses chemical modifications of nanostructured porous Si for stabilizing the material in biologically relevant media while providing an extended release of a therapeutic *in vitro*. This chapter also demonstrates the utility of the porous silicon optical signatures for effectively monitoring drug release from the system and its applications for development of a self-reporting drug delivery device.

In chapter three, the concept of providing a triggered release of a therapeutic from porous silicon microparticles through initiation by an external stimulus is demonstrated. The microparticles are chemically modified, and the release is enhanced by a short application of ultrasound to the particulate system. The effect of ultrasound on the drug release and particle size is discussed.

Chapter four presents a new method for sustaining the release of a monoclonal antibody from the porous matrix of porous SiO₂. The therapeutic is incorporated into the films through electrostatic adsorption and a slow release is

observed *in vitro*. A new method of quantifying the extent of drug loading is monitored with interferometry.

The last chapter of the thesis provides a basic *in vivo* toxicity study of various porous Si microparticles for intraocular applications. Three types of porous Si particles are fabricated and studied in a rabbit eye model. The toxicity studies were conducted by collaborators at the Shiley Eye Center, La Jolla, CA. This work, demonstrates the feasibility of developing a self-reporting, extended release drug delivery system using porous Si microparticles for intraocular applications.

CHAPTER ONE

INTRODUCTION

1.1 Introduction to Porous Si

Porous Si was first fabricated in the 1950's by Arthur Uhlir of Bell Laboratories. He was attempting a method of electrolytic etching in order to shape and polish semiconducting materials.^{1,2} His efforts to electrolytically polish Si failed, and instead, he formed an intricate mesoporous variation of Si. The potentials for a porous semiconducting material seemed to go unrecognized until the latter part of the 1980's and into the 1990's when photoluminescence from porous Si was discovered.³ Since then, porous Si has been investigated for numerous applications including microelectronics,⁴ optoelectronics,⁵⁻⁷ chemical⁸⁻¹⁵ and biological¹⁶⁻¹⁹ sensors, and biomedical devices.²⁰⁻²⁵

1.2 Fabrication of Porous Si

The work defined in this thesis concentrates on the use of nanoporous Si, a product of an electrochemical anodization of single crystalline Si wafers with a hydrofluoric acid electrolyte solution. Pore morphology and pore size can be varied by controlling the current density, the type and concentration of dopant, orientation of the crystalline Si, and the electrolyte concentration in order to form macro-, meso-, and micropores.¹ For the present research described here, (100)-oriented, boron doped Si was electrochemically etched using a 48% aqueous HF and ethanol electrolyte (3:1 by volume) to produce the porous nanostructure

(Figure 1.1). Pore sizes ranging from ~1-20 nm and various porosities were obtained. While the formation of porous Si is not completely understood, a general mechanism has been proposed (Figure 1.2).²⁶ The electrochemically driven reaction consists of a boron doped single crystalline silicon working electrode, a hydrofluoric acid electrolyte, and Pt counter electrode. Upon application of current, Si is attacked by a fluoride ion of the hydrofluoric acid electrolyte solution. Consequent attack of the Si occurs which replaces the reactive hydrides on the Si surface releasing elemental hydrogen. Pore formation occurs as the Si atoms are displaced in the form of SiF_4 and SiF_6^{2-} . The electron micrograph (Figure 1.3) displays the porous nanostructure as a cross-sectional view of a typical porous Si film.

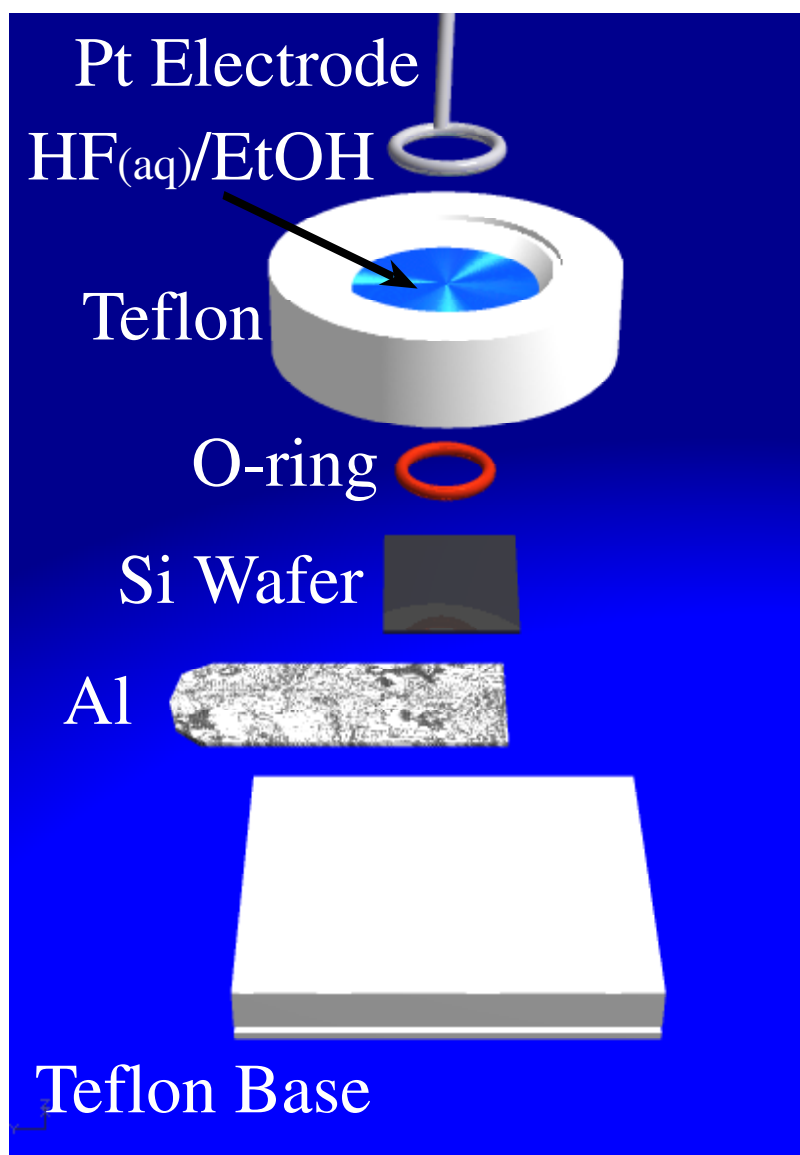


Figure 1.1 Schematic of the electrochemical setup.

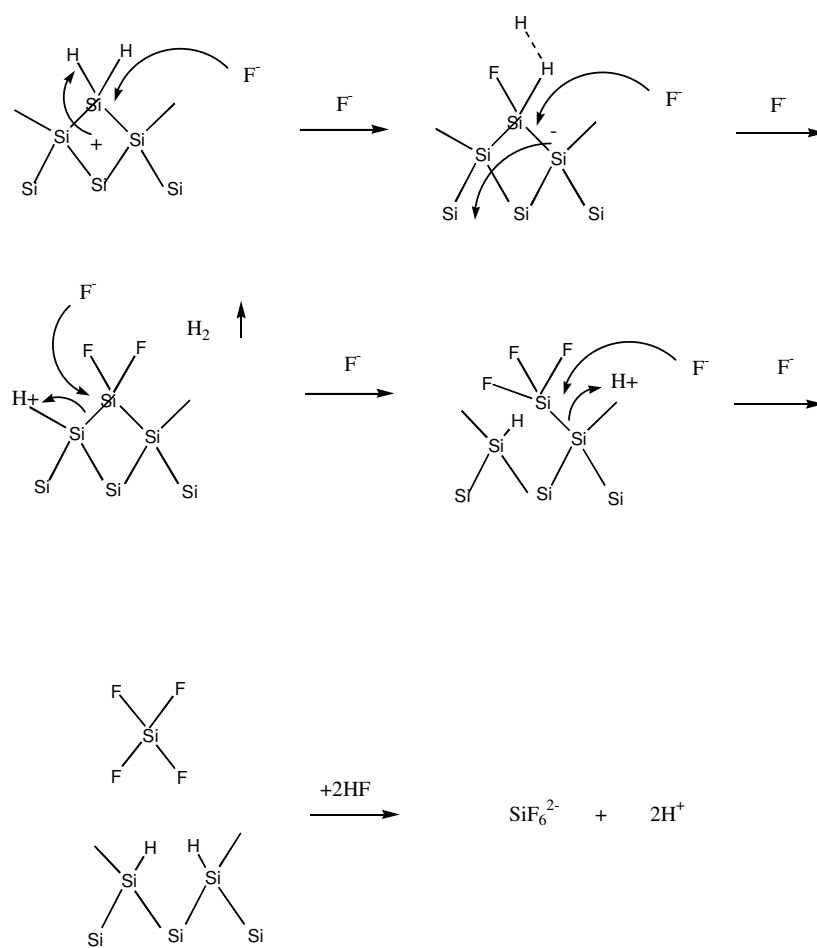


Figure 1.2 Mechanism of porous Si pore formation (adapted from reference 28).

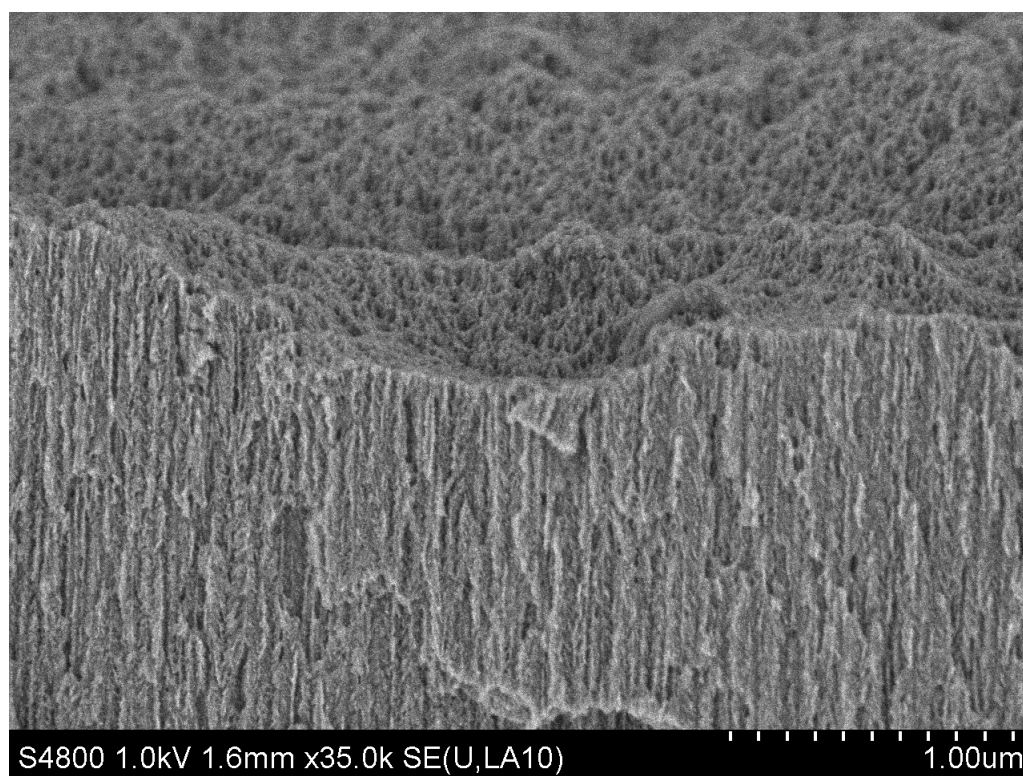


Figure 1.3 Scanning electron microscope image of a single layer porous Si film. This image was taken at a $\sim 60^\circ$ angle to obtain cross-sectional view while simultaneously observing pore entrances from a top view.

1.3 Optical Properties of Porous Si

Varying the etch conditions by applying either a constant current pulse or sinusoidal modulation of current yields a tunable signal in the reflectivity spectrum. Upon illumination of the films with white light at an angle incident to the film, an interference pattern can be observed with the aid of a CCD spectrometer. Depending on the electrochemical etch conditions, various nanostructures can be fabricated within the film including a rugate filter or Fabry-Pérot film (Figure 1.4).²⁷ For Fabry-Pérot interference, the peak maxima and minima represent constructive and destructive interference, respectively, from the porous silicon/air and porous silicon/crystalline silicon interfaces. The interference patterns can also be tuned to resonate as a single reflectance signal known as a rugate (Figure 1.5). A Si-based rugate filter can be fabricated by applying a sinusoidal variation of current during the etch process, and this type of spectral peak can be conveniently tuned to resonate many different wavelengths in the visible spectrum.

The observable changes in the Fabry-Pérot interference patterns upon incorporation of analyte into the film allows one to monitor the infusion or diffusion of analyte from the porous matrix. The observable changes obey the Fabry-Pérot interference relationship represented by equation 1 for normal incidence, where m is the spectral order of the fringe at wavelength λ .²⁸ The composite refractive index of the film corresponds to n , and L is the thickness of

the film. The constant 2, is a result of the pathlength of light that travels into and out of the film to the spectrometer for analysis. In this work, the effective optical thickness is referred to as $2nL$.

Equation 1.1 $m\lambda = 2nL$

As analyte infuses or diffuses from the porous matrix, the refractive index (n) of the film changes, thus shifting the interference patterns in a predictable fashion. For Fabry-Perot films, Fourier transform analysis of the reflection spectrum (intensity vs. frequency) gives a single peak that corresponds directly to the film's effective optical thickness, and in turn, correlates to the amount of drug loading or release. As for porous Si rugate films, due to the sophistication of the computer generated sinusoidal waveform applied during fabrication of the rugate nanostructure, the Fourier transform is essentially built into the material and does not require the aid of a Fourier transform for analysis of spectral shifts.

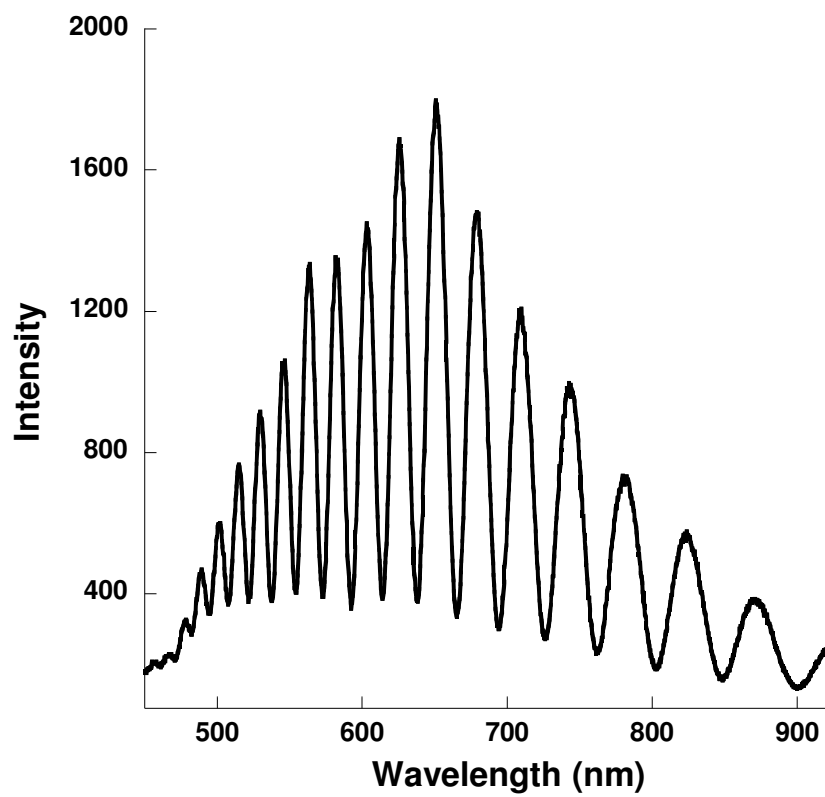


Figure 1.4. Reflectance spectrum of a Fabry-Pérot film.

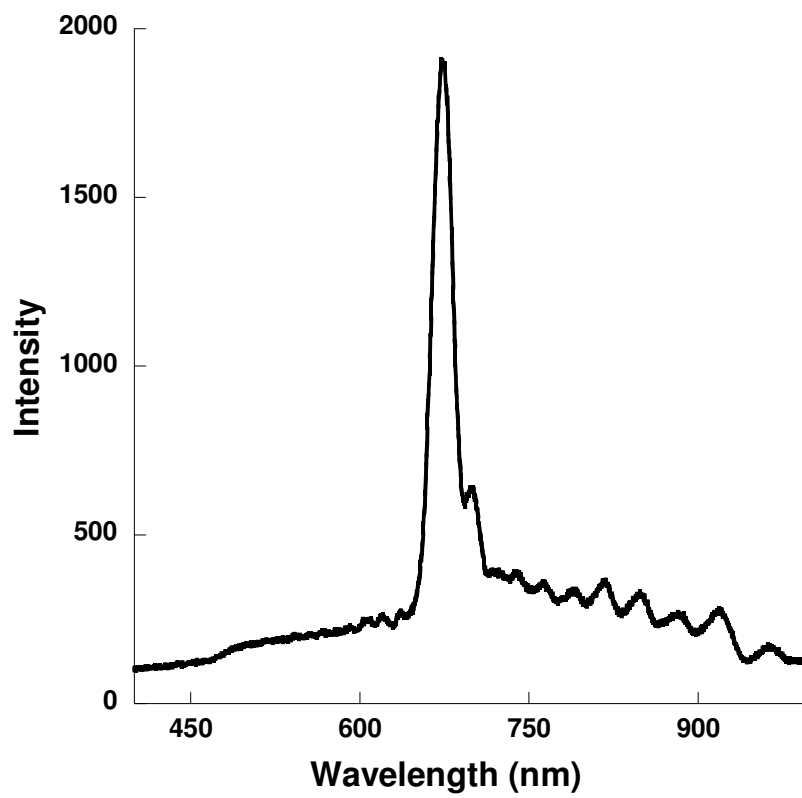


Figure 1.5. Reflectance spectrum of a rugate film.

1.4 Drug Delivery Materials and Devices

Drug delivery is an expanding field in medicine that seeks to improve the efficacy of the drug by either extending the time at which the drug is considered therapeutically relevant or localizing the drug directly to the diseased site. Controlling the rate at which drug is delivered to the patient through a sophisticated delivery device would eliminate the need for multiple administrations and increase the efficacy of the therapy. Localization also enhances the drug efficacy while reducing the side effects that are commonly observed upon drug administration. Two of the most common drug delivery methods are intravenous injection and oral administration. For both methods, the drug must overcome many factors in order to retain its efficacy. A few key issues that affect the efficacy of the therapeutic include the ability of the drug to withstand harsh biological conditions present *in vivo*, how efficiently the therapeutic agent can reach its target, and the solubility of the drug. Many effective drugs are deemed unviable due to the challenges presented in the body.

1.4.1 Polymeric Devices

Polymers are currently some of the most highly used materials for drug delivery and have been actively used since the 1970's.²⁹ Their chemical and physical properties designate polymeric systems as good candidates for

biomaterials. Many natural and synthetic polymers provide biodegradability, biocompatibility, mechanical strength, and elasticity. Generally these systems operate by incorporation of drug into the polymeric matrix. The release rate is typically dependent on the degradation properties of the polymer.

PLGA (polylactic-co-glycolic acid) microparticles have been FDA approved for delivery of therapeutic agents to treat diseases such as prostate cancer, endometriosis, and growth hormone disorders.³⁰⁻³² PMMA (polymethyl methacrylate) adhesives are effective for delivery of drug to the intestinal wall,³³ and other polymer and polymer blends are used as coatings for drug eluting arterial stents.³⁴⁻³⁶

Other polymers that have also made a significant impact for medicinal research are a class known as hydrogels, commonly referred to as ‘smart’ polymers. These ‘smart’ polymers respond to external stimuli such as a change in pH,^{37, 38} temperature,^{39, 40} electric fields.^{41, 42} Changes in the environment influence the physical and chemical properties and have been utilized in a manor to provide controlled drug release.

1.4.2 Porous Materials and Reservoir Devices for Drug Delivery

Porous materials and reservoir devices have also been investigated for controlled drug delivery systems. These materials utilize the void volume to house

therapeutics and release them upon either natural of drug from the reservoirs or provide a triggered release upon application of an external stimulus.

1.4.2.1 Porous Silica

Biocompatible silica is an extensively studied type of porous material for drug delivery, and there are many thorough reviews on the topic.^{43, 44} The large surface area of porous silica provides a convenient means by which enhanced drug loading and/or surface modification can occur. Even though porous silica is regarded to have a relatively easy route of synthesis, pore size is limited by the templating precursors in the condensation reaction.⁴⁵ Functionalization of these materials have been obtained with various grafting techniques that are done both, during and post-condensation reactions.^{10, 46, 47} Through silanization chemistries, therapeutic⁴⁸ and diagnostic agents⁴⁹ have been covalently attached to the walls of the material. Similarly, Lin and co-workers have developed a silicious material capable of releasing a cytotoxic drug from the porous matrix.¹⁰ This system used CdS nanoparticle as caps to physically block the entrance of the pores. Through chemical initiation, the CdS caps were removed and a triggered release of drug was observed in vitro.

1.4.2.2 Porous Ceramics

Ceramics are typically non-metallic inorganic materials, in various forms of oxides, and are generally thermally fabricated. These materials have demonstrated a sustained release *in vitro*. However, they generally require some form of bio-functionalization in order to reduce their toxicity.^{50, 51} A comprehensive study of ceramic materials *in vivo* has yet to be published.

