Drug delivery

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Mesoporous Silica Nanoparticles as a Delivery System for Hydrophobic Anticancer Drugs**

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A critical obstacle and challenge for cancer therapy concerns the limited availability of effective biocompatible delivery systems for most hydrophobic therapeutic anticancer drugs. It is particularly important to improve the aqueous solubility of drugs, as low drug solubility in aqueous media hampers the ability of drugs to be administered through the intravenous route. Since many important anticancer agents have poor water solubility, the development of novel delivery systems for these molecules without the use of organic solvents has received significant attention. Nanoparticles offer great potential and a promising approach to deliver therapeutic agents into targeted organs or cells and they have been actively developed for application in cancer therapy.[1,2] We have incorporated a representative hydrophobic anticancer drug, camptothecin (CPT), into the pores of fluorescent mesoporous silica nanoparticles (FMSNs) and delivered the drug into a variety of human cancer cells to induce cell death, a procedure suggesting that the mesoporous silica nanoparticles might be used as a vehicle to overcome the insolubility problem of many anticancer drugs.

CPT and its derivatives are considered to be among the most promising anticancer drugs of the 21st century.[3] Although studies have demonstrated their effectiveness against carcinomas of the stomach,[4] colon,[5] neck,[6] and bladder,[7] as well as breast[8] and small-cell lung cancers,[9] and leukemia,[10] in vitro, clinical application of CPT in humans has not been achieved to date because the poor water solubility of the drug requires changes to the physicochemical characteristics. The need to formulate water-soluble salts of CPT (that is, alkaline solutions for intravenous injections) led to chemical modifications of the molecule with loss of antitumor activity and significant alterations in the toxicological profile of the drug.[10–13] Although derivatives such as irinotecan have produced good clinical results,[14] irinotecan was shown to have far lower cytotoxicity to cancer cells than CPT (10%), and CPT remains the most potent compound.[15]

Among a variety of drug-delivery systems, mesoporous silica materials[16] have several attractive features for use in the delivery of water-insoluble drugs. These particles have large surface areas and porous interiors that can be used as reservoirs for storing hydrophobic drugs. The pore size and environment can be tailored to selectively store different molecules of interest,[17,18] while the size and shape of the particles can be tuned to maximize cellular uptake. Unlike polymer-based nanoparticles, these robust inorganic materials can tolerate many organic solvents.[19] Silica-based materials have been successfully used as drug-delivery vectors,[20,21] gene transfection reagents,[22] cell markers,[23] and carriers of molecules.[24] Here, we describe the preparation of fluorescent mesoporous silica nanoparticles that are highly stable in aqueous solution and their use for the delivery of the hydrophobic anticancer drug CPT.

The FMSNs were prepared by using a base-catalyzed sol–gel process at high temperature with a modification of published procedures.[23,25,26] In a typical synthesis, fluorescein isothiocyanate (FITC) was first treated with 3-aminopropyltriethoxysilane (APTS) in ethanol. The mixture was then added, along with tetraethylorthosilicate, to cetyltrimethylammonium bromide solution at 80 °C. The surfactants were removed from the pores by refluxing the nanoparticles in acidic methanol, the success of which was confirmed by Fourier transform infrared spectroscopy (FTIR; see Supporting Information). Electron microscopy and X-ray diffraction (XRD) analysis showed that the particle shape and hexagonal arrays of the pores in the FMSNs remained intact after the surfactant-removal process (Figure 1). The nanoparticles were roughly spherical in shape and smaller than 130 nm in diameter. An average pore diameter of around 2 nm was observed by using transmission electron microscopy (TEM) and an interplanar spacing of $d(100) \approx 4$ nm was calculated from the XRD pattern.

It is necessary for efficient cellular uptake of the particles that the FMSNs remain dispersed and do not aggregate in the buffer solution. The observed aggregation is caused by interparticle hydrogen-bonding interactions between the amine groups (from the unreacted APTS) and the silanols (Scheme 1A). By modifying only the surfaces of the FMSNs with trihydroxysilylpropyl methylphosphonate (THMP) after particle formation,[27] we reduced the aggregation and increased the stability of the particles in aqueous solution (Scheme 1B, see Supporting Information).

