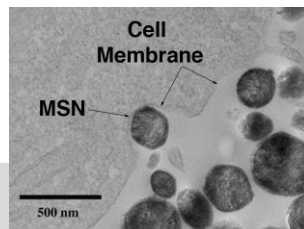


DOI: 10.1002/adfm.200601191

Mesoporous Silica Nanoparticles for Drug Delivery and Biosensing Applications**

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Recent advancements in morphology control and surface functionalization of mesoporous silica nanoparticles (MSNs) have enhanced the biocompatibility of these materials with high surface areas and pore volumes. Several recent reports have demonstrated that the MSNs can be efficiently internalized by animal and plant cells. The functionalization of MSNs with organic moieties or other nanostructures brings controlled release and molecular recognition capabilities to these mesoporous materials for drug/gene delivery and sensing applications, respectively. Herein, we review recent research progress on the design of functional MSN materials with various mechanisms of controlled release, along with the ability to achieve zero release in the absence of stimuli, and the introduction of new characteristics to enable the use of nonselective molecules as screens for the construction of highly selective sensor systems.



1. Introduction

Recent breakthroughs in the synthesis of mesoporous silica materials with controlled particle size, morphology, and porosity, along with their chemical stability, has made silica matrices highly attractive as the structural basis for a wide variety of nanotechnological applications such as adsorption, catalysis, sensing, and separation.^[1–6] In addition, we and others have discovered that surface-functionalized mesoporous silica nanoparticle (MSN) materials can be readily internalized by animal and plant cells without posing any cytotoxicity issue in vitro.^[7,8] These new developments render the possibility of designing a new generation of drug/gene delivery systems and biosensors for intracellular controlled release and imaging applications. Herein we review recent research efforts in developing new MSN-based materials with different surface functionalities targeted for the abovementioned applications.

2. Results and Discussion

2.1. Characteristics of Mesoporous Silica Nanoparticles

Since the discovery of MCM-41 by Mobil scientists,^[9] significant research progress has been made in controlling and modifying the properties of mesoporous silica materials. For example, several key structural characteristics of the material, including the size and morphology of pores^[4–6,9] and particles^[1,2] can be regulated. For example, we have synthesized MCM-41-type MSNs with a variety of shapes and sizes ranging from 20 to 500 nm, and with pore sizes ranging from 2 to 6 nm, as depicted in Figure 1. Functionalization of these materials with a variety of organic groups inside of the mesopores or on the external surface of the particles^[10,11] have been demonstrated.

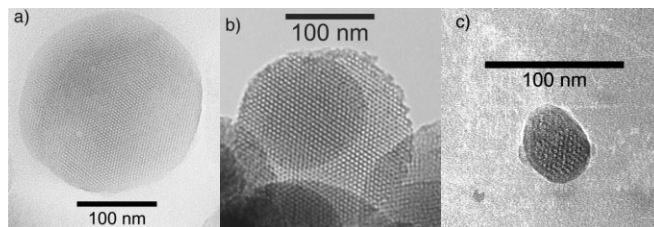


Figure 1. Transmission electron microscopy images of three spherical MSNs with different particle and pore sizes: a) Particle size ca. 250 nm; pore diameter ca. 2.3 nm. b) Particle size ca. 200 nm; pore diameter ca. 6.0 nm. c) Particle size ca. 50 nm; pore diameter ca. 2.7 nm. Figure 1a reproduced with permission from [10]. Copyright 2003 American Chemical Society.

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



Prof. Victor S.-Y. Lin received his Ph.D. from the University of Pennsylvania in 1996. After working as a Skaggs postdoctoral fellow at the Scripps Research Institute, he joined the faculty at Iowa State University in the fall of 1999. He is currently a Professor of Chemistry at Iowa State University and an assistant program director of the Chemical and Biological Sciences program at the U.S. DOE Ames Laboratory. His research focuses on the design of functional mesoporous materials for biomedical and biotechnological applications, such as biosensor design, drug delivery, and gene transfection. His group also specializes in the synthesis of mesoporous heterogeneous catalysts for various reactions, such as cooperative catalysis, carbonyl activation, biodiesel production, and other biorenewable applications.



Igor I. Slowing received his License degree in Chemistry with honors from San Carlos University in Guatemala in 1995. Shortly after graduating he was appointed as lecturer for organic chemistry in San Carlos and Del Valle Universities in Guatemala. In 2003 he left his teaching work to pursue a Ph.D. degree in Chemistry at Iowa State University. He is currently a Ph.D. candidate in organic chemistry working in Professor Victor Lin's research group. In year 2006 he obtained the Cotton-Uphaus award for early excellence in research. His research focuses on the chemical functionalization and structural control of mesoporous silica nanoparticles for their application in the intracellular delivery of drugs and biomolecules.



Dr. Brian G. Trewyn received his B.Sc. in Chemistry and Microbiology from the University of Wisconsin-La Crosse in 2000 and his Ph.D. from Iowa State University in the spring of 2006. In 2006, he received an Honorable Mention to the Zaffarano prize for graduate student research at Iowa State University. He joined the Department of Chemistry at Iowa State University as an assistant scientist and is currently working with Professor Victor Lin. His research centers on the development of controlled release and drug delivery devices using mesoporous silica nanomaterials, the effects of these materials on cellular development and propagation, and the difference in development between normal and cancer cells with the ultimate goal of controlling the release of antitumor drugs in cancer cells.



Supratim Giri was born in West Bengal, India. His career in chemistry began when he joined the undergraduate chemistry program in Presidency College, Calcutta in 1997. He graduated with a Bachelor of Science degree majoring in chemistry in 2000. In the same year he obtained admission to the Indian Institute of Technology, Kanpur for a two year M.Sc. program. After obtaining his M.Sc degree from IIT Kanpur, he joined the Ph.D. program in Iowa State University in fall 2002. In Iowa State University, he joined Professor Victor Lin's laboratory as a graduate assistant. His research interest is on the development of magnetic-nanoparticle-capped MSN materials for intracellular drug delivery.

The cylindrical mesopores of MSNs are arranged in a hexagonal structure, forming well-defined channels that are parallel to each other. These characteristics are typically observed by powder X-ray diffraction and transmission electron microscopy. Such unique features lead to high surface areas ($900\text{--}1500\text{ cm}^2\text{ g}^{-1}$), and large accessible pore volumes ($0.5\text{--}1.5\text{ cm}^3\text{ g}^{-1}$), usually measured by nitrogen sorption analysis. The simple polycondensation chemistry of silica allows for covalent attachment of a wide variety of functional groups, commercially available as substituted trialkoxy- or trichloro-silanes, either by co-condensation during the initial synthesis of the material, or by post-synthesis grafting. By using these different methods, the loading and location of functional groups can be controlled. Decoration of the mesoporous channels and/or the external particle surface of MSNs with various functional groups allows a wide range of manipulation of the surface properties of these materials for controlled release delivery and biosensing applications.

