

Synthesis and Functionalization of a Mesoporous Silica Nanoparticle Based on the Sol–Gel Process and Applications in Controlled Release

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Received October 26, 2006

ABSTRACT

Mesoporous silica nanoparticles (MSNs) are introduced as chemically and thermally stable nanomaterials with well-defined and controllable morphology and porosity. It is shown that these particles possess external and internal surfaces that can be selectively functionalized with multiple organic and inorganic groups. On the basis of these characteristics, the biocompatibility of silica, and their efficient uptake by mammalian cells, MSNs are proposed as the basis of nanodevices for the controlled release of drugs and genes into living cells.

Introduction

The discovery of highly ordered mesoporous silica materials by scientists at the Mobil Corporation in 1992 was quickly recognized as a breakthrough that could lead to a variety of important applications.¹ Uniform cylindrical pores with diameters tunable in ranges between 2 and 30 nm and, consequently, the large surface area of these materials (700–1500 m²/g), along with the high chemical

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and thermal stability and easy functionalization of silica, make them ideal for use as supports for adsorption, catalysis, chemical separations, and biotechnology devices. After the discovery of the above-mentioned materials, MCM-41 (*Mobil Crystalline of Materials*), significant research efforts have been underway to achieve control over the characteristics of mesoporous silica with special emphasis on pore size and morphology. Through this vast research, new families of mesoporous silica material, such as SBA,² MSU,³ and FSM,⁴ were developed with characteristic porosities and particle shapes. Most of those materials consisted of particles with sizes in the micrometer scale. Recently, mesoporous silica nanoparticles (MSNs) with well-defined and controllable particle morphology were developed in our research group in the pursuit of biocompatible materials to be used in controlled release and drug delivery systems.⁵ This Account describes our synthetic approach to obtain functional MSNs and the henceforth controlled release and biological relevant applications and the relevant contributions of ours and other research groups to attain such a goal.

Synthesis and Functionalization of MSNs

The synthesis of mesoporous silica is based on the formation of liquid-crystalline mesophases of amphiphilic molecules (surfactants) that serve as templates for the *in situ* polymerization of orthosilicic acid. The synthesis can be performed either in acidic or basic conditions, and the source of silica can be fumed silica, sodium silicate, or a tetra-alkyl oxide of silane. The first material reported by the Mobil researchers (designated as MCM-41) was micrometer-sized particles with hexagonally ordered mesopores. The morphology of the particles was variable, with a very small amount of hexagonally shaped nanoparticles.⁶

Later works were performed to control the morphology of the particles by manipulation of the pH during synthesis⁷ or by the addition of cosolvents.⁸ We developed a simple and fast alternate route to MCM-41 that leads to uniform nanosized spherical particles (MSNs).⁹ The synthesis of MSNs is performed at a low surfactant concentration to make the assembly of the ordered mesophases strongly dependent upon the interaction between the cationic surfactant and the growing anionic oligomers of orthosilicic acid, which in turn limits the assembly of mesophases to small sizes. In the synthetic procedure, the surfactant cetyltrimethylammonium bromide (CTAB) is initially dissolved in basic aqueous solution and the

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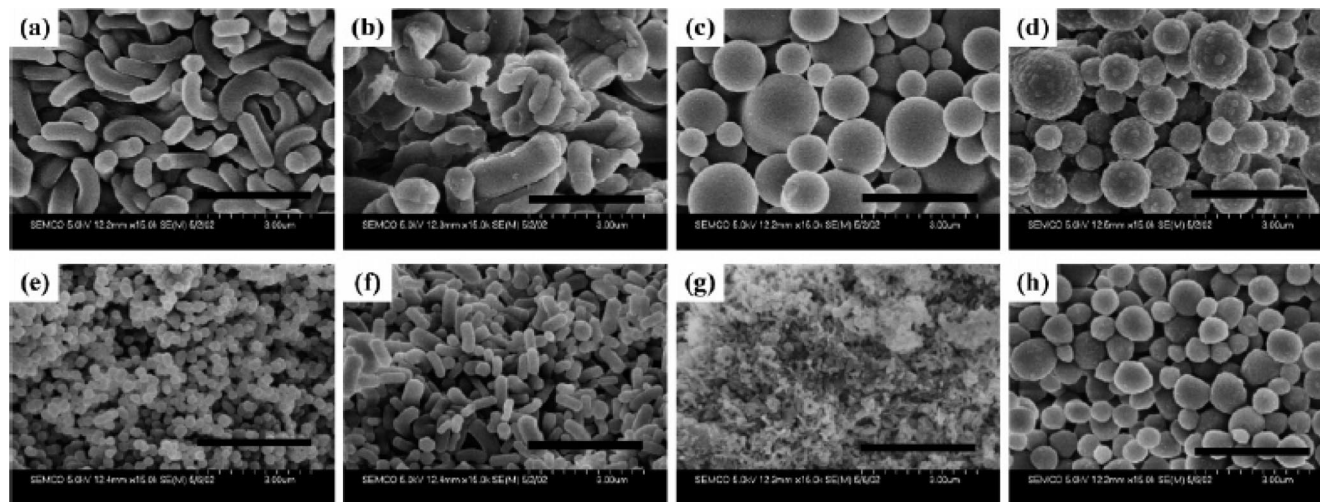