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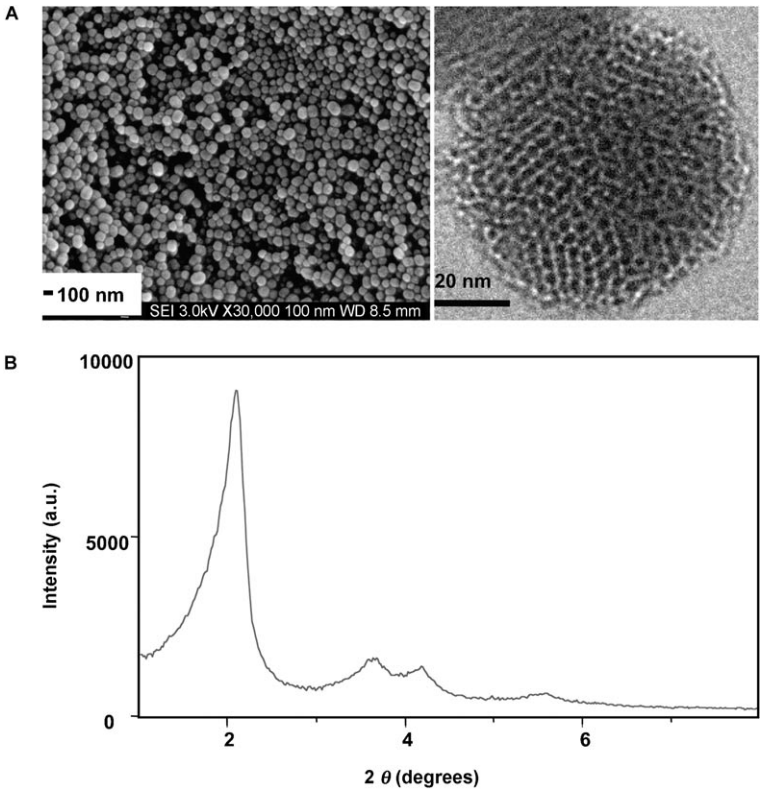
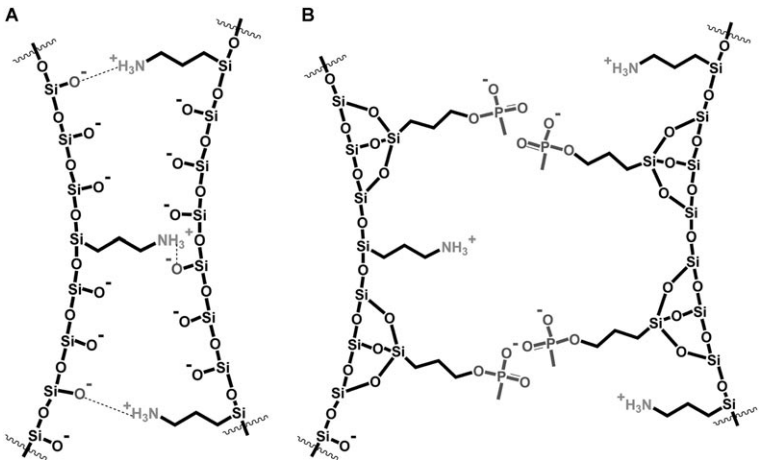


Figure 1. Characterization of the FMSNs. A) Scanning electron microscopy (left) and transmission electron microscopy (right) images of the FMSNs. B) The nanoparticles show the typical XRD patterns of MCM-41-type hexagonal mesoporous silica.



Scheme 1. A) Aggregation between the nanoparticles was caused by the interparticle hydrogen bonding between the surface silanol groups and the amine groups. B) Surface modification with THMP increased the electrostatic repulsion between the nanoparticles and decreased the aggregation.

The uptake of the nanoparticles by various cancer-cell lines was observed by using fluorescence and confocal microscopy. Cancer cells were incubated with FMSNs and then washed with phosphate-buffered saline (PBS) solution to remove the nanoparticles that were not taken up by cells. The emission of the nanoparticles, which were derivatized with fluorescein molecules, was monitored with fluorescence microscopy (Figure 2A). To further specify the intracellular location of the nanoparticles, Acridine Orange (AO) and lysoSensor green DND-189 were used to stain the cells that were incubated with the FMSNs. AO specifically stains acidic organelles such as lysosomes and endosomes red but stains other cellular regions green,[28] while lysoSensor specifically stains lysosomes green. The fluorescence of the nanoparticles overlapped mostly with the red fluorescence of AO (Figure 2B) and with the green fluorescence of lysoSensor (Figure 2C). This result suggests that the FMSNs were mainly taken up into the acidic organelles.

