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Mesoporous silicate materials as substrates for molecular machines and drug delivery

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Abstract

Mesoporous silica thin films and nanoparticles prepared by surfactant-templated sol–gel techniques are versatile substrates that can be easily derivatized with active molecules to create functional materials. By exploiting the chemical and physical differences that exist in different regions of the mesostructure, active molecules can be deliberately placed using one-pot techniques, or they can be tethered to the exposed surfaces post-synthetically. The methods available for functionalization have been used to design operational machines including nanoimpellers based on the dynamic photoisomerization of azobenzene, and nanovalves based on the switchable motion of supramolecular rotaxanes and pseudorotaxanes. The ability of nanoimpellers and nanovalves to control the release of molecules from the pores of mesoporous silica materials is demonstrated using luminescence spectroscopy. These machines can be designed to operate under a range of external stimuli, including light, electrical (redox) or chemical (pH, competitive binding) energy, making them useful systems for a variety of controlled release applications. Mesoporous silica nanoparticles not functionalized with molecular machines are capable of delivering water-insoluble anticancer drugs to cancer cells. Carefully designed nanoimpellers and nanovalves supported on mesoporous silica nanoparticles offer the ability to develop sophisticated drug delivery vehicles for a wide range of drug molecules.

Keywords: Mesoporous silicates; Functional materials; Molecular machines; Drug delivery

1. Introduction

The sol–gel process is a bench-top technique that is widely used for synthesis of inorganic glasses [1]. The process involves the use of metal alkoxides, which undergo hydrolysis and condensation at room temperature, to process a material into various forms including thin films, nanoparticles, or monoliths. We focus herein on the sol–gel processing of tetraethylorthosilane (TEOS) to produce silica thin films and nanoparticles. The as-synthesized glass contains an amorphous network of micropores that is interconnected throughout the silica matrix, enabling small molecules to diffuse in and out of the material [2]. Mesostructured silica was first reported in 1992 [3] and is achieved through the use of templating surfactants; the surfactants are removed when the sol–gel process is complete to achieve mesoporous silica materials.

Because the sol–gel process is sufficiently benign, molecules encapsulated in a sol–gel matrix retain the same characteristic properties as they exhibit in solution and the fabrication of doped sol–gel materials has become an active field of interest. The first biological applications of silica sol–gel materials involved encapsulation of large biomolecules such as enzymes and other proteins [4,5]. Despite being trapped in the silica matrix, biological molecules [4–12] and even cells [13] retain their catalytic, recognition, and transduction functions making these doped materials ideal for a broad range of biosensing applications. Due to the microporosity of the silica matrix, encapsulated biomolecules are accessible to their outside environments and the response of the trapped molecules to various external analytes can be monitored [2].

Another useful characteristic of sol–gel silicates derives from the mesoporosity that is generated when templating agents are used. In this review article, we focus on the use of mesoporous silica thin films and particles as supports for the formation of functional materials designed especially for drug delivery applications. We begin with a brief discussion of the synthe-

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sis of mesostructured silica nanoparticles and thin films, and provide a description of the spatially separated, chemically different regions that exist within the as-synthesized materials. Various strategies that are used to place active molecules into specific regions of the mesostructure are described, leading to the development of operational molecular machines. Active molecules that undergo large-amplitude motions are attached to the surface of mesostructured silicates, and the motion of these machines is investigated spectroscopically. Two types of machines are discussed: nanoimpellers and nanovalves, both of which are designed as mechanisms for controlling the release of guest molecules trapped in the pores of mesostructured silicates. Mesoporous silica nanoparticles are promising vehicles for drug delivery applications because they are not cytotoxic, they are taken up by cancer cells, and they provide a versatile means of carrying a wide variety of drugs (including water-insoluble drugs) into cells. We discuss the ability of unmodified mesoporous silica nanoparticles to traffic water-insoluble molecules into cells, and describe our work aimed at designing active silica nanoparticles that can act as sophisticated drug delivery vehicles capable of releasing molecules on demand.

2. Synthesis of mesostructured silicate materials

2.1. Silica particles

Mesoporous silicate particles have been a focus of great interest due to the materials' large surface areas and the ease in modifying both the particle and the pore size [14]. Various templating mechanisms and pore swelling agents have successfully been used on these silica materials to give a wide range of well-defined pore sizes and mesostructures. The capability of introducing organic functionalities on the pore walls and surfaces makes the mesoporous silica suitable as host materials to incorporate guest molecules [15,16]. Although the original research and applications of these host–guest materials were primarily geared towards catalysis purposes, recent progress in synthesizing mesoporous nanoparticles has developed new potential in the biomedical field. The particle sizes of the mesoporous materials are varied depending on the synthesis conditions, and the pore sizes are determined by the templating surfactant that is used [17,18]. Syntheses that use base to catalyze the silica condensation and small cationic templating surfactants yield mesoporous silica nanoparticles less than 300 nm in diameter with ∼2 nm pores (Fig. 1). These materials are suitable for biomedical applications and have been successfully used as gene transfection reagents, cell markers, and carriers of molecules [19–22].

