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Porous silicon oxide–PLGA composite microspheres for sustained ocular delivery of daunorubicin

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ABSTRACT

A water-soluble anthracycline antibiotic drug (daunorubicin, DNR) was loaded into oxidized porous silicon ($pSiO_2$) microparticles and then encapsulated with a layer of polymer (poly lactide-co-glycolide, PLGA) to investigate their synergistic effects in control of DNR release. Similarly fabricated PLGA–DNR microspheres without $pSiO_2$, and $pSiO_2$ microparticles without PLGA were used as control particles. The composite microparticles synthesized by a solid-in-oil-in-water emulsion method have mean diameters of 52.33 ± 16.37 μm for PLGA–$pSiO_2$_21/40–DNR and the mean diameter of 49.31 ± 8.87 μm for PLGA–$pSiO_2$_6/20–DNR. The mean size, 26.00 ± 8 μm, of PLGA–DNR was significantly smaller, compared with the other two ($P < 0.0001$). Optical microscopy revealed that PLGA–$pSiO_2$–DNR microspheres contained multiple $pSiO_2$ particles. In vitro release experiments determined that control PLGA–DNR microspheres completely released DNR within 38 days and control $pSiO_2$–DNR microparticles (with no PLGA coating) released DNR within 14 days, while the PLGA–$pSiO_2$–DNR microspheres released DNR for 74 days. Temporal release profiles of DNR from PLGA–$pSiO_2$ composite particles indicated that both PLGA and $pSiO_2$ contribute to the sustained release of the payload. The PLGA–$pSiO_2$ composite displayed a more constant rate of DNR release than the $pSiO_2$ control formulation, and displayed a significantly slower release of DNR than either the PLGA or $pSiO_2$ formulations. We conclude that this system may be useful in managing unwanted ocular proliferation when formulated with antiproliferation compounds such as DNR.

1. Introduction

Proliferative vitreoretinopathy (PVR) is the most frequent cause of failure of retinal reattachment surgery [1]. Previous studies have shown that daunorubicin (DNR) is effective in inhibiting PVR formation [2], and also has been shown to be effective for the treatment of experimental PVR [3–5]. However, DNR has a short half-life in the vitreous and also a narrow therapeutic concentration range, which would require too frequent injections to allow intravitreal DNR to be a practical therapeutic [6,7]. A drug appropriate for the control of PVR needs to inhibit cell proliferation effectively and maintain a therapeutic level in the targeting tissue for a minimum 2 months, which is the median time for PVR development [8]. Porous silicon (pSi) is a nanostructured material with a surface area of 400–800 $m^2\,g^{-1}$ that is commonly produced from bulk single crystal silicon by electrochemical anodization in hydrofluoric acid [9]. An oxidized form of pSi that retains the porous nanostructure and displays a lower reactivity with redox-active drugs [10] can be prepared by thermal oxidation of pSi. From a biological and biomedical perspective, pSi and $pSiO_2$ are attractive materials as they are both biocompatible and biodegradable, meaning that they are able to undergo complete degradation in the body to produce silicic acid ($Si(OH)_4$), a nontoxic soluble form of silicon [11]. It has been established that $Si(OH)_4$ is readily cleared from intraocular fluid [12]. Furthermore, surface chemistries such as silanol condensation and hydrosilylation are available for this material that allow adjustment of degradation rate in biological systems [13–15]. It has been shown that therapeutic payloads can be loaded into the pores of pSi or $pSiO_2$ by adsorption or surface grafting [10,14,16,17]. These properties, in addition to the very large internal surface area [18], render pSi a versatile drug delivery platform [19]. In previous works, we reported the possibility of using pSi and $pSiO_2$ microparticles as an intraocular drug delivery

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system. Whereas pSi was found to react with and degrade redox-active DNR, $pSiO_2$ formulations were inert with respect to chemical reaction with the drug [20]. In a study with the $pSiO_2$ formulation, DNR was loaded into $pSiO_2$ microparticles using two methods, covalent attachment and physical adsorption [10]. The study revealed an obvious difference in the release profiles for the two drug-loading strategies. Covalently loaded particles released <1% of the loaded DNR within 8 days in excised rabbit vitreous while particles loaded by physical adsorption released >75% of loaded DNR within the same time period. A subsequent in vivo study demonstrated localized retinal toxicity from adsorption loaded particles due to rapid release of drug [10]. Particles prepared by covalent loading of DNR did not show retinal toxicity during a 3-month observation period, but initial data indicated very low free drug levels in the rabbit vitreous. Poly(DL-lactide-co-glycolide) (PLGA), a food and drug administration (FDA)-approved biodegradable polymer, has been widely investigated for drug delivery applications due to its customizable degradation rates, favorable mechanical properties and biodegradability [21–25]. We reasoned that combining the porous silicon drug delivery platform with PLGA might increase the effectiveness of the pSi drug delivery system for ocular application of DNR. Indeed, Liu et al. [26] fabricated a series of DNR-loaded PLGA nanoparticles using a modified double-emulsion solvent evaporation/diffusion method and achieved the sustained release of DNR for >2 weeks. Fan et al. [27] investigated PLGA–pSi composite microspheres, synthesized by a solid-in-water (S/O/W) emulsion method for a 30-day delivery of bovine serum albumin (BSA) for orthopedic tissue engineering applications. These prior studies demonstrated that both PLGA and pSi contribute to the control of release of a payload. We hypothesized that a PLGA coating would reduce the initial burst release of DNR from $pSiO_2$, and extend the therapeutic duration. In the current study, we loaded DNR into $pSiO_2$ microparticles by infiltration and then coated the drug-loaded $pSiO_2$ particles with PLGA. We aimed to investigate the release properties of $pSiO_2$ and PLGA composites with the goal of identifying an effective means for intravitreal delivery of DNR.

