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Nanoparticles Containing High Loads of Paclitaxel Silicate Prodrugs: Formulation, Drug Release, and Anti-cancer Efficacy

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Abstract

We have investigated particle size, interior structure, drug release kinetics, and anticancer efficacy of PEG-*b*-PLGA-based nanoparticles loaded with a series of paclitaxel (**PTX**) silicate prodrugs [PTX-Si(OR)₃]. Silicate derivatization enabled us to adjust the hydrophobicity and hydrolytic lability of the prodrugs by the choice of the alkyl group (R) in the silicate derivatives. The greater hydrophobicity of these prodrugs allows for the preparation of nanoparticles that are stable in aqueous dispersion even when loaded with up to ca. 75 wt% of the prodrug. The hydrolytic lability of silicates allows for facile conversion of prodrugs back to the parent drug, **PTX**. A suite of eight PTX-silicate prodrugs was investigated; nanoparticles were made by flash nanoprecipitation (FNP) using a confined impingement jet mixer with a dilution step (CIJ-D). The resulting nanoparticles were 80–150 nm in size with a loading level of 47–74 weight percent (wt %) of a PTX-silicate, which corresponds to 36–59 effective wt % of free **PTX**. Cryogenic transmission electron microscopy images show that particles are typically spherical with a core-shell structure. Prodrug/drug release profiles were measured. Release tended to be slower for prodrugs having greater hydrophobicity and slower hydrolysis rate. Nanoparticles loaded with PTX-silicate prodrugs that hydrolyze most rapidly showed *in vitro* cytotoxicity similar to that of the parent **PTX**. Nanoparticles loaded with more labile silicates also tended to show greater *in vivo* efficacy.

Keywords

nanoparticles; paclitaxel; silicate; prodrug; flash nanoprecipitation; drug release; anticancer efficacy

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Notes

The authors declare no competing financial interest

Supporting Information

Nine figures and one table of supporting data (particle size, dialysis controls, release profiles, cytotoxicities, and loading levels). The material is available free of charge via the Internet at <http://pubs.acs.org>.

1. Introduction

The enhanced permeation and retention (EPR) effect serves as a primary basis for considering the use of nanotherapeutics in cancer therapy. In contrast to the blood vessels supplying most normal organs, tumor blood vessels lack a continuous endothelial cell coverage and basement membrane. Delivery vehicles in the colloidal size range (including polymeric nanoparticles, liposomes, and micellar systems) are typically excluded from normal organs but are able to passively accumulate in tumors because of this leaky vasculature. Lack of well-developed lymphatic drainage enhances retention of these vehicles once in the tumors. The EPR effect can result in decreased toxicity and increased efficacy of an encapsulated drug.¹

A number of barriers diminish the translational success of nanomedicine.² A key challenge is the limited intra-tumoral distribution of drug delivery carriers, since their transport within the tumor is highly inefficient. Distal regions receiving lower drug exposure often harbor the more aggressive tumor cells. Thus, achieving therapeutic drug concentrations in these under-supplied regions would represent a significant advance. Many recent studies have shown that particles that are in the sub-100 nm size range demonstrate improved tumor accumulation and, more importantly, improved tumor penetration.^{3,4,5} While many techniques are available to fabricate nanocarriers in the 100 nm size range, it is extremely difficult to achieve reasonable drug loading in such small particles. Most colloidal carriers can be loaded to a mere 10 wt%.

Flash nanoprecipitation (FNP), a technique pioneered by Prud'homme,⁶ enables the synthesis of sub-100 nm nanoparticles with very high drug loading.^{6–9} FNP requires rapid turbulent mixing of organic solutes dissolved in a water-miscible organic solvent (e.g., tetrahydrofuran, acetone, acetonitrile) with the antisolvent, water, in a confined space. When the solvent and antisolvent streams meet and mix, the solutes rapidly precipitate into nanoparticles due to supersaturation. The encapsulating agent used in FNP is typically an amphiphilic block copolymer (BCP), such as poly(ethylene glycol)-*b*-poly(lactic-co-glycolic acid) (PEG-*b*-PLGA). The hydrophobic block of BCP co-precipitates with the drug, while the hydrophilic block provides steric stabilization on the nanoparticle surface.

