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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 (S/O/W) 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 microsphere contained multiple $pSiO_2$ particles. In vitro release experiments determined that control PLGA-DNR microspheres completely released DNR within 38 days and control pSiO2-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 pSiO2 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 it 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 anti-proliferation compounds such as DNR.

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Keywords

Porous silicon oxide; Poly (dl-lactide-co-glycolide); Daunorubicin; Ocular drug delivery

Introduction

Proliferative vitreoretinopathy (PVR) is the most frequent cause of failure for retinal detachment surgery [1]. Previous studies have shown that daunorubicin (DNR) is effective in inhibiting PVR formation [2], and it 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 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 ($Si(OH)_4$) that is 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 allows 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] renders 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 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 less than 1% of the loaded DNR within 8 days in excised rabbit vitreous while particles loaded by physical adsorption released more than 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, its favorable mechanical properties, and its biodegradability [21–25]. We reasoned that combining the porous silicon drug delivery platform with PLGA might increase the effectiveness of the porous silicon drug delivery system for ocular application of daunorubicin. Indeed, Jie 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. D Fan et al [27] investigated PLGA/porous silicon 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 oxidized porous silicon 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.

Materials and Methods

1. Synthesis of Porous Silicon Oxide Microparticles

Porous silicon oxide ($pSiO_2$) 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 counter-electrode. The wafer was etched at a constant current density of 90.2 mA/cm^2 for 200 seconds. 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 seconds 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 minutes 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 and then maintained at 800°C for 2 hours. The furnace was allowed to cool to room temperature for an additional 3 hours prior to removal of the samples. Thereafter, the particles were dispersed into ethanol and filtered through nylon filtration membranes with the sizes of 5 μm, 20 μm 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 uniform nanostructure with a pore size of 15–20 nm as measured from scanning electron microscopic (SEM) images.

2. Drug loading using physical adsorption route

10 mg $pSiO_2$ microparticles were added into a 1.5mL eppendorf tube containing 0.5 mL of 10 mg/mL daunorubicin hydrochloride (Tocris Biosciences, Minneapolis, MN) in DPBS. The particles were then vortexed for 2 hours 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 up to 900°C in a nitrogen atmosphere with a purge rate of 10 mL/min using a Q600 simultaneous TGA/DSC 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 32.5μg/mg and 41 μg/mg.

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 supplemental figure 1). Briefly, 10mg DNR-loaded $pSiO_2$ was mixed with 1 mL 10% PLGA (75:25) (Sigma Chemicals Co. St. Louis, MO) solution (dissolved in dichloromethane) with vortex 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 2h 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 3 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 following:

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

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 remove of the particulate.

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

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 ESEM, Philips Corp, Netherlands). Microparticles were mounted on an aluminum stub using adhesive carbon tape and sputter-coated with a mixture of gold and palladium (60:40) in an argon atmosphere under low pressure using a Dynavac Mini Coater. In addition, to observe the cross-section of the different microsphere

formulation, the microspheres (1 mg for each) were embedded in Neg-50 Frozen Section Medium (Thermo Scientific, Waltham, MA), sectioned with a thickness of 6μm using a cryostat (Cryostat HM550, Thermo Scientific, Waltham, MA). The cross-sectional images of the microspheres were analyzed by light microscopy (EVOS FL Auto, Life Technologies, Carlsbad, CA).

5. In vitro release profiles and determination of DNR concentration

The rate of the DNR release from the microparticles was evaluated in Hank's Balanced Salt Solution (HBSS). Briefly, 3 mg of dried DNR-loaded $pSiO_2$ particles, PLGA-$pSiO_2$-DNR microspheres and PLGA-DNR microspheres were suspended in 1.2 ml of HBSS, respectively. The samples were placed in an incubator at 37°C and continuously stirred with a mini lab roller. At various pre-determined time points, 1 mL of the supernatant was withdrawn after centrifugation at 10,000 rpm for 5 min, and replaced with an equal volume of fresh release medium.

The amount of DNR released was measured by spectrofluorometer (SpectraMax M5, Molecular Devices Corp. USA.). The fluorescence intensity of DNR in the solution was measured at a 590 nm emission wavelength and a 470 nm excitation wavelength. The concentration of DNR was calculated from a standard curve, prepared by measuring the fluorescence intensity of known concentrations of free DNR. The linear range was determined to be from 50 ng/mL to 1500 ng/mL. The samples with the drug concentration higher than 1500ng/mL were diluted before detection. Similarly, some samples with the drug concentration lower than 50ng/mL was concentrated by evaporation before detection.

6. Cytotoxicity study with the in vitro DNR release samples

A human endothelial-like immortalized cell line EA-HY926, derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the lung carcinoma cell line A549 (CRL-2922; ATCC) was used to assess the cytotoxicity of DNR using a water-soluble tetrazolium salt (WST-1) assay (Roche Diagnostics Corp., Indianapolis, IN))[29]. Briefly, EA-HY926 cells were seeded in a 96-well plate at a cellular density of 5000 cells per well in DMEM media (ATCC, Manassas, VA) with 10% FBS (ATCC, Manassas, VA) and antibiotics (ATCC, Manassas, VA). After 1 day of incubation at 37°C in 5% CO_2 to allow the cells to attach, the cell culture medium was replaced with a mixture of 75% (by volume) culture medium and 25% (by volume) of the supernatant from in vitro release studies of PLGA-$pSiO_2$_21/40-DNR microspheres. 100uL of test sample was added into each well with EA-HY926 cells and the plate was allowed to incubate for 5 days at 37 °C 5% CO_2. On day 5 of incubation, 10uL of the WST-1 compound (premixed by the manufacturer) was added into each well and the plate was placed back in the incubator for 2 hours. The plate was read at an absorbance of 440 nm every 30 minutes for 2 hours, with the plate being placed back in the incubator between readings. The optical density readings (OD) were calculated as % cell viability of the controls, which had culture medium without DNR. The positive controls were created using fresh prepared commercial DNR with the equivalent concentrations.