2. Materials and methods

2.1. Synthesis of porous silicon oxide microparticles

$pSiO_2$ microparticles were prepared by electrochemical etch of highly doped, (100)-oriented p-type silicon wafers (boron-doped, 0.99 mΩ cm resistivity; Siltronix Inc., Archamps, France) in a 3:1 (v/v) solution of 48% aqueous hydrofluoric acid to ethanol (Thermo Fisher Scientific, Pittsburg, PA) as described previously [10]. A silicon wafer with an exposed area of 8.04 cm^2 was contacted on the backside with a strip of aluminum foil and mounted in a Teflon etching cell fitted with a platinum counterelectrode. The wafer was etched at a constant current density of 90.2 mA cm^{-2} for 200 s. The resulting porous layer was then lifted off by electropolishing in a 1:29 solution of 48% aqueous hydrofluoric acid to ethanol (Thermo Fisher Scientific) for 120 s at a current density of 6.2 mA cm^{-2}. The etching and electropolishing procedure was repeated 20 times per wafer. The resulting porous layers were ultrasonicated (model FS5 dual-action ultrasonic cleaner; Thermo Fisher Scientific) in ethanol for 30 min to form the microparticles. These pSi microparticles were converted to $pSiO_2$ by oxidation in a furnace chamber (Thermo Fisher Scientific). The pSi particles were placed in a ceramic boat and the temperature was ramped from room temperature to 800 °C at a rate of 10 °C min^{-1} and then maintained at 800 °C for 2 h. The furnace was allowed to cool to room temperature for an additional 3 h prior to removal of the samples. Thereafter, the particles were dispersed into ethanol and filtered through nylon filtration membranes with the sizes of 5, 20 and 40 μm, respectively. Finally, two samples with the size population of 6–20 μm and 21–40 μm (marked as $pSiO_2$_6/20 and $pSiO_2$_21/40, respectively) were collected and dried in a vacuum oven overnight. The particles showed a uniform nanostructure with a pore size of 15–20 nm as measured from scanning electron microscopy (SEM) images.

2.2. Drug loading via physical adsorption

10 mg $pSiO_2$ microparticles were added into a 1.5 ml Eppendorf tube containing 0.5 ml of 10 mg ml^{-1} daunorubicin hydrochloride (Tocris Biosciences, Minneapolis, MN) in Dulbecco's phosphate-buffered saline. The particles were then vortexed for 2 h at room temperature and rinsed briefly with water three times. The resulting particles were dried and stored in a sealed vial at 4 °C.

The drug loading of $pSiO_2$ was determined by thermogravimetric analysis (TGA). The DNR-loaded samples (~3 mg) were placed in a 90 μl alumina sample cup. Samples were heated at a constant rate of 10 °C min^{-1} up to 900 °C in a nitrogen atmosphere with a purge rate of 10 ml min^{-1} using a Q600 simultaneous TGA/differential scanning calorimetry apparatus (TA Instruments, Newcastle, DL). As determined by TGA, the mass loading of DNR for $pSiO_2$_6/20 and $pSiO_2$_21/40 was 32.5 and 41 μg mg^{-1}.

2.3. Preparation of DNR-loaded $pSiO_2$ particles coated with PLGA (PLGA–$pSiO_2$–DNR) and DNR-loaded PLGA microspheres (PLGA–DNR)

$pSiO_2$ particles coated with PLGA were prepared by a modified S/O/W emulsion method [28] (see Supplementary Fig. 1). Briefly, 10 mg DNR-loaded $pSiO_2$ was mixed with 1 ml 10% PLGA (75:25) (Sigma Chemicals Co., St Louis, MO) solution (dissolved in dichloromethane) by vortexing for 20 min. The mixture was added drop by drop into 50 ml 2.0% poly(vinyl alcohol) (PVA, Mw 89,000–98,000) aqueous solution and stirred with a homogenizer (T18 Ultra Turrax, IKA) at 6,000 rpm to form an emulsion (oil in water, O/W). The emulsion was then transferred into 1% PVA aqueous solution (50 ml) and stirred at 1200 rpm for 2 h to evaporate the organic solvent. The suspension was centrifuged at 10,000 rpm (Allegra® 25R Centrifuge, Beckman Coulter, Inc.) for 10 min and the supernatant was collected for determination of drug loss. The PLGA–$pSiO_2$–DNR microspheres were washed with distilled water for three times, 1 min each time. Finally, the product was lyophilized and stored at 4 °C. PLGA–DNR particles were prepared using a similar procedure as in the fabrication of PLGA–$pSiO_2$–DNR microsphere fabrication, except that DNR instead of DNR-loaded $pSiO_2$ particles was mixed with PLGA/DCM.

The drug loading of PLGA–$pSiO_2$–DNR microparticles or PLGA–DNR microspheres was calculated as follows:

$$\text{drug loading}(\mu g\ mg^{-1}) = (\text{drug total} - \text{drug loss})/\text{mass total}.$$

The drug total was the total amount of drug used to fabricate the microsphere composite; and the drug loss was the drug detected from the aqueous phase after removal of the particulate.

The drug loading of PLGA–$pSiO_2$_6/20–DNR, PLGA–$pSiO_2$_21/40–DNR, and PLGA–DNR was 1.34, 1.52 and 2.25 μg mg^{-1}, respectively. Drug-loading efficacy = actual drug-loading/total drug used × 100%. Different formulation of particulate had different drug loading efficiency (Table 1).

2.4. Morphological characteristics of $pSiO_2$ particles, PLGA–$pSiO_2$–DNR microspheres and PLGA–DNR microspheres

The shape and surface morphology of the microparticles were characterized by scanning electron microscopy (Phillips XL30