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A sustained intravitreal drug delivery system with remote real time monitoring capability

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ABSTRACT

Many chorioretinal diseases are chronic and need sustained drug delivery systems to keep therapeutic drug level at the disease site. Many intravitreal drug delivery systems under developing do not have mechanism incorporated for a non-invasive monitoring of drug release. The current study prepared rugate porous silicon (pSi) particles by electrochemical etching with the current frequency (K value) of 2.17 and 2.45. Two model drugs (rapamycin and dexamethasone) and two drug-loading strategies were tested for the feasibility to monitor drug release from the pSi particles through a color fundus camera. The pSi particles ($k = 2.45$) with infiltration loading of rapamycin demonstrated progressively more violet color reflection which was negatively associated with the rapamycin released into the vitreous ($r = -0.4$, $p < 0.001$, pairwise). In contrast, pSi with K value of 2.17 demonstrated progressive color change toward green and a weak association between rapamycin released into vitreous and green color abundance was identified ($r = -0.23$, $p = 0.002$, pairwise). Dexamethasone was covalently loaded on to the fully oxidized pSi particles that appeared in vitreous as yellow color and fading over time. The yellow color decrease over time was strongly associated with the dexamethasone detected from the vitreous samples ($r = 0.7$, $p < 0.0001$, pairwise). These results suggest that engineered porous silicon particles may be used as a self-reporting drug delivery system for a non-invasive real time remote monitoring.

Statement of Significance

The current study, for the first time, demonstrated proof of concept that engineered porous silicon photonic crystal may deliver therapeutics in a controlled fashion while at the same time might offer a non-invasive remote monitoring of its payload release in a living eye. Porous silicon photonic crystal changes color which is in association with its payload release into vitreous. With further optimization, the color change may be harnessed to inform eye care professionals of real time drug concentration in the eye and allow them to make informed decision to re-dose the patients.

1. Introduction

With improved health care and advanced technology for medical discoveries, human life expectancy is steadily increasing. However, a worldwide aging population also presents us with challenges in medical care including eye disease management. For example, age-related macular degeneration has become a major health problem globally with an estimated 25 million people suffering from this disease. [1] Diabetes is also rising with aging; an estimated 285 million people have diabetes in 2010 and prevalence of diabetic retinopathy is nearly 25% if their diabetic history exceeds 15 or more years. [2] For those with chronic and refractory illness originating from the back of the eye, topical or systemic drug administration has been problematic due to either under-therapeutic drug level at the disease site or systemic side effects. Therefore, intravitreal injection of therapeutics has become a standard care procedure for these diseases. [3–6] Intravitreal injection has an advantage in comparison to systemic, topical, and periocular routes in that it places the therapeutic near the disease site—bypassing the various ocular barriers. With the

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availability of small gauge needles and advanced microsurgical techniques, intravitreal injection is significantly less invasive than surgical drug depot implants such as Ozurdex, Vitrasert, and Retisert. [7–9]

Although intravitreal injection is effective and well tolerated, ubiquitous short vitreous half-life of injectable therapeutics necessitates repeated injections, which introduces high risk of severe complications [10]. To extend vitreous half-life and alleviate the need for frequent injections, a variety of drug delivery systems have been proposed, including liposomes [11], nano/microspheres [12], micelles [13] as well as porous silicon particles [14–17]. With extended residence time in the eye comes a need to understand the complex ocular pharmacokinetics of the drug and delivery system, which demands a large number of animals and significant investment in research effort and resources. A drug delivery system that offers non-invasive, real-time ocular drug monitoring would greatly facilitate the determination of dosing intervals. Porous silicon can be prepared in the form of multi layers (in particular, rugate filters) of varying optical density, yielding an intense reflectivity peak at a predetermined wavelength [18–20]. The wavelength of the reflectivity peak depends on the refractive index of each layer, which in turn depends on the amount of material (drug, silicon, silica, etc) contained within each of the porous layers. This property has been used to monitor the loading and release of various biomolecules [21–24], and in addition to monitor the degradation of the porous silicon or porous silica host material in aqueous media [25–27]. Along with the spectral shift, the intensity of light reflected from these structures also changes as they degrade and/or release a molecular payload. The intensity of reflected light is determined by three factors: (1) the differential index (index contrast) of the layers, (2) the angle of observation relative to the angle of incident light and the surface normal, and (3) the degree of surface roughness of the particle [28,29]. We have previously demonstrated that daunorubicin loading and release can be monitored by such reflectance measurements *in vitro* [26], and that these changes are manifested in an apparent spectral change that can be monitored by digital photography [25,26]. The human eye is a unique organ in that it is composed of clear media that is easily probed optically and non-invasively via an external imaging system. In this work we hypothesized that we could monitor the change in spectral properties of intravitreally administered, drug-loaded porous silicon particles, and use either the intensity or color of light reflected from the particles as a measure of intravitreal drug release using a consumer digital camera coupled to a fundus camera system. In the current study, we used porous silicon particles with two types of drug loading strategies and model drugs rapamycin and dexamethasone to test our hypothesis for this pSi sustained drug release system [30–32].