2.2. Thin films

Highly ordered mesostructured thin films are prepared from a solution at room temperature through a process called 'evaporation-induced self-assembly' (EISA) [23,24]. This process uses a one-pot sol comprised of a silica precursor and a templating surfactant in an ethanol/water solvent. A glass or silicon substrate is dipped into the solution and a thin film of liquid is pulled with the substrate as it is retracted. Preferential evaporation of the ethanol during film deposition drives formation of surfactant micelles, which further assemble into a liquid crystalline mesophase. Condensation of the silica monomers around the surfactant micelles begins, and as this process continues a surfactant templated, mesostructured silica film is eventually obtained. It is possible to template different final mesostructures including 2D hexagonal which is formed when cetyl trimethyl ammonium bromide (CTAB) surfactants are used (Fig. 2). The final film is 100–200 nm thick. Because of the continuous nature of the dip coating process, the lengths and widths of the materials are limited only by the size of the substrate used. While thin films are impractical materials for drug delivery applications, their macroscopic size enables easy handling and makes them convenient substrates for fundamental experimentation. We have established new techniques for functionalization and performed preliminary studies on molecular machines using thin films as the silica supports. The basic knowledge that has been developed has been very useful in developing new, biologically relevant systems that use mesoporous silica nanoparticles as supports.

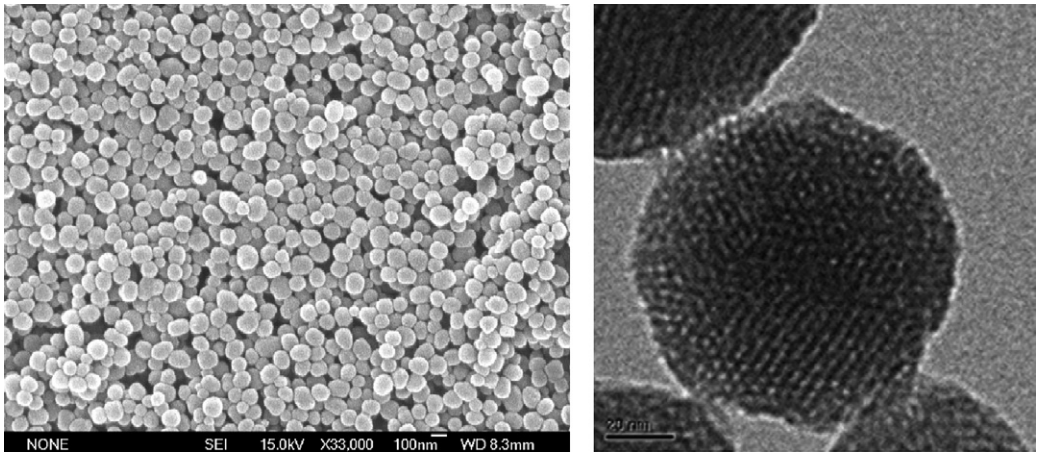

Fig. 1. Scanning electron micrograph (left) and transmission electron micrograph (right) images of the mesoporous silica nanoparticles.

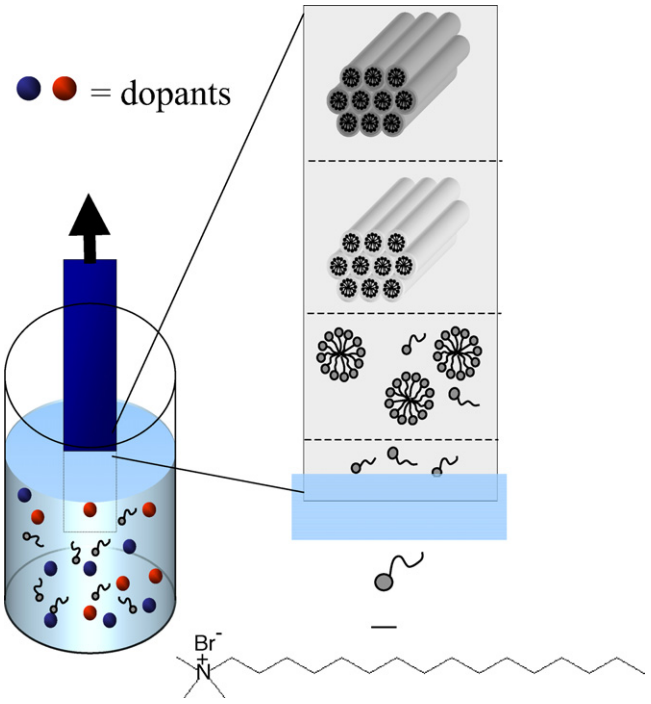

Fig. 2. Evaporation-induced self-assembly during dip coating to form a 2D hexagonal mesostructure. A substrate is dipped into a sol comprised of a CTAB templating surfactant, silica precursor, and dopant molecules in an ethanol/water solvent. As the substrate is pulled out, evaporation of the ethanol drives the formation of surfactant micelles, which further arrange themselves into a liquid crystalline mesophase. Condensation of the silica around the surfactant mesophase locks the structure into place. Various dopant molecules can be incorporated in the EISA process; the chemical properties of the dopant molecules determine their location in the final mesostructure.

3. Designed placement of active molecules in mesostructured materials

Optical or mechanical activities are achieved in mesostructured silica thin films and particles by incorporating active molecules with desired functionalities into the material. In general, the structure that is obtained has chemically and physically different regions in which functional molecules can be placed selectively. Spatial separation of different active molecules can be efficiently achieved using one-pot methods for functionalization. In one-pot functionalization, material synthesis and functionalization are achieved concurrently: active molecules are added directly to the sol solution and arrange themselves within the developing mesostructure as the material forms. Alternatively, post-synthetic approaches to functionalization are useful for derivatizing the surfaces of thin films and particles. The details and utility of the different designed placement strategies are discussed in upcoming sections.