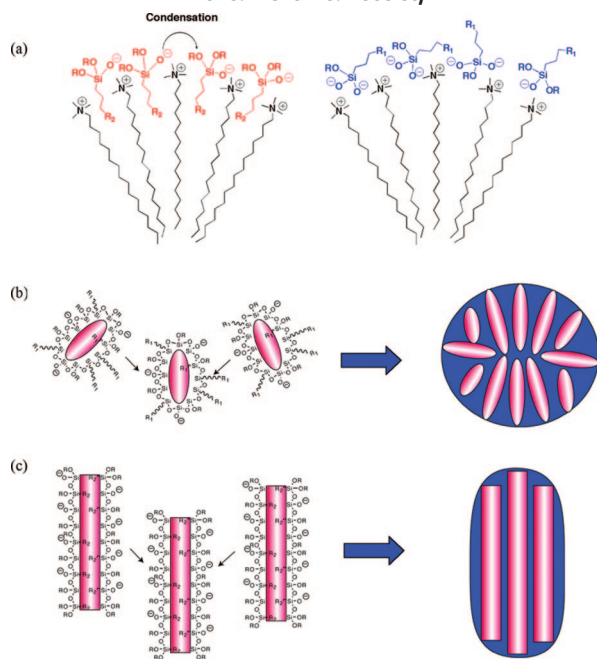
FIGURE 1. Field emission scanning electron microscopy (FESEM) images of (a) 3-aminopropyl-MSN, (b) *N*-(2-aminoethyl)-3-aminopropyl-MSN, (c) 3-[2-(2-aminoethylamino)ethylaminopropyl]-MSN, (d) 3-ureidopropyl-MSN, (e) 3-isocyanatopropyl-MSN, (f) 3-cyanopropyl-MSN, (g) allyl-MSN, and (h) nonfunctionalized MSN. All images are presented using the same scale, with the scale bar = 3 μm . Reproduced with permission from ref 9. Copyright 2003, American Chemical Society.

mixture is vigorously stirred at elevated temperature. Tetraethylorthosilicate (TEOS) is added, and the solution is kept stirring at an elevated temperature for 2 h. After the reaction is complete, the as-synthesized product is filtered and washed with abundant water and methanol. After drying under vacuum, the organic surfactant is removed by either acid wash or calcination. The inorganic silica framework that is left may have a hexagonal, disordered, or cubic pore structure, depending upon the specific synthetic conditions.

When a material is made of discrete, small, and uniform particles, it is possible to clearly distinguish two large surfaces: an internal and an external one. The most popular way of covalently functionalizing MSNs is by grafting the nanoparticles postsynthesis with organotrialkoxysilanes or organotrichlorosilanes. This reaction is performed on surfactant-removed mesoporous silica in nonpolar anhydrous solvents to avoid a reaction of the organosilanes with anything but the silica material. The reaction takes place between the silanol groups on the surface of the silica and the organoalkoxysilanes/organotrichlorosilanes. Unfortunately, it has been found that materials functionalized via this grafting method contain an inhomogeneous surface coverage of organic functional groups.¹⁰ Silanols located on the exterior surface and at the openings of the mesopores are kinetically more accessible than silanols located on the interior pore walls; thus, most organic functional groups added to mesoporous materials through this postsynthetic grafting method have been shown to be located on the exterior surface or congregated at the mesopore opening. However, this method of functionalizing silica is particularly advantageous for exterior surface selectivity. Given the special characteristics of the method of synthesis of the MSN, it is possible to selectively functionalize these surfaces.¹¹ This is especially the case when the grafting is performed before the surfactant is removed from the mesopores. After the grafting has been performed, the surfactant can

be removed by acid wash or, in the case of certain functionalities, by controlled calcination.¹² This control over the location of grafting, i.e., selective for the external surface, may enable the interaction of the MSN with the environment through the surface functionalization while not influencing the pore surface properties.