2.2. Cellular Uptake of MSNs

After our first study showing that MSNs were readily internalized by eukaryotic cells without detectable toxic effects^[7] in vitro (Fig. 2), further studies were performed in order to understand the mechanism of cellular uptake of these materials. Mou and co-workers^[12,13] have shown that the endocytosis of fluorescein-labelled MSNs by 3T3L1 and mesenchymal stem

cells was clathrin-mediated and that the particles were able to escape the endolysosomal vesicles. Our recent work with HeLa cancer cells has demonstrated that the uptake efficiency and the uptake mechanism of the MSNs can be manipulated by the surface functionalization of the nanoparticles.^[8] We observed that functionalization of the external surface of MSNs with groups for which cells do express specific receptors, like folic acid, notably enhances the uptake efficiency of the material by cells. We also found that the functionalization of the particles with groups that alter their ζ -potentials affects not only the efficiency of their internalization, but the uptake mechanism and the ability of the particles to escape the endolysosomal pathway. Hoekstra and co-workers have shown previously that non-phagocytic eukaryotic cells can endocytose latex beads up to 500 nm in size, and that the efficiency of uptake decreases with increasing particle size.^[14] They demonstrated that the highest efficiency was achieved with particles sized around 200 nm or smaller, whereas little, if any, uptake was observed for particles larger than 1 μm . Such information leads us to believe that MSNs can be efficiently employed as carriers for intracellular drug delivery as well as cell tracers and cytoplasmic biosensors.

2.3. Mesoporous Silica for Drug/Gene Delivery

It is well recognized that an efficient delivery system should have the capability to transport the desired guest molecules without any loss before reaching the targeted location. Upon reaching the destination, the system needs to be able to release the cargo in a controlled manner. Any premature release of guest molecules^[15] poses a challenging problem. For example, the delivery of many toxic antitumor drugs requires “zero release” before reaching the targeted cells or tissues.

However, the release mechanism of many current biodegradable polymer-based drug delivery systems relies on hydrolysis-induced erosion of the carrier structure. The release of matrix-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 that can trigger undesirable modifications of the structure and/or function of the encapsulated molecules, such as protein denaturation and aggregation. In contrast, surface functionalized mesoporous silica materials offer, as mentioned before, 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 for hosting molecules with various sizes, shapes, and functionalities.

2.3.1. Release Controlled by the Physical and Morphological Properties of the Materials

The primordial systems employing MSNs for drug delivery took advantage of the high surface areas and pore volumes of these silica materials. Guest molecules are simply adsorbed on the mesopore surface. No functional group acts as a gate to control the release of the loaded substances. The release was controlled either by the size or the morphology of the pores.

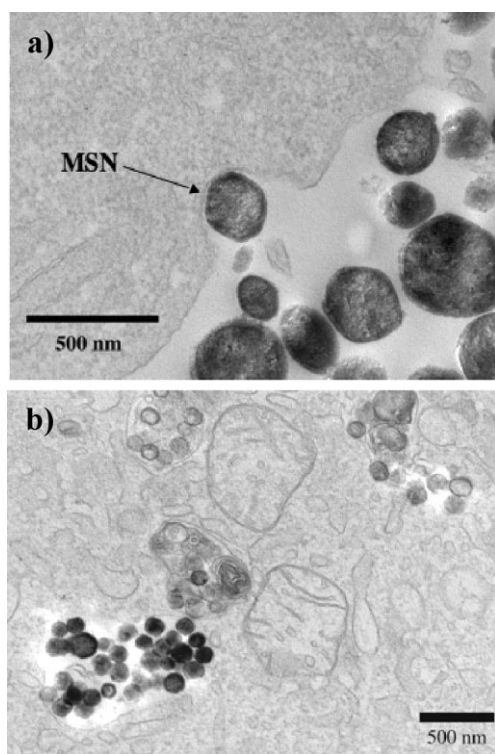


Figure 2. Transmission electron microscopy images of human cervical cancer (HeLa) cells set in contact with MSN. a) An MSN entering through the cell membrane. b) An MSN trapped in vesicles inside the cell [7].

One such system loaded ibuprofen into two MCM-41 materials with different pore sizes and studied the release in a simulated body fluid.^[16] The results showed that the MCM-41 type mesoporous structure with channel-like pores packed in a hexagonal fashion was able to load large quantities of drug molecules and release them over a relatively long period of time. No significant difference was observed between the release profiles of materials with different pore sizes.

Since the pore size appeared not to be a significant factor controlling the release of small drug molecules, we wondered if the pore and particle morphology of the mesoporous silica materials would have an impact on the controlled release properties. In order to address that question we developed a room-temperature ionic liquid (RTIL)-templated MSN system (RTIL-MSN) for controlled release of antimicrobial agents. We synthesized four different RTILs through previously published methods. Subsequently, a series of RTIL-MSN materials with various particle morphologies including spheres, ellipsoids, rods, and tubes, were prepared with these RTILs and utilized as templates.^[2] By changing the RTIL template, the pore morphologies were tuned from MCM-41-type hexagonal mesopores to rotational Moiré-type helical channels, and to wormhole-like porous structures. We investigated the controlled release of the pore-encapsulated ionic liquids by measuring the antibacterial effect on *Escherichia coli* K12.^[2]

First, we measured the antibacterial activities of two RTILs in homogeneous solutions, and compared them with those of the two RTIL-MSNs templated with the RTILs studied in the homogeneous solution, one spherical with hexagonal mesopores and the other tubular with wormhole mesopores, at 37 °C in *E. coli* cultures. Although both RTILs displayed very similar antibacterial activities, the spherical, hexagonal mesopore RTIL-MSN exhibited a superior (1000-fold) antibacterial activity to that of the tubular, wormhole RTIL-MSN. The result was attributed to the fact that the rate of RTIL release via diffusion from the parallel hexagonal channels would be faster than that of the disordered wormhole pores. This work categorically demonstrated the essential role of the morphology of mesoporous silica nanomaterials on controlled release behavior.^[2]

2.3.2. Release Controlled by Chemical Properties of the Materials

Disulfide-Reduction-Based Gating: As mentioned before, an efficient delivery system should have “zero premature release” characteristics. Therefore it is highly desirable to design delivery systems that can be activated by external stimuli in order to release the guest molecules at the sites of interest. To achieve this goal, our group developed a series of stimuli-responsive MSN-based controlled release delivery systems.^[10] As depicted in Figure 3, we first constructed a gated MSN system, where 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 untimely release, allowing it to meet the abovementioned paradigm of “zero premature release”. The release was trig-