7. Statistical Analysis

For the size comparison between the different microspheres, t-test was used. For in vitro drug release, the data were normalized using the initial drug loading dose. The drug release from the different formulations was compared using Kruskal-Wallis one-way analysis of variance. For the cytotoxicity study, OD values were normalized by the OD value of MediumHBSSCells (control) and expressed as the percent of the control. The normalized OD values were compared across the samples using all pairs Tukey-Kramer HSD. All analyses were performed using JMP statistical software (version 11; SAS Institute Inc, Cary, NC) and p-value smaller than 0.05 was considered to be significant.

Results

1. Characterization of $pSiO_2$ and $pSiO_2$/PLGA composite particles

Figure 1 shows the SEM image of $pSiO_2$_21/40 (Figure 1A) and $pSiO_2$_6/20 (Figure 1B). These microparticles were fabricated using ultrasonication of fresh prepared pSi film. Both the film and particles, displayed a similar nanostructure with pore size of 15–20 nm (Figure 1C, 18.67±4.23 nm) before and after ultrasonication (see supplemental figure 2), indicating that the nanostructure was largely preserved during ultrasonication. The overall aspect and the morphology of the PLGA-$pSiO_2$-DNR and PLGA-DNR microspheres were characterized by optical and scanning electron microscopy. Figure 2 shows the optical microscope image of PLGA microspheres without any drug (2A), PLGA-DNR microspheres (2B) and the composite PLGA-$pSiO_2$_21/40-DNR microspheres (2C). These images indicate that the drug or the drug-loaded $pSiO_2$ particles were fully encapsulated in the transparent PLGA spheres. Figures 2D–2F show SEM images of the corresponding microspheres in Figures 2A–2C. PLGA-$pSiO_2$_6/20-DNR displayed a similar size distribution to that of PLGA-$pSiO_2$_21/40-DNR (Figure 3), with the mean diameter 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. However, the mean size of PLGA-DNR was significantly smaller (26.00±8 vs. 49.31±8.87 μm, $p<0.0001$; and 26.00±8 vs. 52.33±16.37 μm, $p<0.0001$; Figure 4) though the PLGA microsphere fabrication procedure was the same.

The majority of the microspheres had sizes between 10 and 80 μm, as shown in Figures 2 and 3. A close-up examination of the microspheres revealed many micron-sized pores on the surface for all the samples, ranging from 0.5 to 2 μm (Figure 5). There was no evidence of DNR aggregates on the surface of the microspheres from the SEM analysis. The cross-sectional images of these particles revealed that multiple porous silicon particles were present in a PLGA microsphere though in general $pSiO_2$ particles were larger in PLGA-$pSiO_2$_21/40-DNR (Figure 6D) than in PLGA-$pSiO_2$_6/20-DNR composite microsphere (Figure 6C). In PLGA-DNR (Figure 6B) and PLGA microspheres (Figure 6A), daunorubicin aggregates were seen in the former and the latter were much smaller but uniform in the cross-sectional view.

2. In vitro drug release

The release profiles of DNR from the different microparticle formulations are given in Figures 7. DNR release from $pSiO_2$ particles of either size showed a large burst release, with

the drug concentration in the eluent solution dropping from 20 μg/mL at the initial sampling to < 100ng/mL on day 14, with over 90% of the total drug payload leached out within the first 2 days. The size of the $pSiO_2$ particles seems not to be a significant factor. In contrast, DNR release from PLGA-DNR as well as from the $pSiO_2$-PLGA composite microspheres (PLGA-$pSiO_2$_6/20-DNR and PLGA-$pSiO_2$_21/40-DNR) was much slower. All three formulations showed a much smaller burst release within the first two days, with approximately 50% of the payload released (Figure 7). After the first 2 days, the PLGA-DNR formulation maintained a steady state of DNR release for up to three weeks, which then tapered off as the material approached 100% release (Figure 7). By contrast, DNR release from the $pSiO_2$-PLGA composite microspheres (PLGA-$pSiO_2$_6/20-DNR and PLGA-$pSiO_2$_21/40-DNR) was gradual and sustained for a period of >60 days. Around 90% of the payload leached out over the course of 70 days. The DNR release characteristics were similar for these two formulations of composite microspheres, with DNR release from the PLGA-$pSiO_2$_6/20-DNR formulation a bit faster in the first 5 days (Figure 7).

