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Supramolecular Chemistry

Photoresponsive Cyclodextrin-Covered Nanocontainers and Their Sol-Gel Transition Induced by Molecular Recognition**

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Stimuli-responsive nanomaterials have attracted much attention as controlled nanodevices, actuators, and biomedical materials because of their ability to change their physical or chemical properties in response to external triggers.[1–3] In particular, nanocontainers with stimuli-responsive functions can provide unique benefits for precise, controlled release under specific conditions.[4] However, although traditional nanocarriers such as liposomes and polymer micelles have suffered from their instability, mesoporous silica particles (Si-MPs) provide a rigid framework with a porous reservoir that can encapsulate a large amount of guest molecules. Therefore, the use of Si-MPs that have a gate function for controlled release can provide a unique opportunity for biomedical applications.[5] To date, several types of supramolecular nanovalve systems for the controlled release of guests under suitable conditions have been reported to act as stimuli-responsive nanocontainers.[6] For this purpose, the surfaces of nanocontainers have been tethered with supramolecular functional motifs that are switchable by various stimuli, for example, redox and pH changes, enzymes, and heat, which can open and close the pore on demand.[6] Several research groups have reported mesoporous silica systems with photoresponsive gate functions.[6a,7] In addition, Si-MPs capped with "gatekeepers" such as nanoparticles, polymers, and dendrimers also exhibited controlled release characteristics in response to stimuli.[5b,8]

Herein, we describe a novel cyclodextrin(CD)-covered nanocontainer system that can exhibit photoresponsive release characteristics and contains a photocleavable linker. In addition to the photocontrolled release properties, we report the formation of the nanocontainer hydrogel that is induced by molecular recognition. The CD-covered nanocontainer system consists of the Si-MPs as a reservoir for guest molecules, an *o*-nitrobenzyl ester moiety as a photocleavable linker, and the CD "gatekeeper" that can close the gate of the pore of Si-MPs (Figure 1).

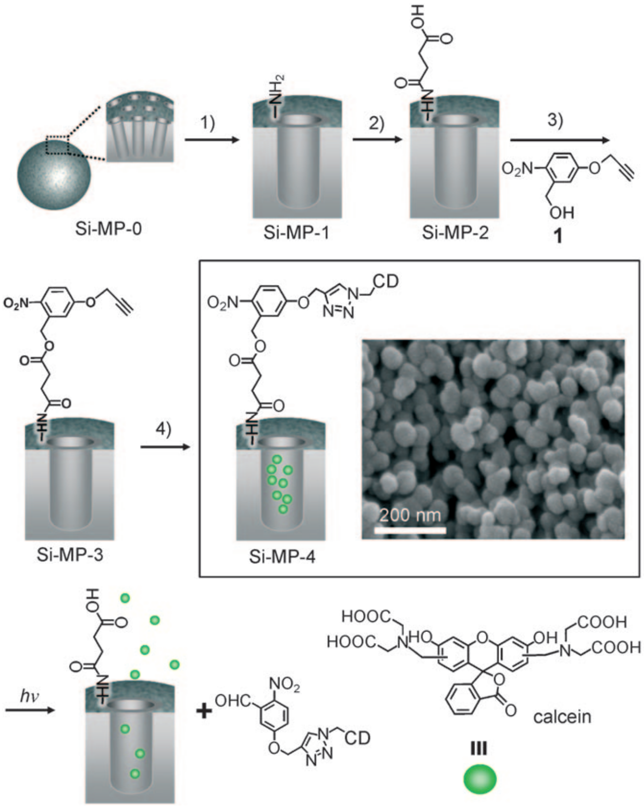

Figure 1. Schematic illustration of the synthesis of cyclodextrin-covered nanocontainers (Si-MP-4) and the photocontrolled release of guest molecules from the pores. The pores of Si-MP-0, Si-MP-1, and Si-MP-2 are filled with CTAB. The inset shows FE-SEM images of Si-MP-4. Conditions: 1) 3-aminopropyltriethoxysilane; 2) succinic anhydride and triethylamine; 3) removal of CTAB, *N*,*N′*-diisopropylcarbodiimide and 4-(dimethylamino)pyridine; 4) calcein, $CuSO_4$, sodium ascorbate, and mono-6-azido-β-CD.

