Published in final edited form as:

Small. 2011 July 18; 7(14): 2061–2069. doi:10.1002/sml.201100438.

Cisplatin-Loaded Porous Si Microparticles Capped by Electroless Deposition of Platinum

Jennifer S. Park,

Department of Bioengineering, University of California – San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0358, USA

Joseph M. Kinsella,

Department of Chemistry and Biochemistry, University of California – San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0358, USA

Danielle D. Jandial,

Division of Gynecologic Oncology, University of California Irvine Medical Center, 101 The City Drive, Building 56 Room 206, Orange, CA 92868, USA

Stephen B. Howell, and

John and Rebecca Moores Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093, USA

Michael J. Sailor

Department of Bioengineering and Department of Chemistry and Biochemistry, University of California – San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, USA

Michael J. Sailor: msailor@ucsd.edu

Abstract

The loading and release of the anti-cancer drug platinum *cis*-dichlorodiamine (cisplatin) from mesoporous silicon (pSi) microparticles is studied. The pSi microparticles are modified with 1-dodecene or with 1,12-undecylenic acid by hydrosilylation, and each modified pSi material acts as a reducing agent, forming a deposit of Pt on its surface that nucleates further deposition, capping the mesoporous structure and trapping free (unreduced) cisplatin within. Slow oxidation and hydrolytic dissolution of the Si/SiO₂ matrix in buffer solution or in culture medium leads to the release of drugs from the microparticles. The drug-loaded particles show significantly greater toxicity toward human ovarian cancer cells (in vitro), relative to an equivalent quantity of free cisplatin. This result is consistent with the mechanism of drug release, which generates locally high concentrations of the drug in the vicinity of the degrading particles. Control assays with pSi particles loaded in a similar manner with the therapeutically inactive *trans* isomer of the platinum drug, and with pSi particles containing no drug, result in low cellular toxicity. A hydrophobic prodrug, *cis,trans,cis*-[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂], is loaded into the pSi films from chloroform without concomitant reduction of the pSi carrier.

© 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Correspondence to: Michael J. Sailor, msailor@ucsd.edu.

This Full Paper is part of the Special Issue dedicated to Chad Mirkin in celebration of 20 years of influential research at Northwestern University.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

1. Introduction

Cisplatin and other platinum anticancer drugs are commonly employed in the treatment of metastatic diseases including ovarian, testicular, head and neck, breast, and bladder cancer.^[1] Platinum drugs exhibit their potent activity by forming intra- or interstrand cross-links to N7 sites on purine base pairs of DNA, unwinding or bending the strands to an extent sufficient to allow recognition by cellular proteins that trigger apoptosis.^[2–5] The DNA-binding conformation of cisplatin is thought to be generated in the low-chloride environment of the intracellular space by replacement of one of the chloride ligands with a water molecule.^[6] Although cisplatin and its second-generation cousins (carboplatin, oxaliplatin, nedaplatin, satraplatin) are standard chemotherapeutic agents, the efficacy of these drugs is limited by nephrotoxicity, neurotoxicity, cellular resistance, and deactivation of the drug by plasma proteins. Most of the newer generations of platinum drugs, either in the clinic (carboplatin, oxaliplatin) or in FDA (USA Food & Drug Administration) clinical trials (nedaplatin, picaplatin, and satraplatin) are designed to reduce nephrotoxicity or to provide a means of oral delivery.

An approach to improve therapeutic efficacy and reduce systemic toxicity of many anti-cancer drugs is to use a carrier that can sequester the drug and deliver it more specifically to the diseased tissues. The carrier performs dual functions of protecting the drug from uptake or deactivation by the immune system, and protecting healthy tissues from the toxic effects of the drug. In the particular case of the cisplatin family, the free drugs are particularly susceptible to deactivation upon contact with serum proteins containing nucleophilic sulfur or oxygen rich groups.^[7] This concept has been established clinically in such drug delivery systems for doxorubicin (liposomal vehicle, Doxil) and paclitaxel (albumin-based nanoparticle, Abraxane).^[8, 9] Cisplatin delivery systems such as liposomal particle formulations (SPI-77, Lipoplatin) and hydroxypropylmethacrylamide (HPMA) co-polymers are in Phase II clinical trials.^[10–16] Other cisplatin drug delivery systems have been explored using polymers such as polylactic acid (PLA),^[17] poly(acrylic acid)-*co*-methyl methacrylate,^[18] hyaluronic acid,^[19] chitosan,^[20] and gold nanoparticles.^[21]

Lin and co-workers pioneered the use of a porous silica nanoparticle as such a protective drug carrier, and they developed one of the first capping strategies that yielded an effective means to sequester and release therapeutic molecules from this material.^[22, 23] The main focus has been on particles with sizes less than a few hundred nanometers in order to develop drug carriers that can circulate in the blood stream.^[24, 25] However, larger (micrometer-scale) particles can carry a much larger drug payload, and such formulations are of interest for localized injection applications—for instance, intra-ocular^[26–28] or intraperitoneal^[29] drug delivery. Several clinical trials have demonstrated improved efficacy of cisplatin against ovarian cancer when the drug is administered intraperitoneally, compared with systemic (intravenous) delivery. The employment of a device to sequester the drug and release it slowly in the peritoneum could potentially further improve its efficacy while reducing its toxic side effects.