1.4.2.3 Microreservoir Devices

Recently, microreservoir devices have been used to store and controllably release drug from the wells or reservoirs present on the device.^{52, 53} Generally, these reservoirs are microfabricated on a silicon substrate and would be incorporated into the body as an implant. The reservoirs are capped with a bio-erodible material^{54, 55} or metallic membrane^{56, 57} and have been shown to provide both a pulsatile and sustained release. Triggered release has been demonstrated by applying an external stimuli such as an electric field.^{56, 58} These devices are commonly referred to as a ‘pharmacy on a chip,’ providing delivery of multiple therapeutics at various release rates from a single device. One disadvantage of these sophisticated materials is the requirement for surgical implantation to incorporate the device *in vivo* as opposed to a more preferred, non surgical and minimally invasive method.

1.4.3 Nanotechnology for Drug Delivery

Nanotechnology for medicinal applications has shown immense promise to revolutionize the process by which therapeutics are administered. Many delivery systems comprised of nanoparticles for drug delivery focus on localized diagnostic and therapeutic treatments for cancer. Most often, these nanoparticles⁵⁹ or nanospheres⁶⁰⁻⁶² have been adapted with a homing moiety.^{63, 64} These systems are typically circulated in the body by intravenous injection with intent to perform one or more of the following duties 1.) locate the diseased site for diagnostic purposes,^{16, 65} 2.) deliver a therapeutic payload directly to the site or 3.) seek and destroy malignant cells directly (ablation therapy).⁶² In recent years, a great deal of effort has been focused on using nanotechnology to provide advancement of drug delivery and design.

1.5 Porous Si for Drug Delivery

Providing a controlled and localized release of therapeutics within the body are key objectives for increasing the efficacy of therapeutics and reducing the risks of potential side effects.⁶⁶⁻⁷⁰ The ease of processing, tunable pore sizes and volumes, high surface area within the material, and convenient routes to

chemical modification, are a few of the qualities that can be utilized to address these issues for designing effective drug delivery devices.

1.5.1 Biocompatibility of Porous Si

Some of the initial work of Leigh Canham has focused extensively on the prospective applications of porous Si for drug delivery and tissue engineering. Some of his preliminary work found porous Si to be biocompatible⁷¹⁻⁷⁷ and bioresorbable^{25, 78-81} material. The biocompatibility and degradation properties of the material are considered an ideal support for a controlled drug delivery system in which the porous Si device can be implanted and complete dissolution of the delivery device could be obtained *in vivo*. Porous Si has been shown to dissolve completely into the bioresorbable form of silicic acid, $\text{Si}(\text{OH})_4$, which would not require any further surgeries or medical procedures for removal or recovery of the delivery device. There are many conditions that affect the lifetime of biomaterials *in vivo*. In order to be considered a good candidate for controlled release in the body, the material has to be able to withstand the harsh biological conditions in the body such as salinity, pH, and enzymatic activity while retaining the physical and chemical properties that deem the material biocompatible.

1.5.2 Surface Chemistry of Porous Si

The surface chemistry plays a large role in controlling the degradation properties of the material *in vivo*. Immediately after Si is electrochemically etched, the reactive hydride-terminated surface can provide a versatile surface at which many different chemical functionalities can be carried out to improve stability in aqueous media, longer lifetimes *in vivo*, and extended drug release rates.^{25, 82-84} Both thermal and chemical methods for oxidation are used. Oxidation imparts hydrophilicity to the porous structure which enables incorporation and adsorption of hydrophilic drugs or biomolecules within the pores. Another method for chemical modification is by way of hydrosilylation reactions of the hydride-terminated porous Si surface with terminal alkenes. A variety of organic and inorganic components can be grafted to the surface,^{82, 85, 86} while calcified forms of porous Si have been developed to create bioactive forms of silicon for applications in drug delivery.^{80, 87}

1.5.3 Tunable Pore Sizes and Surface Area

A unique property of porous Si is the ability to easily tune the pore sizes and volumes during the electrochemical etch.⁸⁸ Other porous materials require some advanced design protocol to control pore size. Control over the porosity and pore size is obtained by adjusting the current settings during the electrochemical

processing of porous Si. Typically, greater current density used in fabrication produces larger pores. Large pores are desirable when incorporating sizable molecules or drugs within the pores. Not only is pore size and porosity important regarding the range of molecules to be incorporated within the pores, but it also determines degradation rates of the porous Si.²² Smaller pores provide more surface area and expose more sites upon which aqueous media can attack. With smaller porous filaments within the film, greater dissolution rates of porous Si are attained. This provides a convenient means to control degradation rates of porous Si. The current density used in the electrochemical etch determines the pore size, shape, and porosity of the films and in turn determines the dissolution rate of the porous Si. One important feature of porous Si is the ability to tune the degradation rates of the material as the porosity and type of Si determines the lifetime of the material *in vivo*.

1.5.4 Optical Properties for Potential In Vivo Monitoring

Many materials have been developed to release drugs, but few can ‘self-report’ on the amount of drug loading or release. Using the unique optical properties of the porous Si films and particles allow for a self-reporting drug delivery system to be devised. Incorporation of analyte into the porous Si film causes the interference patterns to shift to higher wavelengths which is dependent on the composition/refractive index of the guest molecule. On the other hand,

when drug diffuses from the porous matrix, the interference patterns shift to lower wavelengths yielding information on the release of drug from the porous matrix. The shifts are dependent upon the composite refractive index of the film and as molecules, specifically drug molecules, enter or leave the porous nanostructure, the optical signature shifts in a predictable fashion to assist with the reporting capabilities for determining the extent of drug loading and release from the porous framework.

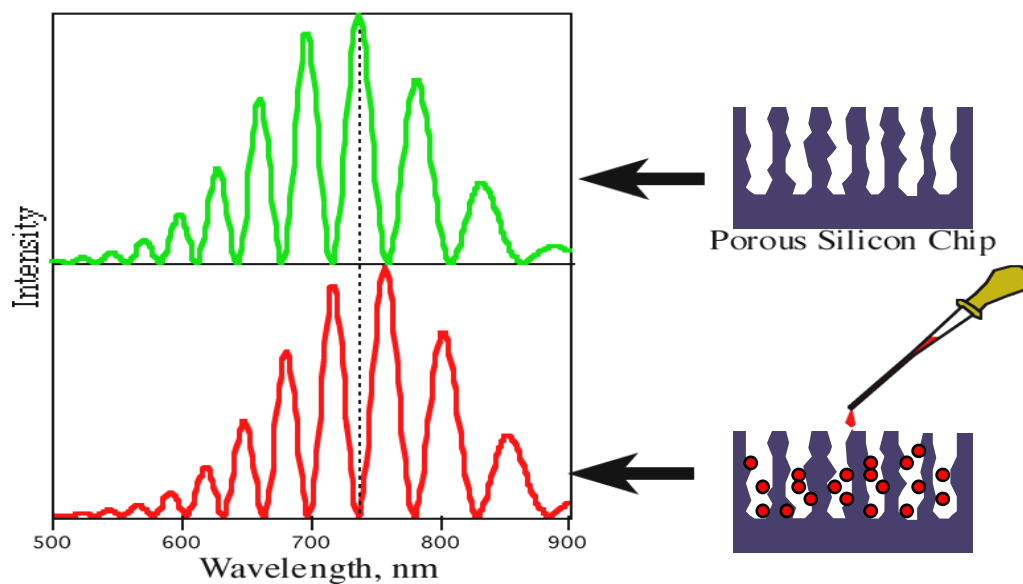


Figure 1.6 Schematic demonstrating the change in a Fabry-Pérot reflectance spectrum upon addition of analyte (drug molecules) into the porous matrix. This refractive index change of the composite film induces a red shift of the Fabry-Pérot fringes. The reverse process can also be monitored yielding a blue shift of the spectrum.

It has been previously reported that the optical features of porous silicon can penetrate human tissue (up to 1 mm in thickness) demonstrating the feasibility of porous Si as a candidate material for an in vivo self-reporting system.¹⁰ These optical properties are particularly useful for intraocular drug release either in the form of porous Si particles or films in the eye. These materials could provide a novel means to monitor intraocular drug release non-invasively. Porous Si microparticles are currently being investigated as self-reporting delivery devices for treatment of age-related macular degeneracy and other eye diseases, which is discussed in more detail in chapter five.



Figure 1.7 Light microscope image of porous Si particles.

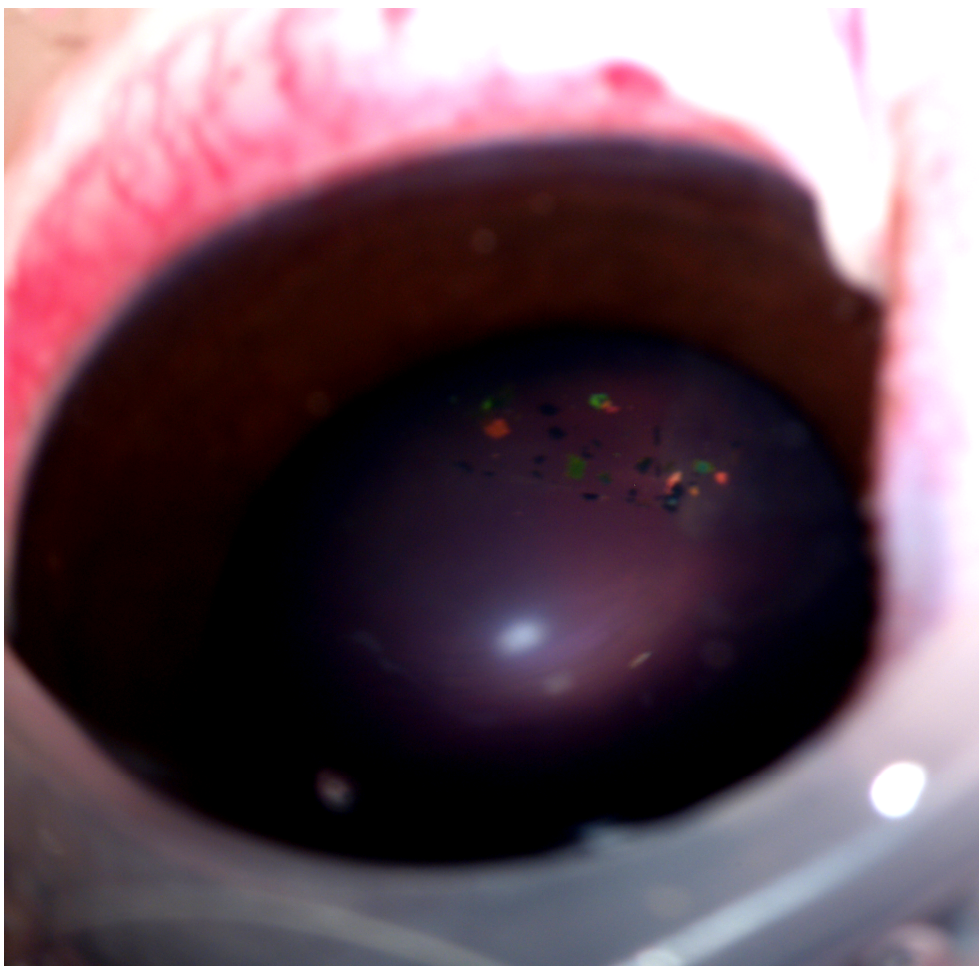


Figure 1.8 Images of porous Si microparticles in a rabbit eye. The particles (bright specs in center of image) can be seen floating the vitreous.

1.5.5 Current medical applications of Porous Si

Various forms of porous Si are being investigated and are also currently in clinical trials for investigating the suitability and efficacy as a medical device or biomaterial in the body. To date, it has been shown feasible to incorporate anti-cancer therapeutics,^{89, 90} anti-inflammatory agents,^{20, 91} analgesics,⁹¹ and medicinally relevant proteins and peptides.⁷¹ Designing effective drug delivery devices comprised of porous Si has taken many forms including porous Si particles,⁹²⁻⁹⁴ films,²⁰ implants,^{95, 96} composites materials,⁹⁷ and microneedles.^{98, 99}

1.5.5.1 Porous Si Particles

Drug delivery with micro and nanoparticles are of particular interest because of the convenience for administration of the therapy. Particles have the potential for percutaneous or intravenous administration depending on the size of the particles. Foraker et al. have shown that using insulin loaded porous Si particles enhances the delivery of insulin across Caco-2 cell monolayers,⁷¹ while Salonen and co-workers have investigated the drug release kinetics from porous Si microparticles for applications in oral drug delivery.¹⁰⁰ Their work investigates the interactions between different drug candidates and various surface modifications of the porous Si substrate. Magnetic porous Si particles for delivery of payloads has also been demonstrated *in vitro*.⁹³

1.5.5.2 Cancer Therapies with Porous Si

Anti-cancer therapeutics have been successfully incorporated into porous Si. Drug delivery systems with cisplatin doped calcium phosphate films grown onto porous Si has been demonstrated in simulated body fluid,¹⁰¹ while doxorubicin loaded porous Si films have shown cytotoxic effects towards human colon adenocarcinoma cell lines.⁹⁰

Some of the most advanced studies that are currently in clinical trials use porous Si as a brachytherapy delivery device for the treatment of cancer.^{96, 102} Porous Si particles (20 μm in size) were incorporated by a percutaneous implantation of the radioactive material (^{32}P doped porous Si) providing local radiation to the lesion. The dissolution of the device is observed as the Si implant is hydrolyzed to silicic acid, a bioresorbable form of Si, thereby requiring no further surgery to recover the delivery devices from the body.

1.5.5.3 Porous Si Composite Materials for Drug Delivery

Porous Si composite materials have also been investigated as new materials for drug delivery devices. Developing hybrid materials generate materials with completely different chemical and physical characteristics. Commonly, polymers and porous Si have been combined to develop new hybrid

materials yielding improved control over the drug release kinetics and improved stability in aqueous media. Porous Si used as either a template for developing polymeric photonic structures or incorporating both the porous Si matrix with a polymer into a new hybrid material shows great promise for improving the mechanical stability and control over release rates of the delivery system. Polymeric systems that have been incorporated into porous Si include polylactide, polydimethylsiloxane,¹⁰³ polyethylene, polystyrene,¹⁰⁴ polycaprolactone,¹⁰⁵ and poly(N-isopropylacrylamide).¹⁰⁶



Figure 1.9 Image of a porous Si-polycaprolactone composite.

1.6 Summary

Establishment of successful ‘smart’ drug delivery systems requires networking of various disciplines and materials. The foundation of this thesis focuses on utilizing porous Si nanostructures for development of effective drug delivery devices.

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CHAPTER TWO

ENGINEERING THE CHEMISTRY AND NANOSTRUCTURE OF POROUS SI FABRY-PEROT FILMS FOR LOADING AND RELEASE OF A STEROID

2.1 Abstract

A method for engineering the surface chemistry and pore dimensions in porous Si films for the purpose of controlling the loading and release of a hydrophobic drug is described. Loading of the steroid dexamethasone is confirmed by Fourier transform infrared spectroscopy, and the release rates are characterized by observation of the appearance of the drug in solution (UV-visible absorption spectroscopy), and by measurement of the Fabry-Pérot fringes in the optical reflectivity spectrum of the porous Si film. Optical reflectivity changes are shown to provide a measure of the release rate of the drug that is amenable to *in-vivo* diagnostic applications. Fresh porous Si films are prepared by electrochemical etch and subsequently modified by hydrosilylation with 1-dodecene. The dodecene modified samples are found to be more robust in aqueous environments and to exhibit slower release rates of the drug relative to freshly-etched porous Si. Whereas the relatively large dexamethasone molecule is found to infiltrate the freshly-etched samples, it does not enter the chemically modified films, due to steric crowding from the dodecyl species. In order to achieve a high degree of loading into these modified films, the pores are enlarged before hydrosilylation by treatment with an aqueous solution containing HF and dimethyl sulfoxide. The pore-expanded, chemically modified samples are found to admit approximately 70% of the dexamethasone that can be admitted into an unmodified (freshly etched) sample. Diffusion of the steroid from the modified, pore-

expanded films into phosphate buffered saline solution is found to be slower than from the unmodified sample by a factor of ~20, with 90% of the drug delivered in 3 days for the chemically modified films compared to 3 hours for the unmodified films.

2.2 Introduction

Localization, controlled release, and monitoring the concentration of drugs within the body are key challenges in the design of effective targeted drug therapies.¹⁻⁵ In addition, a delivery system that can sustain drug release over long periods of time in order to eliminate multiple administrations is desirable. Various nanostructured materials systems have been developed to meet these challenges,⁶⁻¹⁰ and porous Si in particular possesses several properties that have been found to be advantageous. Porous Si is biocompatible¹¹⁻¹⁷ and bioresorbable,¹⁸⁻²² it has tunable pore sizes and volumes,²³ a high surface area,²⁴ and unique optical properties that allow *in-vivo* monitoring.^{9, 25}

A unique attribute of porous Si is its ability to optically report on the loading of a molecule within the porous nanostructure. The optical interference spectrum from Fabry-Pérot layers or from multilayers has been used extensively in sensor applications, where changes in the optical reflectivity spectrum indicate the capture of a chemical or biological species.²⁶⁻³⁷ However, this property has not been used to monitor release of a drug loaded in a porous Si film. In this chapter,

the relatively hydrophobic steroid, dexamethasone, is trapped and slowly released from a chemically modified porous Si Fabry-Pérot film. A method to open the pores to a desired diameter for more efficient drug loading in these optical structures is presented. The Fourier-transform of the optical reflectivity spectrum is shown to provide a simple means to monitor drug loading and release.

2.3 Experimental

2.3.1 Sample Preparation

Dexamethasone (98%), 1-dodecene (95%), and phosphate buffered saline solution (Dabelcco's) were purchased from Aldrich Chemicals. Aqueous HF (48%) and dimethyl sulfoxide (99.9%) were supplied by Fisher Scientific and Alfa Aesar, respectively. Porous Si samples were prepared from single-crystalline p-type Si (boron doped, 3 Ω -cm resistivity, <100> orientation, obtained from International Wafer Service, Inc.) by anodic electrochemical etch in a 1:1 (v/v) solution of 49% aqueous HF in $\text{CH}_3\text{CH}_2\text{OH}$ with a current density of 33.3 mA/cm^2 for 5 minutes. All samples were rinsed with ethanol and dried under a stream of nitrogen after etching. The drug loading solution consisted of 6×10^{-2} M dexamethasone in methanol. For preparation of the non-chemically modified samples, drug loading of the pores was carried out immediately following the electrochemical etch. 25 μL of dexamethasone solution in methanol was pipetted

onto a freshly-etched porous Si layer with an area of 1.2 cm^2 . After solvent evaporation, the films were briefly rinsed with deionized water to remove any excess drug remaining on the surface that had not infiltrated the pores.

The chemically modified porous Si samples were etched in the same manner described above. Chemical pore expansion was carried out after the electrochemical etch. Pore expansion was accomplished by soaking the freshly-etched porous Si film in a 9:1 (v/v) 49% aqueous HF:DMSO solution for 30 minutes prior to hydrosilylation. The sample was rinsed with ethanol and dried under a stream of nitrogen. The sample was then submerged in 1-dodecene in a Schlenk flask, and oxygen was removed from the solvent by three freeze-pump-thaw cycles³⁸. The flask was heated for 2 hours at 120°C in a nitrogen atmosphere. After removal from the reaction vessel, the sample was rinsed with dichloromethane and ethanol to remove excess 1-dodecene. Drug loading of the pore expanded, hydrosilylated samples was carried out following the same method as described for the freshly-etched samples.

2.3.2 Characterization of Porous Si Films

Transmission-mode Fourier-transform infrared (FTIR) spectra were obtained with a Nicolet-Magna 550 spectrometer. A Digital Instruments Nanoscope IIIa equipped with a multimode scanning probe was used to acquire atomic force microscope (AFM) images of the pores. The AFM was operated in

tapping mode. Reflectivity spectra were obtained using an Ocean Optics S2000 spectrometer fitted to an optical microscope via fiber optics. Light from a tungsten source was coupled into the microscope optics using a coaxial fiber optic bundle.

2.3.3 Drug Diffusion Studies from Porous Si Films

Absorbance measurements of drug diffusion into phosphate buffered saline (PBS) solutions at pH = 7.4 were obtained with a Hewlett Packard 8452A diode array spectrophotometer. After preparation, each sample was cut to a size of $\sim 8 \text{ mm}^2$ and placed at the bottom of a Sigma 3 mL quartz cuvette containing the PBS solution, with the surface of the film facing upwards. The appearance of dexamethasone in solution was determined by monitoring the UV absorbance band at 242 nm. UV absorbance measurements were taken concurrently with optical reflectivity measurements. This setup consisted of a drug-impregnated hydrosilylated sample submerged under 4 mL of PBS solution. Immediately after acquisition of the reflectivity spectrum, a 3 mL aliquot of the PBS solution was extracted, and its absorption spectrum was obtained with the UV spectrophotometer. The aliquot of solution was then returned to the sample vessel.

2.3.4 Fourier Transforms of Optical Reflectance Spectra

The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm. The data spacing is approximately 0.4 nm. The x-axis was inverted, the spectrum redimensioned to 4096 data points, and a linear interpolation was applied such that the data were spaced evenly in units of nm^{-1} . A Hanning window was applied to the spectrum, the spectrum was zero padded to the power of two, and a discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was applied. The position of the peak in the Fourier transform spectrum then corresponds to the value $2nL$ from eq. 2

2.3.5 Determination of Porosity and Film Thickness by Fitting the Spectral Reflectivity Data to the Bruggeman Model

The Bruggeman effective medium model was used to calculate the porosity and thickness of the films before and after the pore expansion procedure.³⁹ A two-component Bruggeman approximation was employed to calculate the porosity (fraction of Si) of the porous Si films. Reflectivity data from two measurements were used: the reflectivity spectrum of the sample in air and

the reflectivity spectrum from the sample submerged in a liquid of known refractive index (i.e. ethanol).²⁶ The difference between the two spectra can be attributed to the change in effective optical thickness as the medium in the pores changes from air to liquid, with the assumption that all the void spaces in the film are filled. A fit to the Bruggeman equation then yields a unique solution for both the porosity and the thickness of the sample. Independent measurements of porosity (gravimetric method⁴⁰) agreed with the optical fit to within 10%. The values of refractive index for Si and air used in the Bruggeman fit were 4.1 and 1, respectively.