3.1. Structural regions for separating active molecules

On the molecular level, the structures that are formed have three distinct regions that are important in designing functional materials by one-pot methods [25–27]. The regions are shown in Fig. 3. The silica network that holds the material together is termed the *framework*. The thickness of the framework can

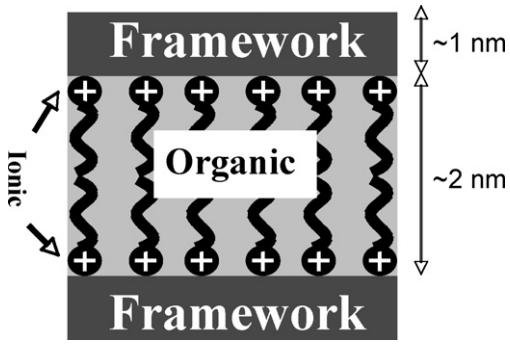

Fig. 3. The spatially separated regions of mesostructured sol–gel films templated by ionic surfactants.

be controlled by both the sol–gel chemistry and/or the speed of the dip coating process. The region containing the hydrophobic long-chain hydrocarbon tails of the surfactant is called the *organic* region. For 2D hexagonal mesostructures, the organic region is a cylindrical tube and the dimensions are controlled by the length of the hydrocarbon tail of the surfactant used in the preparation. In CTAB-templated materials, the organic region is about 2 nm in diameter. The third region that contains the ionic head group of the surfactant and the counter ions is called the *ionic* region. It is a few Angstroms thick and forms the interface between the framework and the organic region. The thickness of the ionic region cannot be readily controlled, but the composition can be modified by the choice of surfactant and the salts that are present in the initial sol.

3.2. One-pot strategies for placing active molecules in desired regions

Spatial separation of active molecules in a mesostructured material is often accomplished by including the dopants in the initial sol, and allowing them to self-assemble into a particular region during material synthesis. We have used three one-pot synthetic strategies for placing the active molecules in the different regions of mesostructured materials, and each of them will be discussed individually below.

3.2.1. Philicity strategy: physical immobilization based on differences in solubility

Physical immobilization of molecules in a particular region of the mesostructure is the most straightforward one-pot functionalization strategy. The deliberate placement utilizes the site 'philicity' (or local solubility) of the molecules. Hydrophobic molecules will reside in the organic region of the final material, ionic molecules in the ionic region, and neutral polar molecules in the framework. The appropriate molecules are simply included in the initial sol and self-assemble into a desired region during material synthesis. The most common use of this strategy involves hydrophobic molecules which are incorporated inside the micelles among the hydrocarbon tails such that they ultimately reside in the organic region of the final structure. The utility of this placement strategy is demonstrated by our work involving pyrene dopants to probe micelle formation [28,29],

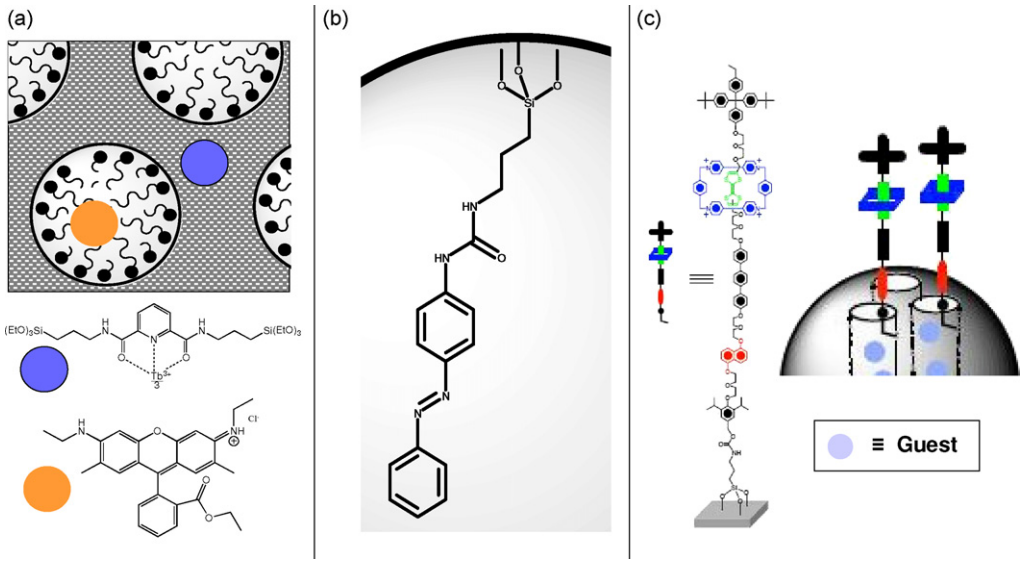

Fig. 4. Functionalized sol–gel materials. Active molecules are placed into specific regions of the material using the placement strategies described in the text. (a) Spatial separation of an electron donor and acceptor pair is achieved by placing a Terbium complex (blue) in the silica framework using the bonding strategy and a Rhodamine 6G acceptor (orange) in the organic region using the philicity strategy. (b) An azobenzene derivative capable undergoing a large-amplitude isomerization upon absorption of light is attached to the interior of a pore wall using the co-condensation strategy. (c) A supramolecular nanovalve is attached near a pore orifice using the post-synthesis grafting strategy. The nanovalve can control the release of luminescent guest molecules, which are introduced into the pores by backfilling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and also immobilization of Rhodamine 6G molecules as acceptors to study energy transfer [30] as will be discussed in the next section.