There are relatively few studies on the loading of platinum therapeutic compounds into larger (micrometer-scale) inorganic silicon-based particles. Silica nanomaterials are most commonly prepared by sol–gel routes, for example, mesoporous silica particles in the size range of 0.5–1 μm (e.g., MCM-41 and SBA-15) can be built by surfactant templated sol–gel synthesis methods,^[30, 31] and these structures have been loaded with cisplatin.^[32] A secondary route into mesoporous silicon-based structures is by electrochemical or chemical etching of crystalline silicon. Microparticles based on mesoporous silicon have been used to sequester or deliver insulin (across Caco-3 cell monolayers),^[33] dexamethasone,^[34] and doxorubicin.^[35, 36] Attractive features of mesoporous silicon for controlled release drug

delivery include the ability to electrochemically program the pore size^[37] and surface area,^[38] the availability of convenient surface modification chemistries,^[39] and the biodegradable^[40] and bioresorbable^[41–45] properties of the material.^[41, 46–50]

Porous Si (pSi) is a good reducing agent. It has Si–H species on the surface, and the skeleton consists of elemental Si. The reduction potential of either of these species is sufficient to reduce many organic molecules. For example, pSi has been found to interfere with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay by directly reducing MTT into formazan,^[51] and it can reduce benzoquinone to hydroquinone.^[52] Therefore, in using pSi as a drug delivery carrier, the redox chemistry of the material with the drug of interest must be considered, especially for molecules that are easily reduced. In this study, we use this redox chemistry to sacrifice cisplatin, reducing it to metallic platinum that then traps additional free cisplatin in the pores through the metallization reaction. The basis for this chemistry was worked out by Ogata and co-workers, who demonstrated deposition of various metals using pSi as an electroless reducing agent.^[53, 54] Once formed, the metal nanoparticles promote the further reduction of cisplatin, in a manner similar to the process developed by Mirkin and co-workers for deposition of silver on gold nanoparticles.^[55, 56] We find that the cisplatin drug trapped under the Pt metal cap can be released in its active form by dissolution of the pSi host matrix, and we study the effect of this localized drug release mechanism on ovarian cancer cells.

2. Results and Discussion

2.1. Preparation of Drug-Loaded pSi Microparticles Capped with Metallic Pt

The pSi particles are composed of hydrophobic Si–H groups covering a silicon skeleton, either of which can undergo galvanic displacement reactions with noble metals in aqueous media.^[53, 54, 57–59] We hypothesized that this reaction could be harnessed to trap cisplatin underneath a metal cap in the appropriate pSi matrix, Scheme 1. Two challenges with the use of as-formed pSi for such a reaction is that the metallization reaction is very rapid, and the surface hydrides make the inner pore walls very hydrophobic and impermeable to water unless a surfactant is present. In order to attenuate the reaction and to provide a more hydrophilic pore surface, we grafted organic species to pSi using a thermal hydrosilylation reaction, which reacts with surface Si–H species to form a Si–C bond.^[39, 60] Two alkenes were grafted in this study: undecylenic acid (Equation 1) and dodecene (Equation 2).

(1)

(2)

When undecylenic acid was used, the carboxylic acid groups on the resulting surface provided a relatively hydrophilic material that was readily dispersed in water. The cisplatin drug used in these studies was first hydrolyzed to the aquo form in order to increase the interaction of the drug with this carboxylated surface and facilitate drug loading. Use of dodecene generated a hydrophobic material that was not as readily dispersed in or infiltrated by water. Steric constraints appear to limit the extent of the hydrosilylation reaction, and Fourier transform infrared (FTIR) measurements indicated the presence of residual Si-H species on the pSi surface after reaction with either alkene.^[35, 61] These residual hydrides can act as reducing species in the galvanic displacement reaction with cisplatin.

Incubation of the carboxylic-acid-terminated pSi microparticles in 3.5 mM aqueous cisplatin or transplatin solution at 37 °C led to removal of significant quantities of soluble platinum from solution (inductively coupled plasma optical emission spectroscopy, ICP-OES, Table 1). Soluble silicon, presumed to be in the form of orthosilicate species, is detected in the solution after the reaction (ICP-OES). The noble metal appears to be deposited on the pSi particle surface in patches, rather than in a conformal layer (Figure 1). High-magnification electron micrographs revealed Pt nodules on the order of 10–200 nm sitting on the pSi surface containing pores of 6–8 nm (Figure 1B). Energy dispersive X-ray maps of Si and Pt (Figure 1D) confirmed the heterogeneous distribution of Pt on the pSi particle surface, and X-ray photoelectron spectroscopy (XPS) analysis confirmed that the nodules consist primarily of elemental platinum, with an oxidation state of zero (Supporting Information, Figure S1). The Pt 4f 7/2 signal in the XPS spectrum at 71.1 eV is in the accepted range for a Pt(0) compound (70.8–71.4 eV), lower than the energy expected for Pt(II) species (>72 eV). A Pt 4f 5/2 peak is also observed at 74.4 eV. Control experiments using pSi particles treated with pure water showed no detectable Pt by XPS. The observed morphology of the Pt deposits is consistent with previous results on reactions of Pt and other noble metals with pSi,^[53, 54, 57–59, 62, 63] and it has been described in terms of a localized deposition mechanism by Ogata and co-workers.^[54] In Ogata's local cell model, the oxidation half-reaction corresponds to Si and Si-H species oxidizing to SiO₂ (Equation 3), and it occurs heterogeneously on the pSi particle surface. This anodic reaction is spatially separated from the cathodic metal deposition process (Equation 4), which occurs preferentially on growing metallic nuclei. Electrons transfer between the two redox-active regions through the conductive pSi substrate.

The reduction kinetics of Equation 4 are sufficiently slowed by the presence of the surface grafted alkyl species that excess free platinum drug can diffuse into a pore before it becomes capped with metallic Pt.

A control experiment was performed using a fully oxidized pSi sample (Equation 5) that contains only SiO_2 ^[64] and thus is not able to participate in the galvanic displacement reaction.

(5)

The results are summarized in Table 1 (“Porous SiO_2 ”). Oxidized pSi has been shown to be an effective carrier for cisplatin by Coffey, Canham, and co-workers.^[65] In that work, the pSi layer was partially converted to hydroxyapatite (calcium phosphate) by electrochemical means using an electrolyte containing cisplatin. The drug was entrained in the resulting hydroxyapatite layer as a consequence of the electrocorrosion process. This prior work did not report the observation of elemental Pt deposits in the hydroxyapatite matrix. In the present work, we found that oxidized pSi particles will absorb relatively small quantities of cisplatin from aqueous solution. This is presumed to be a simple physical absorption process, and 25% of the adsorbed drug was recovered upon digestion of the particles. No evidence of metallic Pt was observed on these oxidized pSi particles (SEM, XPS analysis).