2. Materials and methods

2.1. Synthesis of porous silicon (pSi) microparticles

Porous Si (pSi) microparticles were prepared by anodic electrochemical etch of highly doped, (100)-oriented, p-type silicon wafers (Fiber Optic Center Inc., boron-doped) in an electrolyte consisting of a 3:1 (v:v) solution of 48% aqueous hydrofluoric acid (HF) and ethanol (Fisher-Scientific, Pittsburg, PA). A Si wafer with an exposed area of 8.0 cm^2 was contacted on the backside with a strip of aluminum foil and mounted in a Teflon etching cell that was fitted with a platinum counter-electrode. The wafer was etched using a current density waveform [26]: $J = A_0 + A\cdot\cos(kt + \alpha)$, where J is applied current density, A_0 is current density offset (mA/cm^2), A is current density amplitude (mA/cm^2), k is frequency (s^{-1}), t is time (s), and α is phase shift (s^{-1}). The values used for A_0, A, and α were 90.2 mA/cm^2, 12.4 mA/cm^2, and 0, respectively. The current density waveform generates a porosity modulation in the porous silicon layer that acts as a 1-dimensional photonic crystal. The photonic crystal displays a sharp peak (stop band of the photonic crystal) in the optical reflectance spectrum whose wavelength is directly proportional to k. In order to obtain particles that displayed colors having good contrast against the rabbit fundus, k values were systematically changed in order to generate reflectance peaks in the blue to green region of the spectrum (450–560 nm). The waveform was etched into the silicon wafer for a total of 400 s. The resulting porous layer was then removed from the silicon substrate by replacing the electrolyte with a 1:29 (v:v) solution of 48% aqueous HF and ethanol and then applying an anodic pulse (6.2 mA/cm^2) for 120 s. This latter step, referred to as "electropol ishing" or "liftoff" in the literature, undercuts the porous layer and detaches it from the silicon substrate. The etching and electropolishing procedure was repeated 20 times per wafer. The particles were harvested every 4 etches and the resulting porous layers were dispersed in ethanol and placed in small vials, which were subjected to ultrasonication (Model FS5 dual action ultrasonic cleaner, Thermo Fisher Scientific, Pittsburg, PA) for 30 min to form the microparticles. After ultrasonic treatment, the supernatant was removed and the particles were resuspended in fresh ethanol. The particles were washed with ethanol three times until the supernatant was transparent.

2.2. Reflectance measurements to select the optimal particle color

The particle synthesis was optimized to obtain colors in the yellow or green region of the spectrum, so they would display maximal contrast against the red color of the retina in the digital images. Because the wavelength of the stop band is sensitive to the refractive index of the material filling the pores, the optical reflection spectrum was measured in various media (air, deionized water, and aqueous PBS buffer solution) [33] and the k value from above was systematically varied to obtain stop band wavelengths that could be predicted to fall in the yellow–green region of the spectrum when the particles were immersed in vitreous fluid. The k values 2.45 and 2.17 were selected for the current studies, which, after oxidation and immersion in rabbit vitreous, appeared violet and green, respectively. The particle thickness and open porosity were also calculated by optical measurements of the reflectivity spectrum on the porous layer as a function of liquid infiltration (SLIM, or Spectroscopic Liquid Infiltration Method) [33,34].

2.2.1. Rapamycin loading by infiltration and loading efficiency

Microparticles with k values of 2.45 and 2.17 were used for rapamycin (Rap) infiltration loading. The resulting drug-loaded particles are denoted as particle 2.45-inf-RAP and 2.17-inf-RAP, respectively. The $pSiO_2$–C8 formulation was prepared from pSi by air oxidation followed by silanization with an organosilane containing a pendant C8 hydrocarbon, methoxy(dimethyl)octylsilane: The samples were placed in a ceramic boat inside a muffle furnace (Thermo Fisher Scientific, Pittsburg, PA), ramped from room temperature to 600 °C at a rate of 10 °C/min, held at 600 °C for 1 h and cooled back to room temperature. In order to generate Si-OH terminated surfaces for further coupling of the organosilane, the partially oxidized pSi particles were treated with 4% (v/v) aqueous hydrochloric acid (diluted from 37 wt% aqueous HCl; Sigma–Aldrich), shaken for 1 h at room temperature and washed with deionized water. Approximately 40 mg hydroxyl-terminated $pSiO_2$ microparticles were suspended in ethanol, transferred to a Schlenk flask and dried under vacuum overnight. Then 8.3 mmol of methoxy(dimethyl)octylsilane (98 wt%; Sigma–Aldrich) per gram of pSi microparticles were dissolved in anhydrous toluene and added with a syringe under nitrogen flow to yield an 8 wt% final solution. The flask was heated at 120 °C in a nitrogen

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