3. Cytotoxicity assay in vitro

To confirm the bioactivity of DNR released from the PLGA-$pSiO_2$ composite microspheres, PLGA-$pSiO_2$_21/40-DNR eluents containing the released DNR were added to cultured cells. An equivalent concentration of commercial DNR was used as a positive control and cell culture medium as well as medium:HBSS (75:25) were used as negative controls. HBSS was used because the in vitro drug release experiments were performed in HBSS. The OD values from each well were divided by the mean of the controls, which were the wells of medium:HBSS (75:25) plus cells and expressed as percent of cell viability of the controls. The data are presented in Figure 8, which demonstrates that the cell viability for medium + cells was 111% and the cell viability for medium + HBSS was slightly lower at 100%. This is to be expected, as cells are expected to be less viable in diluted medium. The data are summarized in Table 2. For the positive controls and the test samples, the expected trend of lower cell viability with higher DNR concentration was observed. Comparing the positive controls with the test samples, sample with DNR concentrations of 71.5 ng/mL displayed equivalent cytotoxicity as the commercial DNR equivalent. The cytotoxicity of 228.5 ng/mL of DNR sample from the $pSiO_2$-PLGA composite showed greater cytotoxicity than the commercial DNR equivalent (Table 2). The positive controls were set up according to the available sample DNR concentration and the concentration was escalated up by half-logs. So the highest positive control concentration was 731.25 (actually measured by mass spectrometry) which had the most cytotoxicity and was significantly different from all the other samples (Table 2)

Discussion

In this study, $pSiO_2$-PLGA composite microspheres were fabricated with the goal of developing an ocular drug delivery system. It is encouraging that DNR released from $pSiO_2$-PLGA composite particles retains its full biological function in inhibiting cell proliferation. Though the concentration of 228.5 ng/mL from in vitro release sample showed more potent cytotoxicity than its positive control, the difference magnitude was within the ranges between its equivalent control and the next higher control concentration. Due to the limited

sample size and spread of data variance, this difference needs to be further investigated in a future in vivo study. The current results suggest that a single intravitreal injection of $pSiO_2$-PLGA-DNR microspheres containing 5 μg of DNR should not be toxic and can provide better vitreous pharmacokinetics than the single intravitreal bolus injection of 5 μg of DNR that is typically administered [4, 6] [4] to inhibiting PVR in experimental studies. One study has shown that splitting one 5 ug intravitreal injection into two smaller doses administered at different times to extend the vitreous drug presence can enhance the therapeutic effect. Machemer and colleagues reported that a single intravitreal dose of 15 nmol per eye on the 3rd day following intravitreal cell injection was not effective in preventing retinal detachment. However splitting the dose into 10 nmol and 5 nmol injected 4 hours apart was effective [4]. This suggests that sustained drug exposure at the disease site can drive the minimum effective concentration of a drug much lower. Indeed, it has been reported that exposure to 700nM of DNR for 1 hour inhibits 50% of fibroblast proliferation, but cell proliferation is completely inhibited by exposure to lower concentrations (500 nM) for longer times (5 hours) [6]. Therefore, a delivery vehicle such as PLGA-$pSiO_2$ composite microspheres that can sustain the release of daunorubicin should improve the therapeutic effect.

From the in vitro drug release studies, the DNR-loaded PLGA-$pSiO_2$ composites demonstrated sustained release of DNR for a 70 day period. Composites made with larger $pSiO_2$ particles (size range 21–40 microns) released drug more slowly and for a longer period of time. Stable sustained release was observed between days 14 and 70 (Figure 7). However, between days 0–14 there were two phases of release, one immediate burst release within day 1 and then a much less pronounced period of high drug release between days 2–14. We assign the initial burst release to loosely bound daunorubicin at or near the surface of the PLGA-$pSiO_2$ composite, as a consequence of the two-step emulsification method used in this work. The initial DNR burst release from all three types of microspheres was similar because they were fabricated in the same way. The loosely bound daunorubicin is expected to be released quickly into a dissolution medium and generate a massive burst release. Indeed, nearly 50% of the loaded drug was released during the first day. More thorough washing of the composite microspheres after the drug loading step may reduce this initial burst release and improve the release profile. The second phase of release from the PLGA-pSiO2 composite microspheres (between days 2–14) was moderate compared with the initial burst release. We hypothesize that this phase of release was a typical first-order kinetic process. Though a thicker PLGA coating may extend the drug release profile to longer times, such engineering will not achieve a zero-order release [30]. However, compared with $pSiO_2$ alone or PLGA alone, the PLGA-$pSiO_2$ composite provides a more extended daunorubicin release profile with a more consistent steady state drug concentration. Comparison of the PLGA-$pSiO_2$ composite particles with different size of pSiO2 particles, the one with smaller pSiO2 particles had a slightly faster drug release which may be contributed to the merely difference of pSiO2 particle size because daunorubicin-loaded pSiO2 particles were small and numerous in the cross-sectional view of PLGA-$pSiO_2$_6/20-DNR composite microsphere (Figure 6). At the end of the release period, the concentration of drug in the release medium containing the PLGA-DRN microspheres dropped 10 fold, from ~ 500 ng/mL to ~50 ng/mL within a week. This is attributed to the rapid degradation of

PLGA at the end stage of dissolution [31, 32]. It has been reported that PLGA experiences rapid degradation when its molecular weight reduces from 51 to 8 kDa, which usually develops between 19 and 33 days of incubation [33].

In the current study, micron-scale voids were found on the surface of PLGA-pSiO2 composite microspheres. These voids on the surface of the microspheres may be responsible for the increased rate of release seen in days 2–14, acting as outlets for the first-order release of drug trapped in the $pSiO_2$ particles. Therefore, engineering these pores may provide another layer of control to optimize the drug release profile and warrants further exploration.