The carboxy-terminated surface appears to facilitate drug loading by providing a readily wetted pore surface. This interpretation is supported by a control experiment in which the pSi particle was capped by hydrosilylation with the hydrophobic species 1-dodecene (no carboxylic acid group at the end, Equation 2). In this experiment, a similar quantity of elemental Pt was deposited on the particles (by SEM, XPS and ICP-OES, Table 1), but very little free drug was trapped in the porous matrix (Table 1). Thus a hydrophobic pSi particle will still undergo the galvanic displacement reaction to produce metallic Pt caps, but little of the drug solution can infiltrate the pores, so no drug is trapped.

2.2. Quantification of Drug Loading in Pt-Capped pSi Particles

The mechanism by which the pores of the pSi particles are capped involves sacrificial reduction of the Pt-containing compounds cisplatin or transplatin, which generates the elemental platinum of the caps. Thus the drug-loaded particles consist of two forms of Pt: 1) elemental Pt, with an oxidation state of 0; and 2) the trapped drug, with an oxidation state of +2. We quantified the amounts of elemental Pt and trapped drug Pt in two separate assays. In the first assay, we quantified the total amount of free drug consumed by the reaction (which generated both the elemental Pt of the caps and the soluble Pt of the trapped drug) by measuring the concentration of free platinum in the drug loading solution before and after the loading procedure (using ICP-OES). In the second method, the amount of soluble Pt trapped in the pSi particles was determined by digestion of the pSi composite particles in aqueous 1 M KOH and quantification of soluble Pt by ICP-OES. The digestion conditions and sample workup for this second method will not solubilize elemental Pt; we assume the results of this method yield the quantity of active, soluble drug that can be released once the capping metal or the pSi host matrix is removed. The results of both assays are summarized in Table 1. For cisplatin and transplatin, we found that the majority of drug in the loading solution is reduced to elemental platinum, and only ~0.5% of the total mass of the drug is trapped in its soluble form.

2.3. Loading of Pt Pro-drug in Hydrophobic pSi Particles

As was discussed above, pSi particles hydrosilylated with 1-dodecene are very hydrophobic and do not exhibit significant loading of cisplatin or transplatin from aqueous solutions. In order to test the ability of this hydrophobic formulation to hold a platinum drug, the more hydrophobic drug, *cis,trans,cis* (*c,t,c*)-[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂] was synthesized. This compound is considered a pro-drug, because it undergoes intracellular reduction to form cisplatin.^[66–68] It exhibits very low water solubility, and so it was loaded into the 1-dodecene-modified pSi particles from a chloroform solution that contained 10% water. If water was not included in the loading solvent, no elemental Pt was observed on the resulting particles. Water supports the oxidation half-reaction that converts Si to SiO₂ (Equation 3) and in its absence, the Pt drug apparently cannot be directly reduced by pSi. When the drug was loaded into undecylenic-acid-modified pSi particles from a pure chloroform solution, no elemental Pt was observed on the resulting particles, and most (83%) of the drug was recovered from the particles in a soluble form upon digestion in aqueous base (Table 1). Addition of water to the chloroform loading solution results in metallic Pt deposits similar to those observed from the loading of cisplatin from aqueous solution, and only 6% of the drug was recovered in its soluble form (Table 1).

2.4. Release of Drug from Metal-Capped pSi Particles into Physiologically Relevant Buffers

The amount of cisplatin released from Pt-capped pSi particles into three buffer solutions was quantified (Figure 2a): phosphate buffered saline (PBS) that contained no Ca²⁺ or Mg²⁺ (0.9% w/v), a hypertonic PBS solution (5% w/v), and fetal bovine serum (FBS). All solutions were maintained at 37 °C for a period of 192 h and then assayed for soluble Pt by ICP-OES. In that time period, particles incubated in FBS released the largest quantity of drug. The ICP-OES measurements also detected significant quantities of soluble Si, presumably in the form of orthosilicates. We propose that fetal bovine serum degrades the particles more rapidly due to the presence of amine-based nucleophiles, which can attack and dissolve porous Si and SiO₂ more readily than hydroxyl-based nucleophiles.^[69] The time dependence of drug release into FBS was monitored in the first 15 h of incubation. The data indicate that 10–20% of the loaded drug is released in a burst at the beginning of the experiment, suggesting that this amount is only weakly bound or trapped. The remaining drug is released more slowly; ~50% of the loaded drug is released after 192 h.

Similar experiments using the hydrophobic Pt(IV) prodrug loaded into undecylenic-acid-modified pSi particles showed that less drug (<10%) was released over the same time period, and ICP-OES indicated that the amount of free silicic acid released into solution was lower than with the formulations that contained Pt caps. This Pt(IV) pro-drug formulation did not contain a capping Pt layer, and it was loaded with the hydrophobic drug from a non-aqueous solvent. Because the Pt redox chemistry does not occur under those conditions, minimal quantities of silicon oxides are present in the final formulation (confirmed by FTIR). Presumably the more hydrophobic pSi/drug matrix in this formulation slows the rate of pSi dissolution and drug leaching by protecting the pSi matrix from water infiltration. Buriak and co-workers have demonstrated the stable nature of porous Si modified via hydrosilylation,^[70] and the greater stability of dodecene and undecylenic acid surfaces relative to oxide in aqueous media have been demonstrated.^[71]

2.5. Toxicity of Drug-Loaded Microparticles Toward Human Ovarian Cancer Cells

The toxicity of the undecylenic-acid-modified, cisplatin-loaded pSi particles toward the 2008 human ovarian cancer cell line was assessed using sulforhodamine B cellular viability assay. The data are compared with control experiments using free cisplatin, free transplatin, empty pSi particles, and transplatin-loaded pSi microparticles in Figure 3. The data are presented based on mass of platinum drug for all the formulations. Figure S2 and S3 in the

Supporting Information show the toxicity data over a larger range of concentration. Administration of free transplatin (up to 4.5 $\mu\text{g}/\text{well}$), empty porous Si particles, or solid platinum particles (0.5–1 μm diameter, concentration up to 150 $\mu\text{g}/\text{well}$) resulted in low toxicity (>80% survival over entire concentration range studied). As expected, free cisplatin exhibited substantially greater toxicity than free transplatin, with an IC_{50} (50% inhibitory concentration) of 2.9 μg and <40% survival at 4.5 μg of drug dosed under the conditions of the experiment.