3.2.2. *Bonding strategy: active molecules as framework structural materials*

The approach taken to deliberately place an active molecule in the framework region of the material is to make the molecule itself a building block that is covalently bonded within the silica framework [26,27,30]. The active molecules are precisely positioned in the final mesostructure because they are directly incorporated into the inorganic framework of the material. This strategy requires that the molecule must itself undergo hydrolysis and condensation in order to incorporate into the framework and it also must allow templating by the micelles to occur before the final framework is formed.

The utility of the bonding strategy was demonstrated in an energy transfer study in which a 2D hexagonal thin film templated by CTAB was simultaneously doped with Rhodamine 6G acceptor molecules and a Terbium donor complex (Fig. 4a) [30]. Luminescence spectroscopy showed that R6G was incorporated in the surfactant micelles and that the Tb complex, derivatized with an alkoxysilylated ligand, was incorporated in the silicate framework. Steady-state luminescence spectra, lifetime measurements of R6G luminescence and lifetime measurements on the Tb complex's emission provided evidence of energy transfer. The Tb luminescence lifetime measurements were used to calculate the distance between Tb and R6G in mesostructured films according to the Förster model. This study demonstrated that the philicity and bonding strategies could be used to spatially separate donor and acceptor molecules based on self-assembly.

3.2.3. *Bifunctional strategy: chemical bonding at the framework–ionic interface*

The ideal strategy for placing an active molecule at the interface between the framework and the ionic region is to chemically bond it to the outside of the framework. This type of functionalization can be achieved using bifunctional active molecules: molecules with one end that is a trialkoxysilane and the other end that is hydrophobic [26,27,30]. The bifunctional strategy is highly versatile, as it is relatively easy to derivatize one end of an active molecule with alkoxysilane moieties capable of chemically bonding with the silica through the use of silane linkers. The length of the silane linker will determine the distance between the framework wall and the active part of the molecule. The utility of the bifunctional strategy is demonstrated by our work in which the pore interiors of mesostructured materials are functionalized with azobenzene-based molecular machines [31,32]. These machines undergo a large-amplitude wagging motion upon light excitation, and can act as nanoimpellers able to impel unbound guest molecules out of the nanopores (Fig. 4b) [31].

3.3. *Post-synthesis strategies for functionalization*

Other approaches to silica functionalization that occur post-synthetically are widely useful, and are discussed in this section. They are frequently necessary if calcination of the materials to remove surfactant is required.

3.3.1. *Post-synthetic grafting strategy*

There are cases when it is necessary to use a two-step process for material functionalization: synthesis of an unmodified mesostructured silicate followed by its derivatization [16,33,34].

In general, post-synthesis grafting is useful for functionalizing mesoporous silicates with active molecules that are incompatible with the chemistry of the initial sol. Additionally, it is often desirable to functionalize accessible silica surfaces, such as the surface of nanoparticles or areas near pore orifices. Post-synthesis grafting involves the same types of silane linkers that are commonly utilized in the bifunctional strategy. The organic functionality that is introduced upon modification with a silane linker is typically used for further derivatizations. For example, we have used alkoxysilane linkers attached post-synthetically to the surface of mesoporous silicates to position the rotaxanes and pseudorotaxanes that function as nanovalves (Fig. 4c) [35–39].

3.3.2. Backfilling strategy

Backfilling is a simple strategy for introducing active molecules into the empty pores of mesostructured silica. Empty mesopores are filled by exposing a material to a solution or vapor containing the active molecules and allowing them to diffuse in. Backfilling does not result in chemically modified materials, but is nonetheless widely useful. For example, use of luminescent guest molecules introduced to a material by backfilling is a critical way to probe the performance of nanovalves and other molecular machines [35–40]. Also, drug molecules have been loaded into mesoporous silica nanoparticles and the ability of the particles to carry the drugs into cells has been demonstrated [22].

4. Molecular machines

When molecules that undergo large-amplitude molecular motions are attached to mesostructured silicate thin films and particles, molecular machines capable of doing useful work can be designed. In this section, two different types of machines, nanoimpellers based on the dynamic *trans–cis* photoisomerization of azobenzene, and nanovalves based on the switchable properties of supramolecular rotaxanes and pseudorotaxanes will be discussed. Both types of molecular machines are useful for controlling the release of molecules trapped in the pores of mesostructured silicate materials.

4.1. Photo-driven nanoimpellers

Azobenzene-functionalized mesoporous inorganic materials have been of great interest due to the photo-responsive behaviors of the azobenzene moieties. Our earlier photophysical studies showed that *cis*/*trans* conformers of azobenzene derivatives can be switched at specific wavelengths of light when they are attached in the pores of the mesostructured silica frameworks [32]. Also, it has been demonstrated that the pore sizes of mesoporous silica materials can be varied by azobenzene derivatives existing in the *trans* or *cis* conformation [41–43]. Based on those merits of azobenzene functionalities, we recently developed photoactive nanoimpellers for the controlled movement of molecules through and out of the pores [31]. The controlled transport can occur through ~2 nm diameter pores of ~400 nm sized MCM-41-type silica nanoparticles.