The other common method for synthesizing organically functionalized mesoporous silica materials is the co-condensation method. This functionalization method is a direct synthesis method, in which the organoalkoxysilane is introduced to the basic, aqueous CTAB and TEOS solution during the condensation, therefore named the co-condensation functionalization method. With this synthetic approach, it is possible to control the morphology of the particles by the addition of functional co-condensing reagents (Figure 1). We showed that the degree of functionalization and particle morphology is dependent upon the concentration, molecular size, and hydrophobicity/hydrophilicity of the co-condensing reagents. In fact, the use of 12.8 mol % co-condensing reagents led to MSNs with homogeneously distributed functional groups with surface coverages varying between 13 and 33% as determined by solid-state nuclear magnetic resonance (NMR) of ²⁹Si.¹³ The co-condensing reagents are organo-substituted trialkoxysilanes (organoalkoxysilanes), and their influence on particle morphology depends upon the abilities of their organic groups to stabilize or destabilize the micelles during the formation of the MSN (Scheme 1). Nonpolar groups tend to stabilize the formation of long individual cylindrical micelles by intercalating their hydrophobic groups into the micelles, interacting with the hydrocarbon tails of the surfactant templates, and thereby reducing the charge density of the head groups. This interaction favors the condensation between the "micelle-oriented" trialkoxysilyl groups in the basic aqueous solution. The resulting "side-on" growth of the silicate-coated cylindrical micelles gives rise to rod-

Scheme 1. Reproduced with Permission from Ref 9. Copyright 2003, American Chemical Society²

^aR, methyl or ethyl groups; R₁, hydrophilic functional groups; R₂, hydrophobic functional groups.

like nanoparticles (see Figure 1a). When the nature of the functional group of the co-condensing organoalkoxysilane reagent is more hydrophilic, there is no further stabilization of the micelles. The polar groups are not favored by the interaction with the surfactants; therefore, they tend to show little "side-on" condensation, which in turn inhibits the formation of long micelles and renders small spherical particles resembling the ones obtained in the absence of the co-condensing agent. In addition to the organoalkoxysilane reagents having an effect on the particle morphology, the pore structure is dependent upon the co-condensing reagent.

With this method, it is also possible to obtain monodispersed, multifunctionalized MSNs with the ability to tune the relative ratios of the functional groups of interest (Figure 2).¹⁴ As mentioned before, the degree of hydrophobicity of an organoalkoxysilane precursor in basic aqueous solutions of CTAB does not only influence its loading in the resulting MSN under this co-condensation condition but also determines the pore and particle morphology of the final mesoporous material. The use of two organoalkoxysilanes with different structure-directing abilities as precursors of our co-condensation reaction allows us to use the precursor with the *stronger structure-directing ability* to create the desired pore and particle morphology and employ the second precursor for achieving the functionality of interest. We achieved this goal by synthesizing multifunctional MSNs with two organic functional groups: 3-[2-(2-aminoethylamino)ethylamino]propyl- (AEP) and 3-cyanopropyl- (CP) trialkoxysilanes (TMSs). As monofunctional organic precursors, AEP forms large spherical MSNs, while CP formed smaller rod-shaped MSNs (Figure 1). By varying the ratio of AEP to CP, we determined that AEP had the stronger structure-directing

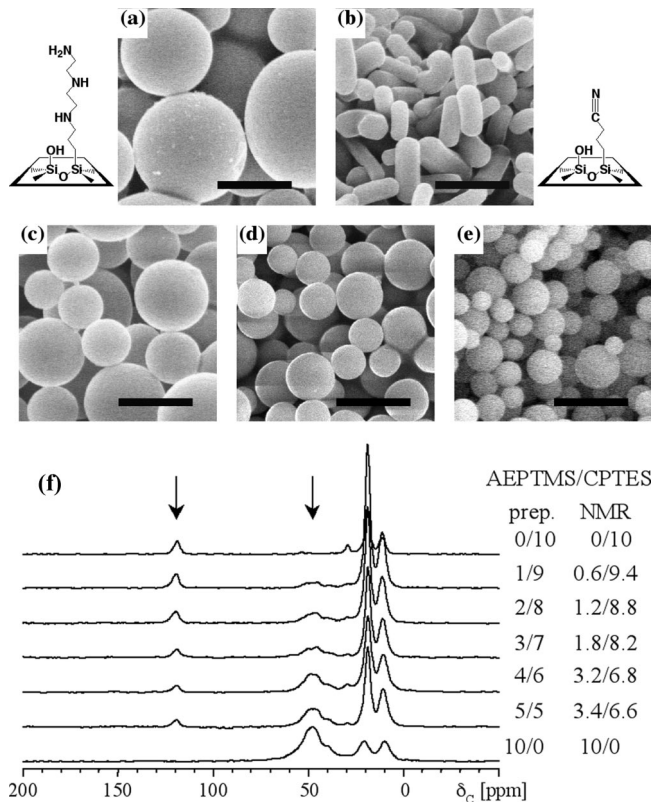


FIGURE 2. FESEM images of (a) *N*-(2-aminoethyl)-3-aminopropyl-MSN (AEP-MSN), (b) 3-cyanopropyl-MSN (CP-MSN), (c) 5:5 AEP/CP-MP, (d) 3:7 AEP/CP-MP, and (e) 1:9 AEP/CP-MP. Scale bar = 1 μm. (f) Cross-polarization magic-angle spinning (¹³CPMAS) spectra of AEP/CP-MPs. Arrows show the resonances that are unique for each species and thus were used for quantitative analysis. The numbers represent the molar ratio between two components used for preparation and obtained from analysis of NMR spectra. Reproduced with permission from ref 14. Copyright 2003, Royal Society of Chemistry.