The nanoparticles were loaded with drug molecules by soaking them in a dimethylsulfoxide (DMSO) solution of CPT overnight. The size of the CPT molecule is approximately 1.3 nm × 0.6 nm, as determined by the program SPARTAN, and is small enough to fit into the pores of the FMSNs. After the organic solvent was removed by centrifugation and high vacuum, the loaded nanoparticles were sonicated and washed twice with PBS to ensure that any weakly adsorbed drugs on the surface were removed (Figure 3). The release profile in solution showed negligible release of CPT when the CPT-loaded nanoparticles were soaked in aqueous solution (see Supporting Information). Absorption measurements by UV/Vis spectroscopy determined that 50 mg of the FMSNs could store approximately 80 nmol of CPT.

A homogeneous suspension of the CPT-loaded FMSN in PBS was added to PANC-1 cells to determine if the nanoparticles were able to transport the hydrophobic CPT into the cancer cells. In a control experiment, a suspension of the same concentration of CPT in PBS was added to the cells. As CPT emits strong blue fluorescence under UV excitation and its excitation wavelength is different from that of the FMSNs, we used fluorescence microscopy to monitor

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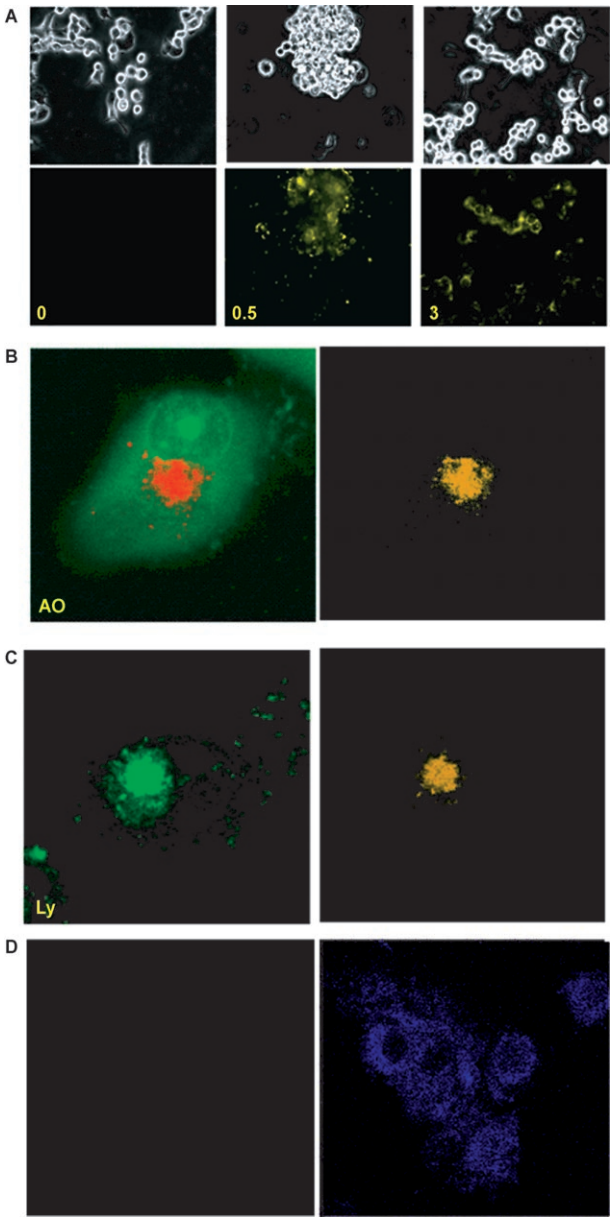

Figure 2. Uptake of the nanoparticles by cancer cells. A) Upper panels: normal microscopy images of PANC-1 cells; lower panels: fluorescence microscopy images after the indicated number of hours. Similar results were observed in other cell lines (data not shown). B) PANC-1 cells stained with Acridine Orange (AO, left) and the fluorescence of the nanoparticles within the same cell (right). C) PANC-1 cells stained with lysoSensor green DND-189 (left) and the fluorescence of the nanoparticles within the same cell (right). D) Fluorescence of CPT after the cells were incubated with CPT-loaded FMSNs (right) for 3 h. No fluorescence was observed within the cells that were incubated with a suspension of CPT in PBS (left).

the distribution of the CPT in the cancer cells. The cells that were treated with CPT-loaded FMSNs showed strong blue fluorescence (Figure 2D, right) after 3 h of incubation, while those that were treated with a suspension of CPT in PBS remained nonfluorescent (Figure 2D, left). This observation indicated that the FMSNs were able to transport and deliver CPT inside the cancer cells.