The bifunctional strategy was used to attach azobenzene derivatives onto the inner pore walls of silica particles. This method involved the coupling reaction of 4-phenylazoaniline (4-PAA) with isocyanatopropylethoxysilane (ICPES) followed by co-condensation with tetraethylorthosilicate (TEOS) [41–44]. The templating surfactant was solvent extracted by refluxing the as-synthesized particles in acidic methanol. In the final form, the particles contained azobenzene derivatives with one end bonded to the inner pore walls and the other end free to undergo photoisomerization (Fig. 5).

Photo-controlled release of molecules was monitored by luminescence spectroscopy of the probe molecule Coumarin540A. The fluorescence intensity of the probe released into solution was recorded as a function of time (Fig. 6, top). No probe molecules were released from unexcited particles. When the particles were illuminated with 457 nm light, a wavelength where both *cis* and *trans* conformers absorb, the dyes were expelled from the pores and the increase of fluorescence in solution was recorded.

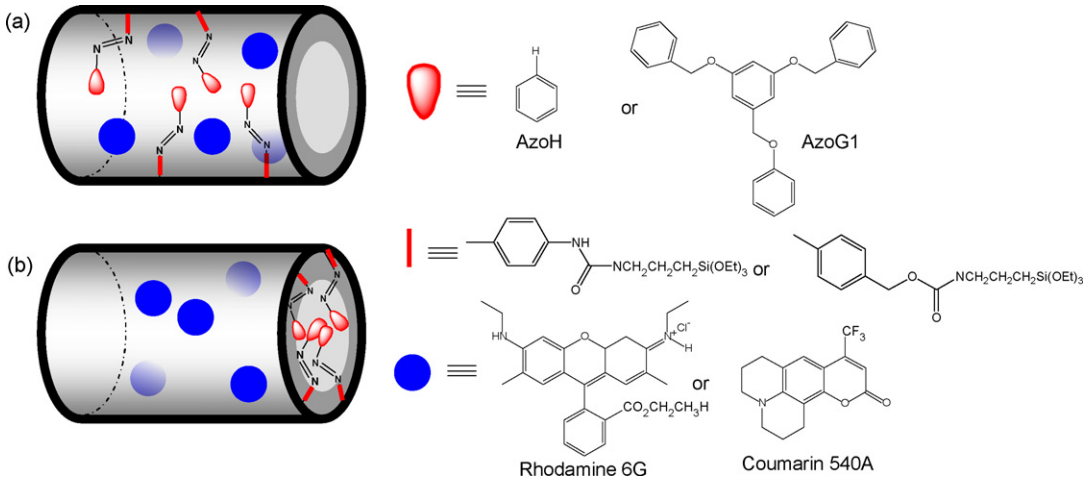

Fig. 5. (a) Azobenzene derivatives that function as nanoimpellers are attached to the pore interior using the co-condensation strategy. (b) Large AzoG1 molecules that act as gatekeepers are attached near pore orifices using the post-synthesis modification method.

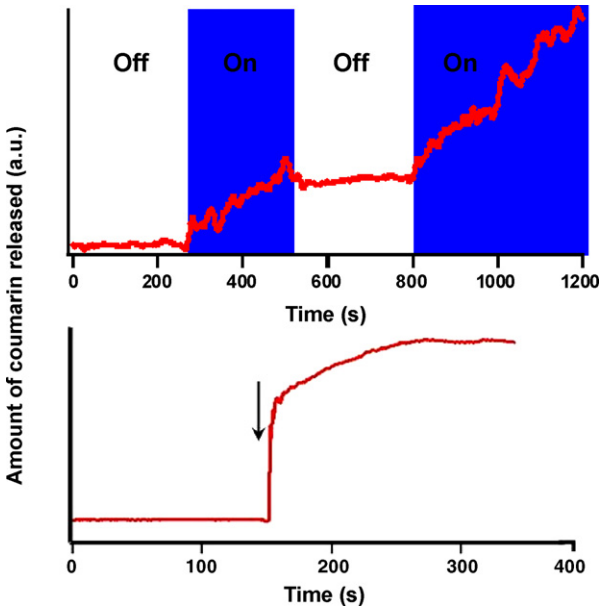

Fig. 6. Release of coumarin dyes from mesoporous silica nanoparticles modified with AzoG1 (top) and redox-active supramolecular nanovalves (bottom). The release of the dye into solution is monitored by fluorescence spectroscopy [31,37].

The photo-controlled release of molecules from the particles is probably driven by the dynamic wagging motion of the azobenzene machines. In the dark, dye molecules are trapped in the pores because the static azobenzene machines clog the inner pore channels and prevent the escape of molecules. Under 457 nm excitation, however, the reversible conversion of two conformers occurs continuously, causing the azobenzene terminus to move back and forth expelling the probe from the pores.

4.2. Supramolecular nanovalves

We have demonstrated the ability of supramolecular rotaxanes and pseudorotaxanes to function as nanovalves when tethered on the surface of mesoporous silica nanoparticles [35–40,45]. In these molecular machines, the moving part consists of a ring (cyclic molecule) that slides along a thread-like molecule between one or more binding stations. The thread is attached near the pore orifices using the post-synthesis grafting strategy. The movable ring acts as a gatekeeper; when it is positioned at the pore opening it blocks guest molecules from entering or escaping from the interior of the pore, but when it moves off the thread or to a position more distant from the pore opening, the guest molecules can move in or out of the pore. Other gatekeeping designs including the photoactivated intermolecular dimerization of tethered coumarins [46], and chemically removable CdS caps [47] have been demonstrated. These systems regulate the release of trapped guests by breakage of covalent bonds to unblock pores.