In this work, we evaluated the use of FNP to fabricate nanoparticles of paclitaxel (**PTX**), a widely used anticancer agent. Studies have shown that FNP-loaded, PEG-*b*-PLGA-based nanoparticles loaded with **PTX** alone are not stable;^{9–12} rapid Ostwald ripening of the freshly prepared nanoparticles leads to precipitation of **PTX** from the 90–95% aqueous dispersion in which the initial FNP formulations are produced^{7, 8, 11, 12}. This phenomenon is likely due to a sufficient water solubility and inherent crystallinity of **PTX**. It is paradoxical that although **PTX** is too hydrophobic a drug to be dissolved in aqueous medium and therapeutically administered, it is still too hydrophilic to be efficiently encapsulated and stabilized as a nanoparticle by FNP.

To render **PTX** more hydrophobic, we have developed a series of **PTX**-prodrugs containing silicate esters [i.e., **PTX**-O-Si(OR)₃].^{13, 14} The silicate moiety is hydrolytically labile and serves as a prodrug to release the parent compound in biological media. We have shown that

both the hydrophobicity as well as the rate of hydrolysis of the prodrug can be tailored. The goal of the current study was to evaluate the use of the FNP technique coupled with the silicate ester prodrug technology to fabricate nanoparticles suitable for **PTX** delivery. Using *in vitro* and *in vivo* models, we have identified factors affecting the regeneration of **PTX** from **PTX**-silicate prodrug-loaded nanoparticles.

2. Materials and Methods

2.1 Materials

The polymer PEG-*b*-PLGA (MW: 5k–10kDa) and suite of **PTX**-silicate prodrugs (see Table 1) were synthesized as previously reported.^{13, 15} Paclitaxel was obtained from Phytogen Life Sciences. Water (H₂O, HPLC grade), tetrahydrofuran (THF, HPLC grade), and acetonitrile (ACN, HPLC grade) were purchased from Sigma-Aldrich and used as received. CDCl₃ was purchased from Cambridge Isotope Laboratories, Inc. Ethanol (anhydrous) was purchased from Decon Labs, Inc. Monosodium phosphate and disodium phosphate were purchased from Fisher Scientific. Phosphate buffered saline was prepared by dissolving 3.12 g of monosodium phosphate, 20.74 g of disodium phosphate, and 9 g of sodium chloride in 1 L of water to achieve a pH = 7.4. The 10 mM acetate buffer was prepared by dissolving 0.21 g of glacial acetic acid, 0.52 g of anhydrous sodium acetate, and 9 g of sodium chloride in 1 L of water to achieve a pH of 5.0. Slide-A-Lyzer dialysis mini capsules (MWCO: 10 kDa, 0.5–1 mL) were purchased from Thermo Scientific.

2.2 Nanoparticle Preparation and Characterization

The nanoparticles were fabricated by flash nanoprecipitation (FNP)¹⁶ using a CIJ-D mixer.¹⁷ In brief, to achieve a ca. 50 equiv. weight% of **PTX**, the molecular weight of the prodrug was factored into consideration by use of the following equation.

$$\text{mass of prodrug to obtain 50 wt\% PTX} = \text{mass PTX (mg)} \times \left(\frac{\text{MW prodrug}}{\text{MW PTX}} \right)$$

Specifically, an amount of **PTX**-prodrug containing the equivalent of 25 mg of free **PTX** and 25 mg of PEG-*b*-PLGA were dissolved in 2.5 mL of THF and impinged against 2.5 mL of deionized water in a CIJ-D mixer and further diluted into 45 mL of water. Blank polymer nanoparticles (i.e., containing no **PTX**-silicate) were also prepared and used as a control group for *in vitro* and *in vivo* cytotoxicity studies. PEG-*b*-PLGA (25 mg) was dissolved in 2.5 mL of THF and impinged against 2.5 mL of deionized water and further diluted into 45 mL of water.

The size of the freshly prepared nanoparticles was measured by dynamic light scattering (DLS) on a DelsaTM Nano C (Beckmann Coulter) instrument. Samples for DLS were prepared by diluting the nanoparticle suspension with water immediately after CIJ-D mixing. The intensity average size, d_I , and polydispersity index PDI calculated (via the cumulants method) by the instrument were recorded. Measurements were taken in triplicate.