A PLGA coating layer is known to act as an effective barrier in preventing the premature release of drugs into aqueous media [30] [34–36] [37]. In the PLGA-$pSiO_2$ composites of the present study, the drug-loaded $pSiO_2$ particles act as drug reservoirs in the core of a PLGA microsphere, and drug leaching is slowed compared with PLGA-only microspheres.

The drug release profile may also depend on the nature of the $pSiO_2$ particles within the PLGA microspheres [38–40]. A relatively large drug-containing $pSiO_2$ particle will have a smaller surface area and tend to degrade and leach drug more slowly than multiple smaller $pSiO_2$ particles, and this is observed in the data. For the PLGA-$pSiO_2$_6/20-DNR samples, the smaller drug-loaded $pSiO_2$ microparticles (of nominal sizes between 6 and 20 microns) were more numerous in a given PLGA microsphere, and they therefore presented a larger specific surface area and a thinner PLGA coating compared with the 21–40 micron sized $pSiO_2$ particles of the PLGA-pSiO2_21/40-DNR formulation.

The faster DNR release characteristic seen with the PLGA-only microspheres can also be due to the formation of phase-segregated aggregates of DNR in PLGA which were shown in figure 6B. Larger aggregates of DNR cannot form in the nanoscale pores of $pSiO_2$ particles due to the small physical size of these pores. Burst release from PLGA microspheres is a common phenomenon [27, 34, 36, 41, 42]. The initial burst release is hard to completely eliminate but could be reduced by using larger drug molecules and increased mass fraction of PLGA [26, 27]. With the current PLGA microsphere technique, the higher drug loading usually leads to a higher initial burst release [26], which might be attributed to the high accumulation of drug on the surface or near the surface of the microspheres [43].

5. Conclusion

In the current study, $pSiO_2$/PLGA microspheres were fabricated by an S/O/W emulsion method to combine the advantages of a biodegradable polymer with the drug release characteristics of biocompatible porous silica for extended release of DNR. Compared with the infiltrative DNR- loaded $pSiO_2$ particles or the PLGA-DNR microspheres, the PLGA-$pSiO_2$-DNR composite microsphere system demonstrated a 5-fold and 2-fold longer duration of DNR release, respectively. This system is encouraging and may be of value in managing unwanted ocular proliferation through a single intravitreal injection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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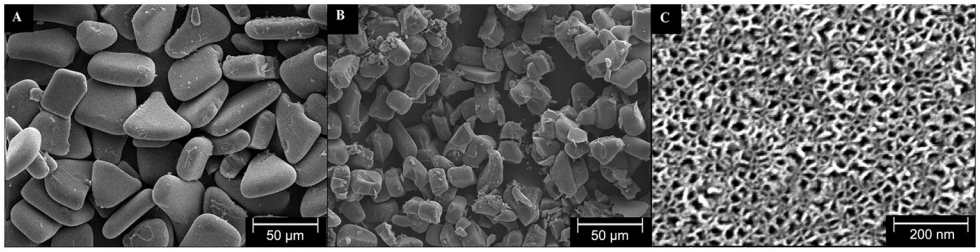

Figure 1.

Scanning electron microscope (SEM) images of the two different $pSiO_2$ microparticle sizes used in this study: (A) $pSiO_2$_21/40 and (B) $pSiO_2$_6/20. (C) Plan-view image of the pore structure on the (100) surface of the particles.

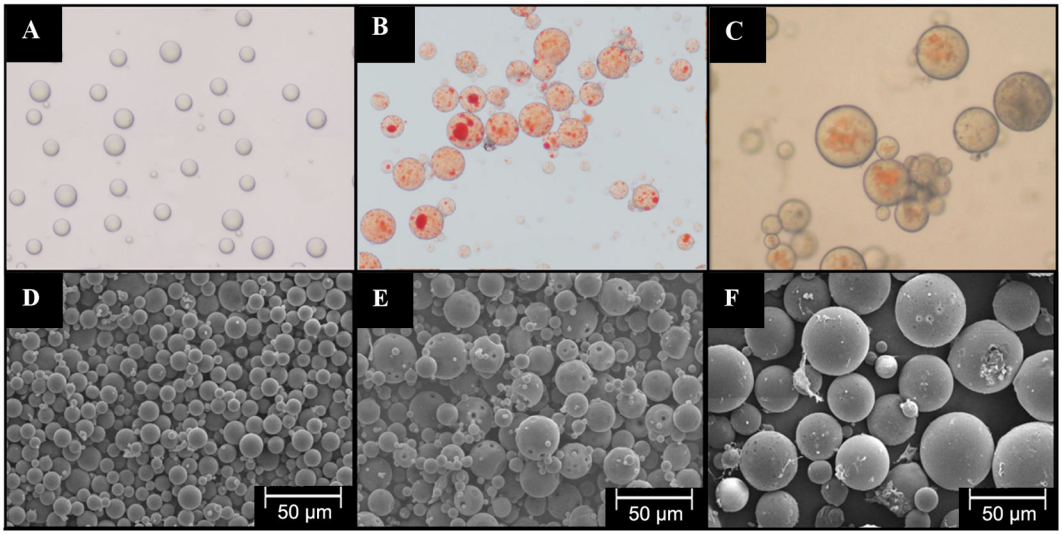

Figure 2.