Delivery of CPT into the cancer cells led to growth inhibition and cell death. This cytotoxic effect of CPT-loaded FMSNs was tested on several cancer-cell lines. As shown in Figure 4, nonloaded FMSNs were not toxic to any of the cells tested, a result indicating good biocompatibility of the nanoparticles. However, growth inhibition of different human cancer cells was observed when the cells were treated with either the suspension of CPT-loaded FMSNs in PBS or a solution of CPT in DMSO. The survival of three pancreatic cancer-cell lines (PANC-1, Capan-1, and AsPc-1), a colon cancer-cell line (SW480), and a stomach cancer-cell line (MKN45; Figure 4) was decreased by CPT-loaded FMSNs. The cytotoxic efficacy of the CPT-loaded FMSNs was very similar to that of CPT dissolved in DMSO. By contrast, CPT suspended in PBS did not show any cytotoxicity to cancer cells, even at high concentrations. This is consistent with our observation that CPT suspended in PBS was not taken up by the cells due to its insolubility, while CPT loaded in FMSNs was quickly taken up (Figure 2D). Thus, the FMSNs effectively delivered the hydrophobic CPT into the cancer cells with minimal leakage into the buffer solution and culture medium.

To check whether the cell death induced by the CPT-loaded FMSNs was due to the cytotoxicity of the drug, the mechanism of cell death was investigated. After PANC-1 cells were incubated for 24 h with 1) CPT-loaded FMSNs suspended in PBS, 2) CPT dissolved in DMSO, or 3) nonloaded FMSNs suspended in PBS as the control, they were double stained with propidium iodide and Hoechst 33342.[29] Nuclear fragmentation and chromatin condensation were observed in most of the cells treated with CPT-loaded FMSNs (Figure 5A) and similarly in the cells treated with CPT in DMSO (Figure 5B), while cells treated with 10% DMSO, which induced necrosis, were stained red with propidium iodide (Figure 5D). These results indicate that the CPT-loaded FMSNs induced apoptotic cell death. Further confirmation of apoptosis came from the detection of DNA fragmentation (Figure 5E) and from the Western-blot result for cleaved caspase-3 in the cells treated with CPT-loaded FMSNs (Figure 5F).

In summary, we have successfully loaded hydrophobic anticancer drugs into mesoporous nanoparticles and delivered them into human cancer cells to induce apoptosis. A similar anticancer effect was observed for CPT in DMSO and CPT-loaded FMSNs in PBS, a result suggesting minimal drug leakage into the buffer solution and cell medium. CPT remained inside the nanoparticles and was released in the hydrophobic regions of the cell compartments to exert the apoptotic effect. At present, about 40% of small-molecule drugs in the pipeline of pharmaceutical companies have low water solubility and therefore cannot be administered by the preferred route or, in some cases, at all.[30] Our results with CPT suggest that the mesoporous silica nanoparticles may offer a solution to this problem in drug development. Mesoporous nanoparticles also offer the possibility of controlled release of anticancer drugs. The pores in the nanoparticles could be closed by constructing an appropriate cap structure. The ability to control the release of anticancer drugs provides mesoporous silica nanoparticles with advan-

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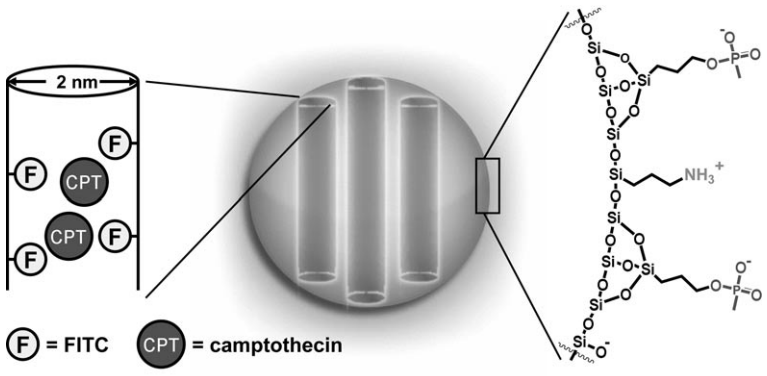

Figure 3. Schematic representation of the CPT-loaded FMSNs (≈130-nm diameter). The 2-nm-diameter pores (not drawn to scale) of the nanoparticles were derivatized with FITC and filled with CPT drug molecules, and the FMSNs surface was modified with THMP.