The cisplatin-loaded particles showed the highest level of toxicity, with IC_{50} values of 0.004, 0.006, and 0.018 μg (based on mass of free drug loaded) for porous silica, undecylenic-acid-conjugated pSi, and 1-dodecene-conjugated pSi particles, respectively. The toxicity of these particulate formulations represent enhancements of between 160- and 700-fold, relative to the free drug.

The higher toxicity observed with the particulate formulations is related to the mode of drug administration in the in vitro cellular assays. The pSi particles settled to the bottom of the wells once they were injected into the microplate, which was also the physical location of the ovarian cancer cells. Thus we ascribe the greater toxicity observed for the pSi–cisplatin formulations to localized release and thus higher drug concentrations in the vicinity of the cells relative to free cisplatin, which was diluted in the cell culture media upon introduction to the microplate wells. The culture plates were quiescent throughout the 5-day incubation period, therefore any drug released from the particles can be expected to be concentrated at the cell surfaces. Consistent with this interpretation, the porous silica formulation, which contained no capping group and released the drug within a few hours of administration, showed the greatest level of toxicity on a Pt mass basis. Control experiments with metallic platinum particles of size 0.5–1 μm confirmed that no cellular toxicity could be ascribed to elemental Pt under the conditions of the experiment.

The formulations loaded with the hydrophobic pro-drug *c,t,c*-[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂] were also tested for cytotoxicity with 2008 human ovarian cancer cells (Figure 4). Free drug was determined to have an IC_{50} of 0.06 μg under the experimental conditions. The drug-loaded particles exhibited a much lower toxicity, consistent with the high stability and low rate of drug release observed with this formulation.

3. Conclusion

The data presented here underscore the importance of redox chemistry in drug delivery devices and systems that use porous Si. In particular, the ability of this material to act as a reducing agent is of key concern.^[35] In the present study, the redox chemistry was used to reduce sacrificial cisplatin to metallic Pt, which then trapped free drug in the remaining porous Si matrix. The majority of the drug is consumed by the capping chemistry and converted to metallic Pt, leading to the trapping of a relatively small fraction of free drug. This free drug can then be released back into solution under the appropriate conditions, and the drug-loaded pSi material was used to kill ovarian cancer cells via localized release of drug. Drug release was triggered by dissolution of the silicon oxide that forms as a by-product of the trapping chemistry. The cellular toxicity studies showed that drug-loaded particles are more toxic than the free drug, and this was ascribed to the localized nature of drug release in the vicinity of cancer cells. The ability of the particles to load and to release the platinum drugs is highly dependent on the hydrophobic or hydrophilic nature of the surface modification chemistry and on the hydrophobic or hydrophilic nature of the drug.

4. Experimental Section

Materials

Stain-etched porous Si microparticles with an average diameter of 4 μm were obtained from Vesta Ceramics LLC (Santee, CA). Cisplatin, transplatin, hydrogen hexachloroplatinate(IV), silicon ICP grade standards (10 000 $\mu\text{g/mL}$ Si), sodium cyanide, hexane, undecylenic acid (99.9% purity), Sulforhodamine B cellular viability assay kit and sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO). PBS without Mg^{2+} and Ca^{2+} was obtained from Mediatech (Manassas, VA). FBS was obtained from Gibco (Carlsbad, CA). RPMI 1640 media with 10% heat-inactivated fetal bovine serum was obtained from Thermo Fisher Scientific (Pittsburg, PA). AgNO_3 was supplied by Mallinckrodt Chemicals (St. Louis, MO). Trichloroacetic acid and glacial acetic acid were purchased from Thermo Fisher Scientific (Pittsburg, PA).

Characterization of Stain-Etched Microparticles

The manufacturer quoted an average particle diameter of 4 μm and that the materials were prepared using a nitric acid–hydrofluoric acid etchant. Brunnauer–Emmett–Teller (BET) nitrogen adsorption analysis yielded 30% porosity and $166 \pm 4 \text{ m}^2/\text{g}$ surface area. Barret–Joyner–Halenda (BJH) analysis of the adsorption data yielded average pore width of 7 nm. Transmission electron microscopy (TEM) images show irregular pores aligned primarily in the $\langle 100 \rangle$ crystallographic direction. Porosity is evenly distributed throughout the particle.

Surface Modification by Microwave-Assisted Hydrosilylation

The native oxide on the as-received pSi microparticles was removed with a brief rinse in a 1:1 solution of aqueous 49% HF:ethanol, to generate a Si–H terminated surface. The particles were rinsed with ethanol, dried, and then thermally hydrosilylated with undecylenic acid or with dodecene. A batch of 50 mg of particles were placed in a 10 mL Pyrex beaker, immersed in 2 mL of the neat alkene (99.9%), and then heated in a commercial consumer microwave oven (Sears Kenmore 700 W) for four 1-min intervals at the 280 W setting. The resulting conjugated particles were centrifuged, rinsed with hexane (3X) and then rinsed with ethanol (3X) to remove unreacted reagent.