ability. All of the particles synthesized with both functional groups were spherical; no rod-shaped MSNs were measured. In addition to directing the particle morphology, the AEPTMS precursor affected the pore properties and structure. As monofunctionalized MSNs, CP had a hexagonally ordered pore structure and AEP had a disordered pore structure. When co-condensed together, a disordered pore structure was measured, providing further evidence that pore morphology is sensitive to the presence of AEPTMS. To quantify the chemically accessible structure-directing functional group, we incorporated the chelating ability of AEP for Cu⁺². With this approach, it is possible to synthesize a wide array of multifunctionalized mesoporous silica materials with *control of both morphology and functionalization*.¹⁴

When the particle morphology and both the degree and type of functionalization are controlled by the co-condensation method, it is also desirable to control the surface concentration of organic functional groups. We reported a new synthetic method to generate organically functionalized MSNs using disulfide-containing organotrimethoxysilanes with different anionic functional groups to electrostatically interact with the cationic surfactant

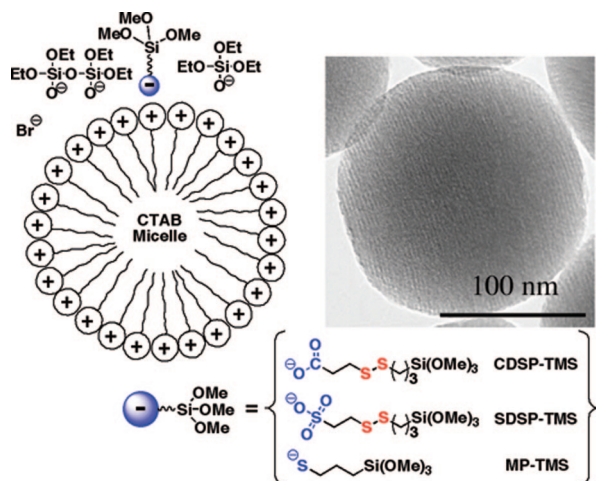


FIGURE 3. Schematic representation of the use of anionic organoalkoxysilanes for controlling the functionalization of the MSN materials. The MCM-41-type mesoporous channels are illustrated by the parallel stripes shown in the transmission electron microscopy (TEM) micrograph of the MSN-SH material. Reproduced with permission from ref 15. Copyright 2005, Royal Society of Chemistry.

template, cetyltrimethylammonium bromide.¹⁵ We investigated the electrostatic matching effects of thiolate-, carboxylate-, and sulfonate-containing organoalkoxysilanes (MSN-SH, MSN-COOH, and MSN-SO₃H, respectively) to CTAB micelles and the effect that matching has on the functional group surface concentration (Figure 3). Upon reduction of disulfide-containing organotrimethoxysilanes, the chemically accessible functional groups were quantified. We determined that the surface concentration of chemically accessible functional groups increased in the order of MSN-SH < MSN-COOH < MSN-SO₃H. We concluded that the greatest concentration of chemically accessible functional groups occurred in the MSN-SO₃H sample because the sulfonate anion is the least hydrated of the three anionic organotrimethoxysilane precursors. With this study, we showed that the surface concentration of organic functional groups in MSNs can be fine-tuned by matching the interfacial electrostatics between the surfactant head groups and desired organic functional groups. It is based on these features that we propose MSNs as agents for controlled delivery and catalytic applications. The internal mesopore surface can serve as a microenvironment in which molecules can be loaded and protected from any contact with the external environment.

Mesoporous Silica-Based Controlled Release Systems

As mentioned above, the structural and chemical properties of MSNs allow for the design of nanodevices capable of transporting, in a protected microenvironment, molecules of interest for their controlled release in a specific target. The discovery that mammalian cells take up and internalize MSNs without cytotoxic effects opened the door to the use of this material as a drug/gene delivery system.^{5,16} In addition to us, Mou and co-workers dis-

covered that various mammalian cells are able to actively internalize MSNs through endocytosis and the nanoparticles are able to escape from endosomes to reach the cytosol.^{17,18} Moreover, we discovered that the functionalization of the external surface of the nanoparticles can affect both the efficiency and the mechanism of their uptake by cells.¹⁸ The types of cells that internalize MSNs include HeLa, Chinese hamster ovary (CHO), rat liver, endothelial, human mesenchymal stem cells, and 3T3-L1.^{5,16-19}

To employ MSNs as an intracellular delivery system, it is important to be able to release the cargo molecules in a controlled manner in the targeted site. Any premature release of guest molecules²⁰ poses a serious problem. In the ultimate scenario, the delivery of toxic antitumor drugs will require “zero release” before reaching the targeted cells or tissues. Unfortunately, the release mechanism of many current biodegradable polymer-based drug delivery systems relies on the hydrolysis-induced erosion of the carrier structure. The release of encapsulated compounds usually takes place immediately upon dispersion of these composites in water. Also, such systems typically require the use of organic solvents for drug loading, which could sometimes trigger undesirable modifications of the structure and/or function of the encapsulated molecules, such as protein denaturation and aggregation. In contrast, the surface functionalized mesoporous silica materials offer several unique features, such as stable mesoporous structures, large surface areas, tunable pore sizes and volumes, and well-defined surface properties for site-specific delivery and hosting molecules with various sizes, shapes, and functionalities.