We prepared Si-MP-0, which has an approximate diameter of 60 nm (pore diameter ≈2 nm), as a nanocontainer for guest molecules (Figure 1).[9] The average pore diameter was measured by using powder X-ray diffraction (PXRD) and Barret–Joyner–Halenda analysis. The structure of the Si-MPs was further confirmed by using transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM; see Figure S1 in the Supporting Information). The TEM and PXRD analyses revealed that the Si-MPs exhibit a well-ordered porous structure with a hexagonal arrangement (Figure S1 in the Supporting Information). The FE-SEM analysis showed that the Si-MPs are not only uniform in size but also possess spherical shapes (Figure S1b in the Supporting Information). The surface functionalization of Si-MPs was monitored by FTIR spectroscopy (Figure S2a in the Supporting Information).[9]

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The surface of Si-MP-0 was functionalized with amine groups by treatment with 3-aminopropyltriethoxysilane to obtain Si-MP-1. The carboxylic acid terminated Si-MP (Si-MP-2) was obtained by the reaction of Si-MP-1 with succinic anhydride in the presence of triethylamine (Figure 1). The surfactant template (*n*-cetyltrimethylammonium bromide, CTAB) was removed from Si-MP-2 by heating the methanol solution of Si-MP-2 (MeOH/HCl 100:1) at reflux for 24 hours.[10] The FTIR spectrum of Si-MP-2 exhibits absorption bands in the regions of amide I (1635 cm^{-1}), amide II (1560 cm^{-1}), and at 1720 cm^{-1}, which arise from the carbonyl stretching modes.[9] Si-MP-3 was prepared by coupling Si-MP-2 with **1**.[9] The product was purified by centrifugation and washing with DMF. The FTIR spectrum of Si-MP-3 showed sharp bands at 2100 cm^{-1} and 3296 cm^{-1} respectively, which arise from the alkyne $C{\equiv}C$ and C–H stretching modes.[9]

Calcein guest molecules were loaded into the pores of silica particles by soaking Si-MP-3 in a solution of calcein in phosphate buffered saline (PBS, pH 7.4). Mono-6-azido-β-CD was then added into the mixture to produce calcein-loaded Si-MP-4, in which the pores were capped by cyclodextrins, by the Huisgen 1,3-dipolar cycloaddition (Figure 1).[9,11] The excess calcein and catalyst were removed by filtration, centrifugation, and washing with PBS buffer solution (pH 7.4). After purification, the resulting particles were resuspended in PBS at pH 7.4. The absorption spectrum of Si-MP-4 showed an absorption of calcein at 504 nm, which indicated that calcein molecules were loaded in the Si-MP-4 pores (Figure 2a). The self-quenching induced by calcein aggregation within the pores of Si-MP-4 results in only a weak fluorescence signal of calcein being observed. The FE-SEM analysis of Si-MP-4 revealed that the particles were spherical, with an average diameter of 60 nm (Figure 1). The loading of calcein in Si-MP-4 was visualized by confocal laser scanning microscopy (Figure S2 in the Supporting Information).

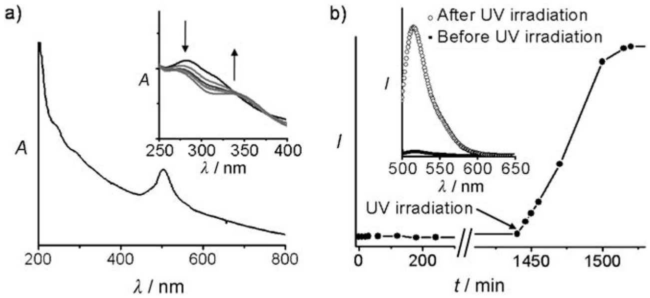

Figure 2. a) Absorption spectrum of Si-MP-4 in PBS (the inset shows absorption spectra of Si-MP-4 in response to UV irradiation). b) Time-dependent change in fluorescence intensity (at $\lambda_{em} = 515$ nm) of Si-MP-4 in PBS ($\lambda_{ex} = 490$ nm). The inset shows fluorescence spectra of Si-MP-4 before and after UV irradiation.