To remove unencapsulated prodrug and free polymer from the nanoparticle suspension, ultracentrifugation was performed on the initial FNP suspension at 50,000 rpm and 4 °C for 30 min. After each round of centrifugation, the supernatant, which contained a considerably higher wt% of polymer than that used in the initial preparation (¹H NMR analysis), was removed using a transfer pipet. The pellet was resuspended in 25 mL of water and the process repeated twice. After the third ultracentrifugation, the pellet was collected and lyophilized using a Freeze Dryer System (Labconco) at −45 °C for 24 h to remove residual water. The overall mass recovery varied following the rounds of three centrifugations for different formulations; it typically ranged from 30–80%.

The prodrug loading level of the lyophilized nanoparticles was further assessed by high performance liquid chromatography (HPLC, Model 508, Beckmann Coulter, CA) or ¹H-NMR analysis. To determine loading by HPLC, 1 mg of lyophilized nanoparticles was suspended in 1 mL of acetonitrile (ACN) and agitated for 24 h, during which time the organic materials (block copolymer and PTX-silicate derived components) were solubilized. An aliquot (30 µL) of the resulting suspension was injected onto a C-18 HPLC column (4.6 × 250 mm with 5 µm packing). The mobile phase consisted of a mixture of acetonitrile and water (75:25) and was delivered at a flow rate of 1 mL/min. The amounts of **PTX** and the PTX-silicate prodrug were quantified by UV detection at 228 nm against a series of calibration curves for **PTX** and the appropriate PTX-silicate, created over a range of concentrations from 3.125 to 100 µg/mL. Alternatively the loading level and efficiency of prodrug incorporation was assessed from ¹H-NMR integrations of various resonances in a solution of the final lyophilized, drug-loaded nanoparticle pellet. A portion (ca. 3 mg) was dissolved in CDCl₃, a solvent in which all components of the loaded nanoparticles are readily soluble. Equations describing these quantification methods are given in the Supporting Information (SI, Figure S7).

2.3 Nanoparticle Morphology

The morphology of the nanoparticles was observed by cryo-TEM. A lacey carbon Cu grid (01881, 200-mesh, Ted Pella, Ltd., Redding, CA) was glow discharged in a vacuum evaporator at 70 mTorr (DV-502A, Denton Vacuum Moorestown, NJ) for 30 seconds to create a hydrophilic surface on the carbon coated side of the grid. A 2–3 µL volume of the fresh nanoparticle suspension was pipetted onto the carbon side at 22 °C in a Mark III Vitrobot chamber (FEI Company, Hillsboro, OR) with a relative humidity of ~100 %. The excess sample was blotted with grade 595 blotting paper (Ted Pella, Ltd., Redding, CA) once or twice using a ~1.5 mm offset parameter and 5 seconds per blot to thin the thin liquid film. Three seconds after blotting, the assembly was plunged into liquid ethane cooled by liquid nitrogen. The procedure was explained in detail previously.¹⁸

The resulting vitrified specimen was transferred to a Gatan 626 cryo-transfer unit (Gatan, Pleasanton, CA) at −194 °C and then characterized at −178 °C in the microscope. A 120 kV FEI Tecnai Spirit BioTWIN was used; images were taken digitally with an EagleTM 2k CCD camera (FEI Company, Hillsboro, OR) in low-dose mode. The images were processed with the TEM Imaging and Analysis software package (version 4.2 SP1, build 816, FEI Company, Hillsboro, OR). To enhance the phase contrast, the images were deliberately

under-focused to 2–4 μm with an objective lens. The degree of under-focus was controlled intentionally both to create Fresnel fringes (white rings on the boundary of nanoparticle structure) and to enhance the contrast between the core (drug) and shell (polymer) regions in nanoparticles.