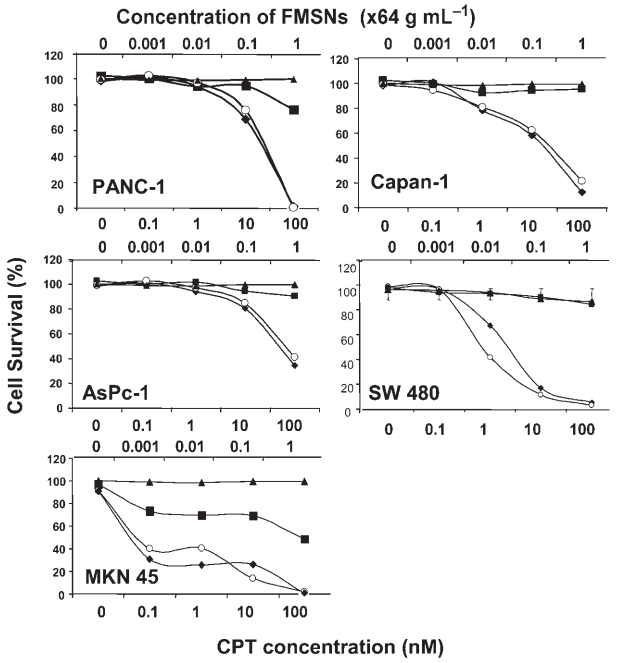

Figure 4. Cell-growth inhibition assay. ▲: nonloaded FMSNs in PBS; ■: CPT in PBS; ○: CPT in DMSO; ◆: CPT-loaded FMSNs in PBS. The concentration of FMSNs is shown on top of each figure, while the concentration of CPT in DMSO, in PBS, or loaded in FMSNs, is shown under each figure.

tages over other drug-delivery systems such as PEGylated liposomal particles or albumin-based nanoparticles. Further work is necessary to compare and contrast the different methods in order to improve the solubility of anticancer drugs with the goal of minimizing toxic effects on healthy tissues while maintaining antitumor efficacy.

Experimental Section

Synthesis of nanoparticles and loading of CPT: All chemicals for the synthesis of the nanoparticles were purchased from Sigma–Aldrich. The FMSNs were synthesized by first dissolving FITC (5.5 mg) in absolute ethanol (3 mL) and then adding APTS (12 μL). In another container, cetyltrimethylammonium bromide (CTAB; 0.5 g) was dissolved in a mixture of distilled water (240 mL) and 2 M sodium hydroxide (1.75 mL); the solution was heated to 80 °C and stirred vigorously. The solution of FITC–APTS was stirred under an inert atmosphere for 2 h before tet-