Upon exposure of the Si-MP-4 solution to UV light ($\lambda = 350$ nm), the absorption signal at 282 nm decreased and the absorption signal at 344 nm increased as a result of photocleavage of the *o*-nitrobenzyl moiety (Figure 1 and 2a). We subsequently investigated the photoresponsive release characteristics of Si-MP-4. The release of calcein guest molecules from the pores of Si-MP-4 was monitored by fluorescence measurements. The fluorescence intensity of the solution of Si-MP-4 at 515 nm was monitored over time (Figure 2). In the dark, only a very weak fluorescence intensity was observed, because of self-quenching of calcein molecules in the pores of Si-MP-4 (Figure 2 inset). As shown in Figure 2b, the calcein molecules entrapped in the pores of Si-MP-4 were not released in the dark because the pore gate was closed by the CD moiety on the particle surface. When Si-MP-4 was exposed to UV light after 24 hrs in the dark, we observed a remarkable increase in the fluorescence intensity, which suggests that the entrapped calcein molecule was released from the pore of Si-MP-4 because the photolysis of *o*-nitrobenzyl ester moiety on the surface of Si-MP-4 triggers the opening of the pore gate of Si-MP-4 (Figure 2a,b). We also observed periodic release of calcein molecules from Si-MP-4 in response to successive UV irradiation over short time periods (Figure S3 in the Supporting Information).

Si-MP-4 can be utilized as a building block for the formation of photoresponsive physical hydrogels because the surface of Si-MP-4 is covered with CDs, which provide the inclusion sites for hydrophobic molecules. In particular, the host–guest interaction between CDs and the polymers that bear alkyl units can be exploited for noncovalent network formation.[12] For this purpose, six-arm poly(ethylene glycol) with dodecyl end groups (6-PEG-C12) that can be included into the hydrophobic cavity of α-CD or β-CD, was prepared by the coupling of six-arm PEG-succinimidyl succinate with dodecylamine (Figure 3a).[9] Upon addition of Si-MP-4 into an aqueous solution of 6-PEG-C12, the mixture formed a hydrogel (Si-MP-4-gel) as a result of the network formation of the inclusion complex between β-CD of Si-MP-4 and the dodecyl group of 6-PEG-C12 (Figure 3b). The Si-MP-4-gel composed of Si-MP-4 and 6-PEG-C12 changed to a sol upon addition of α-CD as a competitive host in which the dodecyl moiety is preferentially included to remove 6-PEG-C12 from the β-CD moiety of Si-MP-4-gel (Figure 3b). The fluorescence intensity of Si-MP-4-sol was not higher than that of Si-MP-4-gel, which suggested that the calcein molecule was not released from the hydrogel system (Figure 4). However, after UV irradiation ($\lambda = 350$ nm) of Si-MP-4-sol, the fluorescence intensity of Si-MP-4-sol-UV increased (Figure 4), which indicated that the calcein molecules entrapped in the pores were released (Figure 3b).

In summary, we have demonstrated that the introduction of a photocleavable linker and a CD “gatekeeper” by click chemistry on the surface of Si-MPs provided a unique route to mesoporous particles, which are capable of releasing guest molecules from the CD-blocked pore under photocontrol. Upon exposure to UV light, the guest molecules were released from the pore because of removal of the CD “gatekeeper”, which was linked on the surface of Si-MP-4 through a photocleavable *o*-nitrobenzyl ester moiety. Furthermore, Si-MP-4 could form a hydrogel through the network formation of the β-CD/6-PEG-C12 inclusion complex. The sol–gel transition and the guest-release characteristics of Si-MP-4-gel were controlled by the combination of the competitive host–guest interaction and photostimulus. We expect that the Si-MPs system described here can be used as a