2.4 Silicate Release Measurements

Lyophilized nanoparticles (15 mg) were redispersed in 15 mL of PBS buffer using micro-tip (1/8") sonication (Misonix Sonicator 3000, NY) in pulse mode for 5 minutes, with an increasing power of 3 to 9 volt within 1 minute. After every 1 minute of sonication, the tube containing nanoparticles was immersed in an ice/water bath to dissipate the heat generated by sonication. After 5 minutes, 500 μL aliquots of the nanoparticle suspension were placed into mini dialysis capsules (MW cutoff size: 10,000 Da, Pierce Biotech, Rockford, IL, US). A total of 30 capsules were immersed, 3 each, in ten 1L solutions of PBS buffer at pH 7.4 and, separately, pH 5.0. The beakers were held at 37 °C and agitated at 100 rpm on an incubated orbital shaker. At predetermined time intervals (0, 1, 2, 4, 8, 12, 24, 48, 72, 96 h), three capsules were removed and frozen at –80 °C. To assay the amount of PTX-silicate and **PTX remaining inside** particles at each time point, the contents of each frozen capsule were lyophilized for 24 h. Acetonitrile (1 mL) was added to each capsule of dried particles and buffer salts, and the resulting suspension was transferred to a 2 mL glass vial. The vial was capped, sealed with Parafilm M® to prevent evaporative loss, and allowed to stand overnight. Undissolved buffer salts remained at the bottom of the vials. An aliquot of the supernatant was analyzed by HPLC and the amounts of **PTX** and PTX-silicate were quantified against calibration curves. To assay the amount of *released* PTX-silicate and **PTX**, each 1 L external buffer solution was lyophilized in twenty-one 60 mL centrifuge tubes. The powdered contents of three sets of seven tubes were consolidated into three separate vials. Each was leached overnight with the same volume of acetonitrile and the supernatant assayed by HPLC as above. Release studies in pH 5.0 buffer were conducted using the same protocols.

2.5 In vitro Cell Growth Inhibition

Cytotoxicity studies were conducted using, MDA-MB-231 Luc+ breast cancer cells (Caliper Life Sciences, Hopkinton, MA). Cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified incubator held at 37 °C. For the cytotoxicity studies, MDA-MB-231 cells were seeded at 5,000 cells/well in a 96 well plate containing 100 μL of MEM supplemented with 5% FBS. Following attachment, cells were treated with free **PTX** or PTX-silicate prodrug-loaded nanoparticles. For free **PTX**, a stock solution was prepared in DMSO (10 mM) and then diluted in regular growth media. Cells were incubated with the **PTX** control or each of the loaded nanoparticle formulations for 4 hours, then washed with fresh medium and finally incubated with fresh medium for an additional 72 hours. Cell viability was determined at the end of the study using the MTT assay. Untreated cells and cells treated with empty nanoparticles were used as controls. The IC_{50} was interpolated from the resulting curves using Graphpad Prism (v5.1, Graphpad Software Inc. La Jolla CA, USA).

2.6 *In vivo* Tumor Growth Inhibition Studies

The entire animal studies were performed in accordance to the policies approved by the University of Minnesota's IACUC. Female Balb/c nu/nu immunocompromised mice were purchased from Charles River Labs (Wilmington MA, USA). For xenograft studies, 7.5×10^5 MDA-MB-231 Luc⁺ cells were suspended in 100 μ L of Dulbecco's phosphate buffered saline (DPBS) and mixed with an equal volume of Matrigel[®]. Subcutaneous injection into mammary pad #9, located near the left flank, was performed with a 26-gauge needle attached to a 0.5 cc syringe.

Based on the prodrug loading levels (Table S1), each nanoparticle formulation was weighed and resuspended in buffered saline such that a 10 microliter volume of the resulting nanoparticle suspension contained 40 μ g of PTX-equivalent dose. Resuspension of the nanoparticles involved initial vortexing (ca. 1 min) followed by five cycles of probe sonication. Each cycle involved sonication for 1 minute at 5W, followed by 30 seconds of off time. Following probe sonication, 10 μ L of nanoparticle suspension was manually injected directly into tumors (average tumor volume ~100 mm). The mice were anaesthetized using isoflurane during nanoparticle administration. Tumor volume measurements were taken with an electronic caliper. Tumor volumes were calculated from the formula: Volume = $(L^2 \times W)/2$. The day when treatments were administered was considered Day 0.