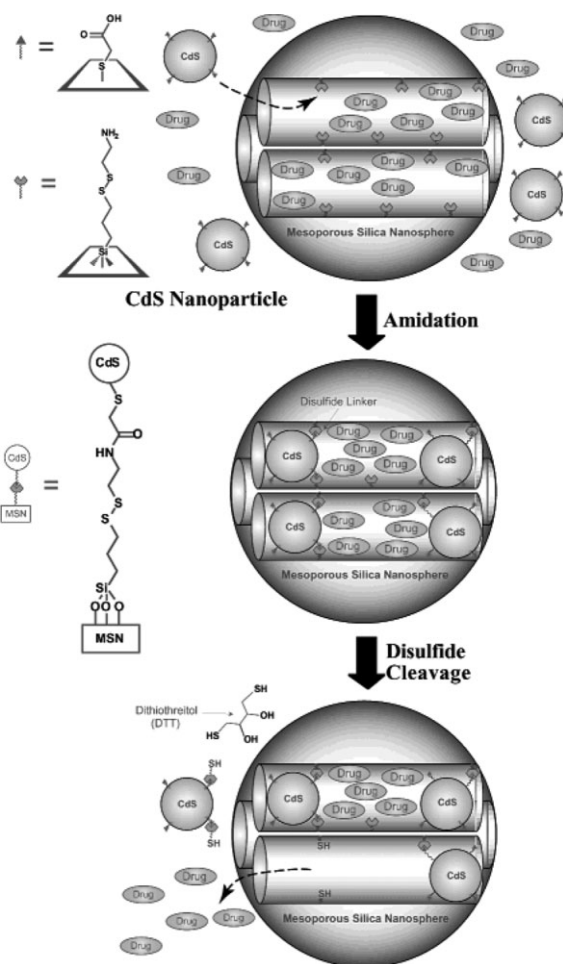


Figure 3. Schematic representation of the disulfide-link-based CdS MSN controlled release system. Reproduced with permission from [10]. Copyright 2003 American Chemical Society.

gered by exposing the capped MSNs to chemical stimulation^[10] that cleaves the disulfide linker, thereby removing the nanoparticle caps and releasing the pore-entrapped guest molecules.

To assemble this system, we first prepared a mercaptopropyl-derivatized mesoporous silica nanosphere material (thiol-MSN) by our reported co-condensation method.^[17] The surfactant-removed thiol-MSN material was treated with 2-(pyridyldisulphanyl)ethylamine^[18] to obtain a chemically labile disulfide-bond-containing, amine-functionalized MSN material (Linker-MSN; Fig. 3). The MCM-41 type of hexagonally packed mesoporous structure of this material was confirmed by transmission electron microscopy (TEM) and the nitrogen sorption analysis confirmed the expected large surface area and 2.3 nm wide mesopores.

The Linker-MSN material was then used as a chemically inert host to soak up aqueous solutions of vancomycin and adenosine 5-triphosphate (ATP). To block the mesopores, we synthesized mercaptoacetic acid coated CdS nanocrystals^[19] (2.0 nm size) as chemically removable caps. As shown in Figure 3, in the presence of drug molecules (ATP or vancomycin) the water-soluble CdS nanocrystals were covalently captured

by the Linker-MSN through the formation of an amide bond. The loading efficiency of vancomycin and ATP was found to be 83.9 and 30.3 mol %, respectively.

To establish the fact that the resulting disulfide linkages between the MSNs and the CdS nanoparticle caps are indeed *chemically labile* and can be cleaved by various disulfide reducing agents, we applied dithiothreitol (DTT) and mercaptoethanol (ME) as chemical stimuli to observe the release of vancomycin/ATP.^[10] High-performance liquid chromatography (HPLC) was used to monitor the released concentration of drug/neurotransmitter in the aqueous solution. Our CdS-capped MSN drug/neurotransmitter delivery system showed no premature release in phosphate buffer saline solution (PBS; 10 mM, pH 7.4) over a period of 12 h in the absence of a disulfide reducing agent, suggesting an excellent capping efficiency.^[10] Addition of disulfide-reducing agents, such as dithiothreitol (DTT) and mercaptoethanol (ME) triggered a rapid release of drug/neurotransmitter. As a result most (85 %) of the loaded molecules were released within the initial 24 h following similar diffusional kinetic profiles for both vancomycin and ATP. Furthermore, in both cases, the amount of drug released 24 h after the addition of DTT showed similar DTT concentration dependencies, indicating the rate of release is dictated by the rate of CdS cap release.^[10]

We also demonstrated the biocompatibility and the utility of our CdS capped MSN delivery systems in vitro with neuroglial cells (astrocytes).^[10] It is known that ATP promotes a receptor-mediated increase in intracellular calcium concentration in astrocytes,^[20] which is an important regulatory mechanism for many cooperative cell activities.^[21] Neuron-free astrocyte type-1 cells were cultured in the presence of surface immobilized CdS capped MSNs loaded with ATP molecules in their pores. Cells were also loaded with a Ca^{2+} chelating fluorescent dye (Fura-2). Upon perfusion application of mercaptoethanol (ME), we observed a pronounced increase in intracellular $[\text{Ca}^{2+}]_i$ (initial Ca concentration) by the color changes (increase of fluorescence) of the pseudo-color images of the cells detected using Attolfluor system with a Zeiss microscope. The application of ME triggered the release of ATP molecules from the MSNs and hence gave rise to the corresponding ATP receptor mediated increase in intracellular calcium concentration.^[10]

In another study, we demonstrated that the release properties of the pore-gated MSN material could be tuned by using different capping agents and trigger molecules. We investigated the release kinetics and mechanism of an ATP-loaded MSN system by applying a series of disulfide-reducing chemicals as triggers to uncage the mesopores. The concentration of the released ATP in solution was monitored by a well-established ATP-induced luciferin/luciferase chemiluminescence assay.^[22] Like in previous studies, ATP was encapsulated within the pores of thiol functionalized MSNs, which were subsequently capped with chemically removable caps, such as cadmium sulfide (CdS) nanoparticles and poly(amidoamine) dendrimers (PAMAM), via a disulfide linkage. Real-time ATP chemiluminescence imaging was used to monitor the release of ATP by detecting the ATP-induced chemiluminescence of firefly luciferase in situ.

Through the use of an intensified charge-coupled device (iCCD) camera attached to a microscope, real-time imaging of the release of ATP with a detection limit of 10^{-8} M and a subsecond time response was demonstrated. Before the addition of reducing agent, the MSNs generated no significant ATP release. In contrast, upon addition of 5 mM DTT, significant levels of ATP were released from the MSNs in approximately 2 min after stimulation. (Fig. 4) 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. Also, we compared the release of ATP from the PAMAM- and CdS-capped MSNs. The results indicated that the dendrimer-capped nanospheres exhibited a prolonged release of ATP with a gradual and plateau-like profile in contrast to the impulsive, fast-release profile of the CdS-capped MSN. It is plausible that the structurally flexible PAMAM could form a dense layer around the MSN and the disulfide linker cleavage would require the trigger molecules to diffuse through this layer of “soft” caps. In essence, we have demonstrated that the release profiles could indeed be regulated in a controllable fashion by choosing proper caps and triggers.^[22]