Physical characterization of PLGA-DNR and PLGA-pSiO_2. Optical microscope images of: (A) PLGA microspheres without DNR, (B) PLGA-DNR microspheres, (C) PLGA-pSiO_2_21/40-DNR microspheres. SEM images of: (D) PLGA microspheres without DNR, (E) PLGA-DNR microspheres, (F) PLGA-pSiO_2_21/40-DNR microspheres.

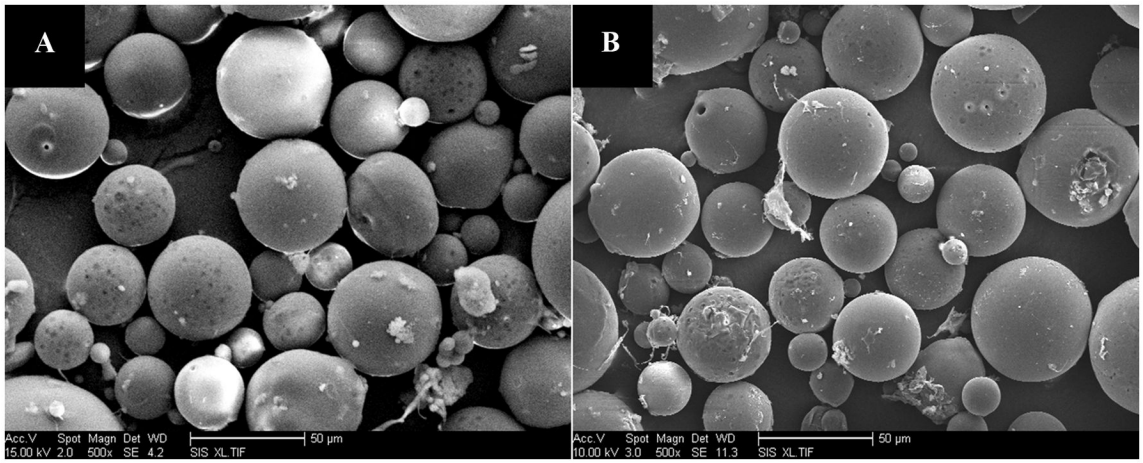

Figure 3.

SEM images of PLGA-pSi_6/20-DNR (left panel) and PLGA-pSi_21/40-DNR (right panel). PLGA-$pSiO_2$_6/20-DNR displayed a similar size distribution to that of PLGA-$pSiO_2$_21/40-DNR. For these two types of composite, The fabricating condition was the same for those two types of PLGA-p SiO_2 composite microspheres: 10mg DNR-loaded $pSiO_2$ ($pSiO_2$_21/40 or $pSiO_2$_6/20) was mixed with 1 mL 10% PLGA (75:25). The ratio of polymer versus $pSiO_2$ was both 1: 10 (w/w).

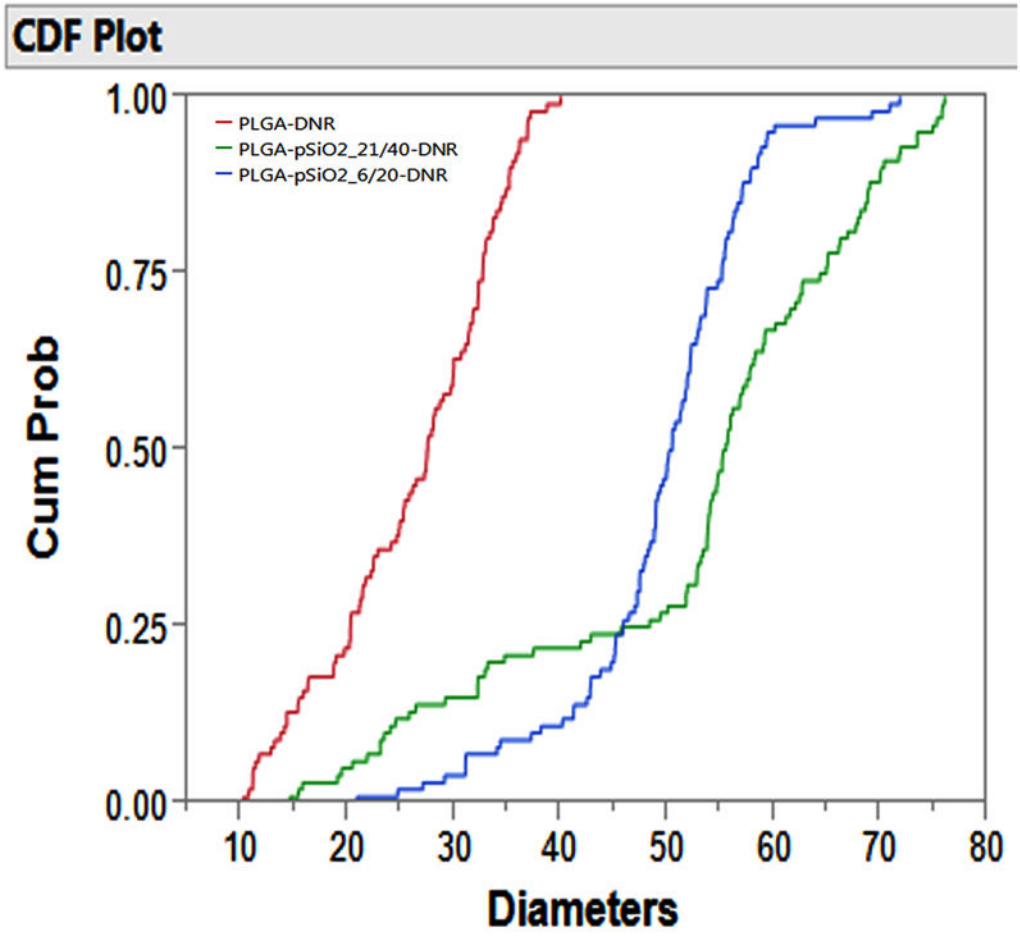

Figure 4.