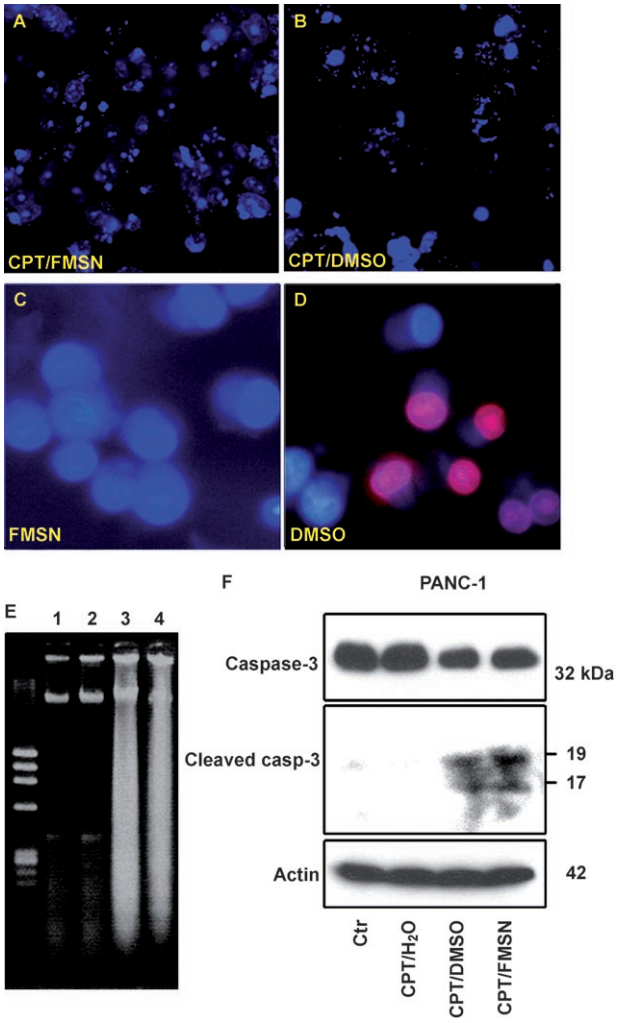

Figure 5. Apoptosis induced by CPT-loaded FMSNs. PANC-1 cells were incubated for 24 h with A) CPT-loaded FMSNs, B) CPT in DMSO, C) FMSNs, or D) 10% DMSO and were then stained with propidium iodide/Hoechst 33342. E) DNA fragmentation assay. PANC-1 cells were treated with 1) PBS, 2) CPT in PBS, 3) CPT in DMSO, or 4) CPT-loaded FMSNs in PBS. F) Western-blot result. Ctrl: control; CPT/H_2O: CPT in water; CPT/DMSO: CPT in DMSO; CPT/FMSNs: CPT-loaded FMSNs. The first antibodies used are caspase-3 and cleaved caspase-3.

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raethylorthosilicate (TEOS; 2.5 mL) was added. Once the temperature of the CTAB solution had stabilized, the ethanol solution containing TEOS and FITC–APTS was added. After 15 min, 3-trihydroxysilylpropyl methylphosphonate (0.63 mL) was slowly added to the mixture. After 2 h, the solution was cooled to room temperature and the particles were filtered and washed with methanol by using a fritted funnel. The particles were allowed to dry at room temperature overnight. To remove the surfactants from the pores of the particles, the particles (850 mg) were dissolved in a mixture of methanol (90 mL) and 12.1 M hydrochloric acid (5 mL) and the solution was refluxed for 24 h. The particles were then filtered and washed thoroughly to remove the surfactants and unbound FITC.

To load drug molecules into the pores of the particles, the FMSNs were soaked in a concentrated solution containing the drug. Typically, the FMSNs (50 mg) were stirred in a solution containing camptothecin (CPT; 5 mg) and DMSO (3 mL). After 24 h, the mixture was centrifuged and the supernatant was removed. By using UV/Vis spectroscopy, the absorption measurements of the original solution and the supernatant were compared to determine the amount of CPT that was loaded inside the FMSNs. The drug-loaded FMSNs were dried under vacuum to remove trace DMSO and then sonicated and washed twice with PBS (pH 7.4) solution to remove CPT that was adsorbed on the surface and not inside the pores.

Cell culture: Human cancer-cell lines PANC-1, AsPC-1, Capan-1 (pancreatic), MKN45 (gastric), and SW480 (colon) were obtained from the American Type Culture Collection and were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO) or RPMI-1640 medium (Cellgro) supplemented with 10% fetal calf serum (Sigma), 2% L-glutamine, 1% penicillin, and 1% streptomycin stock solutions. The media were changed every three days, and the cells were passaged by trypsinization before confluence.

Fluorescence and confocal microscopy: The fluorescence of the nanoparticles at an excitation wavelength of 470 nm was used to confirm the cellular uptake of the FMSN. PANC-1, Capan-1, and AsPc-1 cancer cells were incubated with FMSNs for various time periods and then washed with DMEM medium and PBS to wash off the nanoparticles that did not enter the cells. The cells were then monitored by fluorescence microscopy with an excitation wavelength of 470 nm. To examine the location of the FMSNs within the cells, Acridine Orange (AO; Sigma) and lysoSensor green DND-189 (Invitrogen) were used to monitor the lysosomes and endosomes. Cells were cultured overnight on a Lab-Tek chamber slide system (Nalge Nunc International). After the cells were incubated with FMSNs for 3 h, they were washed with PBS and examined with via confocal microscopy ($\lambda_{ex}=$ 470 nm; Carl Zeiss LSM 310 laser-scanning confocal microscope). The same cells were then incubated with 6 μM AO or 1 μM lysoSensor green DND-189 for 1 h in DMEM without phenol red, washed with PBS, and examined again by confocal microscopy ($\lambda_{ex}=488$ and 440 nm). The emission of the FMSNs was passed through a 560-nm short-pass dichroic mirror. The green emission was passed through a 530-nm band-pass filter and the red emission was passed through a 600-nm long-pass filter; the emissions were simultaneously collected by using two photomultiplier tubes. CPT exhibits intense blue fluorescence under UV light. This property allows the use of fluorescence to study the distribution of CPT inside the cells. PANC-1 cells were incubated with CPT-loaded FMSNs or CPT suspended in PBS (100 nM) for 3 h, washed three times with PBS, and then examined by fluorescence microscopy under UV light.