Surface Modification by Thermal Oxidation

Oxidized particles were prepared from Si–H terminated (freshly etched) particles by thermal oxidation in a tube furnace (Lindberg/Blue M, Fisher Scientific, Pittsburgh, PA) at 800 $^{\circ}\text{C}$ for 2 h. The particles were cooled to room temperature and stored in a desiccator.

Preparation of cis-Pt(NH₃)₂Cl₂ (cisplatin), trans-Pt(NH₃)₂Cl₂, and Pt(IV) Pro-drug Loading Solutions

Aquated cisplatin and transplatin solutions were prepared by removal of the labile chloride ligands by the action of $\text{AgNO}_{3(\text{aq})}$ on the complex. Equimolar (7 mM) solutions of $\text{AgNO}_{3(\text{aq})}$ and the platinum reagent were mixed and allowed to equilibrate for 1 h. A white, cloudy $\text{AgCl}_{(\text{s})}$ precipitate formed and was separated from the loading solution by centrifugation and filtration through a 0.1 μm Durapore PVDF membrane sterile syringe filter (Millipore, inc). The platinum(IV) pro-drug compound *c,t,c*-[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂] was prepared following related published preparations:^[66–68] cisplatin (200 mg) was allowed to react with a solution made from 7 mL of 30% hydrogen peroxides and 5 mL deionized water at 50 $^{\circ}\text{C}$ for 1 h. The resulting solution was chilled in an ice bath, and the precipitate collected by vacuum filtration through a Whatman #2 cellulose filter, rinsed with small quantities of cold water, ethanol, and then dried. This intermediate *c,t,c*-[Pt(NH₃)₂Cl₂(OH)₂] was dissolved in dimethylsulfoxide

(DMSO) and reacted with 2 molar equivalents of decanoic anhydride for 48 h at room temperature. The resulting compound was precipitated from solution by addition of cold deionized H₂O, filtered, and rinsed with ethanol, then diethyl ether. The composition of the product was verified by high-performance liquid-chromatography MS (HPLC-MS; Supporting Information, Figure S4).

Loading of cis-Pt(NH₃)₂Cl₂(cisplatin), trans-Pt(NH₃)₂Cl₂, and Pt(IV) Pro-drug

Hydrosilylated (either dodecyl- or dodecanoic-acid-terminated material) or oxidized pSi particles (75 mg) were added to 40 mL of the aqueous cisplatin solution prepared above (which was 3.5 mM in Pt). The particles were allowed to react for 48 h (at 37 °C) and then separated by centrifugation, rinsed with ethanol (3×) and then dried in a desiccator. The biologically inactive analogue, transplatin (40 mL, 3.5 mM) was loaded into pSi particles using the same procedure. The platinum(IV) pro-drug was loaded into dodecene-modified particles from a loading solution containing a mixture of chloroform and water (10% by volume): pSi particles (75 mg) were mixed with pro-drug (40 mL, 2.5 mM) in the chloroform:water mixture for 48 h, the particles were separated by centrifugation, rinsed with chloroform (3×) and then dried in a desiccator. In order to generate platinum(IV) pro-drug-loaded particles without the Pt cap, undecylenic-acid-modified pSi particles (75 mg) were mixed with pro-drug (40 mL, 2.5 mM) in pure chloroform for 48 h, the particles were separated by centrifugation, rinsed quickly with chloroform (3×) and then dried.

Quantification of Platinum Loading

The quantity of Pt-complex loaded in the pSi microparticles was measured by ICP-OES (Perkin Elmer, Optima 3700DV) or by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Finnigan Element 2 plasma mass spectrometer). Two separate analytical protocols were employed. In the first method, the amount of Pt in the particles was determined by taking the difference between the amount of Pt in the loading solution before addition of pSi particles and the amount of Pt in the supernatant after the 48 h loading procedure was complete (particles were removed from the supernatant by centrifugation). In the second method, the amount of Pt in the particles was measured directly, by digesting the particles in an aqueous solution 1 M in KOH for 7 days (particle concentration 1 mg/mL). This procedure was found to dissolve the pSi particles and release the drug into solution, but not to dissolve the elemental Pt capping the pores. Solid Pt was removed from the digest by centrifugation and filtration (0.1 μm Durapore PVDF membrane syringe filter) prior to ICP-OES analysis. The samples were diluted in 3% (v/v) nitric acid and dissolved Pt was quantified by comparison of the intensity of the elemental Pt emission line (214 nm) in the ICP-OES spectrum to a standard intensity versus concentration calibration curve. For ICP-MS, a 1 ppb ¹¹⁵In internal standard was employed. Samples were measured in triplicate.

In-vitro Drug Release Studies

Release of the Pt drug was quantified in three different physiological buffers (PBS, hypertonic PBS, and FBS) by incubation of 0.5 mg/mL of particles in the respective solution at 37 °C. After 192 h, the solution was separated from the particles by centrifugation, passed through a syringe filter (0.1 μm Durapore PVDF membrane), and the filtrate analyzed for Pt by ICP-OES as described above. The samples were measured in triplicate. A separate set of experiments were performed to verify the quantity of residual Pt drug in the particles and to quantify the time dependence of release in fetal bovine serum: particles (0.5 mg) were added to FBS in 21 separate dialysis tubes with 3500 g/mol cutoff. The tubes were placed in a styrofoam floater and stirred in 300 mL of FBS buffer. At each time point, three dialysis tubes were removed, the particles were collected, digested in 1 M KOH, centrifuged, filtered, and the supernatant processed and analyzed for soluble Pt content by ICP-OES as described above.