2.4 Results and Discussion

2.4.1 Loading of Dexamethasone into Freshly-Etched Porous Si

Dexamethasone was impregnated into the porous matrix immediately following etching. Before loading of drug, the Fourier transform infrared (FTIR) spectrum of the freshly-etched porous Si film displays bands characteristic of surface hydride species (Figure. 2.1a). A band at 2110 cm^{-1} associated with the $\nu_{\text{Si-H}}$ stretching vibrations and one corresponding to the $\delta_{\text{Si-H}_2}$ wag at 915 cm^{-1} is apparent.⁴¹ Dexamethasone can be loaded into the pores of this material by treatment with a $6 \times 10^{-2}\text{ M}$ methanol solution. After evaporation of the solvent, the presence of dexamethasone is confirmed by comparison of the infrared

spectrum of the film (Figure. 2.1b) with a reference spectrum of the pure steroid (Figure. 2.1e).

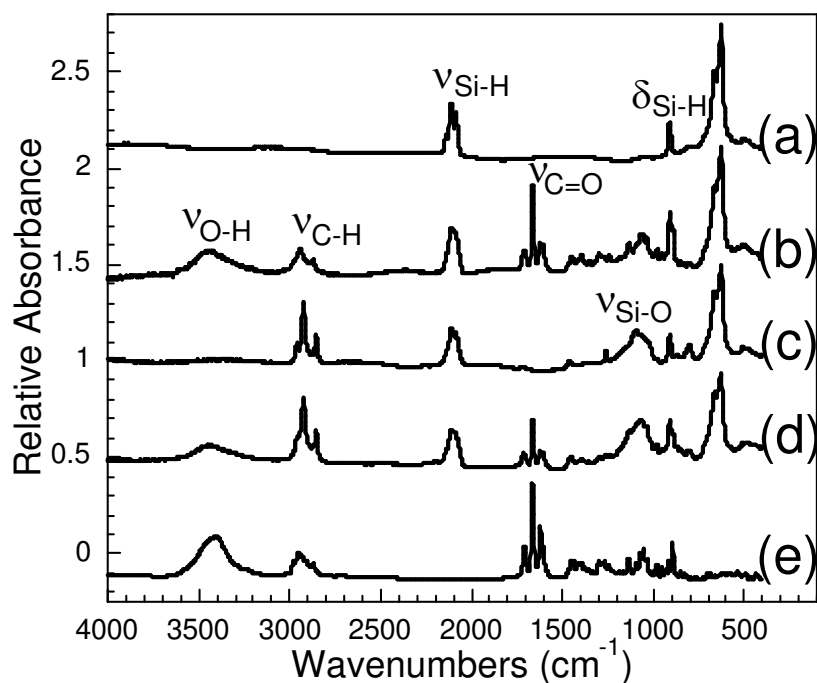


Figure 2.1 Fourier transform infrared (FTIR) spectra of different porous Si films, showing drug loading. **(a)** Freshly-etched porous Si. **(b)** Freshly-etched porous Si after drug loading, displaying the characteristic vibrational bands of dexamethasone. **(c)** Pore expanded, hydrosilylated porous Si. **(d)** Pore expanded, hydrosilylated porous Si after drug loading, also displaying the characteristic vibrational bands of dexamethasone. **(e)** Reference spectrum of dexamethasone. Spectra are offset along the y-axis for clarity.

2.4.2 Preparation of Chemically Modified Porous Si

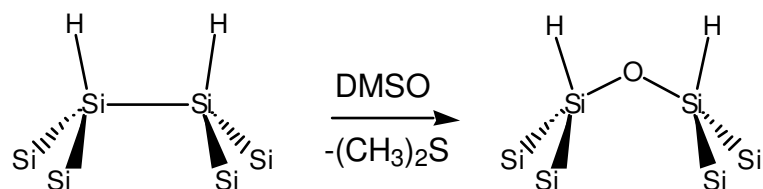
To act as an effective drug delivery material, the host material must be stable in aqueous solutions at physiological pH and salinity for a period of time that is therapeutically relevant. Freshly-etched porous Si is known to be unstable in aqueous media due to oxidation of the reactive surface hydrides.⁴¹⁻⁴³ It has been shown that the stability of porous Si films can be greatly improved through surface alkylation,^{22, 44-46} and in this work hydrosilylation with 1-dodecene is used as a means to increase aqueous stability. Following the procedure outlined above for loading of drug into freshly-etched porous Si, the alkylated porous Si sample was treated with a solution of dexamethasone. However, no detectable vibrational bands for dexamethasone were observed in the FTIR spectrum, indicating that the 1-dodecene-modified porous Si films are no longer able to admit the drug. The results are interpreted in terms of steric crowding in the nanometer-scale pores once dodecyl groups are attached.²⁴

2.4.3 Chemical Pore Expansion

Since the pore size in porous Si prepared from p-type Si wafers depends on the current density used in etching the material, larger pores can be obtained by increasing the current density.⁴⁷ Previous work has focused on this approach, and with the right combination of current density, HF concentration in the etch bath

and wafer resistivity, material with pores sufficient to admit large biomolecules such as bovine serum albumin or immunoglobulin G have been obtained.^{23, 29, 48} The electrochemical approach is convenient, although it is also somewhat problematic since cracking and flaking of the films often results from conditions necessary for the generation of large pores. The mechanical instability of porous Si is directly related to the strain that is induced in the film as it is produced in the electrochemical process.⁴⁹ It was thought that a chemical dissolution approach might produce better results, because porous Si should be chemically attacked preferentially at regions of the film where the most strain exists. Such a mechanism may not hold for the electrochemical route, where pore formation is determined more by local changes in Si conductivity and fluoride ion concentration than by strain.⁵⁰

Equation 2.1



The method used for pore expansion involves slow oxidation of the porous Si surface by dimethyl sulfoxide (DMSO), coupled with dissolution of the newly formed oxide by HF attack (eq. 1 and Scheme 1). A freshly-etched film is soaked in a solution consisting of 9:1 (v:v) 49% aqueous HF:DMSO for 30 minutes prior

to performing the hydrosilylation reaction. DMSO has been shown to act as a mild oxidant for porous Si.^{43, 51} The oxide formed by DMSO is then dissolved by HF, and the net effect is to slowly remove Si atoms, opening the pores in the porous Si matrix. Pore expansion is confirmed by atomic force microscopy (AFM), Figure. 2.2. A Si surface that has been electrochemically etched using a 1:1 49% aqueous HF:ethanol solution (v/v) with a current density of 33.3 mA/cm^2 for 5 minutes displays a pore structure whose feature size (RMS roughness) corresponds to approximately 0.19 nm (Figure 2.2a). A Si surface that has been electrochemically etched and then treated with HF:DMSO displays a significantly rougher topology, with RMS roughness on the order of 0.86 nm (Fig. 2b).



Figure 2.2 Pore expansion procedure. The freshly-etched porous Si film is soaked in a solution containing $\text{HF}_{(\text{aq})}$ and dimethyl sulfoxide (DMSO) for 30 minutes, which partially dissolves the porous matrix, expanding the pores. Following pore expansion, the sample is thermally hydrosilylated in order to stabilize it in aqueous media and to impart a hydrophobic nature to the inner surfaces of the pores (to enhance drug loading).

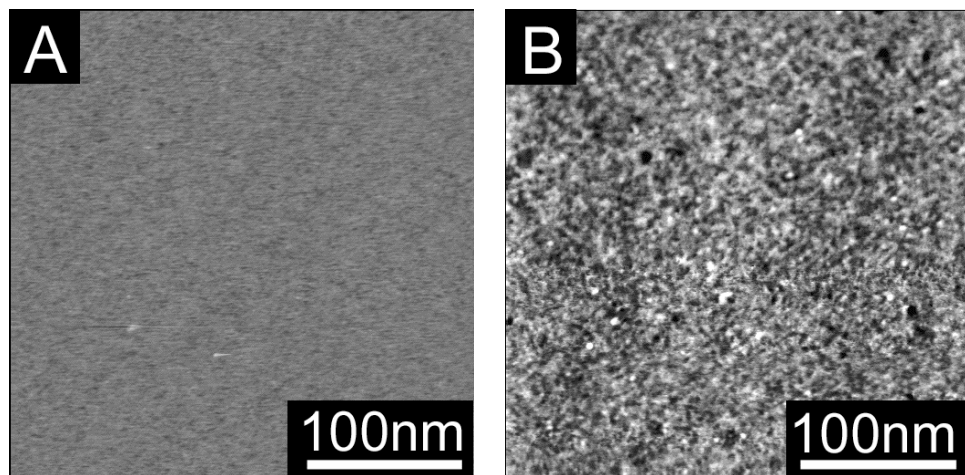


Figure 2.3 Tapping-mode Atomic Force Microscope (AFM) images of porous Si surfaces before (**A**) and after (**B**) pore expansion with DMSO/HF_(aq). Images show an increase in mean pore size upon chemical etching. The larger pores can permit the infiltration of drug molecules and accommodate hydrosilylation chemistries.

2.4.4 Optical Characterization of the Pore Expansion Process

In-situ reflectivity measurements of a porous Si film obtained during the pore expansion procedure confirm the steady removal of Si. The reflectivity spectra of a film (Figure 2.3) displays Fabry-Pérot interference fringes, corresponding to constructive and destructive interference from light reflected at the air/porous Si and porous Si/crystalline Si interfaces.⁵² The peak maxima for each of the spectral features follow the Fabry-Pérot interference relationship represented by eq. 2 for normal incidence, where m is the spectral order the fringe at wavelength λ .⁵² The effective refractive index of the film is given by n , and L is the thickness of the film. The value of n represents a composite refractive index, comprising a porosity-weighted average of Si and the medium filling the pores as discussed previously (Chapter 1.3).⁵³ This average refractive index is expected to decrease as Si is converted to its oxide or is removed from the film and replaced with a material possessing a lower index of refraction, such as air, water, or an organic molecule. The result is a blue shift in the spectral position of the fringes, observed in the present case as Si dissolves and is replaced by the HF/DMSO etchant solution (Fig. 3 and 4).

Equation 2.2

$$m\lambda = 2nL$$

The value of nL from eq. 2, referred to in this work as the optical thickness (OT), is obtained from the position of the peak in the Fourier-transformed plot of reflected intensity versus frequency (Figure 2.3 inset). The refractive index, n , in eq. 2 is a composite index that derives from the refractive index of the Si and of the medium filling the pores. This composite index can be related to the refractive index of the individual constituents, the porosity, and the thickness (L in eq. 2) of the porous Si film by the Bruggeman effective medium model.⁵⁴ A fit to the Bruggeman model is used in this work to provide values of n , L , and porosity, as described in the experimental section.

The optical thickness of a porous Si film undergoing chemical pore expansion was monitored *in situ* as a function of time, Figure 2.4. The measured optical thickness of the film in air (7540 nm, Figure 2.4 -timepoint a) initially undergoes a red shift or increase in optical thickness (8430 nm, Fig. 4 -timepoint b) as a result of an increase in refractive index of the film due to the infiltration of the HF:DMSO solution into the pores. As Si is removed by chemical oxidation a continuous decrease in optical thickness is observed (Fig. 4 -timepoint c), corresponding to an increase in porosity from 56% to 65%.³² This change in porosity is equivalent to removal of 20% of the Si from the porous Si layer (applying a fit to the data using the Bruggeman effective medium model)³⁹.

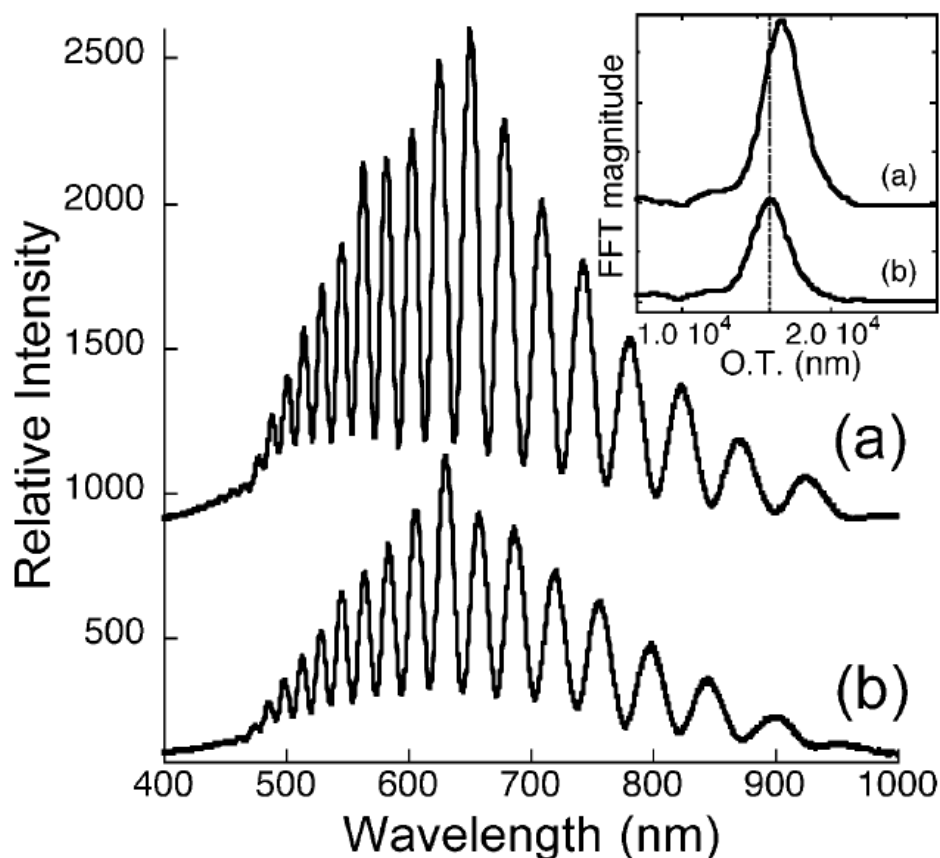


Figure 2.4 Optical reflectivity spectra showing the effect of removal of Si from a porous Si film after 20 min of exposure to the HF/DMSO chemical etchant solution. The spectra are obtained *in-situ*, using a white light source at normal incidence to the film. Inset shows Fourier transforms of the two spectra. The position of the spectral peak reflects twice the optical thickness of the film (nL), which decreases from 8390 nm to 7990 nm, corresponding to removal of 20% of the Si from the porous Si film, and an increase in porosity from 50% to 60%.

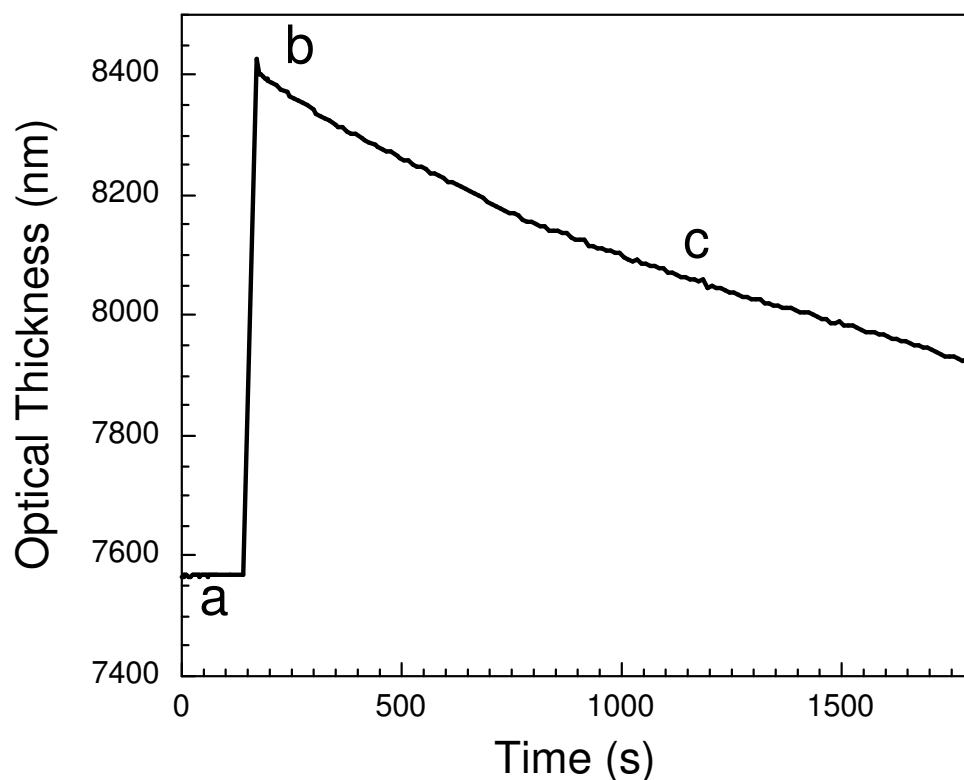


Figure 2.5 Evolution of the optical thickness of a porous Si film undergoing chemical pore expansion. **(a)** represents the optical thickness of the film in air (initial optical thickness 7570 nm). The film is submerged in HF:DMSO solution at point **(b)**, and an increase in optical thickness (to 8430 nm) is observed due to an increase in refractive index as the HF:DMSO solution infiltrates the pores. As the action of the chemical oxidant gradually removes Si from the pores, a continuous decrease in optical thickness is observed **(c)**. There is an approximate increase (~10%) in porosity of the film after the expansion treatment.

2.4.5 Loading of Hydrosilylated Porous Si

The HF:DMSO treatment provides a predominantly hydrogen-terminated surface. Thus the surface can be thermally hydrosilylated in a manner similar to the procedure used to modify the fresh, electrochemically etched material.⁵⁵ The FTIR spectrum of a porous Si film that has been chemically expanded and subsequently alkylated (Figure 2.1c) exhibits vibrational bands characteristic of alkyl termination, with $\nu_{\text{(C-H)}}$ stretching vibrations at 2938 cm^{-1} and various characteristic vibrational bands in the fingerprint region of the spectrum. $\nu_{\text{(Si-H)}}$ and $\delta_{\text{(Si-H)}}$ vibrations associated with surface Si-H species are also apparent at 2110 and 915 cm^{-1} , respectively indicating that not all Si-H sites have reacted.⁴¹ A small band at 1075 cm^{-1} assigned to a $\nu_{\text{(Si-O)}}$ stretching mode of surface oxide⁴¹ is also observed, ascribed to a minor degree of substrate oxidation incurred during the hydrosilylation procedure.

The pores of the DMSO-treated, hydrosilylated material are large enough to allow infiltration of dexamethasone. Thus samples loaded with dexamethasone in the same manner as described for the freshly-etched material display bands in the infrared spectrum characteristic of the steroid (Figure 2.1d).

The optical reflectivity data from drug-impregnated porous Si films, monitored during infusion of the drug, corroborate the FTIR results. As discussed above, shifts in the spectral position of the Fabry-Pérot interference fringes occurs when molecules are loaded into or released from the pores due to a change in the

local refractive index. In the present case, the value of optical thickness, nL, (relative to air) increases by ~100 nm upon loading of dexamethasone into a film with a total nL value of ~7000 nm. Loading of dexamethasone into freshly-etched and pore expanded/hydrosilylated films results in comparable increases in nL of a few percent, corresponding to a loading of approximately 50 milligrams of drug per gram of porous Si. Loading of dexamethasone into material that is pore expanded but not hydrosilylated (i.e. H-terminated) also demonstrates similar loading with an increase of ~100 nm in the optical thickness. The decrease in optical thickness for an alkylated sample that is not pore expanded indicates that dexamethasone does not enter the pores, which is consistent with what we observed with the FTIR spectroscopy. The slight decrease in optical thickness has been correlated to a minor oxidation of the porous silicon during loading.

2.4.6 Drug Release: Diffusion of Dexamethasone

UV absorption spectroscopy was used to monitor the release of dexamethasone from the porous nanostructure into phosphate-buffered saline (PBS) solution. Upon immersion of the drug-impregnated porous Si samples in PBS solution, the solution acquires an absorbance band at 242 nm that increases with time. Separate measurement confirms assignment of the 242 nm band to dexamethasone. Quantification of the amount of drug in solution is obtained using

an experimentally determined extinction coefficient of $72500 \text{ M}^{-1}\text{cm}^{-1}$ for dexamethasone in PBS.

Drug release from freshly-etched samples occurs much more rapidly than from the pore-expanded/hydrosilylated films (Figure 2.5). The increased rate of release can be attributed to the increased rate of degradation of fresh porous Si in PBS solutions. The freshly-etched films display a linear release of steroid over ~3 hours, until the film is completely dissolved. The hydrosilylated films are better able to resist corrosion in PBS buffer and exhibit slower release rates. These samples also display a linear release profile for the first ~3 hours, but this is followed by a slower release period of up to 3 days as the hydrosilylated film degrades. Porous Si films that have been pore expanded but not hydrosilylated display release profiles similar to the freshly-etched material, signifying that the pore expansion process is not responsible for the extended release period observed with the pore expanded/hydrosilylated samples. It is thus concluded that the pore expansion process allows admission of larger molecules, but it has no major effect on the release rate of a molecule once it is loaded.

Release of the drug is also quantified by concurrent measurements involving UV absorbance spectrophotometry of the solution as the porous Si film is monitored by interferometric spectrometry. There is a linear correlation between the absorbance of drug released from chemically modified/hydrosilylated porous silicon and the change in optical thickness of the Fabry-Pérot films (Figure 2.6). As the drug is released (i.e. as the absorbance at 242 nm from the

dexamethasone in solution increases), there is a corresponding decrease in optical thickness of the film. The correlation indicates that the average refractive index of the film decreases as drug is released, consistent with replacement of the drug in the pores with water. The conversion of Si to SiO₂, and/or dissolution of SiO₂ can also cause the observed decrease in refractive index, though the size of the change in refractive index of the chemically modified/hydrosilylated porous silicon observed within the first few hours of aqueous exposure is much smaller in comparison to the amount of change expected for the initial release of drug from the pores.