Photochemical-Based Gating: While we demonstrated the chemically stimulated controlled release from MSNs in an aqueous environment, Tanaka and co-workers reported on a coumarin-modified mesoporous silica material that can be used as a reversible photocontrolled release system for guests in organic solvents.^[23] 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 grafting and the pores were loaded with the steroid cholestane followed by a photodimerization of the coumarin to yield a cyclobutane product that led to the closing of the pores. The release of cholestane was done by exposing the system to UV light with a wavelength of 250 nm to cleave the cyclobutane ring of the coumarin dimer.^[23]

Redox-Based Gating: Apart from the chemical- and photostimuli, an efficient electrochemically controlled delivery system was developed by Zink, Stoddart, and co-workers.^[24] The investigators prepared a pseudorotaxane cap that could undergo conformational changes triggered by solution redox chemistry. The mesopores were loaded with $\text{Ir}(\text{ppy})_3$ (ppy: 2,2'-phenylpyridyl), a fluorescent molecule. The gate opening was stimulated by the addition of an external reducing agent, which caused the pseudorotaxane to disassemble. Following this publication, the investigators further developed the system by introducing reversibility in such a way that the nanovalve could be turned on and off by redox chemistry.^[25] The MCM-41 material was functionalized with a redox-controllable rotaxane with a moveable molecule, tetracationic cyclophane, which could be induced to move between two different recognition sites. In the ground state the cyclophane preferred to encircle a tetrathiafulvalene

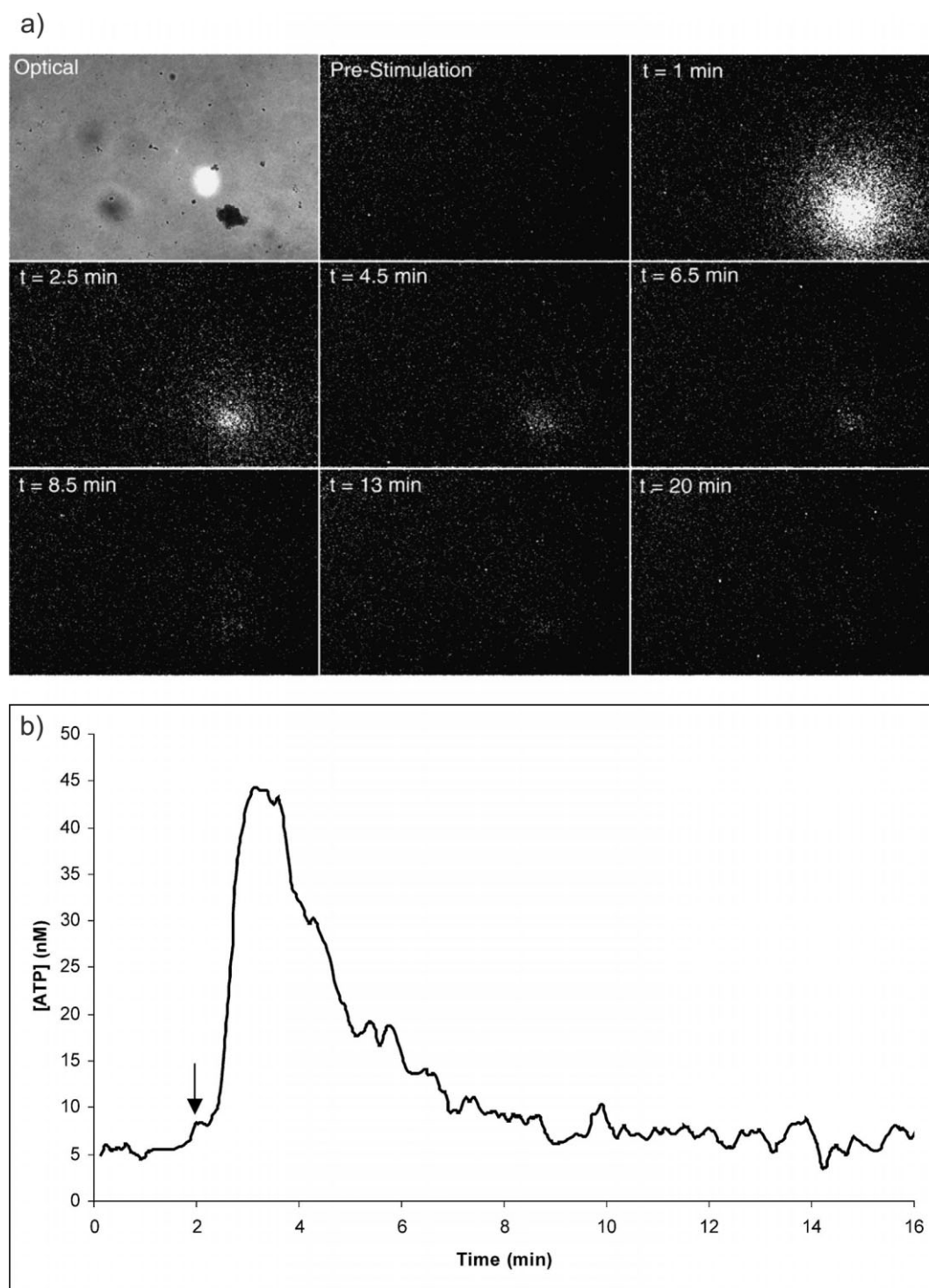


Figure 4. Chemiluminescence signals from ATP released from MSNs. a) The first frame is the optical image of a CdS capped MSN aggregate. The other frames show luminescence images depicting release of encapsulated ATP from the nanospheres stimulated with 5 mM DTT. b) Time course for release of ATP from (a). Arrow indicates point of stimulation. Reproduced with permission from [22]. Copyright 2005 Society for Applied Spectroscopy.

(TTF), located near the pore end of the rotaxane thereby capping the pore. Upon a two-electron oxidation of the TTF group with $\text{Fe}(\text{ClO}_4)_3$ (to give TTF^{2+}) the cyclophane-TTF interaction was destabilized through Coulombic repulsion and the cyclophane traveled to the dioxynaphthalene (DNP) group,

which was separated from the TTF by an oligoethylene glycol chain. This movement of the cyclophane to the DNP uncapped the pore and allowed for the free movement of guest molecules. Reduction of the TTF^{2+} to TTF by ascorbic acid provoked the return of the cyclophane to the TTF, thus re-

capping the pore. This was the first report of a reversibly operating nanovalve that could be turned on and off by redox chemistry.

2.3.3. Additional Properties to Improve Controlled Delivery

Besides the controlled release capabilities, the rich chemistry of silica allows for many other possible manipulations to yield more complex systems, capable of performing more elaborate tasks. Herein, we review two such refinements.