Cellular Toxicity of Drug-Loaded Microparticles

Drug-loaded microparticles were assayed for cellular toxicity using sulforhodamine B cellular viability assay, which measures total biomass by staining cellular proteins. The 2008 human ovarian cancer cell line was grown in RPMI 1640 media with 10% heat-inactivated fetal bovine serum at 37 °C in 5% CO₂. Cells were seeded in standard 96-well plates (5000 cells/well, volume of liquid in each well was 300 µL). After 24 h, the relevant quantity of Pt drug-loaded or control particles were added to the media and cells were allowed to grow for five days. Particles were thoroughly rinsed from the plate with PBS. The surviving cells were then fixed by treatment with an aqueous solution of 50% trichloroacetic acid for 1 h at 4 °C and then stained with 0.4% sulforhodamine B dye. After rinsing the unbound dye with 1% aqueous acetic acid, the bound dye was solubilized in 10 mM Tris-HCl buffer and quantified on a Versamax absorbance microplate reader (Molecular Devices) at a wavelength of 515 nm. The optical density data were processed as previously described.^[72]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project has been funded in part with Federal funds from the National Cancer Institute of the National Institutes of Health J.S.P., J.M.K., D.J., S.B.H., and M.J.S. are members of the Moores UCSD Cancer Center and the UCSD NanoTUMOR Center under which this research was conducted and partially supported by NIH Grant U54 CA 119335 and CA 095298. The authors thank the National Science Foundation (DMR-0806859) for partial support of this project. J.M.K. thanks the American Cancer Society for a postdoctoral fellowship. The authors thank Sean Duncan of the Scripps Institution of Oceanography analytical facility for ICP analyses, and Rocky Mountain Labs for help with the XPS analysis.

References

1. Ho YP, Au-Yeung SCF, To KKW. *Med. Res. Rev.* 2003; 23:633. [PubMed: 12789689]
2. Eastman A. *Cancer Cells Monthly Rev.* 1990; 2:275.
3. Chaney SG, Campbell SL, Temple B, Bassett E, Wu YB, Faldu M. *J. Inorg. Biochem.* 2004; 98:1551. [PubMed: 15458816]
4. Jamieson ER, Lippard SJ. *Chem. Rev.* 1999; 99:2467. [PubMed: 11749487]
5. Rosenberg B, Vancamp L, Trosko JE, Mansour VH. *Nature.* 1969; 222:385. [PubMed: 5782119]
6. Davies MS, Berners-Price SJ, Hambley TW. *J. Inorg. Biochem.* 2000; 79:167. [PubMed: 10830862]
7. Deconti RC, Toftness BR, Lange RC, Creasey WA. *Cancer Res.* 1973; 33:1310. [PubMed: 4515709]
8. Gordon AN, Tonda M, Sun S, Rackoff W. *Gynecol. Oncol.* 2004; 95:1. [PubMed: 15385103]
9. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. *J. Clin. Oncol.* 2005; 23:7794. [PubMed: 16172456]
10. White SC, Lorigan P, Margison GP, Margison JM, Martin F, Thatcher N, Anderson H, Ranson M. *Br. J. Cancer.* 2006; 95:822. [PubMed: 16969346]
11. Harrington KJ, Lewanski CR, Northcote AD, Whittaker J, Wellbank H, Vile RG, Peters AM, Stewart JSW. *Ann. Oncol.* 2001; 12:493. [PubMed: 11398881]
12. Terwogt JMM, Groenewegen G, Pluim D, Maliepaard M, Tibben MM, Huisman A, Huinink WWT, Schot M, Welbank H, Voest EE, Beijnen JH, Schellens JHM. *Cancer Chemother. Pharmacol.* 2002; 49:201. [PubMed: 11935212]
13. Stathopoulos GP, Boulikas T, Vougiouka M, Deliconstantinos G, Rigatos S, Darli E, Viliotou V, Stathopoulos JG. *Oncol. Rep.* 2005; 13:589. [PubMed: 15756428]
14. Rademaker-Lakhai JM, Terret C, Howell SB, Baud CM, de Boer RF, Pluim D, Beijnen JH, Schellens JHM, Droz JP. *Clin. Cancer Res.* 2004; 10:3386. [PubMed: 15161693]