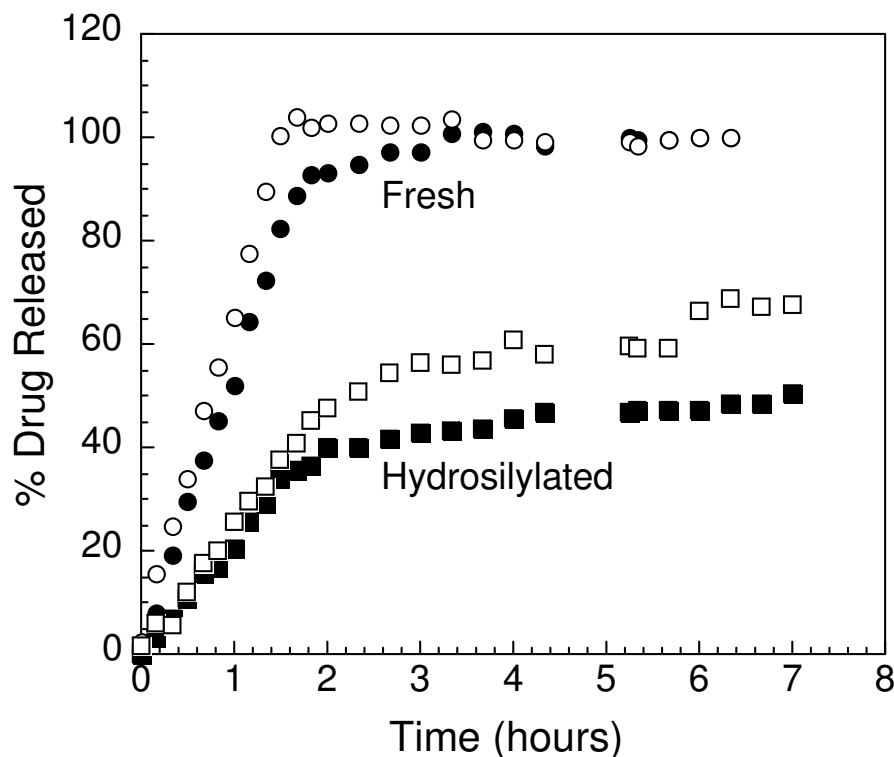


Figure 2.6 Percent of dexamethasone appearing in PBS solution as a function of time, measured by monitoring the UV absorption band at 242 nm in the solution. The circles represent release of dexamethasone from freshly-etched (H-terminated) samples, and the squares represent release of dexamethasone from pore-expanded, hydrosilylated (dodecyl-terminated) samples. The open and solid shapes represent replicate runs on different samples. H-terminated material completely releases dexamethasone after 2 hours, corresponding to complete dissolution of the porous Si layer. After 7 hours only 50-60% of the drug is released from the more stable hydrosilylated material and approximately 3 days until complete drug release is observed. Approximately 30 μg of dexamethasone was loaded and released from the freshly-etched films, and the pore expanded/hydrosilylated samples contained 20 μg dexamethasone per sample.

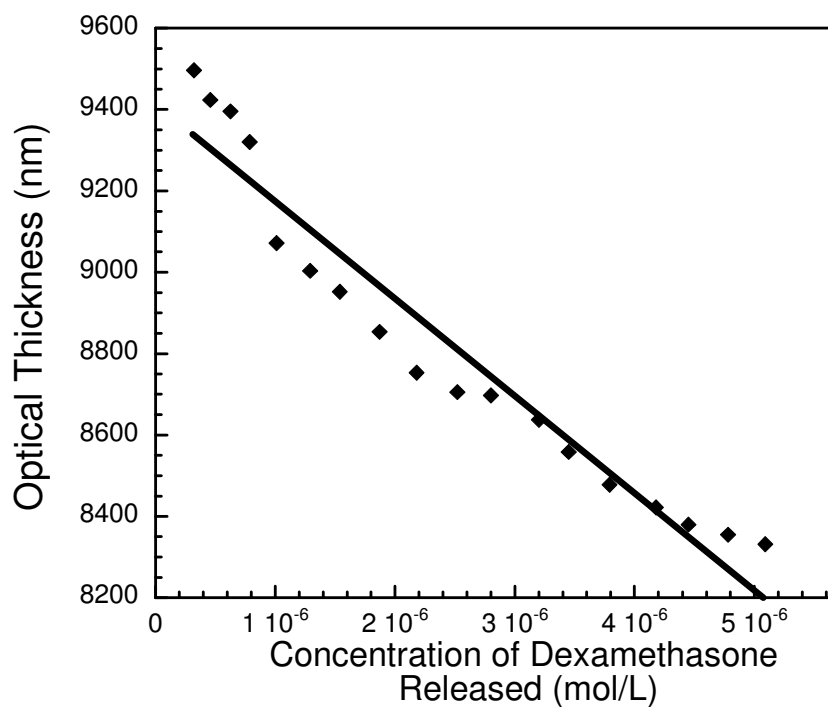


Figure 2.7 Correlation of the optical thickness (nm) of pore-expanded, hydrosilylated porous Si film to concentration of drug appearing in PBS solution over 2 hours. There is a linear correlation between the optical thickness of the film and the amount of drug released. Release of the drug was observed by UV absorbance spectrophotometry of the solution and optical thickness was monitored by simultaneous acquisition of the reflectivity spectrum from the film.

2.5 Conclusions

Loading of dexamethasone can be achieved in electrochemically etched porous Si films. The diffusion rate of the steroid into aqueous solution is rapid from freshly etched films due to the instability of porous Si at physiologic pH. Chemical modification of the porous Si film by thermal hydrosilylation with dodecene increases the stability of the film in aqueous solution but it reduces the average pore size to the point that dexamethasone can no longer be admitted. In order to admit the drug, the pores of the hydrosilylated samples must be expanded prior to chemical modification. Pore expansion is achieved by chemical etching of the porous Si film with a solution of dimethyl sulfoxide containing aqueous HF. The chemically modified, pore expanded material can load 50 mg of dexamethasone per gram of porous Si. The rate of release of drug from these samples is reduced by a factor of ~20 relative to the original, freshly-etched porous Si samples.

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CHAPTER THREE

TRIGGERED RELEASE PARTICULATE SYSTEM: ULTRASOUND-INDUCED DRUG RELEASE OF FROM POROUS SI MICROPARTICLES

3.1 Abstract

The steroid dexamethasone is loaded into porous Si microparticles and released into an aqueous solution by subjecting the microparticle dispersion to an ultrasonic stimulus. The drug-loaded microparticles are prepared from single crystalline Si wafers as follows: First, a porous Si layer is prepared by electrochemical etch of a highly doped p^{++} type silicon wafer. The layer is removed from the crystalline Si substrate with an electropolishing reaction. The free-standing particles are then chemically modified by microwave-induced hydrosilylation of 1-dodecene. Finally, dexamethasone is loaded into the hydrophobic porous matrix from an ethanol solution. Fourier-transform infrared spectroscopy is used to characterize the surface chemistry of the particles and to confirm drug loading. Brief immersion of an aqueous dispersion of the particles in a low-power ultrasound bath causes fragmentation of the microparticles and release of the drug. Dexamethasone release is quantified by UV-visible spectrophotometry: 10 mg of microparticles exposed to ultrasound for 1 min release 90 μg of dexamethasone, whereas microparticles that have not been exposed to ultrasound release 40 μg under otherwise similar conditions.

3.2 Introduction

Ultrasound is a widely used technique best known for its application in medical diagnostic imaging. Higher frequencies (1 - 20 MHz) of ultrasound are employed for non-invasive diagnostic imaging of a wide range of abnormalities in the body and for monitoring fetal development.¹ Recent research has focused on the use of ultrasound in therapeutic applications, such as cataract surgery,² pathogenic tissue ablation,^{1, 3, 4} and enhancement of wound healing.⁴ To effectively treat certain cancers where direct tissue or tumor ablation is desired, lower frequencies and higher intensities of ultrasound are generally used.⁵

The ability of medicinal ultrasound to facilitate *in vivo* drug delivery has also been investigated. Ultrasound can be used to assist drug transport across a membrane,⁶ or it can cause structural changes in a delivery vehicle to induce drug release. Liposomes are most commonly employed;⁷⁻¹⁰ for example, Dromi and co-workers have demonstrated ultrasound-triggered release of the cancer therapeutic doxorubicin *in vivo*.¹¹ Controlling release with an external stimulus allows more effective dosing and can increase the efficacy of a therapy, particularly when the drug carrier can be localized to the diseased tissue. Circulating nanoparticles equipped with a tumor-homing moiety,¹² and directed implants (brachytherapy)¹³ use this approach to provide concentrated amounts of drug to a particular region of the body. Drug delivery systems capable of providing both localized and triggered release hold great promise for many medical applications.

This chapter focuses on using porous Si micro particles to provide a triggered release of drug by applying ultrasound. Porous Si is a nanostructured material that is being investigated for biomedical applications including drug delivery,¹⁴⁻¹⁹ tissue engineering,²⁰ and biological sensors.²¹⁻²⁴ Porous Si has a tunable porous network,^{25, 26} a high surface-to-volume ratio,²⁷ convenient routes for chemical modification,^{28, 29} and it is considered a bioresorbable material.^{29, 30} A unique property of porous Si is that the nanostructure can be prepared to provide a distinct and tunable optical signature³¹⁻³⁵ useful for *in vivo* diagnostics.^{36, 37} Porous Si films can also be fractured into microparticles without loss of the optical signature.³⁸ Ultrasound is a common method used to create small particles from porous Si;³⁹ depending on the conditions, particles as small as a few nm can be obtained.⁴⁰ Ultrasonic cavitation provides a convenient means for destruction of the fragile porous matrix. In this chapter, this methodology is exploited to controllably release a drug from porous Si microparticles *in vitro* (Figure 3.1).

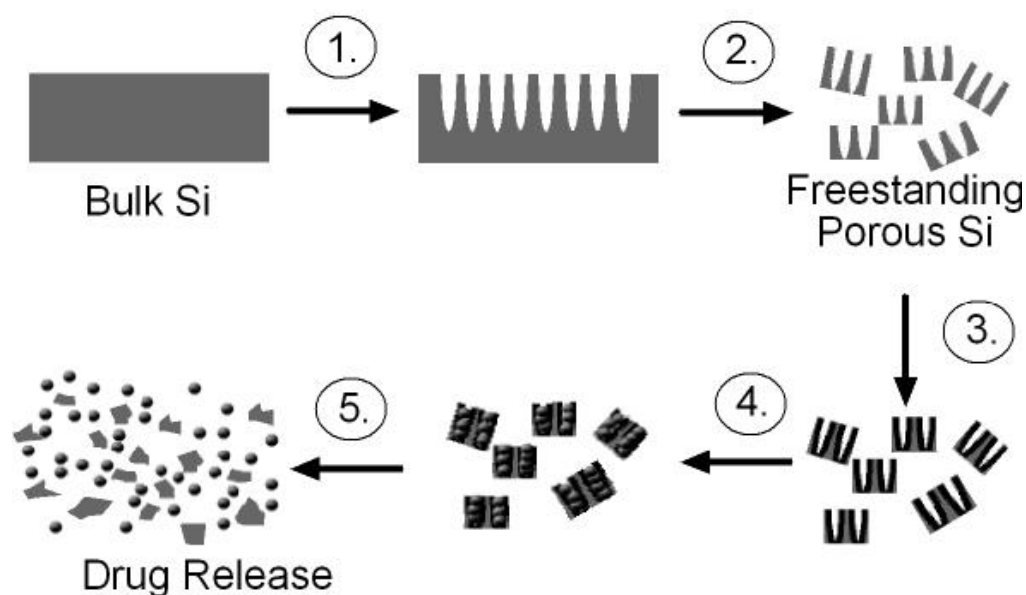


Figure 3.1 Method used to prepare drug-loaded porous Si particles and release the drug by ultrasonic fracture. (1) A nanostructured porous Si film $\sim 75 - 100 \mu\text{m}$ thick is prepared by electrochemical etching of a single crystalline Si wafer. (2) The porous film is removed from the Si wafer by electropolishing and broken into mm-scale particles. (3) The freestanding particles are chemically modified with alkyl groups by microwave-assisted thermal hydrosilylation of the Si-H surface with 1-dodecene. (4) Dexamethasone is loaded into the particles by solution infusion and solvent evaporation. (5) Drug is released into aqueous solution by brief exposure to ultrasound.

3.3 Experimental Methods

Silicon wafers were purchased from Siltronix. 1-dodecene (95%) and dexamethasone (98%) was obtained from Aldrich chemicals. HF (48%) and ethanol was purchased from Fisher Scientific. Phosphate buffered saline solutions were supplied by Mediatech.

3.3.1 Sample Preparation

Silicon wafers were purchased from Siltronix. 1-dodecene (95%) and dexamethasone (98%) was obtained from Aldrich chemicals. Aqueous HF (48%) and ethanol was purchased from Fisher Scientific. Phosphate buffered saline solutions were supplied by Mediatech.

Porous Si microparticles were prepared by electrochemical etch of boron-doped p^{++} -type (resistivity 1 $m\Omega$ -cm) Si wafers using a current density of 130 mA/cm^2 for 20 min. The electrolyte solution consisted of 3:1 (v/v) 48% aqueous HF:ethanol. After etching, the porous Si film was removed from the bulk crystalline Si substrate by applying a current density of ~ 60 mA/cm^2 in a 3.3% HF in ethanol electrolyte. The film was mechanically fractured into plate-like particles approximately 1 mm across and 75-100 μm thick. The particles were rinsed extensively with ethanol and dried in ambient conditions.

3.3.2 Microwave Hydrosilylation of 1-dodecene

Immediately after the electrochemical etch and subsequent removal of the films from the crystalline silicon wafer, the particles were hydrosilylated with 1-dodecene. The particles were placed in a microwave safe vial, submerged in ~ 10 mL of 1-dodecene, and loosely capped with a rubber stopper to avoid pressure buildup during the procedure. The vial was placed in a commercial microwave oven (Sears Kenmore model #66319) located in a fume hood. The sample was heated by microwave on full power for a total of 10 min at 1 min intervals. After each 1 minute interval, the vial was removed from the microwave and pressure was released from the system. After hydrosilylation, the remaining 1-dodecene supernatant was decanted, the particles were rinsed extensively with dichloromethane and ethanol and then dried in ambient atmosphere and temperature.

3.3.3 Drug loading into porous Si microparticles

Dexamethasone was loaded into 10 mg of alkylated microparticles using a solvent evaporation method previously described.⁴¹ The particles were submerged under a solution of 2.25×10^{-2} M dexamethasone in ethanol, and the solvent was allowed to evaporate leaving the dexamethasone residue in the pores

and on the surface of the particles. The particles were briefly rinsed with PBS prior to exposure to ultrasound.

3.3.4 Ultrasound induced drug release from porous Si microparticles

The drug loaded porous Si microparticles were placed in glass vials containing 10 mL aqueous PBS buffer and the vials were placed in an ultrasonic water bath (Fisher Scientific model FS20). Ultrasound was applied for 1 min. The solutions of PBS were extracted from each vial and filtered using Anopore syringe filters (pore size = 0.22 μm). For comparison, control particles (alkylated and loaded with dexamethasone) were also placed in 10 mL PBS solution for 1 min, but not exposed to ultrasound. The PBS solution containing the control particles was also filtered through a syringe filter (pore size = 0.22 μm) prior to analysis of drug content.

3.3.5 Quantification of drug release with UV-vis spectrophotometry

The PBS solutions from the drug release samples were analyzed by UV - visible absorption spectrophotometry. The absorbance at 242 nm, corresponding to the absorption band of dexamethasone, was recorded and fit to a standard curve that had been experimentally established in order to quantify release of dexamethasone from porous Si microparticles.

3.3.6 Microscopy of particles

Scanning electron microscopy (Phillips XL30 environmental scanning electron microscope operating in the secondary electron mode, accelerating voltage of 20 keV) and optical microscopy (Nikon Eclipse LV500, coupled with a Photometrics HQ2 camera) was used to characterize the dimensions of the particles before and after ultrasound exposure. The optical microscopy was performed on particles immersed in ethanol.

3.4 Results and Discussion

3.4.1 Chemical modification of porous Si particles

In order to stabilize the particles in aqueous media and impart a hydrophobic nature to the material, the particles were chemically modified with alkyl groups. Microwave-assisted thermal hydrosilylation was used, following the method of Boukherroub, et. al.⁴² Fourier transform infrared (FTIR) spectroscopy was used to characterize the surface chemistry. Freshly etched particles display characteristic $\nu_{(\text{Si-H})}$ bands at 2113 cm^{-1} and a $\delta_{\text{Si-H}_2}$ band at 918 cm^{-1} indicative of the hydride species present on the surface (Figure 3.1a). After thermal hydrosilylation with 1-dodecene, the FTIR spectrum shows characteristic

vibrational bands associated with $\nu_{(\text{C-H})}$ modes at 2891 cm^{-1} and $\nu_{(\text{C-H})}$ scissors mode at 1481 cm^{-1} (Figure 3.1b). The $\nu_{\text{Si-H}}$ stretching vibrations are still observed in the FTIR spectrum indicating that not all of the surface hydrides react in the thermal hydrosilylation process but a relative decrease in the intensity of the Si-H peak, corresponding to conversion of the Si-H bonds to Si-C bonds is observed. Oxides of silicon are also observed as $\nu_{(\text{Si-O})}$ stretching vibrations present at 1025 cm^{-1} (Figure 3.1b), indicating some surface oxidation occurs during the chemical reaction.

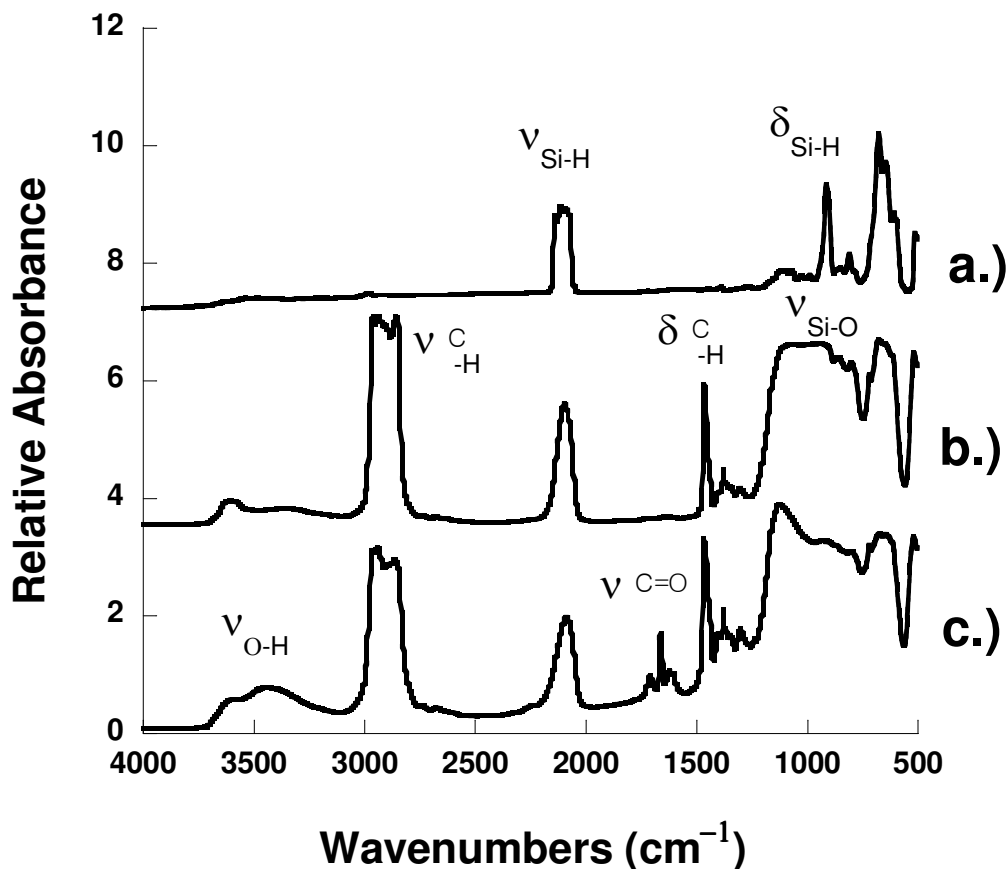


Figure 3.2 FTIR spectra of porous Si particles. **(a)** Freshly etched porous Si displays characteristic vibrational bands at 2113 cm^{-1} for $\nu_{\text{Si-H}}$ and at $\delta_{\text{Si-H}_2}$ 918 cm^{-1} are observed for the surface hydrides present after the electrochemical etch. **b.)** FTIR spectrum after microwave hydrosilylation with 1-dodecene. Characteristic $\nu_{\text{C-H}}$ stretching vibrations at 2891 cm^{-1} and $\delta_{\text{C-H}}$ scissoring at 1481 cm^{-1} . Some surface oxidation occurs during the hydrosilylation and is seen by the $\nu_{\text{Si-O}}$ stretching vibrations present at 1025 cm^{-1} . **c.)** Spectrum after drug loading. The characteristic stretching vibrations for dexamethasone are apparent as $\nu_{\text{C=O}}$ stretching at 1666 cm^{-1} and $\nu_{\text{O-H}}$ at 3484 cm^{-1} along with the stretching bands for the porous Si particles.