Magnetic Manipulation: 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.^[26] In addition to the stimuli-response-controlled release property, we demonstrated that the magnetic-nanoparticle-capped MSN could be led to any specific site of interest by applying an external magnetic field. The system consisted of rod-shaped MSNs functionalized with 3-(propyldisulfanyl)propionic acid to give “Linker-MSNs” that had an average particle size of $200\text{ nm} \times 80\text{ nm}$ (length \times width) and an average pore diameter of 3.0 nm . As a proof of principle, fluorescein was used as the guest molecule. The openings of the mesopores of the fluorescein-loaded Linker-MSNs were covalently capped in situ through an amidation of the 3-(propyldisulfanyl)propionic acid functional groups bound at the pore surface with 3-aminopropyltriethoxysilyl-functionalized superparamagnetic iron oxide (APTS- Fe_3O_4) nanoparticles.^[26]

To demonstrate that the entire Fe_3O_4 -capped MSN (Magnet-MSN) carrier system could be magnetically directed to a target site where the controlled release is intended to take place, two cuvettes were charged with fluorescein-loaded Magnet-MSNs in PBS solution (100 mM , $\text{pH } 7.4$). Both cuvettes were held against the tips of two magnets. As illustrated in Figure 5a, the Magnet-MSNs were attracted to the walls of the cuvettes closest to the tips of magnets.^[26] DTT was added to one of the two cuvettes (left) for the release experiment, while the other cuvette (right) served as a control. After 72 h (Fig. 5b), green fluorescence was clearly observed in the solution to which DTT was added whereas no fluorescence could be observed from the control solution.^[26] These results indicated that fluorescein molecules were indeed released from the Magnet-MSNs by the introduction of the disulfide-reducing trigger.

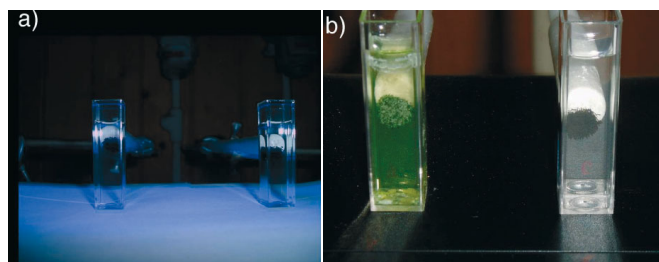


Figure 5. Two cuvettes containing fluorescein-loaded Magnet-MSN dispersed in PBS. DTT was added to the left cuvette, and the pictures were taken a) before addition of DTT, b) 72 h after the addition of DTT. Reproduced from [26].

The kinetics of the controlled release of fluorescein by cell-produced antioxidant and disulfide reducing agent such as dihydrolipoic acid (DHLA) and DTT, respectively showed similar behavior as in the CdS capped MSN case.^[10]

To investigate the endocytosis and biocompatibility of this system, HeLa (human cervical cancer) cells were incubated overnight with Magnet-MSNs to allow the endocytosis of Magnet-MSNs.^[26] The cells that took up Magnet-MSNs were magnetically separated from those that did not. The isolated cells were treated with nucleus staining blue-fluorescent dye 4',6-diamidino-2-phenylindole (DAPI) dye in PBS solution (100 mM , $\text{pH } 7.4$) and placed in a cuvette. When a magnet was moved along the side of the cuvette, the blue-fluorescent HeLa cells were clearly seen to move across the cuvette toward the magnet (Fig. 6a–c) indicating that the Magnet-MSNs were indeed endocytosed by HeLa cells.^[26] To further confirm the endocytosis of Magnet-MSNs, these HeLa cells were examined by confocal fluorescence microscopy. The observed green fluorescence (Fig. 6d) strongly indicated that the mesopore-encapsulated fluorescein molecules were released inside the cells. As previously reported, cancer cell lines express significant amounts of DHLA,^[27] we attributed the efficient intracellular release of fluorescein to the high intracellular concentration of DHLA serving as the trigger.^[26]

Double Delivery Systems: As mentioned above, we demonstrated that PAMAM dendrimers could be used as caps on the pores of MSNs. In addition to serving as a cap, PAMAM could also be used to modify the surface charge properties of MSNs for other controlled release applications, such as gene transfer. To realize this goal, we reported the synthesis of a second generation (G2) PAMAM dendrimer-capped MSN (G2-MSN). This material was used to complex with plasmid DNA (pEGFP-C1) that encoded for enhanced green fluorescent protein (Fig. 7).^[7] We showed that the G2-MSN could bind with plasmid DNA to form stable DNA–MSN complexes at weight ratios larger than $1:5$. By introducing varying ratios of restriction endonucleases to the complex, we also demonstrated that this DNA–MSN complex protects the plasmid DNA from enzymatic digestion. We investigated the gene transfection efficacy, uptake mechanism, and biocompatibility of the G2-MSN with astrocytes, HeLa, and Chinese hamster ovarian (CHO) cells.^[7] Transfection efficacy was demonstrated by incubating the DNA–MSN complex with cells and measuring the expression of green fluorescent protein (GFP) by flow cytometry and the results were compared to other commercially available transfection reagents. We concluded that our material had better transfection efficiency than all tested commercial transfection reagents. Fluorescence confocal microscopy clearly illustrated that the G2-MSN, entered the cytoplasm of neural glial cells. Transmission electron microscopy images of post-transfection cells also provided direct evidence that a large number of G2-MSN–DNA complexes were endocytosed by all three cell types.^[7] Many subcellular organelles, such as mitochondria and Golgi, were observed with MSNs nearby, which strongly suggested that the MSNs were not cytotoxic in vitro.

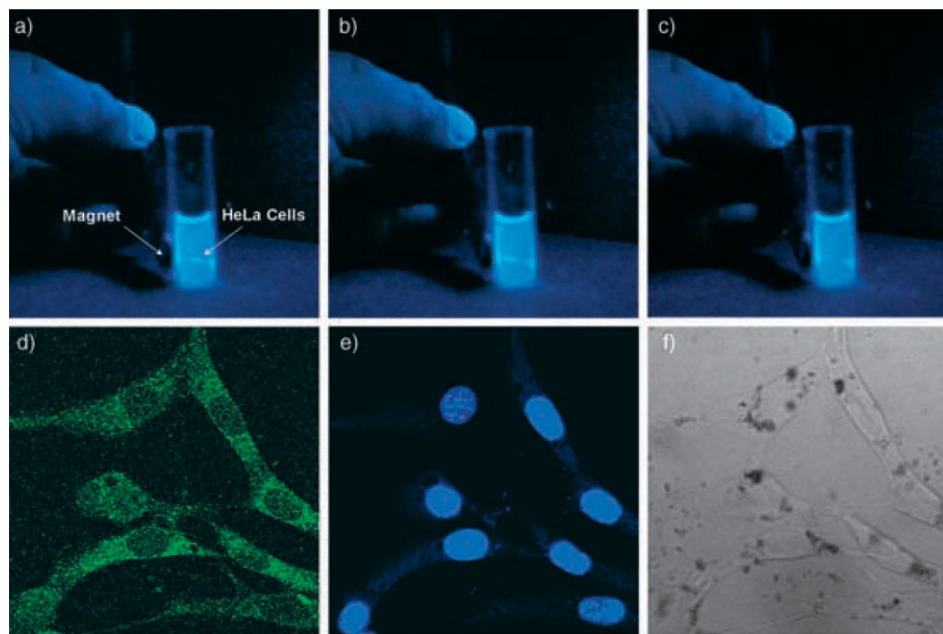


Figure 6. a–c) HeLa cells that have endocytosed fluorescein-loaded Magnet-MSN are suspended in PBS and placed in a cuvette. It can be observed that the cells are being pulled from one side of the cuvette to the other when attracted by a magnet. d–f) Confocal micrographs of HeLa cells after 10 h incubation with fluorescein-loaded Magnet-MSN: d) fluorescein emission upon excitation at 494 nm, e) DAPI-stained nuclei observed upon excitation at 356 nm, f) pseudo-brightfield image of the cells, the dark spots correspond to aggregated Magnet-MSN. Reproduced from [26].