Cell death assay: The cytotoxicity assay was performed by using a cell-counting kit from Dojindo Molecular Technologies, Inc. Cancer cells were seeded in 96-well plates (5000 cells $well^{-1}$) and incubated in fresh culture medium at 37 °C in a 5% CO_2/95% air atmosphere for 24 h. The cells were then washed with PBS and the medium was changed to a fresh medium containing the CPT in DMSO, CPT-loaded FMSNs in PBS, FMSNs or CPT suspended in PBS at the indicated concentrations. After 24 h, the cells were washed with PBS to remove the CPT and FMSNs that were not taken up by the cells, and the cells were then incubated in fresh medium for an additional 48 h. The cells were washed with PBS and incubated in DMEM with 10% WST-8 solution for another 2 h. The absorbance of each well was measured at 450 nm with a plate reader. Since the absorbance is proportional to the number of viable cells in the medium, the viable cell number was determined by using a previously prepared calibration curve (Dojindo Co.).

Apoptosis assay: Cell death was also examined by using the propidium iodide and Hoechst 33342 double-staining method. The cells were stained with propidium iodide/Hoechst 33342 (1:1) for 5 min and then examined with fluorescence microscopy. A DNA fragmentation assay was also performed. PANC-1 cells were treated for 24 h with 1) 100 nM CPT in PBS, 2) 100 nM CPT loaded in FMSNs (suspended in PBS), or 3) 64 $\mu g\,mL^{-1}$ nonloaded FMSNs in PBS. The cells were then washed twice with ice-cold PBS and collected by trypsinization. The cell pellets were resuspended in tris(hydroxymethyl)aminomethane (Tris)/ethylenediaminetetraacetate (EDTA) buffer (500 μL; 20 mM Tris–HCl at pH 8.0, 20 mM EDTA) containing 0.1% sodium dodecylsulfate (SDS) and 0.5 $mg\,mL^{-1}$ proteinase K at 50 °C for 2 h and then treated with RNase A (0.02 $mg\,mL^{-1}$) for 30 min at 37 °C. DNA was extracted by using phenol/chloroform and ethanol precipitation and was dissolved in distilled water, separated on a 2% agarose gel, and stained with ethidium bromide.

Western blot analysis: Proteins were separated by gel electrophoresis on a polyacrylamide gel containing SDS and then transferred to nitrocellulose membranes. The membranes were blocked with Tris-buffered saline (TBS) containing 5% (w/v) skimmed milk. After being washed with TBS containing 0.1% Tween 20 (Sigma), the membranes were incubated overnight at room temperature with caspase-3 antibody (BD Science) and cleaved caspase-3 antibody (Cellsignaling) diluted with TBS. After being washed, the membranes were incubated for 2 h at room temperature with the second antibody (Santa Cruz Biotechnology, CA). Bands were detected with an ECL system (Amersham Pharmacia Biotech K.K., UK).

Statistical analysis: All results are expressed as mean values $\pm$ the standard deviation (SD). Statistical comparisons were made by using Student's t-test after analysis of variance. The results were considered to be significantly different at a P value < 0.05.