We have used luminescence spectroscopy to investigate the operation of supramolecular nanovalves. With the valves open, the pores are loaded with luminescent guests by backfilling and the valves are then closed to trap the guests. A small sample (∼15 mg) of the dye-loaded, capped particles is placed in the corner of a quartz cell, solvent is carefully added to the cell, and the liquid above the solid powder is exposed to an excitation light beam. The luminescence spectrum of the dye dissolved in solution is monitored as a function of time to measure the rate of release of the guest molecules.

4.3. Methods of activating supramolecular nanovalves

4.3.1. Redox activation

A reversible molecular valve able to repeatedly trap and release guest molecules from the pores of mesostructured silica nanoparticles has been demonstrated (Fig. 7) [38]. The gatekeeping component in this system was a [2]rotaxane consisting of a thread with two recognition stations and a movable cyclobis(paraquat-*p*-phenylene) ($CBPQT^{4+}$) ring. The valve could be controllably closed and opened by oxidizing and reducing the thread component of the valve. The two stations of the thread were a dioxynaphthalene (DNP) station located close to the silica surface, and a π-electron rich tetrathiafulvalene (TTF) station positioned further from the silica support. In the ground state, the tetracationic ring resides on the more distant TTF station, and the valve is open. Chemical oxidation of the TTF station to give TTF^{2+} destabilizes its interaction with the $CBPQT^{4+}$ causing the ring to shuttle to the DNP station and close the valve. The valve is opened upon reduction of TTF^{2+}, which enables the ring to shuttle back to the more distant location and thereby unblock the nanopores. Release of the trapped molecules was monitored as a function of time using luminescence spectroscopy (Fig. 6, bottom). Operation of the nanovalve was monitored by the luminescence intensity of naphthalene. In the closed configuration, the $CBPQT^{4+}$ quenched the naphthalene luminescence, and upon activation of the valve an increase in naphthalene emission intensity was observed.

Alternatively, activation of pseudorotaxane-based nanovalves has been accomplished by reducing the $CBPQT^{4+}$ ring [35]. A [2]pseudorotaxane consisting of a 1,5-bis[2-(2-hydroxyethoxy)bisethoxy]naphthalene (BHEEEN) thread encircled by a cyclobis(paraquat-*p*-phenylene) ($CBPQT^{4+}$) ring was mounted onto the surface that was functionalized with ICPES. The valve was opened by reduction of the ($CBPQT^{4+}$) ring by cyanoborohydride. Emission from the BHEEEN was monitored and used as an indicator of the threading–dethreading process of the machines.

4.3.2. Light activation

The [2]pseudorotaxane system discussed above was also operated under light activation [40]. Photosensitizers that become strong reductants and transfer electrons after absorption of a photon such as anthracene carboxylic acid and trisbipyridine ruthenium (II) were attached to the silica. Photo-induced electron transfer from the sensitizer to the $CBPQT^{4+}$ rings opened the valves.

4.3.3. Activation by pH and competitive binding

A pseudorotaxane based on a dialkylammonium thread encircled by a dibenzo[24]crown-8 (DB24C8) ring was tethered to