15. Rice JR, Gerberich JL, Nowotnik DP, Howell SB. Clin. Cancer Res. 2006; 12:2248. [PubMed: 16609041]
16. Campone M, Rademaker-Lakhai JM, Bennouna J, Howell SB, Nowotnik DP, Beijnen JH, Schellens JHM. Cancer Chemother. Pharmacol. 2007; 60:523. [PubMed: 17308894]
17. Aryal S, Hu CMJ, Zhang LF. ACS Nano. 2010; 4:251. [PubMed: 20039697]
18. Yan XL, Gemeinhart RA. J. Control. Release. 2005; 106:198. [PubMed: 15979187]
19. Jeong YI, Kim ST, Jin SG, Ryu HH, Jin YH, Jung TY, Kim IY, Jung S. J. Pharm. Sci. 2008; 97:1268. [PubMed: 17674407]
20. Cafaggi S, Russo E, Stefani R, Leardi R, Caviglioli G, Parodi B, Bignardi G, De Toter D, Aiello C, Viale M. J. Control. Release. 2007; 121:110. [PubMed: 17601625]
21. Dhar S, Daniel WL, Giljohann DA, Mirkin CA, Lippard SJ. J. Am. Chem. Soc. 2009; 131:14652. [PubMed: 19778015]
22. Slowing I, Trewyn BG, Lin VSY. J. Am. Chem. Soc. 2006; 128:14792. [PubMed: 17105274]
23. Lai C-Y, Trewyn BG, Jeftinija DM, Jeftinija K, Xu S, Jeftinija S, Lin VS-Y. J. Am. Chem. Soc. 2003; 125:4451. [PubMed: 12683815]
24. Patel K, Angelos S, Dichtel WR, Coskun A, Yang YW, Zink JI, Stoddart JF. J. Am. Chem. Soc. 2008; 130:2382. [PubMed: 18232687]
25. Bégu S, Durand R, Lerner DA, Charnay C, Tourné-Petel C, Devoisselle J-M. Chem. Commun. 2003:640.
26. Gavini E, Chetoni P, Cossu M, Alvarez MG, Saettone MF, Giunchedi P. Eur. J. Pharm. Biopharm. 2004; 57:207. [PubMed: 15018976]
27. Cheng LY, Sailor MJ, Kayikcioglu O, Kim D, Kozak I, Bergeron-Lynn G, Freeman WR. Investigative Ophthalmology Visual Sci. 2005; 46
28. Cheng L, Anglin E, Cunin F, Kim D, Sailor MJ, Falkenstein I, Tammewar A, Freeman WR. Br. J. Ophthalmol. 2008; 92:705. [PubMed: 18441177]
29. Kawanabe K, Yamamuro T, Kotani S, Nakamura T. J. Biomed. Mater. Res. 1992; 26:209. [PubMed: 1569114]
30. Charnay C, Begu S, Tourne-Petel C, Nicole L, Lerner DA, Devoisselle JM. Eur. J. Pharm. Biopharm. 2004; 57:533. [PubMed: 15093603]
31. Liong M, Lu J, Kovichich M, Xia T, Ruehm SG, Nel AE, Tamanoi F, Zink JI. ACS Nano. 2008; 2:889. [PubMed: 19206485]
32. Tao ZM, Toms B, Goodisman J, Asefa T. ACS Nano. 2010; 4:789. [PubMed: 20131868]
33. Foraker AB, Walczak RJ, Cohen MH, Boiarski TA, Grove CF, Swaan PW. Pharm. Res. 2003; 20:110. [PubMed: 12608544]
34. Anglin EJ, Schwartz MP, Ng VP, Perelman LA, Sailor MJ. Langmuir. 2004; 20:11264. [PubMed: 15568884]
35. Wu EC, Park J-H, Park J, Segal E, Cunin F, Sailor MJ. ACS Nano. 2008; 2:2401. [PubMed: 19206408]
36. Park J-H, Gu L, Maltzahn Gv, Ruoslahti E, Bhatia SN, Sailor MJ. Nat. Mater. 2009; 8:331. [PubMed: 19234444]
37. Collins BE, Dancil K-P, Abbi G, Sailor MJ. Adv. Funct. Mater. 2002; 12:187.
38. Zhang XG. J. Electrochem. Soc. 2004; 151:C69.
39. Buriak JM. Chem. Rev. 2002; 102:1272.
40. Anderson SHC, Elliott H, Wallis DJ, Canham LT, Powell JJ. Phys. Status Solidi A-Appl. Res. 2003; 197:331.
41. Canham LT, Stewart MP, Buriak JM, Reeves CL, Anderson M, Squire EK, Allcock P, Snow PA. Phys. Status Solidi A-Appl. Res. 2000; 182:521.
42. Canham LT, Reeves CL, King DO, Branfield PJ, Crabb JG, Ward MCL. Adv. Mater. 1996; 8:850.
43. Canham LT. Adv. Mater. 1995; 7:1033.
44. Canham LT, Reeves CL, Loni A, Houlton MR, Newey JP, Simons AJ, Cox TI. Thin Sol. Films. 1997; 297:304.

45. Canham LT, Reeves CL, Newey JP, Houlton MR, Cox TI, Buriak JM, Stewart MP. *Adv. Mater.* 1999; 11:1505.
46. Canham LT, Newey JP, Reeves CL, Houlton MR, Loni A, Simmons AJ, Cox TI. *Adv. Mater.* 1996; 8:847.
47. Coffey JL, Whitehead MA, Nagesha DK, Mukherjee P, Akkaraju G, Totolici M, Saffie RS, Canham LT. *Phys. Status Solidi A-Appl. Mat.* 2005; 202:1451.
48. Nagesha DK, Whitehead MA, Coffey JL. *Adv. Mater.* 2005; 17:921.
49. Rosengren A, Wallman L, Bengtsson M, Laurell TH, Danielsen N, Bjursten LM. *Phys. Stat. Sol. A.* 2000; 182:527.
50. Moxon KA, Kalkhoran NM, Markert M, Sambito MA, McKenzie JL, Webster JT. *IEEE Trans. Biomed. Eng.* 2004; 51:881. [PubMed: 15188854]
51. Laaksonen T, Santos H, Vihola H, Salonen J, Riikonen J, Heikkilä T, Peltonen L, Kumar N, Murzin DY, Lehto V-P, Hirvonen J. *Chem. Res. Toxicol.* 2007; 20:1913. [PubMed: 17990852]
52. Harper TF, Sailor MJ. *J. Am. Chem. Soc.* 1997; 119:6943.
53. Tsuboi T, Sakka T, Ogata YH. *J. Appl. Phys.* 1998; 83:4501.
54. Harraz FA, Tsuboi T, Sasano J, Sakka T, Ogata YH. *J. Electrochem. Soc.* 2002; 149:C456.
55. Taton TA, Mirkin CA, Letsinger RL. *Science.* 2000; 289:1757. [PubMed: 10976070]
56. Xu XY, Georgiopoulou DG, Hill HD, Mirkin CA. *Anal. Chem.* 2007; 79:6650. [PubMed: 17663531]
57. Parbukov AN, Beklemyshev VI, Gontar VM, Makhonin II, Gavrilov SA, Bayliss SC. *Mater. Sci. Eng. C.* 2001; 15:121.
58. Coulthard, I.; Sham, TK. "Synthesis of nanophase noble metal systems utilizing Porous Silicon"; presented at Mat. Res. Soc. Symp. Proc; 1997.
59. Coulthard I, Jiang D-T, Lorimer JW, Sham TK, Feng X-H. *Langmuir.* 1993; 9:3441.
60. Boukherroub R, Wojtyk JTC, Wayner DDM, Lockwood DJ. *J. Electrochem. Soc.* 2002; 149:59.
61. Wu EC, Andrew JS, Cheng L, Freeman WR, Pearson L, Sailor MJ. *Biomaterials.* 2011; 32:1957. [PubMed: 21122914]
62. Lin H, Mock J, Smith D, Gao T, Sailor MJ. *J. Phys. Chem. B.* 2004; 108:11654.
63. Lin H, Gao T, Fantini J, Sailor MJ. *Langmuir.* 2004; 20:5104. [PubMed: 15984274]
64. Pap AE, Kordas K, Toth G, Levoska J, Uusimäki A, Vahakangas J, Leppavuori S, George TF. *Appl. Phys. Lett.* 2005; 86
65. Li X, John JS, Coffey JL, Chen Y, Pinizzotto RF, Newey J, Reeves C, Canham LT. *Biomed. Microdev.* 2000; 2:265.
66. Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. *Proc. Natl. Acad. Sci. USA.* 2008; 105:17356. [PubMed: 18978032]
67. Barnes KR, Kutikov A, Lippard SJ. *Chem. Biol.* 2004; 11:557. [PubMed: 15123250]
68. Feazell RP, Nakayama-Ratchford N, Dai H, Lippard SJ. *J. Am. Chem. Soc.* 2007; 129:8438. [PubMed: 17569542]
69. Coffey JL. *J. Lumin.* 1996; 70:343.
70. Stewart MP, Buriak JM. *Angew. Chem. Int. Ed. Engl.* 1998; 37:3257.
71. Lees IN, Lin H, Canaria CA, Gurtner C, Sailor MJ, Miskelly GM. *Langmuir.* 2003; 19:9812.
72. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigrowolff A, Graygoodrich M, Campbell H, Mayo J, Boyd M. *J. Natl. Cancer Inst.* 1991; 83:757. [PubMed: 2041050]