3.4.2 Loading of dexamethasone into alkylated porous Si microparticles

The hydrophobic, chemically modified particles were loaded with the commonly used anti-inflammatory steroid dexamethasone. This was achieved using an organic solvent evaporation method previously described.⁴¹ The particles were immersed in a solution of dexamethasone in ethanol and the solvent was allowed to evaporate, leaving the drug packed into the pores of the material. The particles were briefly rinsed before each trial in order to remove any drug residue on the surface of the particles. The FTIR spectrum of the drug-loaded particles displays bands characteristic of dexamethasone; in particular a $\nu_{(C=O)}$ vibrational mode at 1666 cm^{-1} and $\nu_{(O-H)}$ at 3484 cm^{-1} that corresponds to the steroid.⁴¹

3.4.3 Ultrasonically induced release of dexamethasone from porous Si microparticles.

Drug release was triggered by a brief ultrasound application to the particulate system with an ultrasonicator water bath (frequency $42 \pm 6\%$ kHz). The vials containing the drug loaded porous Si microparticles (in 10 mL PBS) were placed in the sonicator for 1 minute. After the one minute application, the aqueous solution was decanted from the particles and filtered with $0.22\text{ }\mu\text{m}$

syringe filters to remove any trace porous Si particles that could interfere with the absorbance reading taken at 242 nm. Ultrasound has been shown to break down porous Si films into particles, and the size of the particles are dependent on the exposure time of ultrasound.³⁸ An enhanced release of drug was observed as the porous Si matrix was subjected to ultrasonically-induced mechanical stress. The fracturing of the particles exposes the drug impregnated porous framework to the aqueous media. The drug was quantified with UV-visible absorbance spectrophotometry by fitting the absorbance values to a standard curve for dexamethasone. The porous Si particles that were subjected to ultrasonic frequencies released 91 μg of drug while the particles that were not exposed to ultrasound had released 37 μg of dexamethasone (Figure 3.2). Some drug diffusion is observed from the particles that are not exposed to ultrasound. Slower drug release has been observed from alkylated porous Si films.⁴¹ The difference is believed to be attributed to an increase in surface area for the particles, incomplete surface coverage of the hydrosilylation reaction (as demonstrated by the Si-O peak present in the FTIR), and a multidirectional release of drug from the porous Si particles as opposed to the uni-directional release of porous Si films on the bulk Si substrate. During the process of fabricating porous Si particles from porous Si films, the surface area increases as the films fragment into smaller particles. This overall increase in surface area leads to more available sites for oxidation to occur to the unreacted hydrides present on the surface, which in turn, allows for a more rapid infiltration of water into the pores. As the particles are exposed to

ultrasound, the particles fragment further, and expose more area resulting in a faster release of drug from the porous matrix. Incomplete rinsing of the particles can also contribute to the observation of drug in solution. Separate measurements confirm the absorbance at 242 nm is due to the dexamethasone in solution and not fragments of porous Si in solution.

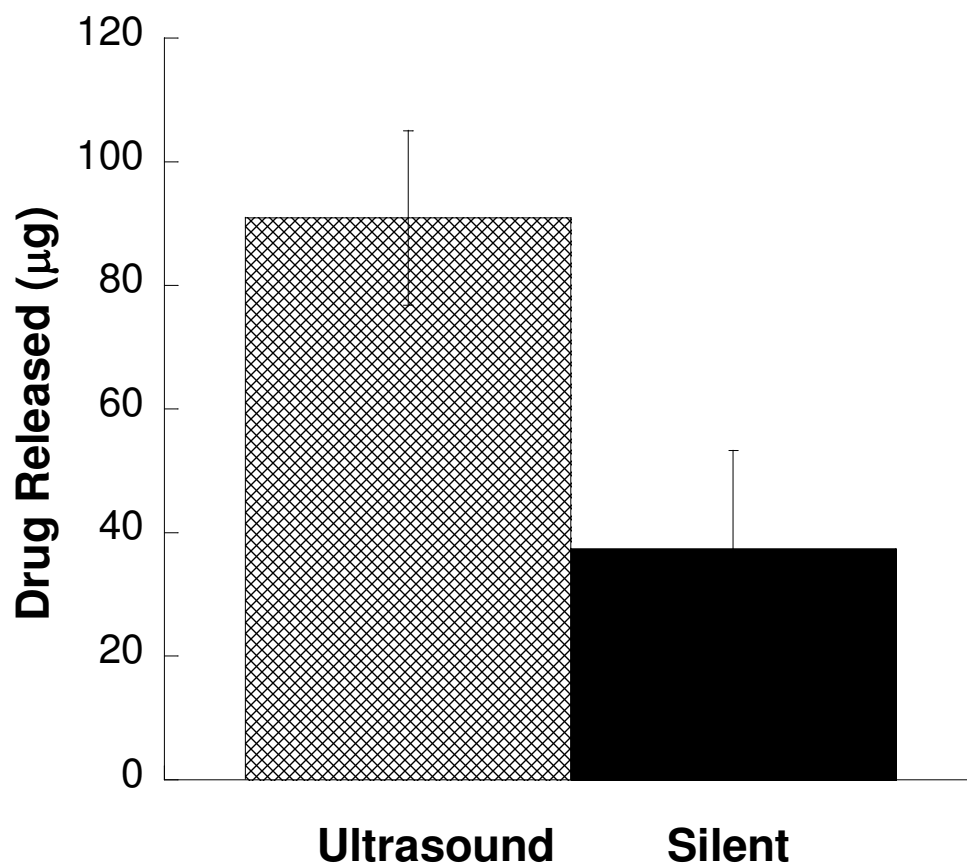


Figure 3.3 Triggered release of dexamethasone from porous Si microparticles. Drug loaded, chemically modified porous Si microparticles were placed in PBS and exposed to ultrasound for 1 minute duration. A triggered release of dexamethasone was obtained, by which, 91 μg of dexamethasone was released. While that particles that were drug loaded, chemically modified that were not exposed to ultrasound demonstrated a 37 μg release of drug from the porous matrix. Samples were run in triplicate, and error bars correspond to standard deviation.

3.4.4 Scanning Electron Microscopy and Optical Microscopy for characterization the porous Si particles.

Optical and electron microscope imaging was used to characterize the shape, size, and thickness of the particles before and after ultrasound. The particles after hydrosilylation and drug loading are roughly $>1000\text{ }\mu\text{m}$ in size and approximately $75 - 100\text{ }\mu\text{m}$ thick. This route of particle production yields a particulate system that has a large size distribution range as the mechanical stability within the particles is not homogenous and no further method was employed to control the particle size (Figure 3.3). Images of the particles after 1 minute of ultrasound demonstrate structural changes in the particle shape and size in comparison to the particles that were not exposed to ultrasound. The particle size is reduced from $\sim 1000\text{ }\mu\text{m}$ to approximately $300 - 700\text{ }\mu\text{m}$, and the particle thickness is roughly the same as the particles that were not sonicated (Figure 3.3B and 3.3E). Particles that were further exposed to ultrasound for a total of 2 minutes continued to fracture due to mechanical stress and decreased in size to $\sim 100 - 250\text{ }\mu\text{m}$ (Figure 3.3C and 3.3F).

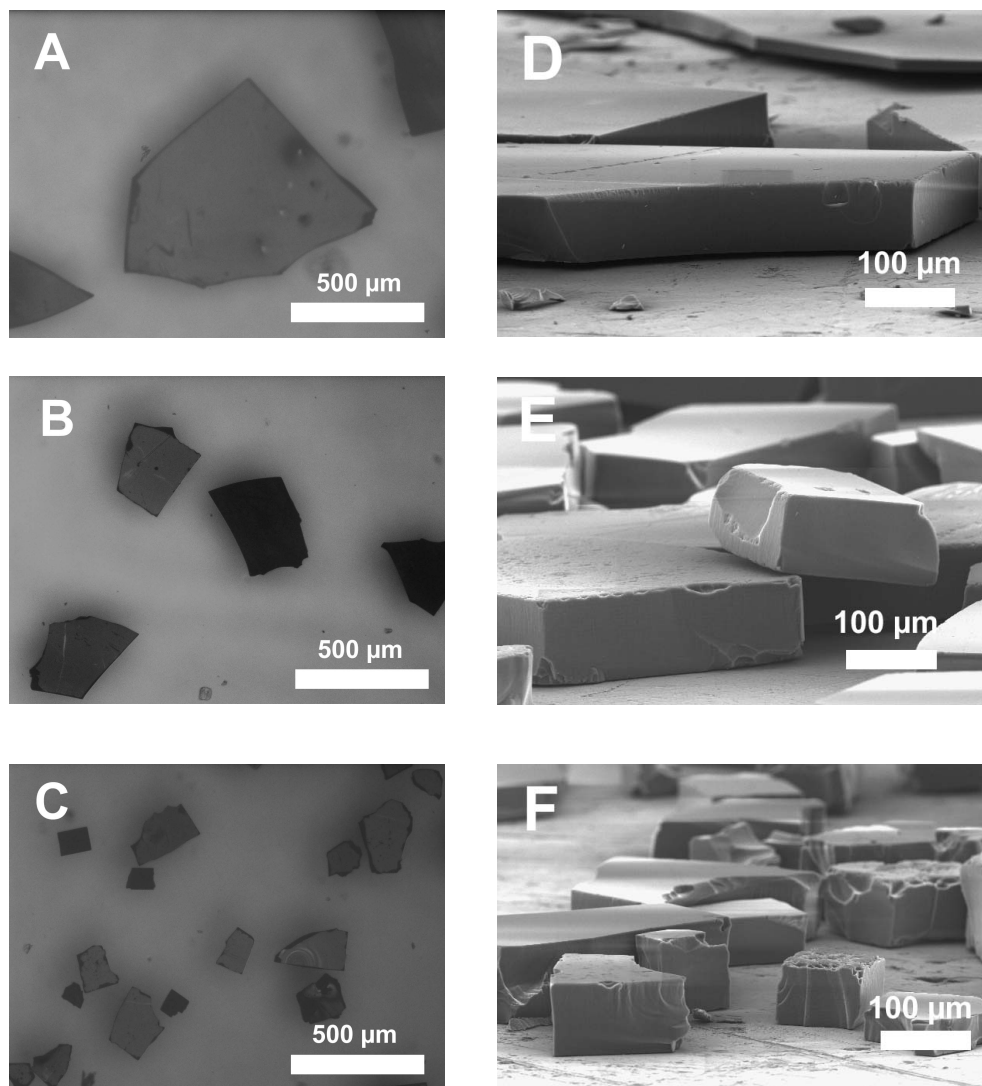


Figure 3.4 Optical and electron microscope images of porous Si microparticles. A.) Optical microscope image of porous Si microparticles before ultrasound exposure. The average particle size is $>1000\ \mu\text{m}$ with a thickness of the particles is $\sim 75\text{--}100\ \mu\text{m}$. The particles vary in size and shape. B.) Optical microscope image of porous Si microparticles after 1 minute of ultrasound with an average particle size ranging between $300\text{--}700\ \mu\text{m}$. The thickness of the particles does not change after sonication. C.) Optical microscope image after 2 minutes of ultrasound. The average particle size ranges between $100\text{--}250\ \mu\text{m}$ with a thickness remaining relatively unchanged. D.) Scanning electron microscope image before ultrasound. E.) Scanning electron micrograph of the porous Si particles after 1 minute of ultrasound. F.) Scanning electron micrograph of the particles after 2 minutes of ultrasound exposure.

3.5 Conclusions

Porous Si microparticles can be fabricated from electrochemically prepared porous Si freestanding films that have been removed from the bulk crystalline wafer and fragmented by ultrasonication. The freshly-etched, hydride-terminated particles can be chemically modified with alkyl groups by thermal hydrosilylation with 1-dodecene using a commercially available microwave for stabilization of the particles for *in vivo* applications. Dexamethasone was loaded into the functionalized porous matrix. Ultrasonic frequencies were used to induce mechanical strain in the porous Si matrix, thereby fracturing the particles by cavitation effects occurring within the porous framework. The drug loaded porous Si microparticles that were exposed to ultrasonic frequencies (42 ± 6 % kHz) released 246 % more drug than the microparticles that were not exposed to ultrasound. Release of dexamethasone from porous Si microparticles is triggered by a short application of ultrasound (1 minute) demonstrating the utility of using a triggered release particulate drug delivery system comprised of electrochemically fabricated porous Si microparticles for *in vivo* applications requiring localized therapeutic delivery.

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CHAPTER FOUR

SUSTAINED RELEASE OF A MONOCLONAL ANTIBODY FROM ELECTROCHEMICALLY FABRICATED POROUS SILICA

4.1 Abstract

Nanostructured porous Si Fabry-Pérot films are used to load and release the monoclonal antibody, bevacizumab (Avastin), *in vitro*. A biodegradable and biocompatible form of mesoporous Si is fabricated by an electrochemical etch of highly doped (boron), p^{++} -type single crystalline Si. The freshly prepared films are oxidized in air at a temperature of 800 °C to yield a hydrophilic porous SiO₂ material. The monoclonal antibody bevacizumab (Avastin) spontaneously adsorbs to the inner pore walls when the porous matrix is exposed to a solution of the drug in buffered (pH 7.4) aqueous solution. Drug loading is monitored by optical interferometric measurements of the Fabry-Perot fringes exhibited in the reflectivity spectrum of the porous Si film. The Bruggeman effective medium model is employed to calculate percent porosity, film thickness, and the extent of drug loading into the porous Si matrix. *In vitro* drug release profiles are characterized by micro bicinconinic acid protein assay. The nanostructured delivery system described here provides a sustained release of the monoclonal antibody bevacizumab where approximately 70% of drug is released in 2 weeks.

4.2 Introduction

Localized and controlled delivery of small molecules, oligonucleotides, steroids, and antibodies are of current interest in the field of drug delivery. There

is increased interest in antibodies due to the relatively recent emergence of a class of FDA approved monoclonal antibody-based therapies.^{1, 2} Of particular interest in this study is bevacizumab (Avastin), a monoclonal antibody that interferes with angiogenesis pathways.³ In combination with more traditional chemotherapies, this drug has shown efficacy in the treatment of various types of cancer. It has also been used alone in the treatment of certain ocular diseases.¹

There remains a lack of effective methods for delivering therapeutically relevant biomolecules. This is partially due to the limitations of current routes of delivery as some biomolecules can be vulnerable to the harsh conditions present in areas such as the gastrointestinal track. Targeting or localizing a drug to the diseased tissue in order to minimize toxicity and other side effects is also a significant challenge. Finally, controlling and extending the release of drug can be essential to increase the efficacy and duration of treatment. Particulate systems such as liposomes⁴, microemulsions⁵, polymeric spheres,^{6, 7} and nano/micro devices⁸⁻¹⁰ have been under investigation to address these issues. In particular, drugs with a short *in vivo* half-life can benefit from encapsulation in a non-toxic, biodegradable, protective carrier.

Biocompatible¹¹⁻¹⁶ and bioresorbable¹⁷⁻²¹ porous Si has been under intensive study for the past decade and is currently engaged in clinical trials as a material that can address the challenges described above.²² Characteristics of porous Si that illustrate the feasibility of this material for therapeutic delivery include a high surface-to-volume ratio,²³ tunable porous nanostructures,²⁴

convenient routes for chemical functionalization,²⁵⁻²⁷ and optically tunable signatures that are applicable to *in vivo* diagnostics.^{28, 29} Many different types of biomolecules have been placed in porous Si or its oxidized form, porous SiO₂. Previous work has shown incorporation of a range of biomolecules including BSA,^{24, 30} enzymes,³¹ Immunoglobulins,^{32, 33} and protein A³³. The work presented in this chapter aims to develop a porous SiO₂ host to effectively sustain the release of the therapeutically relevant antibody bevacizumab.

4.3 Materials and Methods

4.3.1 Materials

Bevacizumab (trade name Avastin[®]) was obtained from Genetech, Inc. Phosphate buffered saline (PBS) solution was purchased from Mediatech. Silicon wafers were acquired from Siltronix, and aqueous HF (48%) and ethanol was purchased from Fisher Scientific. The micro bicinconinic acid (BCA) protein assay utilized in this study was obtained from Pierce Scientific.

4.3.2 Sample Preparation

Porous Si samples were prepared by an electrochemical etch of single crystalline, highly boron-doped (p⁺⁺-type) Si of 1 mΩ-cm resistivity in an ethanolic aqueous HF electrolyte solution (3:1 48% aqueous HF:ethanol by

volume). Samples were etched in a Teflon etch cell that exposed 1.2 cm^2 area of the Si wafer to electrolyte. A constant current density of 417 mA/cm^2 was applied for 1 min. A two electrode configuration was used, consisting of a Pt mesh cathode and a Si anode; the anode was connected to the circuit by means of an aluminum foil contact on the backside of the Si sample. After etching, samples were rinsed with ethanol and dried under a stream of nitrogen. The freshly etched films were thermally oxidized to produce porous SiO_2 , a hydrophilic nanostructured porous matrix. Samples were thermally oxidized in a tube furnace (Lindberg/Blue M furnace, 800°C for 1 hr) in ambient atmosphere. The samples were then allowed to cool to room temperature over a period of several h.

4.3.3 Loading of Avastin into Porous SiO_2 Films

All drug loading solutions were diluted in aqueous PBS, and various concentrations of Avastin were used to determine loading efficiencies (0.125 mg/mL, 0.5 mg/mL, 1 mg/mL). Drug loading into the porous SiO_2 films was carried out in a flow cell apparatus as previously described³⁴ using a flow rate of 1 mL/min for ~ 6 hrs in a closed loop configuration. After loading, the samples were rinsed with PBS solution with the flow cell apparatus in an open loop configuration at a flow rate of 5 mL/min for 10 minutes.

4.3.4 Electron Microscopic Imaging and Interferometric Characterization of the Porous Si Films During and After Drug Loading

The microstructure of the porous SiO₂ samples was characterized by scanning electron microscopy (SEM). Images were obtained using a Phillips XL30 environmental scanning electron microscope operating in secondary electron imaging mode, with an accelerating voltage of 20 keV. Samples were sputter-coated with a thin layer of Cr (~ 10 nm) before imaging.

Loading and release of drug was quantified by optical interferometry. Spectra were collected using an Ocean Optics CCD S-2000 spectrometer fitted with a microscope objective lens coupled to a bifurcated fiber optic cable previously described.³⁰ Both illumination of the surface and detection of the reflected light were performed along an axis coincident with a vector normal to the particle surface. A tungsten light source was focused (spot size approx. 1 mm²) onto the porous SiO₂ surface. Reflected light was collected through the same lens and sent via the second arm of the bifurcated fiber optic cable to the spectrometer for analysis. Reflectivity data were recorded in the wavelength range 400-1000 nm, with a nominal spectral acquisition time of 100 ms.

4.3.5 Analysis of Optical Reflectivity Spectra

Fourier transforms were performed on the optical reflectance spectra of the Fabry-Pérot films to obtain effective optical thickness (EOT) values (product of refractive index and metric thickness, nL) of the porous layers. The wavelength axis of the spectrum from the Ocean Optics spectrometer was calibrated using a least-squares fit of five spectral lines observed from a neon lamp, at 585.3, 614.3, 640.2, 703.2, and 811.5 nm with a nominal data spacing of ~ 0.4 nm. The x-axis was inverted and the spectrum redimensioned to 4096 data points. A linear interpolation was applied such that the data were spaced evenly in units of nm^{-1} . A Hanning window was applied to the spectrum and zero padded to the power of two. A discrete Fourier transform using a multidimensional fast prime factor decomposition algorithm from the Wavemetrics, inc (www.wavemetrics.com) IGOR program library (FFT) was applied. This method yields the value of $2nL$ directly.

4.3.6 Detemination of Film Thickness, Porosity, and Extent of Drug

Loading using the Bruggeman Effective Medium Model

The open porosity, film thickness, and extent of drug loading of the porous SiO_2 films are obtained by applying a least squares fit of the Bruggeman approximation, assuming that the liquid fills all void volume of the film.³⁵⁻³⁹ The

density of Avastin is taken as 1.4 g/mL. The refractive index of the PBS (1.336) was measured with a refractometer (Milton Roy Refractometer). Refractive indices for air and Avastin were assumed to be 1 and 1.4, respectively. The refractive index value of the porous Si film was determined to be 2.02 by the spectroscopic liquid infiltration method (SLIM), as previously described.³⁷ Separate gravimetric measurements^{37, 40} were conducted and agreed within 10% of the optical measurements.

4.3.7 Drug Release from Porous SiO₂ Films

Porous SiO₂ films were loaded with Avastin and placed in vials containing 5 mL PBS (pH 7.4) solution. Drug release was carried out and monitored at room temperature with frequent shaking. The aqueous solution from each vial was exchanged with new PBS solution at time intervals of 0.5, 1, 2, 3, 5, 6, 10, 13 days, and the solutions were analyzed for drug content. Avastin was quantified from standard curves using a colorimetric quantification method (Micro BCA protein assay). The assay was performed on all standards and aliquots of the release samples as described by the vendor (<http://www.pierce-endogen.com/files/0412dh5.pdf>). Absorbance was monitored at 562 nm and fit to a standard curve to determine the mass of Avastin released into solution.

4.4 Results and Discussion

4.4.1 Preparation of Porous SiO₂

Freshly etched porous Si films possess a highly reactive hydride-terminated (Si-H) surface that oxidizes and dissolves in aqueous media.⁴¹⁻⁴³ Thermal oxidation has been found to stabilize the films in aqueous media, and it additionally imparts a hydrophilic nature to the surface allowing more effective infusion of aqueous solutions into the micro and mesoporous material. Air-oxidation of the porous Si samples at 800°C for 1 hr results in near complete conversion of the films to the oxide (SiO₂). This form of SiO₂ is fairly stable in aqueous media.³² Scanning electron microscopy was used to confirm that a substantial portion of the pores were large enough to allow the infiltration of the antibody (~150 kDa). Median pore size was measured to be around 20 nm (Figure 4.1a), and the thickness of the porous SiO₂ film is 12.6 μm (Figure 4.1b). The thickness of the film measured by SEM agreed within 3% of the value determined with the Bruggeman model.