Cumulative distribution plot comparing the size distribution of PLGA-DNR, PLGA-$pSiO_2$_6/20-DNR, and PLGA-$pSiO_2$_21/40-DNR microparticles. Cumm Prob=Cummulative Probability.

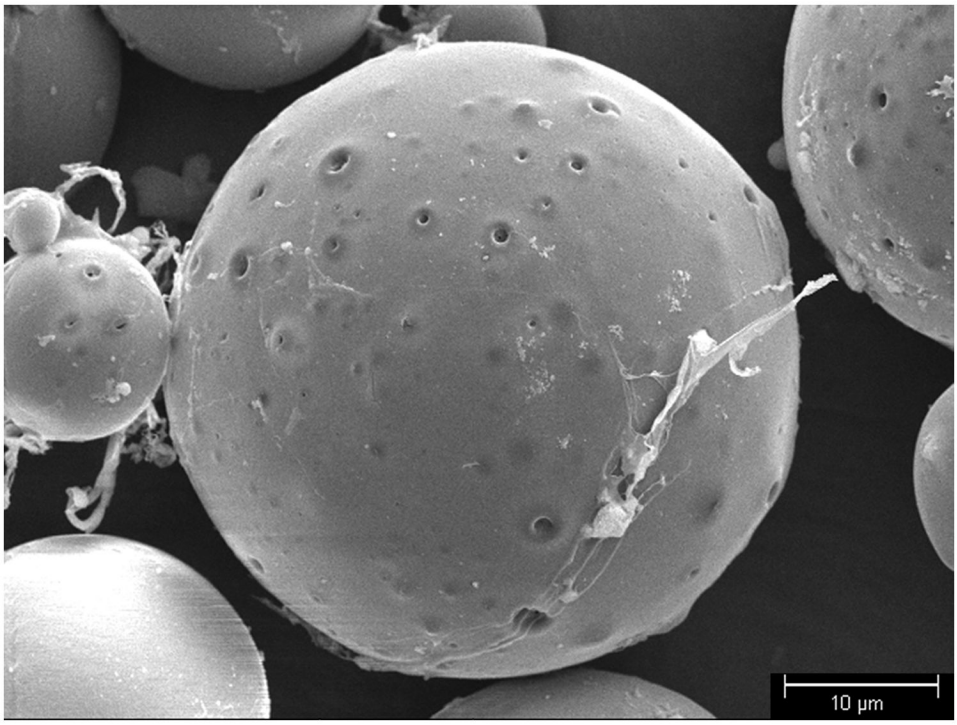

Figure 5.
SEM image of PLGA-pSiO_2_21/40-DNR microspheres, showing the presence of micron-scale voids at the surface of the microspheres.