Figure 1. Porous silicon microparticles analyzed for elemental platinum by scanning electron microscopy (SEM) and energy dispersive X-ray mapping. A) Typical appearance of particles exposed to the Pt drugs (cisplatin, in this case) showing a heterogeneous distribution of platinum nodules on the surfaces. B) Close-up view showing the Pt nodules and the nanostructure of the pSi particle substrate. C) SEM image of a single pSi particle covered with Pt caps, and D) the corresponding elemental map of Pt and Si (as indicated).

Figure 2.

A) Quantity of cisplatin released from undecylenic-acid-modified pSi particles after 192 h in the indicated solutions: PBS is phosphate buffered saline (0.9% w/v), hypertonic PBS is phosphate buffered saline (5% w/v), and FBS is fetal bovine serum physiological buffer. Solutions were analyzed for total drug released into solution by ICP-OES and the data are expressed as micrograms of cisplatin drug released into solution per milligram of particle. B) Percent of cisplatin released into FBS as a function of time for the first 15 h of release. The same particle preparation was used in both (A) and (B): particles were loaded with aquated cisplatin as described in the text and contained $\sim 1 \mu\text{g}$ of cisplatin per milligram of pSi particles. Particles were contained in dialysis tubes and analyzed in triplicate.

Figure 3.

Percent survival of ovarian cancer cells as a function of drug quantity for the indicated drug and drug carrier formulations: free cisplatin, free transplatin, pSi particles modified with undecylenic acid and loaded with transplatin, pSi particles modified with dodecene and loaded with cisplatin, pSi particles modified with undecylenic acid and loaded with cisplatin, and oxidized pSi particles (porous silica) loaded with cisplatin. The x -axis gives mass of drug added to each well in the assay. The cisplatin and the transplatin drugs show minimal toxicity in this dosage range; both are represented by the line near 100% survival (IC_{50} for cisplatin under these conditions is 2.9 μg ; free transplatin shows >80% survival at

4.5 μg , the highest dose studied). Viability tested using sulforhodamine B assay after an incubation period of 5 days. Liquid volume in each well was 300 μL .

Figure 4.

Percent survival of ovarian cancer cells as a function of drug concentration for the hydrophobic Pt(IV) pro-drug c,t,c -[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂], either free or loaded in undecylenic-acid-modified pSi particles as indicated. The x -axis gives mass of drug added to each well in the assay. Viability tested using sulforhodamine B assay after an incubation period of 5 d.

Scheme 1.

Loading of cisplatin in surface-modified pSi microparticles by galvanic displacement/trapping with metallic Pt. Subsequent dissolution of the pSi matrix releases the trapped drug from the matrix.

Table 1

Drug loading data for pSi particles.

Compound	Surface chemistry	Platinum removed from solution ^{a)} [μg/mg]	Soluble platinum trapped in pores ^{b)} [μg/mg]
Cisplatin	Undecylenic Acid	231.3 ± 0.2	1.5 ± 0.8
Cisplatin	1-Dodecene	241.3 ± 0.2	0.06 ± 0.02
Cisplatin	Porous SiO ₂	0.7 ± 0.3	0.17 ± 0.01
Transplatin	Undecylenic Acid	267.0 ± 0.2	1.0 ± 0.5
Pt(IV) pro-drug ^{c)} (no cap)	Undecylenic Acid	5.29 ± 0.05	4.40 ± 0.04
Pt(IV) pro-drug ^{d)}	1-Dodecene	11.11 ± 0.04	0.67 ± 0.01

^{a)} Micrograms of dissolved Pt compound removed from solution per milligram of pSi particles added. Determined by ICP-OES analysis of the soluble Pt drug in the loading solution before and after addition of pSi particles. For cisplatin and transplatin, initial concentration of Pt reagent in solution corresponds to 360 μg/mg; for the Pt(IV) pro-drug, initial concentration of Pt reagent in solution corresponds to 260 μg/mg;

^{b)} Micrograms of Pt per milligram of pSi. Determined by digestion of pSi superstructure in 1 M KOH;

^{c)} Pt(IV) pro-drug is *c,t,c*-[Pt(NH₃)₂(O₂C(CH₂)₈CH₃)₂Cl₂], loaded from chloroform solution, where no reduction of the Pt reagent is observed. This formulation contains no elemental Pt capping the pores ("no cap");

^{d)} Drug loaded from chloroform solution containing 10% H₂O.