Release by Nonfunctionalized MCM-41

Since the beginning of the current decade, there have been several examples of MCM-41-type silicas used for drug delivery and controlled release. In 2001, scientists in Europe loaded ibuprofen into MCM-41 materials with different pore sizes and studied the drug release in a simulated body fluid.²¹ This report demonstrated that the unique MCM-41 mesoporous structure with channel-like pores packed in a hexagonal fashion could be used for loading large quantity of drugs while controlling the rate of release of drugs.

To investigate how the pore and particle morphology of mesoporous silica materials would impact the controlled release properties, we developed a series of room-temperature ionic liquid (RTIL) containing MSN materials with various particle morphologies, including spheres, ellipsoids, rods, and tubes. When the RTIL template was changed, the pore morphology was tuned from the MCM-41 type of hexagonal mesopores to rotational moiré type of helical channels and wormhole-like porous structures. These materials were used as controlled release delivery nanodevices to release antibacterial ionic liquids against *Escherichia coli* K12. Our results indicated that the rate of RTIL release from the MSN material is governed by the

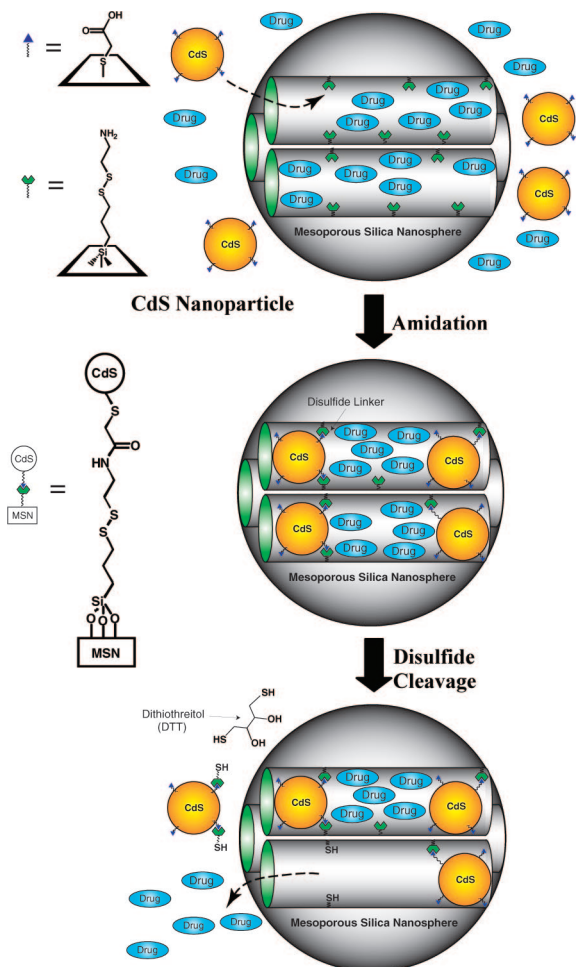


FIGURE 4. Schematic representation of the CdS nanoparticle-capped MSN-based drug/neurotransmitter delivery system. The controlled release mechanism of the system is based on chemical reduction of the disulfide linkage between the CdS caps and the MSN hosts. Reproduced with permission from ref 16. Copyright 2003, American Chemical Society.

particle and pore morphology, leading to different anti-bacterial activity.

Stimuli-Responsive Controlled Release from Capped MSNs

As mentioned previously, it is highly desirable to design delivery systems that can respond to external stimuli and release the guest molecules at specific sites. To achieve this goal, our group developed a series of stimuli-responsive, MCM-41-type MSN-based controlled release delivery systems.^{16,22,23} As depicted in Figure 4, the mesopores loaded with guest molecules were capped by CdS nanoparticles via a chemically cleavable disulfide linkage to the MSN surface. Being physically blocked, guest molecules were unable to leach out from the MSN host, thus preventing any premature release. The release was triggered by exposing the capped MSNs to chemical stimulation¹⁶ that could cleave the disulfide linker, thereby removing the nanoparticle caps and releasing the pore-entrapped guest molecules.

We first prepared a disulfide amine functional mesoporous silica nanoparticle material (linker-MSN) following our reported co-condensation method.²⁴ After synthesis, these spherical nanoparticles were loaded with adenosine 5-triphosphate (ATP) and vancomycin and the pores were covalently capped with acid functional 2.0 nm diameter CdS nanoparticles as chemically removable caps. Loading efficiency of the drug molecules were 83.9 and 30.3 mol %, respectively. Two different reducing agents, dithiothreitol (DTT) and mercaptoethanol (ME), were employed as chemical stimuli to reduce the disulfide linkage, uncap the pores, and release the drug molecules. This system demonstrated negligible premature release in the absence of a reducing agent. The majority (85%) of the total loaded drug molecules were released within the initial 24 h. In addition, we showed that drug release was dependent upon the reducing agent concentration, indicating the rate of release is dictated by the rate of cap removal.