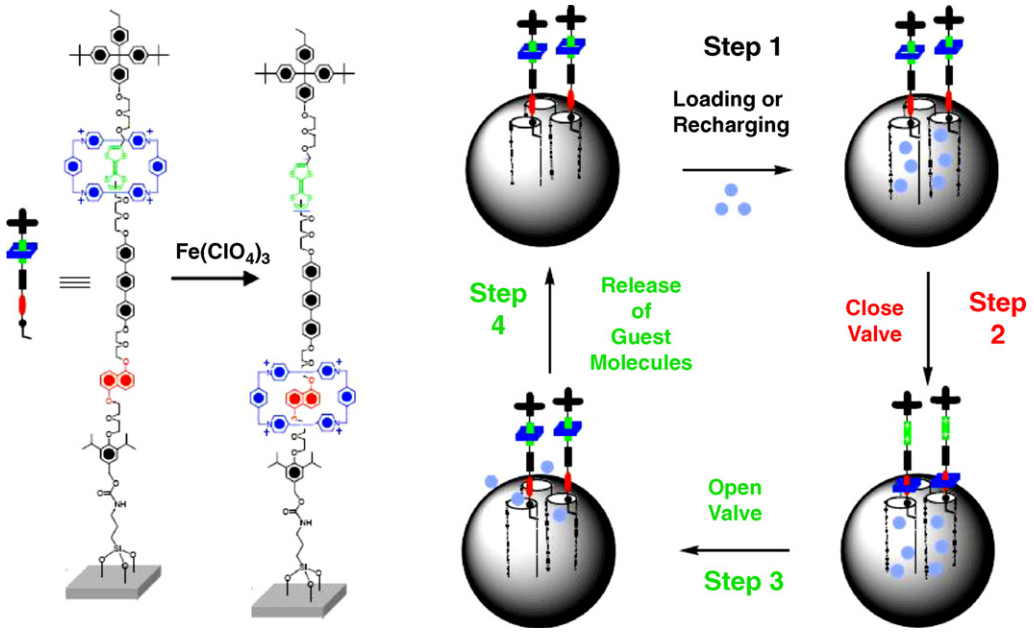

Fig. 7. A reversible molecular valve. The moving part of the molecular valve is a $CBPQT^{4+}$ ring (blue) which shuttles between a TTF station (green) and a DNP station (red) under redox control. The openings of the cylindrical pores are blocked by the $CBPQT^{4+}$ ring when the valve is closed. Guest molecules (light blue spheres) are loaded in Step 1 by diffusion into the open pores when the $CBPQT^{4+}$ ring is located on the TTF station. The valve is closed in Step 2 by oxidation of the TTF unit to its dication, causing the $CBPQT^{4+}$ ring to move to the DNP station, which is much closer to the openings of the pores. The valve can be opened (Step 3) by adding ascorbic acid to reduce the TTF dication back to its neutral state, whereupon the $CBPQT^{4+}$ ring moves back from the DNP station to be relocated around the TTF station, releasing the guest molecules in Step 4. Copyright 2005, National Academy of Sciences: reproduced with permission from reference [38]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

an MCM-41 to generate a supramolecular nanovalve that could be activated by pH and also by competitive binding [36]. This system relied on hydrogen bonding for complexation; the components could be made to dissociate upon deprotonation of the thread by addition of a base. Activation was demonstrated using a variety of bases; the steric size of the activating bases greatly affected the rate of release. Additionally, competitive binding of metal and fluorodialkylammonium cations was used as an alternative activation method for nanovalves based on the same pseudorotaxanes. Introduction of cationic species that strongly complex with the DB24C8 rings caused the nanovalves to open by shifting the equilibrium of the complexation–decomplexation process of the rings and threads, effectively trapping the rings and preventing them from recomplexing with the threads. The binding affinity of the activating cations toward the DB24C8 rings was the major factor affecting the rate of release.

4.4. Optimization of molecular nanovalves

There are several aspects of nanovalve design that can be fine-tuned to optimize the performance of the machines and adjusted so that the valves do not leak. Two of the attachment methods that strongly affect nanovalve performance are the positioning of the silane linker relative to the pore orifices and the length of the silane linker. The relative sizes of the trapped molecules, the moving parts, and the pore orifices are also important. Optimization of the valve requires testing different pore diameters, adjusting the distance of the moving part from the pore opening, adjusting the size of the moving parts, and testing activation mechanisms [37].

5. Drug delivery

The limited availability of effective biocompatible delivery systems for water-insoluble anticancer drugs remains as one of the biggest problems in cancer therapy. Since many important anticancer agents have poor water solubility, the research effort in developing novel delivery systems for these molecules without the use of organic solvents or surfactant mixtures has grown significantly over the years. Nanoparticles offer great potential and a promising approach to deliver therapeutic agents into targeted organs or cells and are being actively developed for application in cancer therapy [48]. Intravenous delivery of the nanoparticle drug carriers is feasible since their small particle size allows them to travel easily in the circulatory system. Surface modifications to the nanoparticles can give targeting functionality [49] and prevent uptake by macrophages [50], thus leading to a more effective therapeutic dosage and less undesirable side-effects to the body.

Among a variety of nanoparticle-based drug delivery systems, mesoporous silica nanoparticles have several advantageous features for use in the delivery of water-insoluble drugs. These materials have large surface areas and porous interiors that can be used as reservoirs for storing the drugs. The pore size and environment can be modified to selectively store different molecules of interest [51,52] while the size and shape of the nanoparticles can be tuned to enhance the cellular uptake process [44]. These robust inorganic materials do not swell in organic solvents and are stable at varying pH conditions [53]. Reports have shown that mesostructured silica nanoparticles are able to undergo cellular uptake and deliver molecules inside of

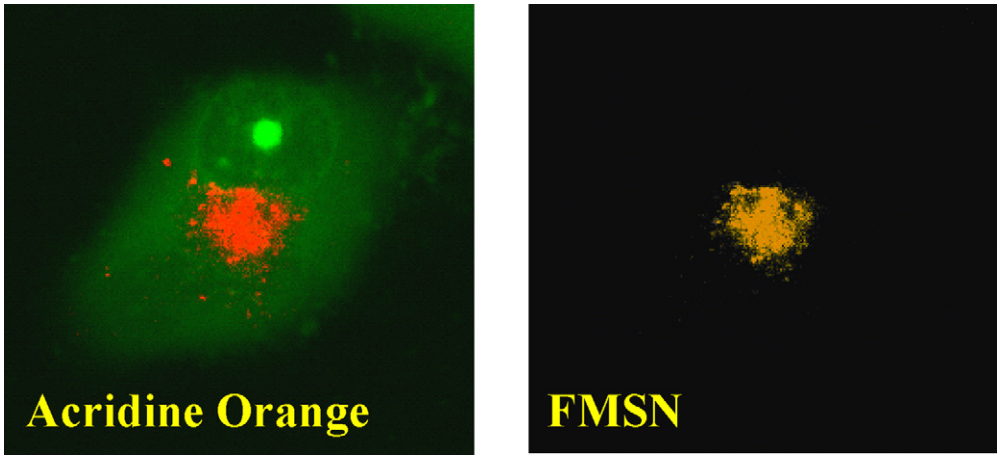

Fig. 8. Uptake of the nanoparticles by cancer cells. PANC-1 cells stained with Acridine Orange (AO, left) and the fluorescence of the nanoparticles within the same cell (right).

cells [19–22,47]. We have recently reported the modification of mesoporous silica nanoparticles using the bifunctional strategy, post-synthesis grafting, and backfilling strategy in order to utilize them as drug delivery vehicles [22]. The modified nanoparticles were able to deliver the water-insoluble drug camptothecin into pancreatic and stomach human cancer cells without the use of molecular machines.