2.3.4. Other Silica Containing Drug Delivery Systems

One common approach to modify characteristics, such as solubility, drug release capability, adsorption of drugs, and specific targeting of silica-based drug delivery systems is to combine the properties of silica with those of other organic or inorganic materials. The functionalization of a silica surface with organic groups is a relatively simple process which can be achieved either by co-condensation during its synthesis or by post-synthesis grafting with a wide variety of commercially available substituted trialkoxy- or trichloro-silanes. A diversity of organic functionalities ranging from the relatively simple alkyl^[28] or amino^[29] groups to species as complex as peptides or antibodies have been placed on the surface of silica.

Another approach for combining the properties of silica with those of other materials is the formation of core/shell hybrid particles. Several core materials, such as gelatin^[30] and magnetite,^[31–33] have been coated with a layer of porous silica. The drugs to be delivered were stored either in the core or in the pores of the outer layer of silica. For example, Shi and co-workers synthesized a core/shell material by coating a Fe_3O_4 nanoparticle with a layer of mesoporous silica.^[33] The mesoporous silica layer thickness appeared to be about 50 nm with a surface area of $283 \text{ m}^2 \text{ g}^{-1}$ and the average diameter of randomly arranged pores was found to be 3.8 nm. The loading of ibuprofen within the mesoporous silica layer was determined to be 12 wt %, while 87 % of the loaded amount was later released in simulated body fluid over a period of 70 h.

More complex multi-layered systems have been prepared by incorporating metals within a biodegradable layer, like a gold

nanoparticle-doped gelatin core to allow the tracking of the particles by luminescence,^[34] or cobalt in alginate/poly-L-lysine to allow for magnetic manipulation of the particles.^[35] Polymeric nanocapsules^[36] or single crystals surrounded by a porous silica shell^[37] have also been employed. Furthermore, this kind of core/shell strategy has been recently employed to provide stability to micellar nanoparticles, where the outer silica shell prevents the micelles from breaking down upon dilution.^[38]

The combination of the core/shell design along with the surface functionalization of silica has been performed. For example, the surface of cobalt ferrite/silica nanoparticles was functionalized with polyethyleneglycol to enhance the biocompatibility of the material.^[36] Even more complex architectures have been achieved at the micrometer scale by sequential assembly of silica and polymeric nanoparticles.^[39] Another alternative of incorporating other nanomaterials to MSNs is to fuse a silica-coated nanoparticle with the surface silicate groups of MSNs. For example, Mou and co-workers covalently fused the amorphous silica layer of a silica-shell/magnetite core nanocomposite material with a fluorescein isothiocyanate (FITC) labelled MSN.^[40]

In contrast to these silica-based core/shell nanoassemblies, the utilization of silica particles with different porous structures as carriers for drug delivery has been exploited. For example, hollow silica micro-^[41] and nanocapsules^[42,43] containing magnetic nanoparticles inside have proven to be able to absorb drugs that can later be released. Also, silica nano-“test tubes” that can be loaded with drugs and capped with nanoparticles have been reported recently.^[44]

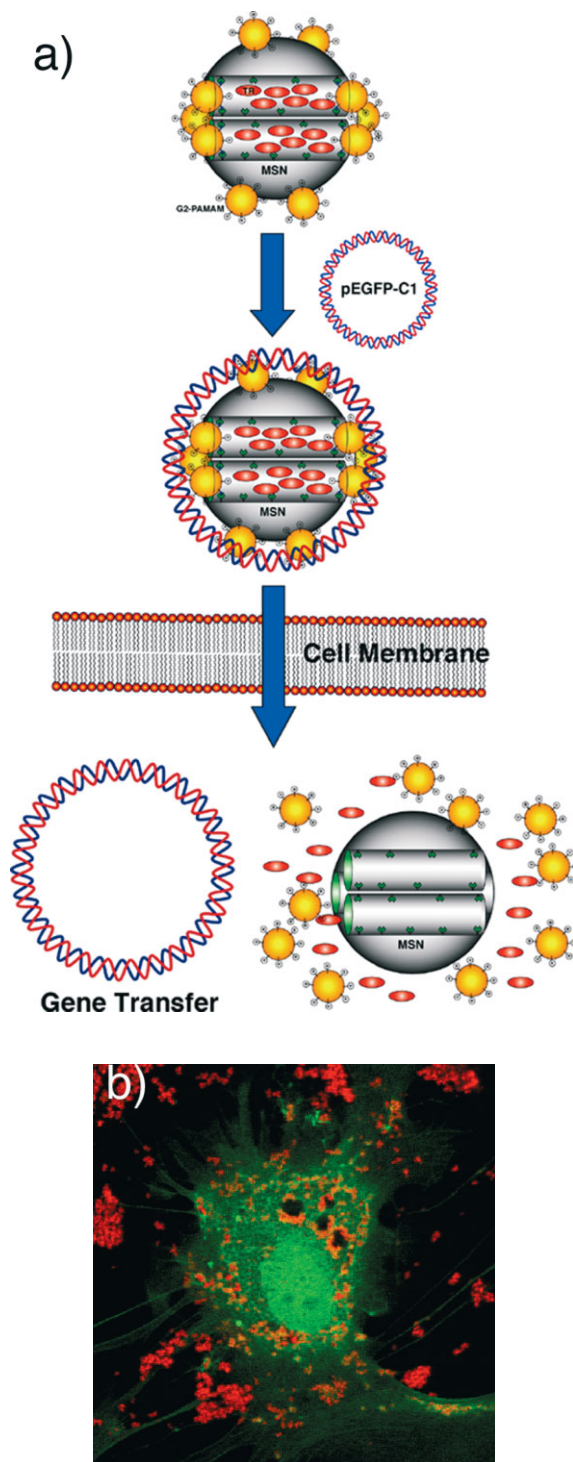


Figure 7. a) Scheme of the DNA-PAMAM coated, Texas Red-loaded MSN (G2-MSN) as a double delivery system. b) Confocal fluorescence microscopy image of Texas Red-loaded G2-MSNs inside a GFP-transfected rat neural glia cell (astrocyte) [7]. Reproduced with permission from [7]. Copyright 2004 American Chemical Society.