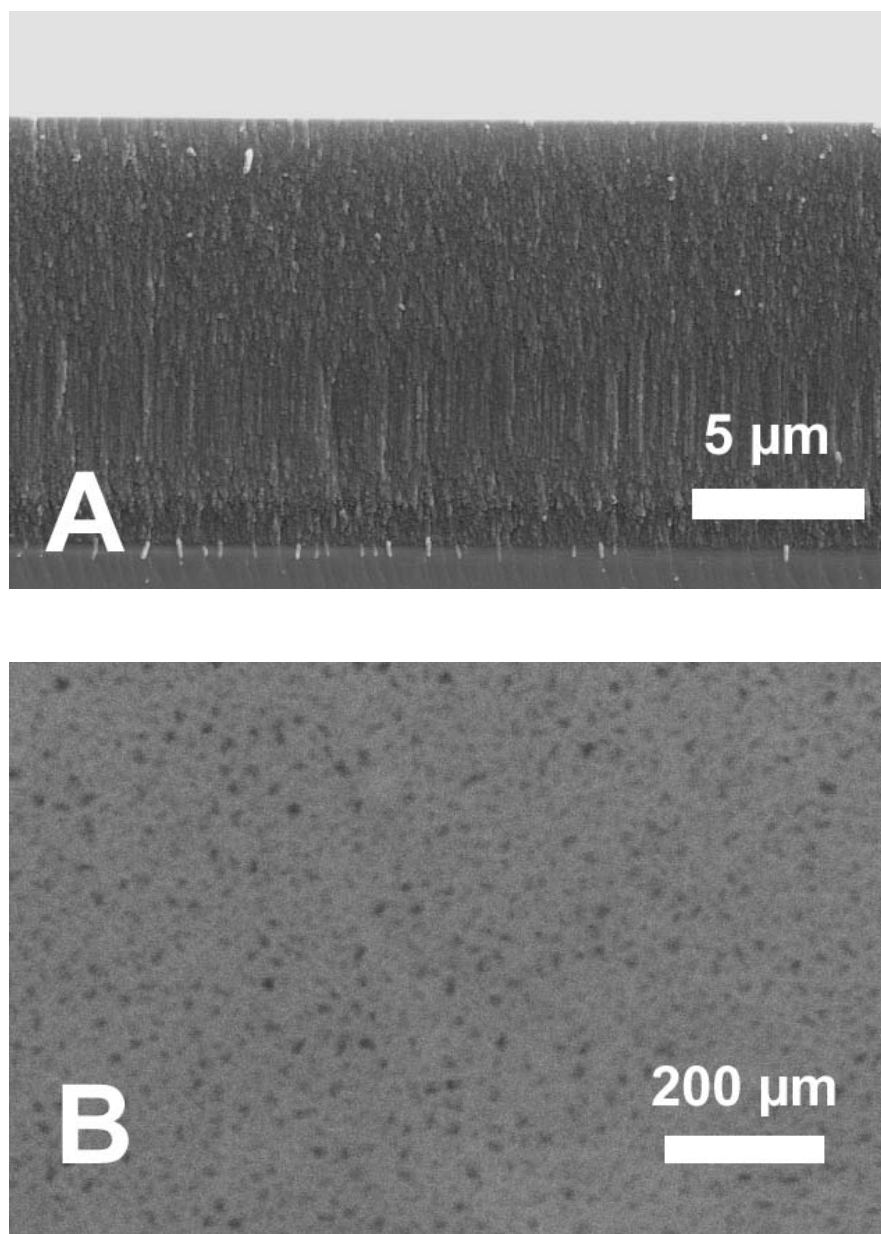


Figure 4.1 Scanning electron microscope image of a porous Si film after conversion to SiO₂. **1a.** Cross-sectional view of an oxidized porous Si film. The pores are aligned in the $\langle 100 \rangle$ direction, and the thickness of the film is 12.6 μm. **1b.** Plan-view of the film. Mean pore size is ~20 nm.

4.4.2 Drug Loading into Porous Si for Drug Release

Avastin was loaded into the porous matrix by introduction of a solution of 1 mg/mL Avastin in PBS to the oxidized porous Si film. A flow cell configured in a circulating loop was used during loading in order to reproducibly load and monitor drug loading with interferometric measurements. The films sat in the circulating flow cell (flow rate 1 mL / min) until drug adsorption was complete (~6 hrs). Immediately after, the films were rinsed with PBS (flow rate 5 mL / min) for 10 min.

4.4.3 Real-time Monitoring of Drug Loading into Porous

Avastin was loaded into the porous matrix by introduction of a solution of Avastin in PBS to the porous SiO₂ film. A flow cell with an optical window was configured in a circulating loop in order to simultaneously introduce and monitor the progress of infusion of the drug. The reflectivity spectra of the porous films contain Fabry-Pérot interference fringes that provide a convenient means of monitoring the rate and extent of drug loading in real-time. The fringe maxima and minima correspond to constructive and destructive interference at the air/porous Si (or liquid/porous Si) and porous Si/crystalline Si interfaces. They follow the Fabry-Pérot interference relationship represented by eq. 1,⁴⁴ where m is the spectral order of the fringe at wavelength λ ,⁴⁴ n relates to the effective

refractive index of the material, and L is thickness of the film (in nanometers). As a drug becomes concentrated in the film, the average refractive index increases because the drug possesses a higher refractive index than the aqueous buffer solution. The result is a red shift of the Fabry-Pérot fringes, which is readily monitored in the Fourier transform of the spectrum (Figure 4.2).

$$m\lambda=2nL \quad (1)$$

The value of $2nL$ from eq. 1 represents the effective optical thickness (EOT). This corresponds to the position of the peak in the Fourier-transform of the reflected intensity versus frequency plot, and yields quantitative values for EOT of the porous SiO_2 films. As concentration equilibrium is reached between the drug in solution and the amount of drug in the pores, the value of EOT reaches a steady-state value (Figure 4.2). After loading with Avastin, the films were rinsed with PBS for 10 min. Monitoring the EOT during the PBS rinse shows no significant decrease in EOT indicating the drug has adsorbed to the pore walls. The effect of solution concentration of Avastin on the efficiency of loading was investigated for three different concentrations: 0.125 mg/mL, 0.5 mg/mL, and 1.0 mg/mL. The greater the amount of drug that adsorbs to the pore walls of the film, the greater the shift in the effective optical thickness was observed (Figure 4.2 and Figure 4.3).

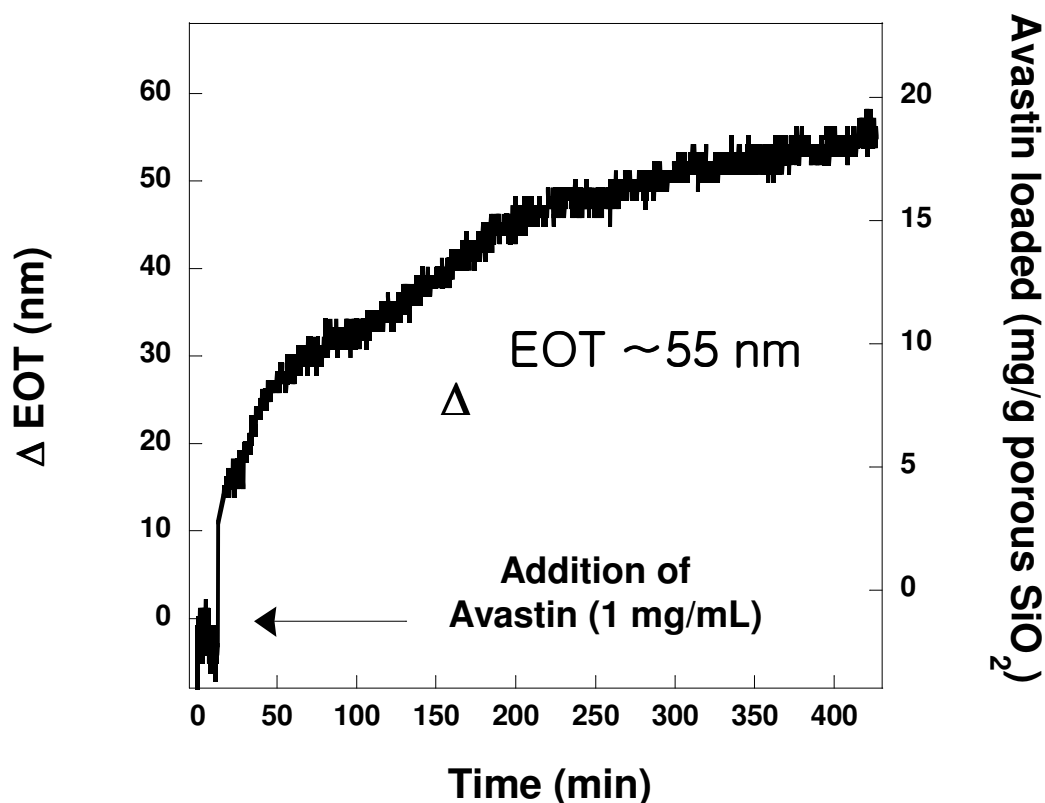


Figure 4.2 Real-time monitoring of drug loading into a porous SiO₂ film using optical interferometry. The quantity EOT (effective optical thickness, $2nL$) displayed on the left axis is related to the amount of drug loaded in the film per gram of porous SiO₂ as shown on the right axis. Baseline was established by flowing PBS through the flow cell apparatus (at time 0 through 15 minutes). bevacizumab solution (1 mg/mL in PBS) was introduced (at time = 15 min), and a steep increase in EOT is observed as drug solution infiltrates the film. At time = 75 minutes, a slower but continual increase in EOT is observed, and this can be attributed to bevacizumab adsorbing to and concentrating on the pore walls of the porous SiO₂ film. As drug loading approaches completion, the value of EOT reaches a limiting value of ~ 55 nm. The sample is then rinsed with PBS to remove any drug residue and weakly adsorbed drug from the film. No observable changes in EOT are observed in the rinsing step indicating that the drug is strongly bound to the film.

4.4.4 Thickness, Porosity, and Amount of Drug Loaded into Porous SiO₂ films

The porosity and thickness was determined by comparing the EOT values obtained from the reflectivity spectrum in air and the spectrum of the film submerged in a liquid of a known refractive index (PBS in this study). The difference between the two EOT values can be attributed to an overall increase in composite refractive index as the liquid filling the pores replaces air. A fit to the Bruggeman model was used in this work to provide values of film thickness (12.3 μm) and porosity (~77%). The difference between the spectra of the film submerged in a liquid of known refractive index (PBS) before and after drug infusion is attributed to an increase in the composite refractive index of the film as drug infiltrates and adsorbs to the pore walls. The mass of drug loading was determined by employing a three-component Bruggeman model. Figure 4.3 displays the data obtained for several concentrations of Avastin.

	Drug concentration in loading solution, mg/mL		
	0.125	0.5	1.0
EOT (nm) in air ^b	29,707 ± 4	30,989 ± 4	30,620 ± 4
EOT (nm) in buffer ^b	36,626 ± 2	38,461 ± 4	37,333 ± 5
EOT (nm) in drug solution ^b	36,642 ± 2	38,494 ± 4	37,393 ± 2
Δ EOT (nm) ^c	14 ± 4	31 ± 7	62 ± 9
mg drug loaded/g porous SiO ₂ ^{b,c,d}	5 ± 1	10 ± 2	21 ± 3
mg drug loaded/mL porous SiO ₂ ^{b,c,e}	12 ± 3	25 ± 6	54 ± 7
Concentration factor ^f	96	50	54

Figure 4.3 Steady-State Avastin Loading Data for Porous SiO₂ films.

^a The porosity of the films is taken to be 77 % and film thickness is 12.3 μ m.

^b Representative values obtained from a single sample. Error corresponds to 95 % confidence interval.

^c Average values obtained from 3 samples.

^d Assuming the true density of the SiO₂ component of the film is 2.6 g/cm³.

^e Based on the bulk volume of the film: the volume of the SiO₂ component + the volume of the pores.

^f Increase in the concentration (in mg/mL) of the drug in the porous material relative to its concentration in the loading solution.

To investigate the extent of drug loading in the pores as a function of concentration, three different concentrations of bevacizumab were used in the loading solutions: 1 mg/mL, 0.5 mg/mL, and 0.125 mg/mL. The total mass loading of drug in mg per mL of porous SiO₂ was determined from the optical measurements, assuming the density of porous SiO₂ is 2.6 g/cm³ and the film porosity is 77%. A concentration factor can be defined as the ratio of the mass of drug in the loading solution per unit volume to the mass of drug in the porous nanostructure per unit volume (Figure 4.2). In this calculation the unit volume of the porous nanostructure is taken as the bulk volume, including the volume taken up by the SiO₂ component. The data are depicted graphically in Figure 4.4. The mechanism by which the drug is incorporated in the material is attributed to electrostatic adsorption. The overall negative charge of the porous SiO₂ surface^{46,}
⁴⁷ attracts the positively charged bevacizumab (pI of IgG = 7.6)⁴⁸ when the drug is loaded into the films in PBS (pH 7.4). Similar behavior has been observed for protein adsorption into porous Si films, and this mechanism has been used to control the loading and release of biomolecules from the porous matrix.^{24, 34}

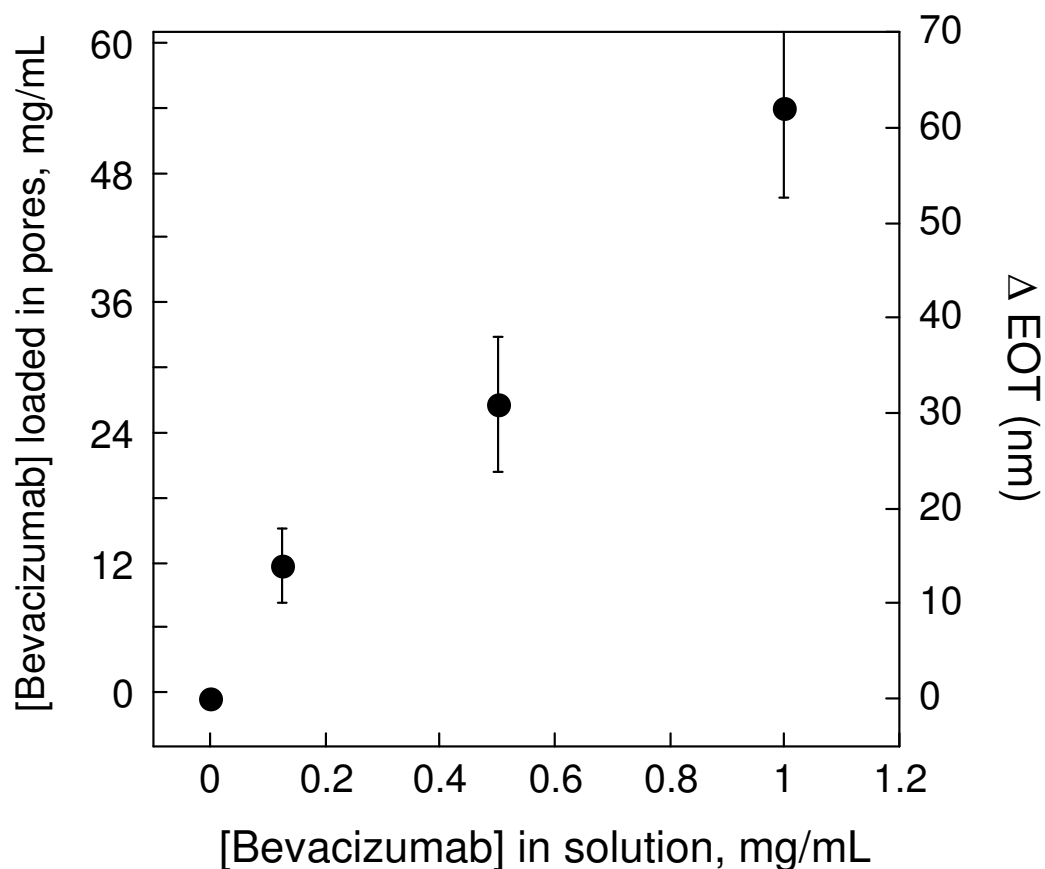


Figure 4.4 Amount of bevacizumab loaded into a porous SiO_2 film as a function of the concentration of the drug in PBS solution. The change in effective optical thickness (EOT, in nanometers, right axis) measured in the optical spectrum is directly related to the mass of drug loaded in the film (left axis). The mass of drug loaded was calculated from the optical response using the Bruggeman model. Higher concentrations of bevacizumab in solution results in larger values of ΔEOT and consequently larger mass loadings in the film. Samples were prepared in triplicate and error bars correspond to 95% CI.

4.4.5 Drug Release from Porous Si

Drug release was quantified by a protein assay (Micro BCA) for each designated time point. Separate measurements confirm the absorbance value observed in the Micro BCA assay was from bevacizumab released in PBS solution, and there was no interference observed from the porous Si platform or other materials used. Approximately $66 \mu\text{g}/\text{cm}^2$ (with a film thickness of $12.3 \mu\text{m}$, and 77% porosity) of bevacizumab was incorporated by loading from a $1 \text{ mg}/\text{mL}$ solution of bevacizumab, corresponding to $\sim 21 \text{ mg}$ of bevacizumab per gram of porous Si which is comparable to other reports of biomolecule loading in porous Si.³⁸ The material showed a sustained release of drug for a period of 13 days (Figure 4.5), at which time only $\sim 70\%$ of drug was released based on the calculated total amount of drug initially loaded in the pores.

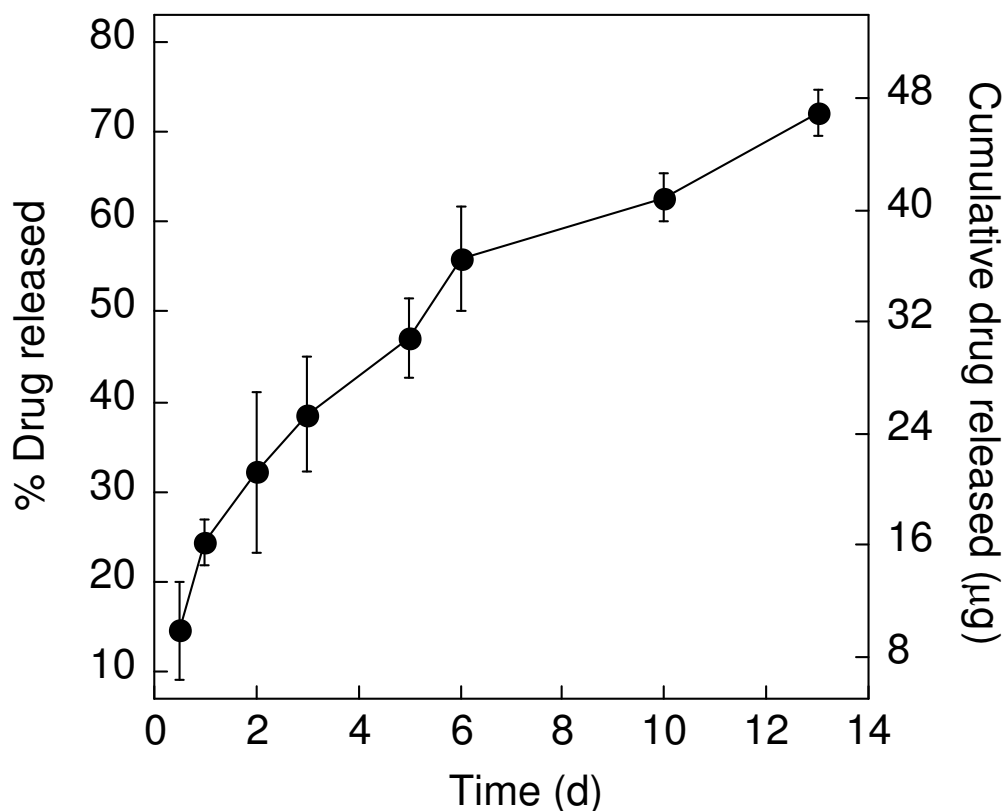


Figure 4.5 . Temporal release profile of bevacizumab from porous SiO₂ film. The percent release and mass of bevacizumab appearing in solution was quantified by Micro BCA protein assay. About ~ 70 % (47 µg) of the drug is released after 13 days. Total drug loading was approximated by fitting the optical interferometric data to the Bruggeman effective medium model. Total drug loading was calculated to be ~ 66 µg (thickness of film ~ 12.3 µm, total area 1 cm²) of bevacizumab per porous SiO₂ film. Samples were prepared in triplicate and error bars correspond to 95% CI.

4.5 Conclusions

The monoclonal antibody bevacizumab (Avastin) can be loaded and released from thermally oxidized porous Si. The degree of loading can be monitored optically by observation of the Fabry-Pérot interference fringes in the reflectivity spectrum of the porous Si film. The optical measurements provide a convenient means to quantify mass of drug loaded in the pores. The negative surface charge of porous SiO₂ allows concentration of the monoclonal antibody in the porous matrix by a factor of up to 100 relative to the solution concentration. The porous SiO₂ matrix releases the drug into aqueous PBS buffer solution slowly, with no initial burst, for a period of > 2 weeks.