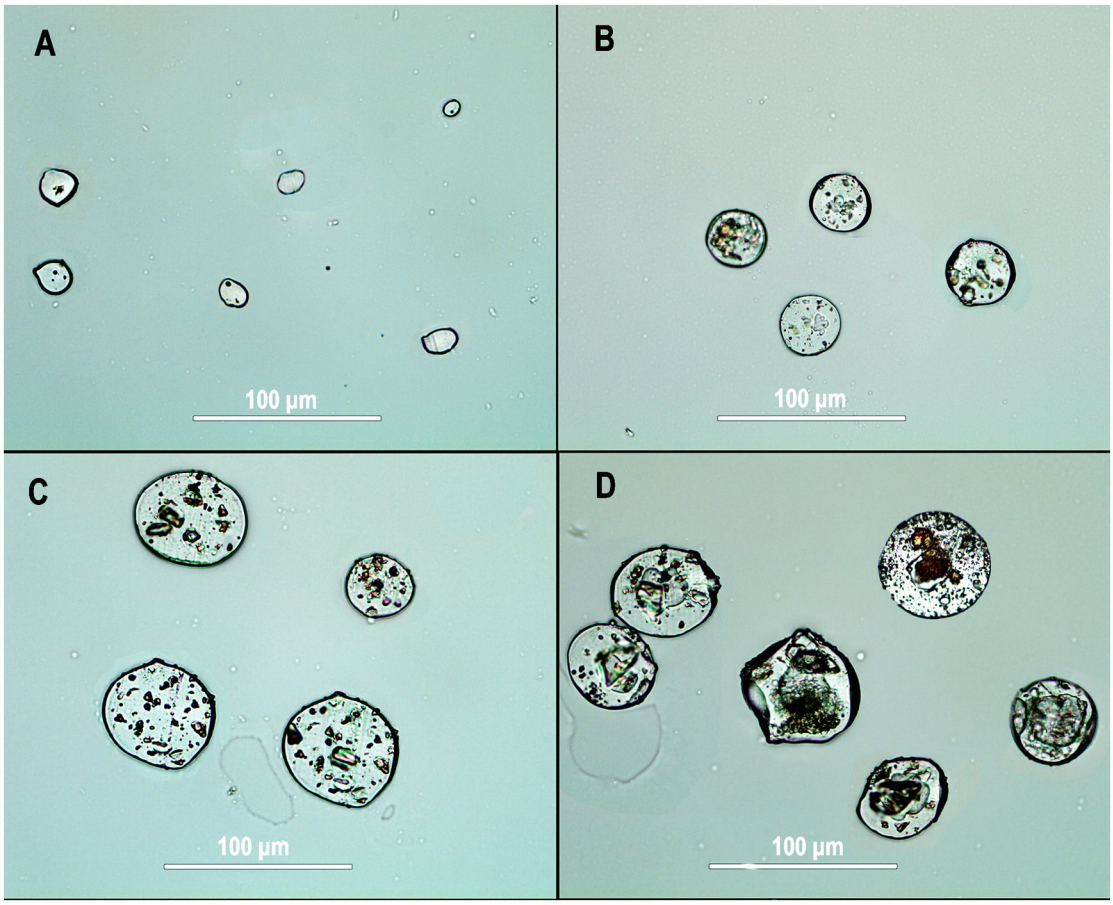

Figure 6.

Cross-sectional views of microspheres of (A) PLGA microspheres without DNR, (B) PLGA-DNR microspheres, (C) PLGA-pSiO_2_6/20-DNR microspheres, and (D) PLGA-pSiO_2_21/40-DNR microspheres.

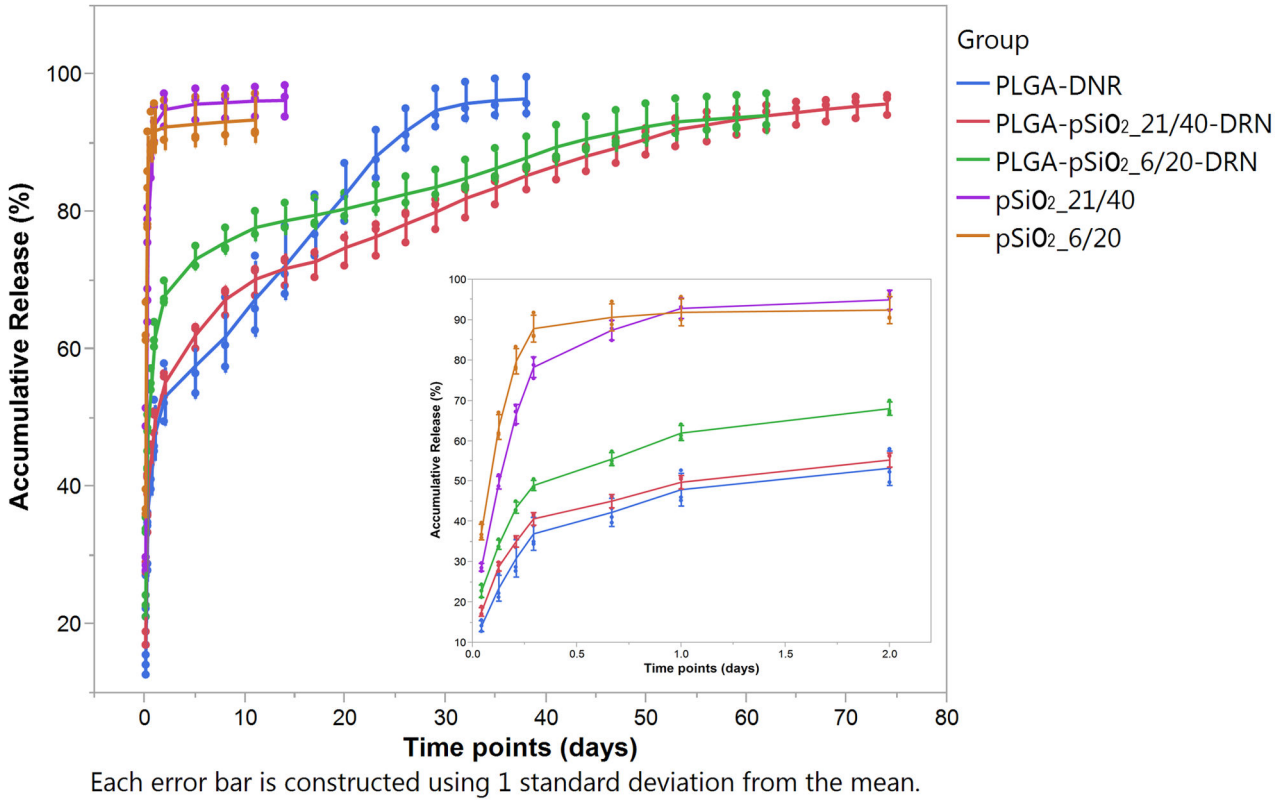

Figure 7.

Cumulative amount of DNR released from the indicated microsphere formulations as a function of time.