3. Results and Discussion

3.1 PTX-silicate Prodrugs

The paclitaxel silicate prodrugs studied (i.e., **1–8**) are shown at the top of Table 1. Their molecular weight, calculated octanol/water partition coefficients (cLogP), and relative rates of chemical hydrolysis (in aqueous acetone containing trifluoroacetic acid) are given in Table 1.¹³ Collectively, these show that the hydrophobicity can be tuned based upon the nature of the alkyl group present in the silicate ester(s) moiety. Greater hydrophobicity of the prodrug allows for the formation of stable nanoparticle suspensions, a property not attainable with FNP-produced, PEG-*b*-PLGA nanoparticles loaded with PTX alone.^{7–12} The size of the alkyl groups in the silicate also affects the relative hydrolysis rates of silicate cleavage. The hydrolytic stability of each prodrug plays a significant role in the overall rate of drug release, as discussed below.

3.2 Nanoparticle Characterization

An initial set of loaded nanoparticles was prepared using the most easily synthesized silicates **1–5** and **8**. The intensity average size (d_I) and size distribution (PDI) for each preparation was measured in triplicate by dynamic light scattering (DLS); the results are given in Table 2. The suspensions of nanoparticles (as initially formulated in 5% THF/water) remained homogeneous and stable for 1 (for **1**) to several (for **2**) to >14 (for **3–5**) days before showing significant aggregation, as determined both by visual inspection and DLS measurements. As the hydrophobicity of the PTX-silicate prodrug increased (higher cLogP), the resulting loaded nanoparticles sizes tended to decrease.

Preliminary release studies with some of these preparations showed “burst-release” behavior²⁰ that is characteristic of samples containing both loaded nanoparticles as well as a portion of free (pro)drug that is not protected by the block copolymer.²¹ We therefore implemented a protocol using ultracentrifugation to pelletize the loaded nanoparticles (3x) followed by final lyophilization to produce dry-powdered preparations of the loaded nanoparticles. This means of preparation largely removed the initial burst phase of the release profiles (see 3.3). Redispersion of the lyophilized powder in water, aided by probe tip sonication, gave nanoparticles that showed evidence of aggregation as judged from the larger sizes revealed by DLS analysis (Table 2). Freeze-drying induced aggregation and resulted in an increase in particle size, as has been reported for other nanoparticle structures.^{7, 8, 22, 23}

The levels of prodrug loaded into these final lyophilized nanoparticles are listed in Table 2. The nanoparticles contained ca. 50–75 wt% of PTX-silicate. Because the supernatant from the centrifugation contained a greater portion of polymer than PTX-derived material (NMR analysis), the final PTX-silicate loading levels of the nanoparticle formulations were typically greater than the wt% of silicate charged in the initial FNP procedure. The very high load level that can be achieved using FNP formulation in combination with hydrophobic prodrugs of **PTX** is one of the main advantages of this nanoparticle formulation and approach. We also noted that the use of probe tip sonication did not lead to significant leakage of **PTX** or the prodrugs (by HPLC). Also little to no hydrolysis of the prodrugs was observed after the final redispersion (by ¹H-NMR).

Cryo-transmission electron microscopy was used to examine various features of the loaded nanoparticle preparations. Selected cryo-TEM images are shown in Figure 1. Samples were selected from each stage: after flash nanoprecipitation (A1 and A2), after lyophilization and centrifugation (B, freeze-dried and centrifuged nanoparticle powder, 0hr *in vitro* drug release), and after 24hr *in vitro* drug release (C). Figures A1 and A2 show the spherical nature of the freshly prepared loaded nanoparticles and their size distribution. In addition, a core-shell morphology is clearly evident in the larger particles, A1. The core is rich in PTX-silicate, which is more electron dense than the PLGA appearing as a shell. It was very difficult to image the PEO halos around each particle due to their very low electron density. They can sometimes be seen in images of particles containing only PEG-*b*-PLGA diblock copolymer (see Figure S9).

Nanoparticles were examined following centrifugation, lyophilization, and redispersion into PBS. These were loaded with PTX-2'-Si(OEt)₃ (**1**), the most hydrolytically labile silicate. When examined immediately after redispersion, the core-shell structure was still intact (Figure 1B). However, the particle size had grown due to aggregation as represented by the chain of particles in the right side of Figure 1B. This is consistent with the increased *d_f* measured by DLS (Table 2). After 24 hr in suspension, another sample of these nanoparticles was imaged. The size had diminished and the cores disappeared (Figure 1C), suggesting that hydrolysis and/or release of **PTX** and/or the PTX-silicate was occurring during that aging period. We confirmed the hydrolysis of the PTX-silicate back to the parent PTX by differences in retention times observed by HPLC and LC-MS. Similar results were observed for particles made from PTX-2'-[Si(OEt)₃]₂ (**8**), which hydrolyzed rapidly, lost

their cores and appear to shrink; while particles made from PTX-2'-Si(O^tBu)₂(OEt) (**4**), with very slow hydrolysis, did not change with 24 hr aging (see Figure S8 ²⁴).