In order to create an effective drug delivery system, the particles have to be small enough to undergo cellular uptake, be highly dispersed in aqueous solution, and contain pores that are large enough to incorporate the drug molecules. The synthetic method used a dilute precursor solution, high temperature, base-catalysis, and CTAB as the mesostructure template. In order to monitor the nanoparticles by confocal microscopy, fluorescein isothiocyanate was conjugated and attached using the bifunctional strategy. The surface of the nanoparticles was derivatized with hydrophilic phosphonate groups by post-synthesis grafting to prevent the nanoparticle aggregation caused by the interparticle hydrogen bonding interaction between the anionic silanol groups and the unreacted cationic amine groups [54].

The solvent-extracted nanoparticles were backfilled with the drug camptothecin (CPT) by stirring them in a dimethyl sulfoxide (DMSO) solution. After the organic solvent was removed using centrifugation and high vacuum, the loaded nanoparticles were washed thoroughly with buffered aqueous solution (PBS) to remove the drug molecules that were adsorbed on the particle surface. The molecules remained in the pores of particles suspended in water. To measure the amount of drug in the pores, the CPT-loaded nanoparticles were again redispersed in DMSO and centrifuged. UV/vis spectra taken on the supernatant showed that 1 μmol of CPT was released from 50 mg of nanoparticles.

A suspension of the drug-loaded nanoparticles in PBS was added to the pancreatic cancer cells (PANC-1). Uptake of the nanoparticles by the cells occurred in a relatively short time; the fluorescent mesoporous silica nanoparticles (FMSN) were observed in the cancer cells after only 30 min of incubation. The intracellular location of the nanoparticles and the CPT drug molecules were monitored using fluorescence confocal microscopy. Acridine Orange was used as the stain and to distinguish the acidic cellular organelles. The fluorescence of the FMSN overlapped the red emission of Acridine Orange, indicating that the nanoparticles were mainly located in the acidic organelles such as the lysosomes (Fig. 8). Additionally, the fluorescence of the CPT drug molecules was observed within the cancer cells. The non-specific distribution of the CPT drug molecules within the cells indicated that the drugs had been released from the pores of the FMSN.

The delivery of the water-insoluble drugs inside the cells by the CPT-loaded nanoparticles led to growth inhibition and cell death (Fig. 9). Growth inhibition of the cancer cells that were treated with either the suspension of CPT-loaded FMSN in PBS or a solution of CPT in DMSO was observed. In contrast, the water-insoluble CPT molecules that could only be suspended in PBS did not show any cytotoxicity to the cells even at high concentrations. The silica nanoparticles alone were not toxic to the cells, demonstrating that the materials are biocompatible at the concentrations used in the experiment. Thus, the FMSN effectively delivered the hydrophobic CPT into cancer cells to induce apoptosis.

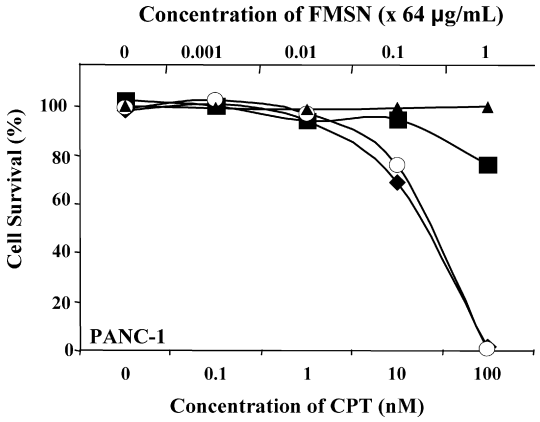

Fig. 9. Cell survival assay. (▲) Non-loaded FMSN in PBS; (■) CPT in PBS; (◯) CPT in DMSO; (♦) CPT-loaded FMSN in PBS. The concentrations of the CPT-loaded FMSNs and non-loaded FMSNs in PBS are shown at the top, while the concentrations of CPT in DMSO and in PBS are shown at the bottom. Reproduced with permission from reference [22].

6. Summary

In summary, mesoporous silica thin films and nanoparticles are versatile materials that can be modified with active molecules to produce operational molecular machines. One-pot functionalization strategies are useful for placing active molecules in chemically different regions of the mesostructure, and post-synthesis approaches to functionalization are useful for derivatizing the exposed surfaces of the materials. We have demonstrated the applicability of the various functionalization strategies for creating active machines including nanoimpellers based on the dynamic photoisomerization of azobenzene, and nanovalves based on the movement of supramolecular rotaxanes and pseudorotaxanes. Nanoimpellers and nanovalves are useful machines for controlling the release of guest molecules trapped within the mesopores of silicate materials.

Mesoporous silica nanoparticles are also useful vehicles for the delivery of hydrophobic anticancer drugs. Nanoparticles loaded with water-insoluble drugs are taken up by cancer cells and release the drugs inside the cells to induce apoptosis. Having established the potential of mesoporous silica nanoparticles for drug delivery, future research directions will be aimed at designing functional nanoparticles that can act as sophisticated vehicles for drug delivery. Mesoporous silica nanoparticles functionalized with biocompatible nanoimpellers and nanovalves are promising materials for the on-demand release of a wide range of drug molecules.

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