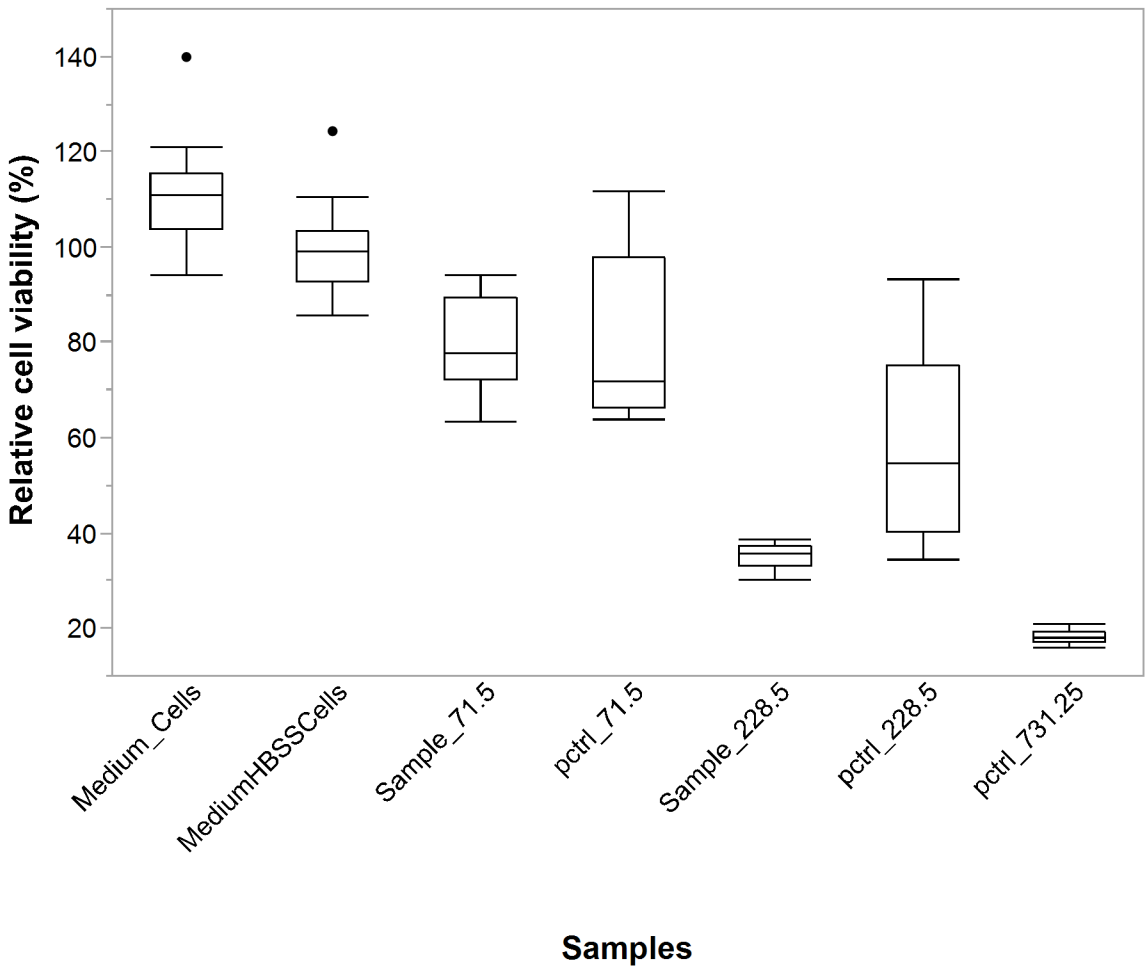

Figure 8.

Viability of EA-HY926 cells (quantified by WST-1) after exposure to the samples with different concentrations of DNR released from PLGA-pSiO_2_21/40-DNR for 5 days. Medium_Cells=medium plus cells; MediumHBSSCells=culture medium 75% plus HBSS 25% plus cells; pctrl=positive control. The concentration unit is ng/mL. In the box plot, the box represents the middle 50% of the data sample. The upper 25% of the data was represented by the upper whisker while the lower 25% of the data represented by the lower whisker. The median line was within the box. The outliers were denoted by the dots above or below the whiskers of the box.

Table 1

Drug loading efficiency for different formulations

Formulations*	Drug-loading efficacy
$pSiO_2$_6/20-DNR	6.5%
$pSiO_2$_21/40-DNR	8.2%
PLGA-DNR	2.74%
PLGA-$pSiO_2$_6/20-DNR	3.08%
PLGA-$pSiO_2$_21/40-DNR	4.64%

Table 2

Cytotoxicity of the in vitro drug release samples and positive controls

Levels					Mean % of the Control
Medium_Cells	A				111
MediumHBSSCells	A				100
pctrl_71.5		B			82
Sample_71.5		B			79
pctrl_228.5			C		58
Sample_228.5			D		35
pctrl_731.25				E	18

Levels not connected by same letter are significantly different. (Comparisons for all pairs using Tukey-Kramer HSD, Alpha=0.05).

Sample: Sample from in vitro release; pctrl: Positive control

Medium_Cells=cell plus 100% medium; MediumHBSSCells=cell plus 75% medium and 25% HBSS; pctrl_71.5=cell plus 75% medium and 25% commercial DNR sample, having a resultant DNR concentration of 71.5 ng/mL; Sample_71.5= cell plus 75% medium and 25% in vitro DNR release sample, having a resultant DNR concentration of 71.5 ng/mL; pctrl_228.5, Sample_228.5, and pctrl_731.25 were made in a same paradigm. HBSS was used as the in vitro drug release medium therefore MediumHBSSCells was used as a control to calculate cell viability for the other test samples.