3.3 *In Vitro* Drug Release Profiles

PTX and PTX-silicate release studies were performed on selected PTX-silicate-loaded nanoparticles using dialysis mini capsules with a semipermeable membrane. Control experiments showed that diffusion of the drug or prodrug alone through the membrane occurred readily. An aqueous suspension of either **PTX** or PTX-2',7-[Si(OEt)₃]₂ (**8**) was placed in the capsule, which was then immersed in buffer (pH = 7.4). Departure of each compound from the capsule was monitored over time. Both compounds were absent after 3 hours (SI, Figure S1). This showed that diffusion of **PTX** or a PTX-silicate could be expected to occur rapidly once each was no longer inside a nanoparticle.

We next studied the release of **PTX** and the PTX-silicate from several of the prodrug-loaded nanoparticle preparations. These studies were conducted at 37 °C and representative plots for release of PTX-2'-Si(OⁿOct)₃ (**2**) from the loaded nanoparticles are shown in Figure 2. The release and retention plots for nanoparticles loaded with **1** or with **2** at pH 7.4 and pH 5.0 are provided in the SI (Figures S2–S5). The amount of total drug/prodrug was measured both inside and outside the dialysis capsules. The amounts released and retained 12, 24, and 48 h are given in Table 3 (for **1** and **2** at pH = 7.4) and Table 4 (for **1–5** at pH = 5.0). These measurements indicated good overall mass-balance. In general, the release rates were faster at the lower pH.

3.4 *In vitro* cytotoxicity of PTX-silicate prodrug-loaded PEG-*b*-PLGA nanoparticles

We have previously reported the cytotoxicity of prodrugs **1–8** alone toward the MDA-MB-231 human breast cancer cell line.¹³ As we described there, “silicates having faster rates of hydrolysis [cf. Table 1] tend to show cytotoxicities similar to those of the parent taxane. This suggests that the silicate esters are hydrolyzed back to PTX either in the culture medium or inside the cell during the course of the assay.” We have now examined the cytotoxicities of the prodrug-loaded nanoparticles.

Cytotoxicity of nanoparticle formulations loaded with six different prodrugs (**1–5** and **8**) was evaluated in the MDA-MB-231 cells and the IC₅₀ data are shown in Table 2. The ethyl-containing silicate prodrugs of PTX **1** and **8** show a cytotoxicity profile similar to that of free **PTX**. The cytotoxicity of the PTX-2'-Si(OⁿOct)₃ (**2**) nanoparticles was considerably lower, consistent with the fact that only a small portion of **2** had been observed to hydrolyze to free **PTX** in the PBS release experiments at pH = 7.4. The nanoparticle formulation containing **3** is more potent than that loaded with **1**, which, interestingly, mirrors the relative IC₅₀ values of **3** not in nanoparticles and **PTX**.¹³ Finally, the formulations containing the hindered and, therefore, hydrolytically stable PTX-silicates **4** and **5** showed very low cytotoxicity, which is consistent with our early observations made with these silicates.¹³ We previously observed that free **3** has a IC₅₀ comparable to PTX. It is possible that **3** may, in fact, have intrinsic PTX-like cytotoxicity. The *in vitro* cytotoxicities of the PTX-silicate loaded nanoparticle formulations are provided in the SI (Figure S6).

Nanoparticle formulations of prodrugs **1** and **8** had a particle size of 420 and 310 nm, respectively, compared to the 200–240 nm size range of other prodrug formulations (Table 2). Thus, differences in the cytotoxicity of the various prodrugs formulations could also be attributed to differences in their cellular uptake. To quantitatively determine cellular uptake, the concentration of nanoparticle treatments would need to be significantly higher (μM range) than their $\text{IC}_{50\text{s}}$, which would likely kill a large fraction of the treated cells.