4.6 References

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CHAPTER FIVE

INTRAVITREAL PROPERTIES OF POROUS SILICON PHOTONIC CRYSTALS: A POTENTIAL SELF-REPORTING INTRAOCULAR DRUG DELIVERY VEHICLE

5.1 Abstract

Porous silicon photonic crystals were injected into living rabbit vitreous for assessment of the use as intraocular drug delivery vehicles. A rugate structure was electrochemically etched into a highly doped p-type silicon substrate to create a porous silicon film that was subsequently removed from the bulk silicon substrate and ultrasonically fractured into particles. In order to stabilize the particles in aqueous media, the freshly etched porous silicon particles were modified by surface alkylation using thermal hydrosilylation with 1-dodecene and by thermal oxidation. Unmodified particles, hydrosilylated particles, and oxidized particles were injected into rabbit vitreous. The stability and toxicity of each type of particle was studied by indirect ophthalmoscopy, biomicroscopy, tonometry, electroretinography, and histology. No toxicity was observed for the unmodified, hydrosilylated, and oxidized porous silicon particles after intravitreal injection during a period of over 4 months. The chemical modification of the fresh porous silicon particles by both methods led to dramatically increased intravitreal stability and slow degradation. The estimated vitreous half-life increased from one week (fresh particles) to five weeks (oxidized particles) and to 16 weeks (hydrosilylated particles). The current study demonstrated the intravitreal biocompatibility of porous silicon photonic crystals and the feasibility of porous silicon as a platform for an intraocular drug delivery system.

5.2 Introduction

There is an important medical need for a minimally invasive, controllable and monitorable drug delivery system that would enable long acting local treatment of intraocular diseases affecting the retina and choroid. In particular, diseases such as choroidal neovascularization (CNV) in age related macular degeneration (ARMD), proliferative vitreoretinopathy (PVR) associated with retinal detachment and trauma, and refractory uveitis would benefit greatly. For those chronic refractory diseases, drug delivery to the vitreous, retina, and choroid is a challenging task due to the formidable obstacles posed by the blood-retinal barrier and the tight junctions of the retinal pigment epithelium. With systemic administration, only small fractions of drug reach the target, requiring large and potentially toxic doses. An ideal method of retinal drug delivery would provide a locally sustained release for prolonged periods of time. Due to the short vitreous half-life of most injectable intravitreal drugs, frequent administrations are necessary.^{1 2} Intravitreal injection has become standard in clinical practice and trials; however, the reported risk of infections is up to 1% which could lead to devastating visual loss.^{2 1} Intraocular implants have provided sustained vitreoretinal drug levels for treating certain retinal diseases; however, this route demands intraocular surgery that is known to cause complications when placing and replacing the implant.^{3 4}

Self-assembling liposomes and a crystalline drug delivery material have been investigated to achieve intraocular long-lasting release of selected antiviral and antiproliferative compounds.⁵⁻⁷ In particular, the lipid prodrug delivery system is not feasible for delivery of large molecular compounds such as polypeptides and proteins, which are becoming increasingly important in the treatment of eye diseases.^{1, 8} Other systems consisting of biodegradable and bioerodible polymeric microparticles have been used to deliver drugs within animal eyes with a certain degree of success.^{9, 10} Many of these particulate systems have been investigated for intraocular drug delivery, but none have demonstrated the capability to provide a non-invasive method to monitor drug release *in vivo*. It is desirable for a drug delivery system to be capable of housing many types of therapeutics, to be biodegradable after injection, to provide controllable drug release, and to allow post-injection monitoring of drug release with a simple optical device in a clinic.

In this chapter, porous Si photonic microparticles are investigated as a material to address the issues of sustained intraocular drug release while providing a means to “self-report” on the drug release through non-invasive methods.¹¹ This work is the first to demonstrate the use of the optical features of the material *in vivo*. The “self-reporting” scheme utilizes the unique optical properties of the photonic crystal to yield qualitative information on the drug release and degradability of the material. The distinctive reflectivity spectrum of the photonic porous Si particle can be observed through human tissue,¹² and it can be used to

monitor the loading and release of various organic or biomolecules including dexamethasone,¹³ DNA,¹⁴ IgG,¹⁵ and bovine serum albumin.¹⁶ We believe that this optical method of monitoring molecular loading and release is well suited for ophthalmic applications. The drug can be housed in the porous matrix while the optical spectrum allows non-invasive measurement of the release rate. This is the first study to characterize the intraocular properties of porous silicon particles that are capable of acting as a self-reporting drug delivery system in living animal eyes.

5.3 Materials and Methods

5.3.1 Fabrication of Porous Silicon Particles

Porous silicon particles were fabricated by an electrochemical etch of single-crystalline, degenerately B-doped p-type silicon (Siltronix Inc., <100> orientation, $\sim 1\text{m}\Omega\bullet\text{cm}$ resistivity) in a 48% aqueous HF : ethanol (3:1 by volume) electrolyte solution. An optical rugate structure was electrochemically etched into the Si wafer using a sinusoidal current modulation of $15\text{-}45\text{ mA/cm}^2$, with 70 repeats and a periodicity of 12.5 seconds. The films were removed from the bulk silicon substrate by electropolishing in a 3.3% HF in ethanol solution using a current density of 200 mA/cm^2 for 2 min. The manufactured porous Si film was generally 20 microns thick (Figure 1) with a porosity of 67% as determined by

gravimetric analysis described previously ¹⁷. The freestanding films were then ultrasonically fractured using an ultrasonic cleaner (5 min.) to produce particles ranging in size from 1-270 microns with over 70% particles falling in the range of 15-30 microns (estimated by optical microscopy). For a 20 micron particle, there is an estimated free volume of $4 \times 10^{-9} \text{ cm}^3$ available for drug loading per particle, with a total free volume of $1.2 \times 10^{-4} \text{ cm}^3$ per particle injection in the rabbit vitreous.

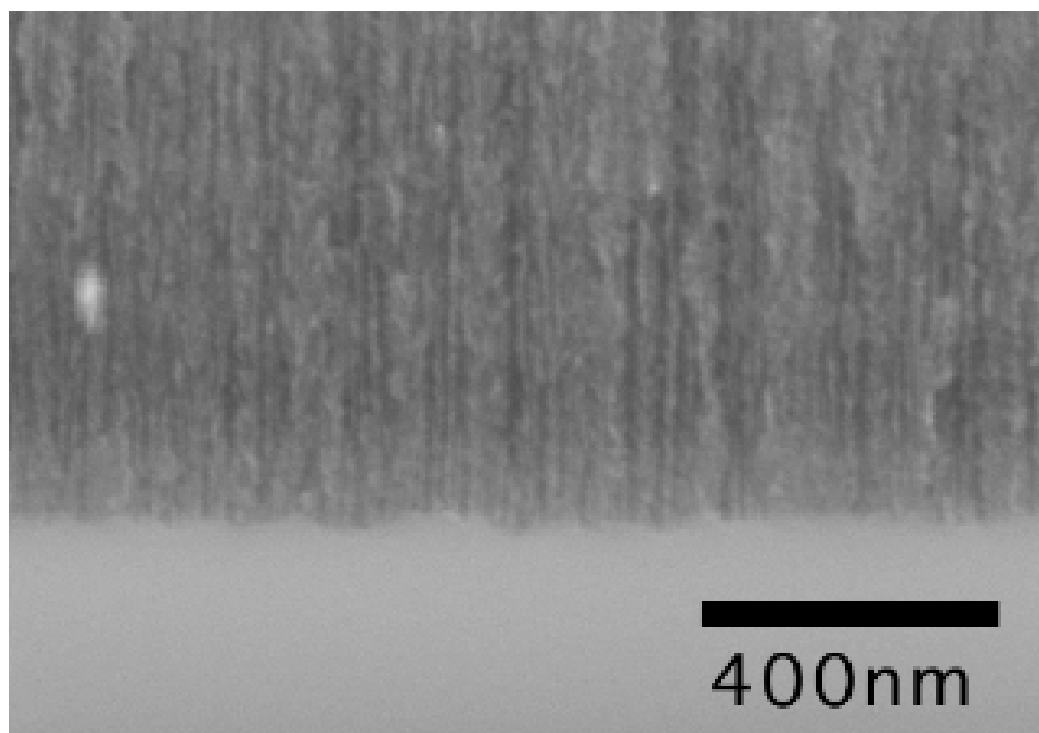


Figure 5.1 Cross-sectional scanning electron micrograph image of an intact porous Si film prior to removal from the bulk silicon substrate and fracture into microparticles. The pores are aligned along the $\langle 100 \rangle$ direction of the original silicon crystal.

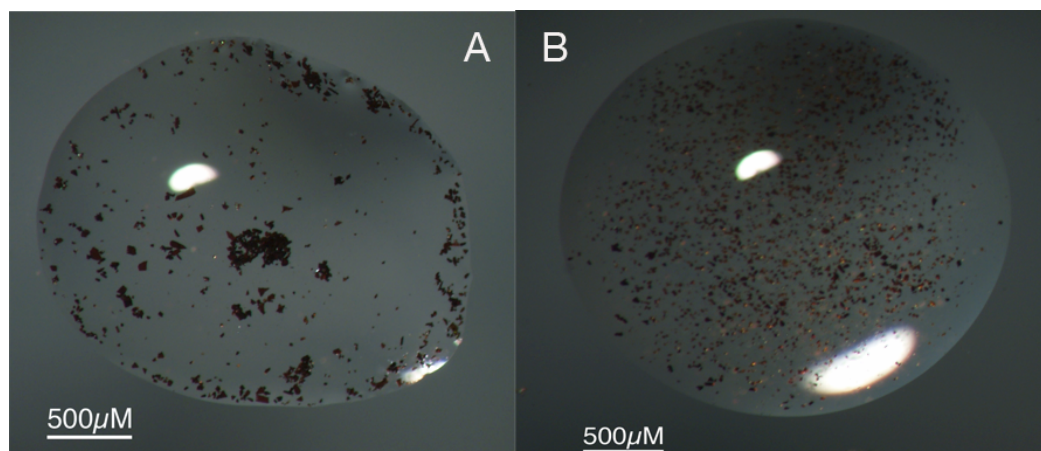


Figure 5.2 (A) Fresh porous Si particles in a droplet of 5% dextrose solution. Particle clumping is observed due to the hydrophobic nature of the unmodified porous Si particles. (B) Oxidized particles in a droplet of 5% dextrose solution. These particles were observed to be more dispersed in solution, presumably because of the hydrophilic nature of the SiO_2 surface.

5.3.2 Chemical Modification of Porous Si Particles

Unmodified porous silicon is known to be unstable in aqueous media because of rapid oxidation of the reactive hydride species present on the surface.^{18,}
¹⁹ In this chapter, two different chemical modification reactions were attempted in order to stabilize the particles. The first method involves surface alkylation by means of thermal hydrosilylation with 1-dodecene,²⁰⁻²² and the second method is thermal oxidation.

5.3.3 Surface Alkylation of Porous Si Particles.

Thermal hydrosilylation was carried out on porous Si particles immediately after their preparation, following the method of Buriak.²³ The particles were placed in a Schlenk flask containing 1-dodecene and 3 freeze-pump-thaw cycles were performed to remove oxygen.²⁴ The reaction flask was filled with nitrogen and the mixture was heated at 120 °C for 2 hours. The particles were rinsed thoroughly with dichloromethane and ethanol and then dried in air. The product was characterized by FTIR, confirming the presence of alkyl species on the surface of the particles.

5.3.4 Thermal Oxidation of Porous Si Particles

Oxidation was carried out on porous Si particles immediately after their preparation. Oxidation was accomplished by heating at 220 °C in an oven in ambient air for 24 hours.

5.3.5 Animal studies

The animal studies presented in this chapter were conducted by collaborators Drs. Lingyun Cheng and William Freeman at Shiley Eye Center, La Jolla, California. The data from the toxicity studies were included as supplementary information. Eleven New Zealand Red rabbits were used to study the safety and stability of the porous silicon particles in the rabbit vitreous. All of the animal handlings were carried out in adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Using injection methods previously published, one eye of each animal was injected with the porous Si particles, and the fellow eye was injected with the same volume of 5% dextrose to serve as the control.²⁵ Three rabbits were injected with fresh (not chemically modified) porous Si particles, five rabbits were injected with hydrosilylated porous Si particles, and three were used to evaluate the oxidized porous Si particles. All of the particles were suspended in ethanol for sterilization. Prior to

injection, the ethanol was evaporated and 1mL of 5% dextrose was added to ~120 mg of the particles. One drop (~6 μ L) of sample was taken for particle sizing and counting by light microscopy (Figure 5.2a, 5.2b). A 25 gauge needle was used to deliver 100 μ L of the suspension (roughly 12 mg particles) into the rabbit vitreous through the pars plana under direct view of a surgical microscope. After intravitreal injection, the eyes were monitored by indirect ophthalmoscope, tonometer, and biomicroscopic slitlamp on day 3 and once each subsequent week thereafter.^{7,26} Fundus photography was carried out in the selected rabbit eyes at different intervals after injection to assess degradation of the porous Si particles. The electroretinogram (ERG) was recorded from all eyes of the animals prior to animal sacrifice, which has been described previously.⁵ After animal sacrifice, the eye globes were enucleated for histology evaluation. The vitreous containing the hydrosilylated porous Si particles was excised from selected eyes, and the particles were examined by scanning electron microscopy.

5.4 Results

5.4.1 Observation of unmodified porous Si particles in the rabbit eye

A 100 μ L aliquot of porous Si particles in 5% dextrose solution was injected into the vitreous of three eyes of three rabbits using a 25 gauge needle. The particles ranged in size from 1 to 270 μ m and the estimated number of particles per

injection was approximately 12,000. The particles were suspended in the vitreous at the injection site (Figure 5.3A) and observed to disperse into the surrounding vitreous during the following 2 to 3 days (Figure 5.3B). No toxic effects were observed, and the particles degraded completely in 3 to 4 weeks (Figure 5.3C). Pathologic examination by light microscopy revealed no indications of toxicity (Figure 5.3D).

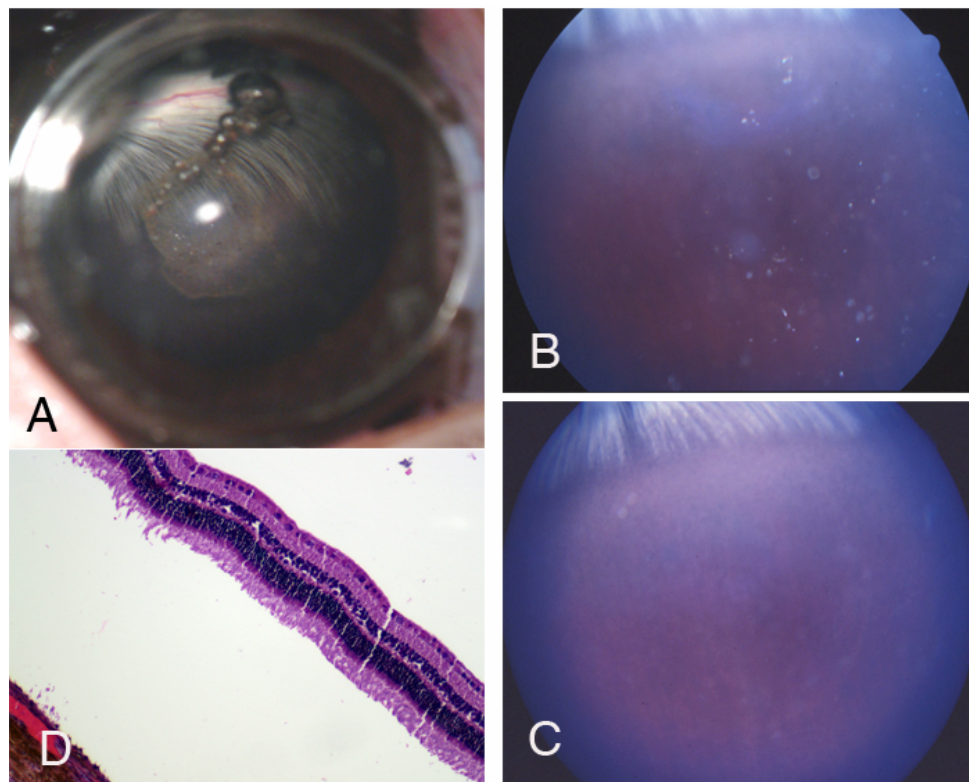


Figure 5.3 A: Photograph taken under a surgical microscope immediately after intravitreal injection of fresh porous Si particles. Particles can be observed suspended in the center of the vitreous. A few small air bubbles mixed with the porous silicon particles are present at the top of the vitreous cavity. B: Fundus photograph taken one week after the injection, showing porous Si particles dispersed in the vitreous. C: Fundus photograph taken 2 weeks after injection, indicating that most of the particles have disappeared and those remaining were barely observable. D: Light microscopic graph showed normal retina detached from the retinal pigment epithelium during the histology processing (25X, H&E staining).

Images obtained from Shiley Eye Center.

5.4.2 Observation of hydrosilylated porous Si particles in the rabbit eye

A 100 μL aliquot of hydrosilylated porous Si particles in 5% dextrose solution was injected into the vitreous of five eyes of five rabbits using a 25 gauge needle. The particles ranged in size from 1 to 300 μm (longest dimension) with an estimated 1900 particles per injection. The hydrosilylated particles became distributed throughout the vitreous within 2 to 3 days while displaying a vivid green color. Degradation was observed to be much slower than for the unmodified porous Si particles (Figure 5.4). Four months after injection the animals were sacrificed, and the particles were analyzed by optical and by scanning electron microscopy. Approximately 50% of the viewable particles appeared blue-green in color (Figure 5.5A). The scanning electron microscope images revealed sharp edges on the particles but a pitted surface, indicating some degree of erosion (Figure 5.5B). The other three rabbits were sacrificed for histopathology. ERG examination, tonometry, and histology did not show any indications of toxicity (Figure 5.5C).

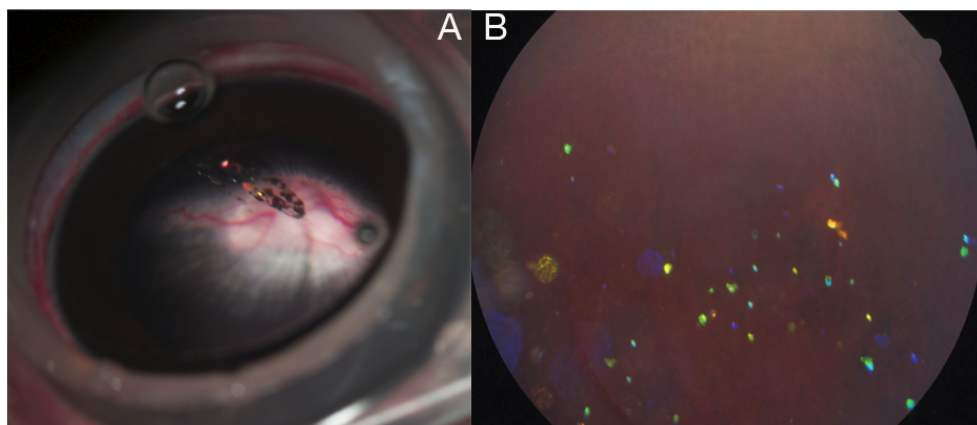


Figure 5.4 A: Photograph taken under a surgical microscope immediately after intravitreal injection of hydrosilylated porous Si particles. Particles can be observed suspended in the center of the vitreous. B: Fundus photograph obtained 3 months after injection. The particles are dispersed in the vitreous and many demonstrated a distinctive blue color corresponding to partial degradation and dissolution. Images obtained from Shiley Eye Center.

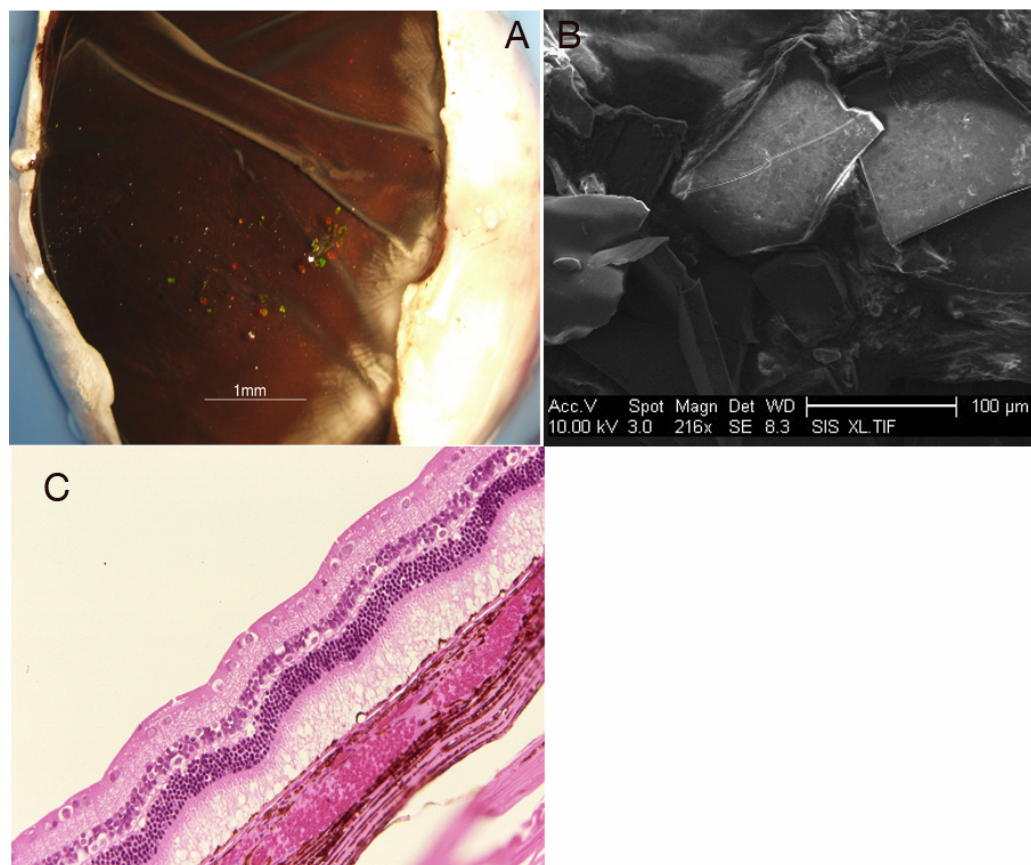


Figure 5.5 A: Surgical microscope image of a dissected rabbit eye cup, with hydrosilylated porous Si particles distributed on a normal looking retina. Photograph was obtained 4 months after injection. Two retina folds are present, caused during dissection. B: Scanning electron microscope image of the hydrosilylated porous Si particles sampled from a rabbit eye 4 months after intravitreal injection. The sharp edges and pitted surface of the particles indicate a very slow erosion process. C: Light microscopic photograph of the retina and choroid from a rabbit eye harvested 9 months after intravitreal injection of hydrosilylated porous Si particles. Normal chorioretinal morphology and structures are observed (62.5x, H&E staining). Images obtained from Shiley Eye Center.