To demonstrate biocompatibility, we cultured neuron-free astrocyte type-1 cells in the presence of our ATP-encapsulated CdS-capped MSNs. Cells were treated with Ca^{+2} -chelating fluorescent dye. Upon perfusion application of ME, we observed a pronounced increase in the intracellular calcium concentration. The application of ME triggered the uncapping of the pores and the release of ATP, hence giving rise to the corresponding ATP receptor-mediated increase in the calcium concentration.

To introduce site-directing capability to the MSN-based delivery system, we designed a MSN material capped with superparamagnetic iron oxide (Fe_3O_4) nanoparticles.²² Similarly, linker-MSN material was synthesized and loaded with fluorescein as a proof-of-principle guest molecule. Once loaded, the pores of the MSNs were covalently capped with 10 nm diameter Fe_3O_4 nanoparticles (magnet-MSN) (Figure 5a).

Magnetic site direction was demonstrated using cuvettes and simple laboratory magnetic retrievers. The magnet-MSNs were directed to one wall of the cuvette, and DTT was dissolved in the buffer in the cuvette. Pictures recorded at 12 and 96 h after the addition of DTT showed uncapping of the pores and release of the loaded fluorescein. The kinetics of release of fluorescein from magnet-MSN by DTT and cell-produced antioxidant dihydrolipoic acid showed similar behavior as in the CdS-capped MSNs explained above.

To investigate biocompatibility of this system, HeLa (human cervical cancer) cells were incubated in the presence of magnet-MSN to allow for the internalization of this material. The cells that internalized magnet-MSN were isolated and controlled site direction was demonstrated by using a magnet to move these magnet-MSN internalized HeLa cells across a cuvette (panels b-d of Figure 5). To confirm that the magnet-MSNs were internalized, we examined the cells with confocal fluorescence microscopy. We observed green fluorescence in the same plane of the cell as the nucleus, indicating that the magnet-MSNs (loaded with fluorescein) were indeed internalized and had released fluorescein from the pores.

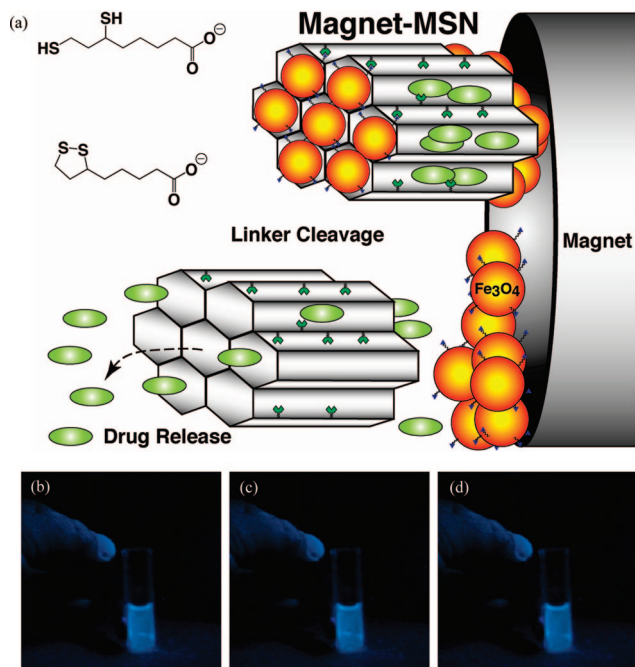


FIGURE 5. (a) Schematic representation (above) of the superparamagnetic iron oxide nanoparticle-capped mesoporous silica nanorod material (magnet-MSN). Panels b–d are single frames of HeLa cells with Fe_3O_4 -capped fluorescein-loaded MSNs traveling across the cuvette, propelled by a magnetic force. Reproduced with permission from ref 22. Copyright 2005, Wiley-VCH Verlag GmbH and Co. KGaA.

We investigated the release kinetics and mechanism of an ATP-loaded MSN system by applying a series of disulfide-reducing chemicals as triggers to uncap the mesopores. The concentration of the released ATP in solution was monitored by a well-established ATP-induced luciferin/luciferase chemiluminescence assay.²³ Real-time ATP chemiluminescence imaging was used to monitor the release of the ATP by detecting the ATP-induced chemiluminescence of firefly luciferase *in situ*. Upon the addition of 5 mM DTT, significant levels of ATP were released from the MSNs in approximately 2 min after stimulation. These results suggested that the majority of release occurs in the first few minutes after disulfide reduction. We also compared the disulfide reduction capabilities of DTT and tris(2-carboxyethyl)phosphine (TCEP). We determined that DTT released more ATP quicker than in the case of TCEP. We attributed this difference to the superior reducing power of DTT.