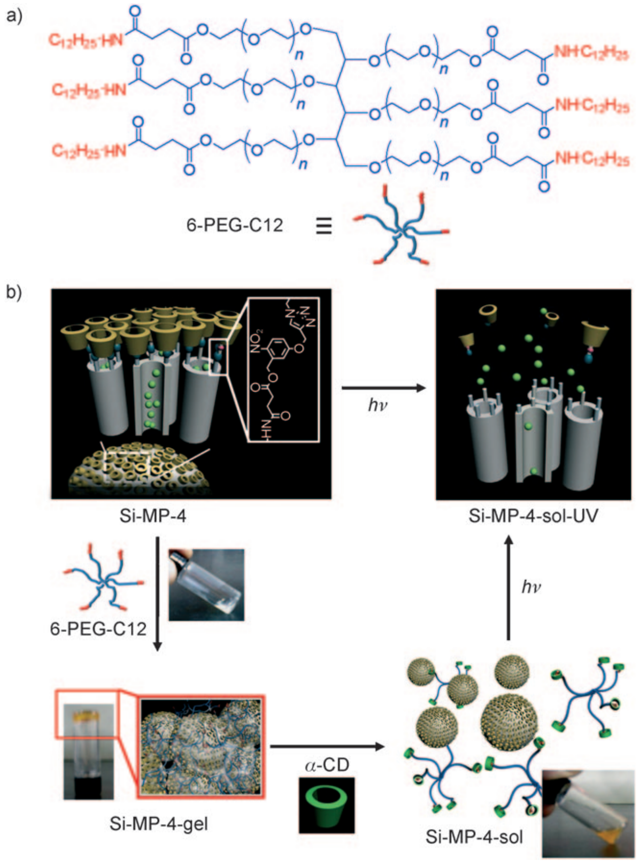

Figure 3. a) Chemical structure of 6-PEG-C12. b) Schematic illustration of the photoresponsive release of guest molecules from the pores of Si-MP-4 and sol–gel transitions induced by molecular recognition.

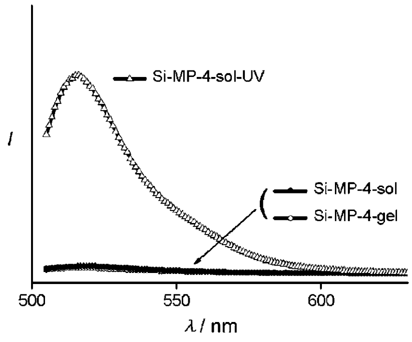

Figure 4. Fluorescence spectra of Si-MP-4-gel, Si-MP-4-sol, and Si-MP-4-sol-UV 2 h after irradiation.

versatile multifunctional photoresponsive nanocontainer that can provide a unique opportunity for sustained and localized "on demand" release in therapeutic systems.

Experimental Section

Loading experiments: Si-MP-3 (20 mg) and calcein (18 mg) were suspended in a mixture of PBS solution (7 mL, pH 7.4) and DMF (2 mL). After stirring for 24 h at room temperature, mono-6-azido-β-CD (23 mg) was then added, followed by $CuSO_4 \cdot 5H_2O$ (20 mg, 80 μmol) and sodium ascorbate (31 mg, 156 μmol). The mixture was stirred at room temperature for 3 days. The resulting particles were then filtered and purified by centrifugation (9500 rpm, 20 min) and washing with H_2O/MeOH.

Release experiments: Si-MP-4 was placed on the bottom of a quartz cuvette which was then filled with PBS (pH 7.4). The photocleavage was induced by irradiation with UV light ($\lambda = 350$ nm), which was then turned off. The fluorescence intensity of a solution of Si-MP-4 at 515 nm was measured as a function of irradiation time.

Fluorescence measurements: All fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer. The emission and excitation slit widths were set at 3 nm with $\lambda_{ex} = 490$ nm.

PXRD experiment: PXRD patterns were recorded at room temperature on a Rigaku model RINT-2000 counter diffractometer with a $Cu_{K\alpha}$ radiation source (Operated at 40 kV, 40 mA).

BET: The pore size was measured at 77 K on a Quantachrome instrument.

TEM analysis: TEM images were obtained using a Philips CM 200 instrument operated at an acceleration voltage of 120 kV. For the preparation of dispersed samples in water, a drop of sample solution (100 $mg\,L^{-1}$) was placed onto a 300-mesh copper grid coated with carbon. About 2 min after deposition, the grid was touched with filter paper to remove surface water. The samples were air dried before measurement.

SEM analysis: Field emission scanning electron microscopy (FE-SEM, Hitachi S-4200) was carried out on a field emission gun FE-SEM instrument (accelerating voltage: 10–15 kV and pressure range: 0.8–0.9 Torr). The FE-SEM samples were prepared by transferring a drop of sample solution onto a 200 mesh carbon-coated copper grid or a silicon wafer. About 5–10 min after deposition, excess water was removed by touching the edge of the substrate with filter paper. The samples were air dried before measurement.

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