2.4. Mesoporous Silica for Biosensing and Cell Tracing

Because of their size and versatile chemistry, nanomaterials are gaining attention as powerful tools for biosensing applica-

tions.^[45–47] The unique surface properties and the small particle sizes of these nanoparticle-based sensor systems allow for the detection of analytes within individual cells both in vivo and in vitro.^[48] In particular, nanoparticles offer several advantages over homogeneous fluorescent and staining dyes. Unlike stains, nanoparticles do not suffer from fluorescent self-quenching and other diffusion-related problems.^[49] Furthermore, the ability to functionalize the surface of nanoparticles with large quantities of cell-recognition or other site-directing compounds makes them ideal agents for cell tracing.^[50–55]

In contrast to other solid nanoparticle biosensors, micro- and mesoporous silica provide two important unique advantages: 1) *High porosity*. The large surface areas and pore volumes allow the encapsulation/immobilization of large amounts of sensing molecules per particle for fast response times and low detection limits. 2) *Optical transparency*. This unique feature permits optical detection through layers of the material itself. Because of these advantages, different types of porous-silica-based materials have been employed for building biosensors. One of them is microporous silicate glass obtained by sol-gel processing.^[56–58] These nanoparticles have long been used for the creation of biosensors using drugs, enzymes, antibodies,^[48,53,54,59] and DNA aptamers as recognition elements.^[52] However, the pores of sol-gel silica lack a high degree of order, which results in wormhole-like porous structure, and consequently slow diffusion of the analytes to the sensing sites. In contrast to microporous silica, mesoporous-silica nanomaterials have much larger surface areas and pore diameters. These features allow for the detection of larger analyte molecules and the incorporation of a great amount of receptors/sensors into the porous matrix to achieve a better detection limit. A faster diffusion of the analytes through the mesoscale pores of these materials to the sensor sites also permits a shorter response time.

Many specific molecular receptors, such as proteins, have been used to achieve analyte selectivity in many mesoporous silica-based biosensors. For example, a glucose oxidase- and horseradish peroxidase-loaded mesoporous silica material has been used as a selective sensor for glucose detection.^[60] In another study, myoglobin and hemoglobin were immobilized in mesoporous silica-modified electrodes to be used as a sensor for H_2O_2 and NO_2^- .^[61,62] Apparently, the reason for using enzymes and proteins as the recognition unit is their high specificity for substrates. However, these biomolecules lack long-term stability and are relatively expensive. To avoid these disadvantages, while maintaining high analyte selectivity, we and others have developed a different approach. Unlike the molecular imprinting approach, we attained the selectivity not by synthesizing size or shape selective recognition receptors, but by controlling the diffusional penetration of analytes into the surface-functionalized mesopores. As described below, new mesoporous-silica-based selective sensory systems by using this strategy have been reported.

To introduce a signal transduction mechanism to the mesoporous silica nanoparticle material, we first prepared *o*-phthalic hemithioacetal (OPTA) functionalized MSNs that would fluoresce upon reaction with primary amines.^[17] This OPTA-MSN material was further derivatized into a selective sensory

system that is able to distinguish between two similarly sized biogenic molecules, dopamine and glucosamine. We grafted propyl-, phenyl-, and pentafluorophenyl groups to the OPTA-MSNs to manipulate the properties, such as hydrophobicity, of the mesopores. The largest difference in the fluorescence responses to the two analytes was observed in the case of pentafluorophenyl grafted OPTA-MSN. This difference was attributed to the π - π acceptor-donor interactions occurring between the aromatic ring of the dopamine and the pentafluorophenyl groups. In this way, we demonstrated that secondary noncovalent interactions can play a significant role in the improvement of the selectivity of a biosensor.^[17]

To further develop a fluorescent sensor suitable to distinguish between several structurally similar neurotransmitters, such as dopamine, tyrosine, and glutamic acid, we exploited the ability of selective functionalization of the exterior and interior surfaces of MSNs.^[63] The molecular recognition system we have developed was synthesized by first functionalizing the pores with OPTA groups. The exterior surface of OPTA-MSN was selectively functionalized with poly(lactic acid) as depicted in Figure 8a.^[63] The layer of poly(lactic acid) acted as a gate-keeper controlling the diffusion of neurotransmitters to the inside of the pores where they were able to react with the surface-anchored OPTA groups. We found that several natural neurotransmitters with different pK_a values, such as dopamine and glutamic acid, indeed exhibited different fluorescence responses while interacting with the OPTA-MSN, indicating that it is possible to achieve a high degree of selectivity by controlling the diffusional penetration of analytes into the mesopores.^[63] We demonstrated that the electrostatic, hydrogen bonding, and dipolar interactions between these neurotransmitters and the poly-L-lactic acid (PLA) layer in pH 7.4 buffer were the dominating factors in regulating the penetration of the neurotransmitters into the mesopores (Fig. 8b). In fact, at pH 7.4, dopamine is positively charged ($pI = 9.7$), whereas tyrosine and glutamic acid are negatively charged ($pI < 7.4$). It has been reported that PLA has an isoelectric point (pI) lower than 3.0 and it is negatively charged in pH 7.4 buffer. In the case of those neurotransmitters (dopamine) with electrostatic attraction for PLA, it is understandable why there would be a faster kinetics of entering the pores than those of the other neurotransmitters, such as glutamic acid, with repulsive forces.^[63]

Another biosensor that was synthesized and studied by Martínez and co-workers involved aminomethylantracene groups grafted onto MSN and used for the recognition and detection of anions (Fig. 9). The bulkiness of the grafted group combined with the steric restrictions provided by the pore size of the material led to the ability of the material to respond in different degrees to ATP, ADP, and AMP (adenosine tri-, di-, and monophosphate, respectively). ATP was able to quench the fluorescence of the material to a larger degree than the other two species, and small anions like chloride, bromide, and phosphate did not produce any response of the sensor. They compared the sensitivity towards ATP of the aminomethylantracene MSNs with aminomethylantracene grafted on fumed silica, and observed a 100-fold improved sensitivity for the MSN-based matrix.^[64,65]