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[1] O. C. Farokhzad, J. M. Karp, R. Langer, *Expert Opin. Drug Delivery* **2006**, *3*, 311.
[2] A. G. Cuenca, H. Jiang, S. N. Hochwald, M. Delano, W. G. Cance, S. R. Grobmyer, *Cancer* **2006**, *107*, 459.
[3] F. M. Muggia, I. Dimery, S. G. Arbuck, *Ann. N.Y. Acad. Sci.* **1996**, *803*, 213.
[4] D. A. Litvak, H. T. Papaconstantinou, K. O. Hwang, M. Kim, B. M. Evers, C. M. Townsend, *Surgery* **1999**, *126*, 223.
[5] S. Takiguchi, T. Shimazoe, A. Kono, *Gan To Kagaku Ryoho* **1994**, *21*, 705.
[6] D. Abigerges, G. G. Chabot, J. P. Armand, P. Herait, A. Gouyette, D. Gandia, *J. Clin. Oncol.* **1995**, *13*, 210.
[7] T. E. Keane, R. E. El-Galley, C. Sun, J. A. Petros, D. Dillahey, A. Gomaa, S. D. Graham, Jr., W. P. McGuire, 3rd, *J. Urol.* **1998**, *160*, 252.
[8] K. D. Miller, S. E. Soule, L. G. Haney, P. Guiney, D. J. Murry, L. Lenaz, S. L. Sun, G. W. Sledge, *Invest. New Drugs* **2004**, *22*, 69.
[9] N. Masuda, M. Fukuoka, Y. Kusunoki, K. Matsui, N. Takifuji, S. Kudoh, S. Negoro, M. Nishioka, K. Nakagawa, M. Takada, *J. Clin. Oncol.* **1992**, *10*, 1225.
[10] R. P. Hertzberg, M. J. Caranfa, K. G. Holden, D. R. Jakas, G. Gallagher, M. R. Mattern, S. M. Mong, J. O. Bartus, R. K. Johnson, W. D. Kingsbury, *J. Med. Chem.* **1989**, *32*, 715.
[11] D. O. Scott, D. S. Bindra, V. J. Stella, *Pharm. Res.* **1993**, *10*, 1451.
[12] A. Guiotto, M. Canevari, P. Orsolini, O. Lavanchy, C. Deuschel, N. Kaneda, A. Kurita, T. Matsuzaki, T. Yaegashi, S. Sawada, F. M. Veronese, *J. Med. Chem.* **2004**, *47*, 1280.
[13] H. Onishi, Y. Machida, *Curr. Drug Discovery Technol.* **2005**, *2*, 169.
[14] C. Fuchs, E. P. Mitchell, P. M. Hoff, *Cancer Treat. Rev.* **2006**, *32*, 491.
[15] K. S. Cunha, M. L. Reguly, U. Graf, H. H. Rodrigues de Andrade, *Mutagenesis* **2002**, *17*, 141.
[16] C. T. Kresge, M. E. Leonowilz, W. J. Roth, J. C. Vartuli, J. S. Beck, *Nature* **1992**, *359*, 28.
[17] B. Munoz, A. Ramila, J. Perez-Pariente, I. Diaz, M. Vallet-Regi, *Chem. Mater.* **2003**, *15*, 500.
[18] Y. J. Han, G. D. Stucky, A. Butler, *J. Am. Chem. Soc.* **1999**, *121*, 9897.
[19] A. Stein, B. J. Melde, R. C. Schroden, *Adv. Mater.* **2000**, *12*, 1403.
[20] M. Arruebo, M. Galan, N. Navascues, C. Tellez, C. Marquina, M. R. Ibarra, J. Santamaria, *Chem. Mater.* **2006**, *18*, 1911.
[21] M. Arruebo, R. Fernandez-Pacheco, S. Irusta, J. Arbiol, M. R. Ibarra, J. Santamaria, *Nanotechnology* **2006**, *17*, 4057.
[22] D. R. Radu, C. Y. Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija, V. S. Y. Lin, *J. Am. Chem. Soc.* **2004**, *126*, 13216.
[23] Y. S. Lin, C. P. Tsai, H. Y. Huang, C. T. Kuo, Y. Hung, D. M. Huang, Y. C. Chen, C. Y. Mou, *Chem. Mater.* **2005**, *17*, 4570.
[24] C. Y. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija, V. S. Y. Lin, *J. Am. Chem. Soc.* **2003**, *125*, 4451.
[25] S. Huh, J. W. Wiench, J. C. Yoo, M. Pruski, V. S. Y. Lin, *Chem. Mater.* **2003**, *15*, 4247.
[26] I. Slowing, B. G. Trewyn, V. S. Y. Lin, *J. Am. Chem. Soc.* **2006**, *128*, 14792.
[27] R. P. Bagwe, L. R. Hilliard, W. Tan, *Langmuir* **2006**, *22*, 4357.
[28] P. G. Canonico, J. W. C. Bird, *J. Cell Biol.* **1969**, *43*, 367.
[29] J. Hasegawa, S. Kamada, W. Kamiike, S. Shimizu, T. Imazu, H. Matsuda, Y. Tsujimoto, *Cancer Res.* **1996**, *56*, 1713.
[30] V. Wagner, A. Dullaart, A. K. Bock, A. Zweck, *Nat. Biotechnol.* **2006**, *24*, 1211.

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