5.4.3 Observation of oxidized porous silicon particles in the rabbit eye

A 100 μ L aliquot of oxidized porous Si particles in 5% dextrose solution was injected into the vitreous of three eyes of three rabbits using a 25 gauge needle. The particles ranged from 10 to 40 μ m and an estimated 30,000 particles were injected. The oxidized particles showed similar dispersion in the vitreous as the unmodified and the hydrosilylated particles. Faster degradation rates were observed for the oxidized particles than for the hydrosilylated particles. Two weeks after injection, 20% of the particles showed evidence of degradation and roughly 80% of them were reflecting purple light (Figure 5.6B). Nine weeks after injection, over 80% of the observable particles lost their vivid reflective property and appeared degraded and brown. The particles had settled into the inferior vitreous or retina (Figure 5.6C). The ERG, tonometry, and histology did not reveal any indications of toxicity (Figure 5.6D).

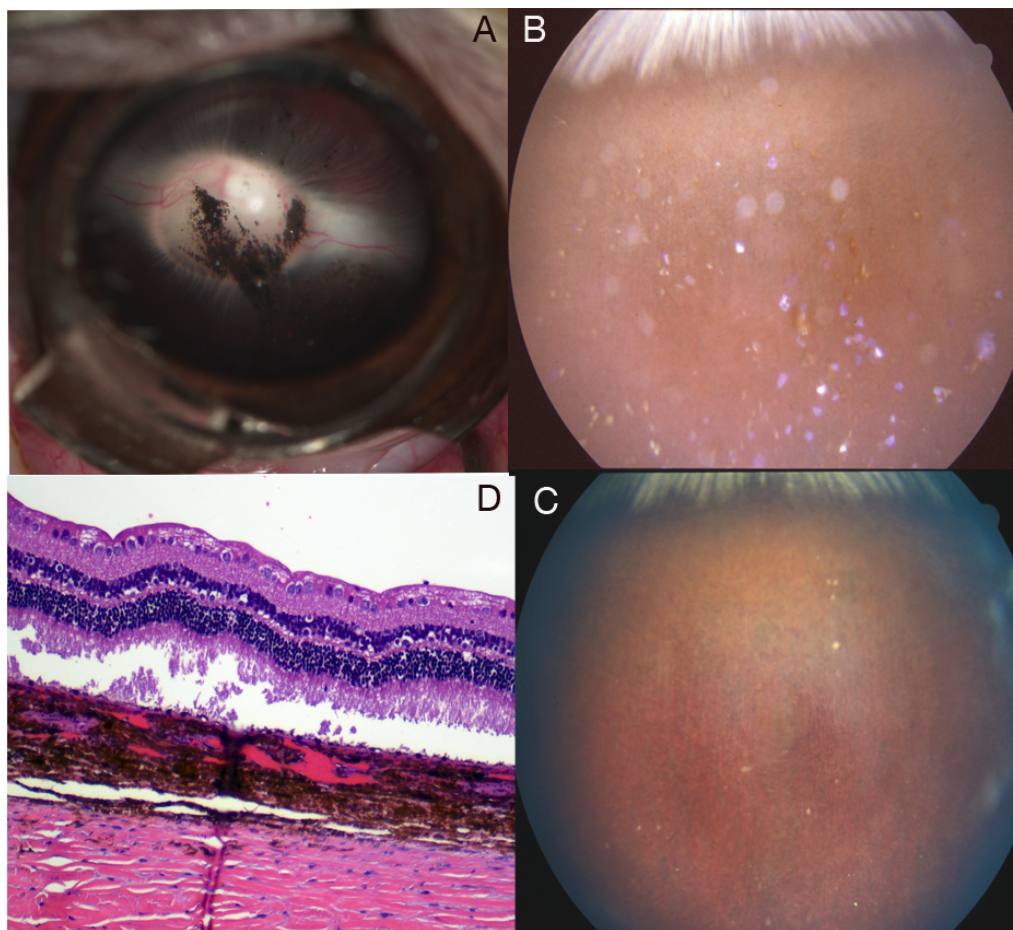


Figure 5.6 A: Photograph taken under a surgical microscope immediately after intravitreal injection of oxidized porous Si particles. Particles can be observed suspended in the center of the vitreous above the optic nerve. B: Fundus photograph of a rabbit eye at 2 weeks after intravitreal injection of oxidized porous Si particles. Many violet particles and a normal fundus can be seen. The particles were initially green upon injection. The violet color indicates that significant oxidation and dissolution of the particles has occurred. Some of the particles have lost their vivid reflectance completely and appear brown in color. C: Fundus photograph of the same rabbit eye, 9 weeks after intravitreal injection of oxidized porous Si particles. Many of the particles have degraded and are no longer observed. The fundus appears normal. D: Light microscopic photograph of the retina and choroid from a rabbit eye harvested 4 months after intravitreal injection of oxidized porous Si particles. Normal chorioretinal morphology and structures are observed with a slight artificial retinal detachment (25X, H&E staining). Images obtained from Shiley Eye Center.

5.5 Discussion

This chapter demonstrates that porous Si particles can be safely injected into rabbit vitreous, and the unmodified particles degrade in three to four weeks without evidence of toxicity. Chemical modification of the particle and pore surface, either by grafting of dodecyl species (hydrosilylation) or by conversion to SiO₂ (thermal oxidation) dramatically increases the stability and vitreous residence time of the particles. This suggests that it is plausible for hydrosilylated or oxidized porous Si particles to be used as a long-lasting intravitreal drug delivery vehicle. Furthermore, by controlling the extent of oxidation or hydrosilylation, we suggest the vitreous residence time of the particles can be manipulated to fit the specific treatment modality.

Porous Si has been studied previously in physiological aqueous solutions and was found to dissolve into the form of orthosilicic acid, which is vital for normal bone and connective tissue homeostasis.²⁷ However, porous Si dissolution has never been studied in vitreous, which is a complex biological solution with constant fluid turn over. This type of condition is not easily duplicated in an in vitro setting. Therefore, the dissolution and the associated potential toxicity must be studied directly in the living eyes.

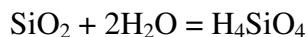
The unique photonic properties of porous Si make this material ideal for drug delivery by imparting a potential self-reporting feature within the delivery system. The wavelength of the spectral peak reflected from porous Si photonic

crystals is dependent on the refractive index (n) of the porous Si matrix as discussed in further detail in chapter 1.3.²⁸ Changes in refractive index of the porous Si layer occurs as aqueous solution ($n = 1.34$) replaces organic molecules or proteins ($n \sim 1.4$) in the pores results in a blue shift of the reflectivity peak, producing an observable color change.²⁹ A spectral blue shift is also expected as the Si matrix ($n \sim 3.5$) is oxidized to SiO_2 ($n \sim 1.7$) or as the SiO_2 matrix dissolves. In the present case, the initial green color of the photonic crystals is observed to turn blue or violet after several days to weeks in vitreous (depending on the surface chemistry) indicating dissolution of the porous matrix. After extended periods in vitreous, some of the particles lose their vivid reflectance and appear brown in color. The brown color is attributed to light absorption by residual Si in a particle whose photonic signature has shifted into the ultraviolet range. It is also possible that the signature spectrum of the photonic crystal no longer exists due to extensive degradation of the periodic nanostructure. This unique signature spectrum of the photonic crystal could be utilized to monitor drug release through the transparent optical medium of the eye using a simple CCD spectrometer device that would provide a non-invasive method to monitor drug release. This would be a major advantage over other drug delivery materials such as biodegradable and bioerodible polymeric microparticles.^{9, 10} It is possible that the injected particles could interfere with vision initially. However, with dispersion and settling of the particles into the inferior vitreous cavity, the possibility of interference will diminish. In clinical practice, many patients who

have asteroid hyalosis seem to not be aware of the presence of the vitreous crystals.³⁰

The fact that certain preparations of the porous Si particles have long vitreous lifetimes and display no apparent toxicity supports the feasibility of the use of porous Si as an intravitreal delivery material. With the advent of many intravitreal injectable therapeutics, such as dexamethasone, pegaptanib (Macugen), bevacizumab (Avastin), and the recently FDA-approved ranibizumab (Lucentis), repeated intravitreal injections can potentially generate serious problems. These procedures impose life quality issues with patients and raise the risk of intraocular infections. If the drugs could be trapped in porous Si microparticles by some encapsulant or by covalent or electrostatic interactions between the drug and the porous Si particles, then the drug would be slowly released as the particles degrade. This would eliminate the necessity of frequent injections.

A 100 μ l intravitreal injection as used in our rabbit studies typically contains ~30,000 porous Si particles, each approximately 50 microns square and 20 microns thick. We estimate that we can load at least ~50 mg of dexamethasone per gram of porous Si material.¹³ If we assume that the porous Si particles display first order dissolution kinetics and that drug release occurs concomitant with particle dissolution, then the steady-state concentration of drug in the eye can be approximated using the dissolution mechanisms of Dove and Crerar.³¹⁻³³ With this model, the dissolution of the porous Si particles can be approximated by this overall reaction.³¹



Where the species H_4SiO_4 represents the water-soluble form of silicic acid. The rate expression for this reaction is dependent on the total surface area of the particles exposed to solution and the mass flow rate of silicic acid out of the system. For our particulate system, we assume that the appearance of silicic acid in solution would correlate with the appearance of drug in solution, and that the total surface area of particles exposed to solution is proportional to the number of particles, N . As the particles dissolve, the drug would be released, and the steady-state concentration of drug in the eye can be calculated based on the following relationship that has been adapted from Dove's model:

$$M_d = [t_{1/2} (\text{drug}) / t_{1/2} (\text{particle})] \times N \times L$$

Where M_d is the mass of free drug in the vitreous, N is the number of particles injected per eye, L is the mass of drug loaded per particle, and $t_{1/2}(\text{drug})$ is the half-life of free drug in vitreous, and $t_{1/2}(\text{particle})$ is the half-life of the particles in vitreous.

We assume that the longer the particle half-life, as demonstrated for the hydrosilylated particles and oxidized particles, the lower the steady-state

concentration of drug. For particles with a 60-day half-life and an initial loaded drug mass of 600 μg in 12 mg of PSi particles ($\sim 30,000$ particles), the steady-state concentration of dexamethasone with a vitreous half-life of 3.48 h³⁴ would be 1 $\mu\text{g/mL}$ in rabbit vitreous (1.4 ml), which is above the therapeutically relevant dose of >5 ng/mL.³⁵ We expect the particles to delivery drug at therapeutically relevant quantities for at least 3 half-lives of the particles (180 days).

Drugs with a longer vitreous half-life such as Avastin (5 days) should be able to further extend the period between injections. For Avastin, we estimate a loading capacity of 20 mg of drug per gram of particles. Thus, the initial amount of drug in a 0.1 cc injection of porous Si loaded with Avastin would be 100 μg of drug. If the half-life of the particles is 60 days, then the steady-state concentration of drug in vitreous would be $\sim 8\mu\text{g/mL}$. The therapeutically relevant dose of Avastin as an intraocular treatment is generally considered $> 22\text{ng/mL}$.³⁶

5.6 Conclusions

In summary, the chapter demonstrates the intravitreal biocompatibility of porous Si microparticles and the feasibility of porous Si as a platform for an intraocular drug delivery system. Further studies to characterize the drug loading, intravitreal release profiles, and non-invasive remote monitoring of drug release

are needed to determine the viability of the approach for effective patient treatment.

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APPENDIX A:
POROUS SILICON – POLYCAPROLACTONE
COMPOSITES

A.1 Porous Silicon – Polycaprolactone Composites

Porous Si composite materials provide a convenient means to alter the physical properties of the nanostructured porous Si films while maintaining the unique optical features built into the porous material. These hybrid materials comprising of a porous Si framework infused with various polymers and hydrogels have been explored for various applications in drug delivery.¹⁻³ These composites are more flexible and durable as opposed to their porous Si only counterparts. In order to trap and controllably release therapeutics from the porous silicon host, we have selected the biocompatible and biodegradable polymer polycaprolactone. This particular polymer provides two advantages to our system: flexibility of the substrate and prolonging the release of therapeutics.

A.2 Fabrication of Porous Silicon Films

Porous silicon films were fabricated by an electrochemical etch of single-crystalline, degenerately B-doped p++ type silicon (Siltronix Inc., <100> orientation, $\sim 1\text{m}\Omega\text{-cm}$ resistivity) in a 48% aqueous HF : ethanol (3:1 by volume) electrolyte solution. A rugate structure was electrochemically etched into the Si wafer using a sinusoidal current modulation of $15\text{-}45\text{ mA/cm}^2$, with 70 repeats and a periodicity of 12.5 seconds. A partial electropolishing step was performed to weaken the mechanical strength at the bulk Si / porous silicon interface. Partial

electropolishing was done by etching in HF: ethanol (3:1 by volume) electrolyte solution at an increased current density ($\sim 400\text{mA}/\text{cm}^2$) for ~ 1 minute.

A.3 Casting of Polymer

Polycaprolactone (PCL) was infused into the porous Si nanostructure by melt casting (of the PCL pellets) at $\sim 120^\circ\text{C}$ for ~ 15 minutes. A color change was generally seen as the polymer melted into the porous structure indicating the infiltration of the polymer into the porous matrix.

A. 4 Removal of Composite from Substrate

The composites were removed from the bulk silicon substrates by a freeze fracture in liquid N_2 . Due to the glass transition temperature of the polymer, we are able to readily extract the newly formed hybrid material from the bulk silicon substrate. The samples were dipped into liquid N_2 for ~ 10 seconds or until the composite released from the substrate and a free standing porous Si – PCL composite film resulted.

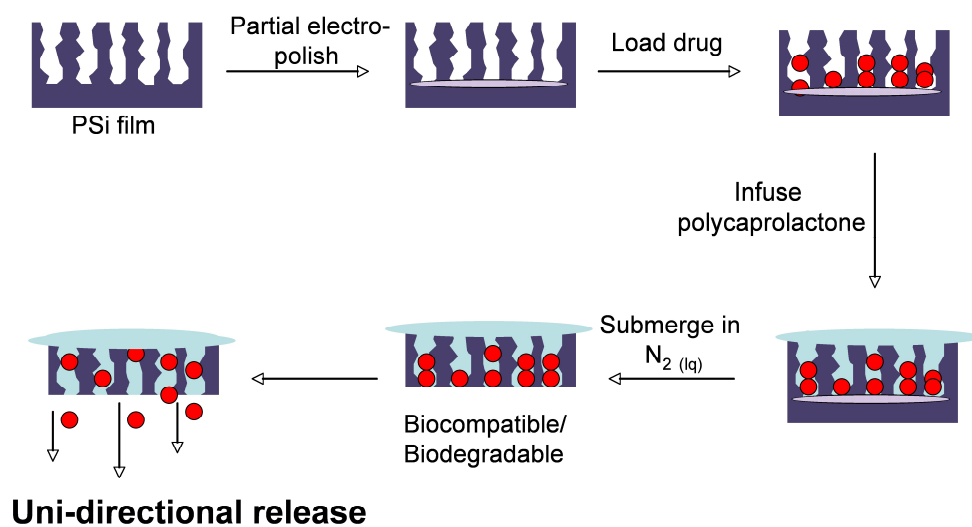


Figure A. 1 General schematic of the fabrication of porous Si – polycaprolactone composites.

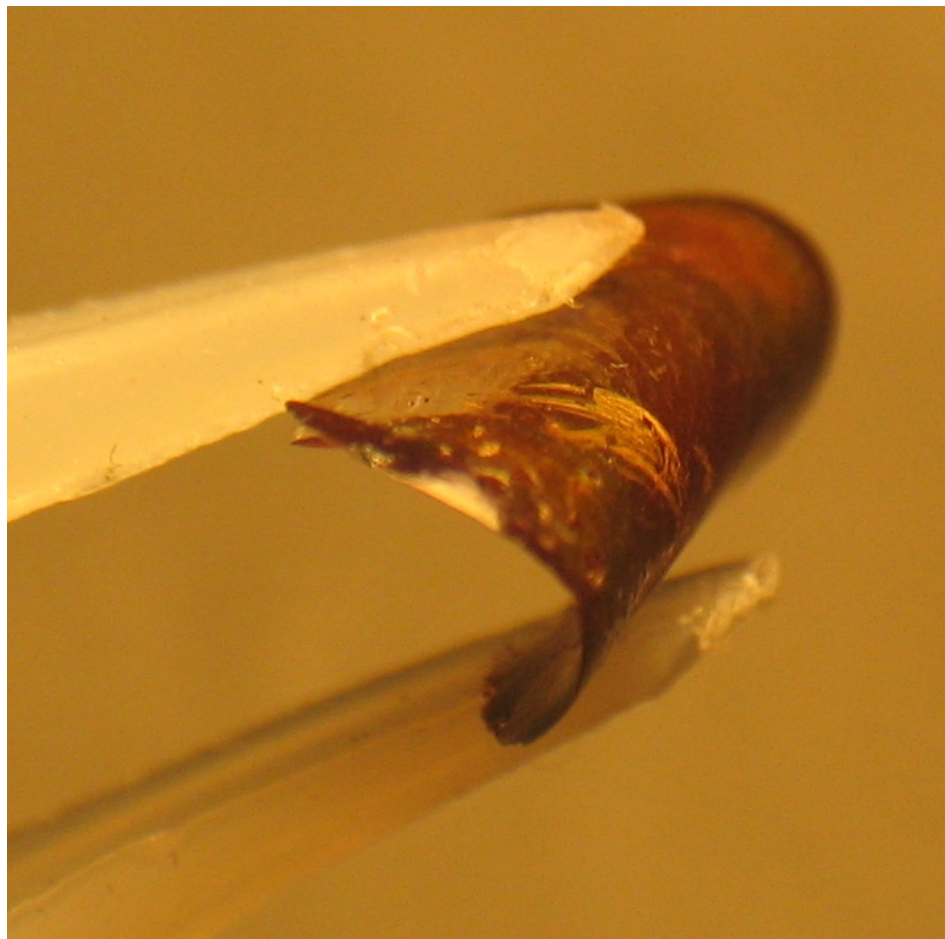


Figure A.2 Porous Si – polycaprolactone composite fabricated from highly doped p-type Si. This composite consists of a rugate porous structure, characteristic of the bright color.

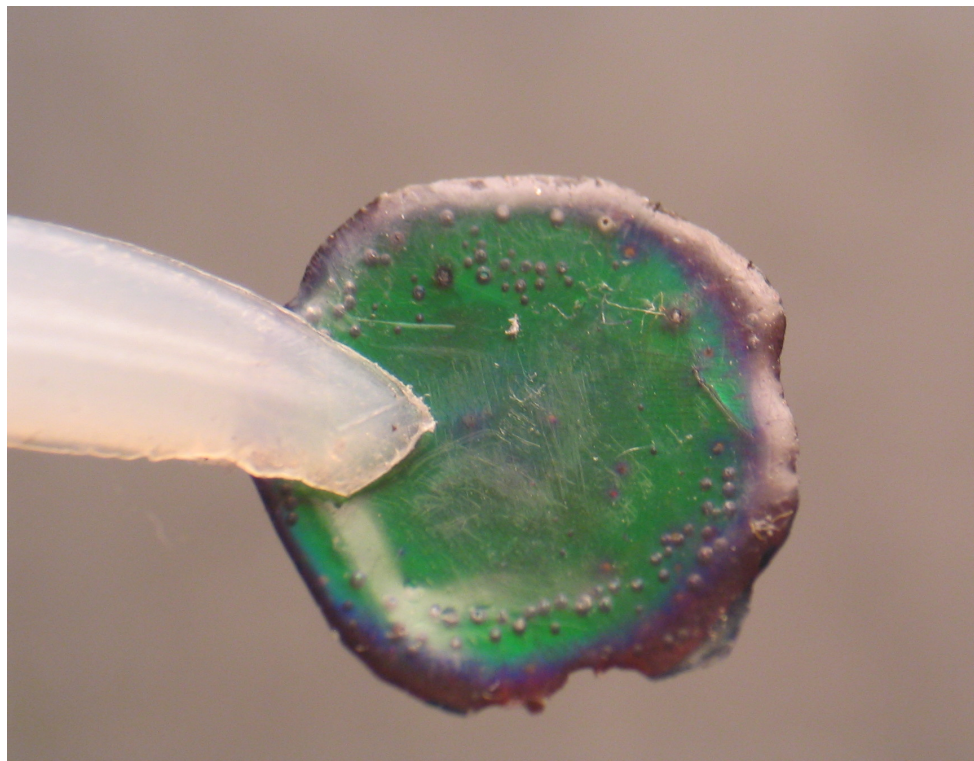


Figure A.3 Porous Si – polycaprolactone composite material. This composite consists of a rugate porous structure as identified by the bright color.

A.5 References

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