3.5 *In vivo* Tumor Inhibition by Direct Tumor Injection of Nanoparticle Formulations

Labile prodrugs that showed significant stability as well as cytotoxicity, either alone¹³ or in the nanoparticle formulation (i.e., **2**, **3**, and **6–8**), were chosen for an intratumoral injection study in a xenograft mouse model. The ratio of the prodrug to polymer in the initial FNP feeds, the total mass of loaded nanoparticles recovered following ultracentrifugation (3x) and lyophilization, the DLS-measured size and size distribution, and the load levels of PTX-silicate (expressed as equivalent wt% of **PTX**) are presented in the SI (Table S1). This study showed (Figure 3) that all nanoparticles slowed tumor growth rates relative to a control using blank nanoparticles. Particles containing PTX-silicates having faster hydrolysis rates [e.g., PTX-2'-Si(OⁿOct)₃ (**2**) and PTX-7-Si(OEt)₃ (**6**), see Table 1] were the most effective in inhibiting tumor growth. These studies also show that even a single dose of these silicate prodrug nanoparticles is capable of inhibiting tumor growth over a prolonged period, pointing to their ability to sustain PTX levels in the tumor tissue. Additional studies evaluating tissue concentrations of PTX and prodrugs are needed to accurately determine the duration of sustained release achieved *in vivo*. Although the prodrug formulations chosen for this study released PTX for only two days based on the *in vitro* studies, compounds **4** and **5** demonstrate 50% release over the course of 9 days. We envision a combination approach utilizing a faster releasing prodrug formulation and a slower releasing formulation to achieve variable drug release rates.

4. Conclusion

The combination of FNP and silicate prodrug strategy enabled the fabrication of nanoparticles in a size range desirable for tumor drug delivery, with high **PTX** loading levels, and sustained drug-release properties. Particle size, drug loading, and drug release rates were a function of the hydrophobicity of the prodrug. The *in vitro* and *in vivo* anticancer activities correlated with the hydrolysis rates of the prodrug. Future studies will examine the *in vivo* efficacy of these formulations following intravenous administration. Overall, the FNP/silicate prodrug strategy is a promising approach for delivering **PTX** while avoiding the side effects associated with the use of large quantities of nanoparticle excipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cryo-TEM images of nanoparticles loaded with PTX-2'-Si(OEt)₃ (**1**). **A1 and A2** freshly prepared (i.e., prior to centrifugation), **B**) lyophilized, immediately following redispersion into PBS after centrifugation, and **C**) lyophilized following redispersion into PBS and aging for 24 h in a dialysis cassette suspended in PBS (pH 7.4).

Figure 2.

Release profiles for nanoparticles loaded with PTX-2'-Si(OOct)₃ (2). **A.** Appearance of 2 and free PTX to the exterior of the dialysis cassette at pH = 7.4. **B.** Appearance of 2 and free PTX to the exterior of the dialysis cassette at pH = 5.0. **C.** Amount of 2 and PTX remaining inside the dialysis cassette at pH = 7.4.

Figure 3.

Intratumoral injection of nanoparticles loaded with PTX-2'-Si(OⁿOct)₃ (**2**), PTX-2'-Si(OⁱPr)₃ (**3**), PTX-7-Si(OEt)₃ (**6**), PTX-7-Si(OⁿOct)₃ (**7**), and PTX-2',7-[Si(OEt)₃]₂ (**8**) at equivalent dose (i.e., the same amount of **PTX** if all prodrug has reverted back to **PTX** and is no longer in the nanoparticles) in comparison to a control with empty nanoparticles.