While we demonstrated chemically stimulated controlled release from MSNs in an aqueous environment, Tanaka and co-workers demonstrated the ability of coumarin-modified mesoporous silica to be used as a photocontrolled reversible guest molecule release vehicle in an organic solvent.²⁵ They showed that the uptake, storage, and release of organic molecules using coumarin-modified MCM-41 can be regulated through the photocontrolled and reversible intermolecular dimerization. The coumarin was covalently attached to the MCM-41 pore walls by postsynthesis grafting, and the pores were loaded with the steroid cholestane, followed by photodimerization of the coumarin by exposure to light greater than 310 nm. The

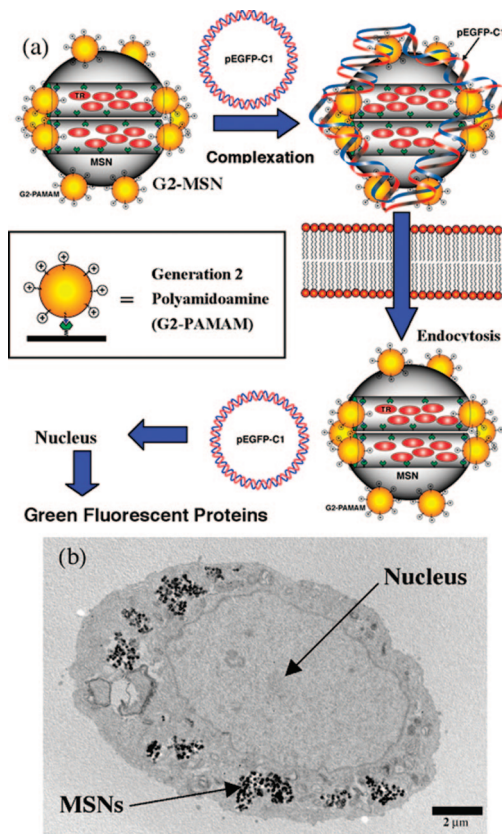


FIGURE 6. (a) Schematic representation of a nonviral gene transfection system based on a Texas Red (TR)-loaded, G2-PAMAM dendrimer-capped MSN material complexed with an enhanced GFP (*Aequorea victoria*) plasmid DNA (pEGFP-C1). (b) TEM micrographs of G2-MSN–DNA complexes (black dots) endocytosed by CHO cells. Reproduced with permission from ref 5. Copyright 2004, American Chemical Society.

resulting photodimerization led to the isolation of pores by blocking the pore entrance with cyclobutane dimers spanning the pore diameter. The cholestane-loaded photodimerized material was then exposed to UV light with a wavelength of 250 nm to cleave the cyclobutane rings of the coumarin dimers, and the subsequent release of the stored cholestane molecules was observed. The report was the first to demonstrate a photocontrolled release of guest molecules from MCM-41 material by reversible photoresponsive dimerization techniques.

In addition to the use of inorganic nanoparticles as caps, we have demonstrated that large organic molecules can also be used to modify the surface properties of mesoporous silicas to achieve controlled release.⁵ Our report was also the first uptake study of MCM-41-type mesoporous silicas into eukaryotic cells. Functional MSNs were capped with second generation (G2) PAMAM dendrimer via the disulfide linkage explained above, and this G2-PAMAM-capped MSN (G2-MSN) was complexed with plasmid DNA that codes for enhanced green fluorescent protein (GFP) (Figure 6a). It was determined that the G2-MSN could bind with plasmid DNA to form stable DNA–MSN complexes and protect the DNA from enzymatic digestion. Transfection efficacy was demonstrated by incubating the DNA–MSN complex with CHO cells,