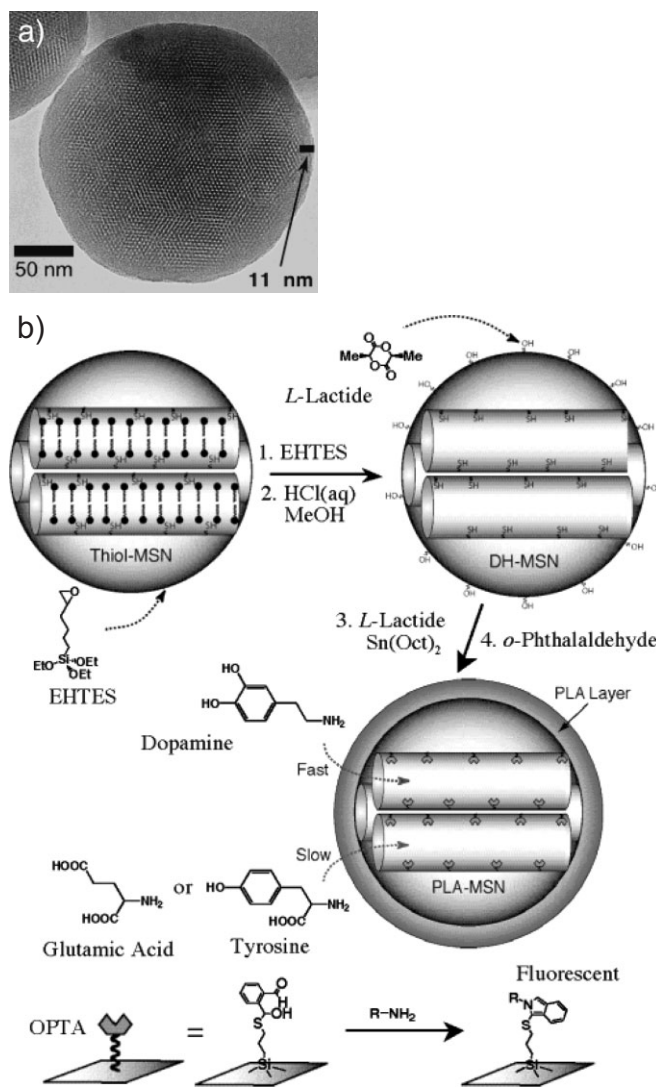


Figure 8. a) TEM image of a OPTA-functionalized, poly(lactic acid) coated MSN (PLA-MSN); an 11 nm thick layer of the polymer can be clearly observed surrounding the MSN core. b) Schematic representation of PLA-MSN for selective detection of dopamine against glutamic acid or tyrosine. Reproduced with permission from [63]. Copyright 2004 American Chemical Society.

The abovementioned core/shell design has also been employed for cell tracing. One such nanocomposites was reported by Lee and co-workers.^[50] They used cobalt ferrite nanoparticles (9 nm in diameter) as magnetic cores, which were further stabilized by polyvinylpyrrolidone (PVP) for dispersion in ethanol. A base-catalyzed condensation reaction using tetraethylorthosilicate (TEOS) and a dye-modified silane compound was carried out on the surface of the PVP-stabilized cobalt ferrites to obtain a fluorescent-dye-functionalized amorphous-silica-shell-coated magnetic-core nanocomposite. The dye-modified silane compound was synthesized by reacting either FITC or rhodamine B isothiocyanate (RITC) with 3-aminopropyltriethoxysilane (APS). In order to enhance the biocompatibility of these composites, a layer of poly(ethylene glycol) (PEG) was covalently attached on the surface of a silica shell. Attach-

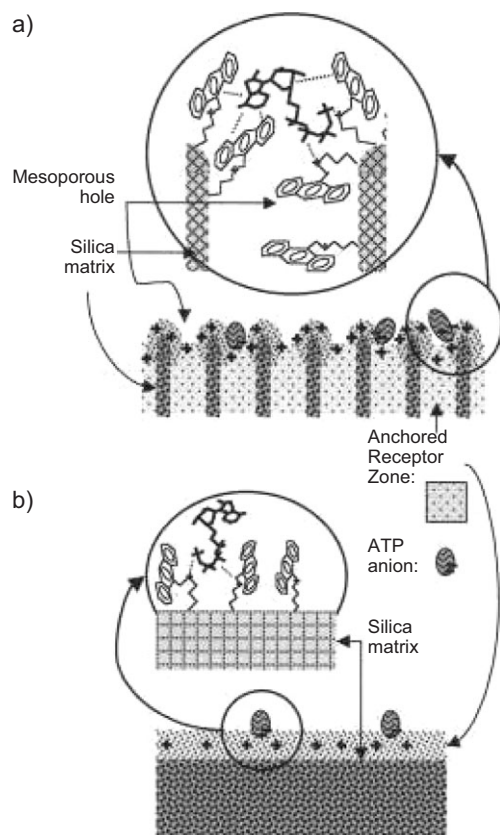


Figure 9. a) Aminomethylanthracene functionalized MSNs for detection of ATP by fluorescence quenching. The MSN system proved to be more sensitive than b) aminomethylanthracene functionalized fumed silica. Reproduced from [64].

ment of a PEG layer had no significant effect on the size of those core/shell nanocomposites and the authors selected 50 nm average-sized organic-dye-labelled Co ferrite/silica (core/shell) nanocomposites for incorporation studies in the cells. Breast cancer cells (MCF-7), after 24 h of growth in the media containing both the PEG modified RITC-core/shell and unmodified nanocomposites (RITC-core/shell without a PEG layer) showed enhanced incorporation of PEG modified systems compared to the unmodified ones, as confirmed by confocal laser scanning microscopy (CLSM) results. When incorporated with a mixture of PEG-RITC-core/shell and PEG-FITC-core/shell particles; the breast cancer cells gave off emission signals at two different wavelengths, orange light from RITC and green light from FITC. The authors also demonstrated by confocal microscopy imaging that the nanocomposites were actually present in the cell cytoplasm without affecting the living nucleus. On a time scale experiment, they figured out that the breast cancer cells were saturated with dye-labeled core-shell nanoparticles within first 30 min of the uptake process. They believed that the internalization process took place via the normal endocytosis mechanism as also observed in the case of mammalian lung normal cells (NL-20) and lung cancer cells (A-549). An uptake quantity of 10^5 nanocomposites per cell was observed by inductively coupled plasma atomic emis-

sion spectrometry (ICP-AES) measurements. This type of functional core/shell nanocomposites are expected to find applications in magnetic cell separation, biological labeling, and detection.

3. Outlook

In conclusion, we have highlighted several recent research breakthroughs on the design of functional mesoporous silica nanomaterials for biological applications. These systems are sufficiently intelligent to respond to specific stimuli and exhibit high selectivity for the detection of biomolecules. While these developments are exciting, several key challenges need to be overcome to answer the following important questions to further advance the applications of these structurally defined nanomaterials. 1) In addition to pharmaceutical drugs and DNAs, can other biogenic molecules, such as enzymes/proteins, be successfully delivered into cells by MSNs? 2) Even though the intracellular delivery of several drugs and DNAs in animal cells by MSNs has been achieved, can these cargoes be also introduced into living plant cells? One must consider that the plant cells possess a harder and more rigid outer layer: the cell wall. 3) What other mechanisms can be introduced to MSNs for different controlled-release applications? 4) What is the long-term biocompatibility of the MSN-based nanosystems in vitro and in vivo? 5) Can one control the circulation properties of these materials in vivo? We envision that many new synthetic methods and clever designs will be developed to bring even more functions to MSNs and provide answers to the questions above. New generations of powerful and smart nanodevices would be introduced to this burgeoning field of research.

Received: December 13, 2006

Revised: January 26, 2007

Published online: April 20, 2007

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