Structures and properties (hydrophobicity and hydrolytic lability) of the PTX-containing silicate prodrugs used in this study.¹³

	hydrophobicity	hydrolytic lability	
	R ¹	R ²	shorthand descriptor	MW	cLogP ^a	k _{rel} ^b	t _{1/2} (min)
PTX	H	H	paclitaxel	854	3.2	N/A	N/A
1	Si(OEt) ₃	H	PTX-2'-Si(OEt) ₃	1016	5.0	18,000	3.7
2	Si(O ⁱ Oct) ₃	H	PTX-2'-Si(O ⁱ Oct) ₃	1269	7.7	5,600	12
3	Si(O ⁱ Pr) ₃	H	PTX-2'-Si(O ⁱ Pr) ₃	1058	5.6	570	120
4	Si(O ⁱ Bu) ₂ (OEt)	H	PTX-2'-Si(O ⁱ Bu) ₂ (OEt)	1072	5.8	5.6	12,000
5	Si(Omenthyl) ₃	H	PTX-2'-Si(Omenthyl) ₃	1347	7.4	1.0	69,000
6	H	Si(OEt) ₃	PTX-7-Si(OEt) ₃	1016	5.1	2,200	30
7	H	Si(O ⁱ Oct) ₃	PTX-7-Si(O ⁱ Oct) ₃	1269	7.8	450	150
8	Si(OEt) ₃	Si(OEt) ₃	PTX-2',7'-[Si(OEt) ₃] ₂	1178	6.3	15,000 (R ¹) 2,100 (R ²)	4.6 (R ¹) 33 (R ²)

^a cLogP values from ALOPS 2.1.¹⁹

^b hydrolysis was performed in homogenous solutions of 90:9:1 volume ratio of acetone-*d*₆:D₂O:CF₃CO₂H and the *k*_{rel} values are benchmarked against the silicate **5**, which showed the slowest rate of silicate cleavage¹³.

Table 2

Size, prodrug loading levels, and cytotoxicities of nanoparticles loaded with PTX-silicates.

Prodrug	Before centrifugation		After centrifugation, lyophilization, and resuspension				
	d_f (nm)	PDI	d_f (nm)	PDI	PTX-silicate loading (wt%) ^a	PTX loading (wt%) ^b	Loading efficiency (%)
PTX	-	-	-	-	-	-	-
1	140	0.10	420	0.25	63.0	53.0	106
2	83	0.16	210	0.23	65.0	43.7	87.4
3	140	0.17	200	0.34	58.0	46.8	93.6
4	86	0.09	240	0.36	73.5	58.6	117
5	95	0.08	200	0.31	73.5	46.6	93.2
8	120	0.13	310	0.65	60.4	43.8	87.6
							77
							71
							220
							14
							1000
							650
							80

^a PTX-silicate prodrug loading is the wt% of encapsulated silicate derivative in the loaded nanoparticles following centrifugation;

^b PTX loading is the wt% of PTX that can be regenerated from silicate derivative in the loading nanoparticles following centrifugation. It is calculated using PTX-silicate loading and the MW of PTX and silicate derivatives.

Table 3

Release profiles over time of the percent of PTX plus PTX-silicate prodrug released into PBS at pH = 7.4.

Time (h)	1 [PTX-2'-Si(OEt) ₃]		2 [PTX-2'-Si(O ⁿ Oct) ₃]	
	%Released ^a	%Retained ^a	%Released ^a	%Retained ^a
12	39±6	51±5	33±8	57±3
24	52±6	50±6	41±8	49±9
48	63±8	34±5	56±13	–

^aTotal amount of the PTX-silicate plus free **PTX**, as measured by HPLC analysis.

Release profiles over time of the percent of PTX plus PTX-silicate prodrug released into acetate buffered saline at pH = 5.0.

Table 4

Time (h)	1 [PTX-2'-Si(OEt) ₃]		2 [PTX-2'-Si(O' Oct) ₃]		3 [PTX-2'-Si(O'Pr) ₃]		4 [PTX-2'-Si(O'Bu) ₂ (OEt)]		5 [PTX-2'-Si(Omenthyl) ₃]	
	% Released ^a	% Retained ^a	% Released ^a	% Retained ^a	% Released ^a	% Retained ^a	% Released ^a	% Retained ^a	% Released ^a	% Retained ^a
12	76±11	25±12	45±5	46±6	36±5	65±4	32±4	69±10	35±7	65±8
24	99±6	0.4±1.3	58±7	36±17	42±8	55±11	40±5	60±13	49±9	50±14
48	--	--	74±10	27±20	50±15	37±7	53±11 ^b	46±13 ^b	89±17 ^c	10±9 ^c

^aTotal amount of the PTX-silicate plus free **PTX**, as measured by HPLC analysis.

^b 216 hr .

^c 168 hr.