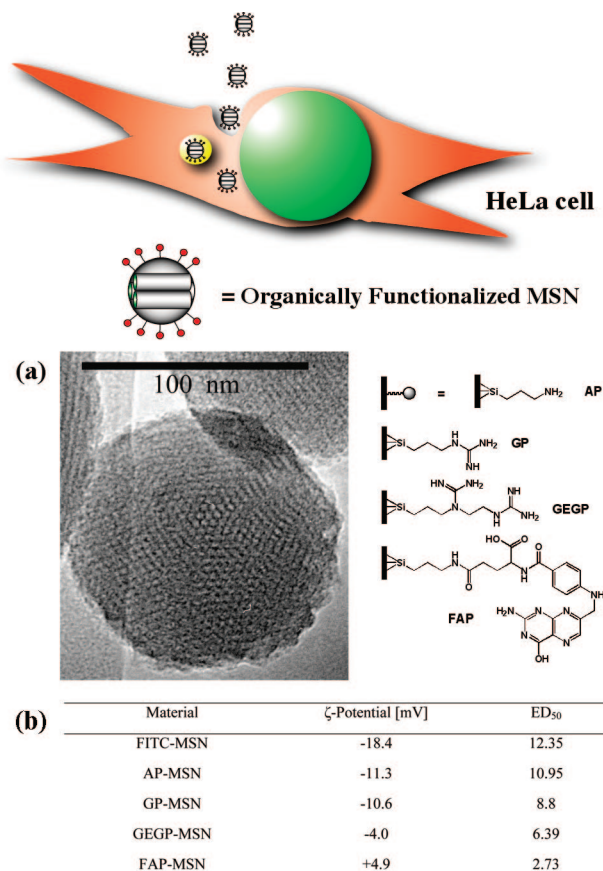


FIGURE 7. Schematic representation of the endocytosis of organically functionalized MSNs by a human cervical cancer cell line (HeLa). (a) TEM image of a fluorescein-functionalized MSN (FITC-MSN). (b) ζ potentials and ED₅₀ for the cellular uptake of the MSNs. Reproduced with permission from ref 18. Copyright 2006, American Chemical Society.

measuring the expression of GFP, and comparing it to commercially available transfection reagents. Transmission electron micrographs of post-transfection cells also provided direct evidence that a large number of G2-MSN-DNA complexes were internalized (Figure 6b).

Mou and co-workers^{17,19} studied the mechanism of cellular uptake of fluorescein-labeled MSNs in 3T3-L1 and mesenchymal stem cells, showing that the endocytosis of the material was time- and concentration-dependent and clathrin-mediated. In a recently published communication, we reported the cellular uptake of surface functionalized fluorescein-labeled MSNs and discovered that surface functionalization may have an effect on the mechanism of cellular uptake and the more negatively charged MSNs were able to escape from endosomes, while less negatively charged MSNs remained trapped within endosomes (parts a and b of Figure 7).¹⁸

Very recently, we reported the internalization of organically and inorganically surface-functionalized MSNs by plant cells and the delivery of DNA and chemicals.²⁶ We loaded the pores with a chemical inducer, capped the mesopores with gold nanoparticles, and coated the exterior with a plasmid that codes for GFP when induced by the chemical inducer. These Au-capped MSNs were internalized by plant cells by bombardment; the surface-

bound plasmid DNA was released; and with the addition of a chemical uncapping trigger in the media, the inducer was released. Gene expression was observed 48 h after bombardment.

Apart from the chemical stimuli, an efficient electrochemical redox-activated system based on mesoporous silica was developed by Zink, Stoddart, and co-workers.²⁷ The investigators demonstrated a supramolecular nanovalve tethered to the opening of the mesopores that can be turned on by redox chemistry. The mesopores were loaded with a fluorescent molecule and contained these molecules by using a pseudorotaxane as a gatekeeper. The opening of the nanovalve was stimulated by the addition of an external reducing agent, which causes the pseudorotaxane to disassemble. After this report, Zink, Stoddart, and co-workers further developed a reversibly operated nanovalve that can be turned on and off by redox chemistry.²⁸ This reversible nanovalve is similar to the original nonreversible, but in this case, a large molecule caps the free end of the tether, thus keeping the cyclophane in contact with the tether. This movement of the cyclophane uncaps the pore and allows for the free movement of guest molecules. Reduction of the oxidizing molecule by ascorbic acid permits the return of the cyclophane to the mesopore opening and recapping. This was the first report of a reversibly operating nanovalve that can be turned on and off by redox chemistry.

Conclusion and Outlook

This Account describes in detail the recent progress of synthesizing and functionalizing MSNs and using these nanomaterials as controlled release and biological cell delivery vehicles. With the synthesis and functionalization techniques discussed here, it is now possible to synthesize functionalized MSNs with control over particle and pore morphology and degree of functionalization. The studies reported here established the ability to load drugs and analytes into the mesopores and release by controlling the morphology and stimuli responsiveness. The studies reported here have also shown that numerous pore-capping agents can be incorporated to encapsulate drugs and chemicals in the mesopores, both organic and inorganic.

As progress continues with the synthesis of different functional mesoporous nanomaterials, there is a need to tailor these novel materials for studying the numerous chemical pathways of biological systems. One such avenue is investigating selective endocytosis of functionalized MSNs in mammalian and plant cells. If selective endocytosis is achieved, then drugs may be able to be delivered to selective cell types. Also, intracellular controlled release is a significant avenue of research that needs to be addressed, if biogenic molecules, such as drugs, genes, and imaging agents, can be released by intra- or extracellular stimuli after selective endocytosis. Furthermore, if the pore sizes of MSNs can be increased, the controlled release of therapeutic proteins, enzymes, and large polynucleotides can be achieved. The loading and release of

these large biomolecules could lead to therapeutics and metabolic applications. These research breakthroughs may lead to new treatments for gene therapy, protein deficiencies, and metabolic disorders.

The authors thank the U.S. National Science Foundation (CHE-0239570) and the U.S. DOE Ames Laboratory (W-7405-Eng-82) for the support of their research.

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