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Development of mesoporous silica nanomaterials as a vehicle for anticancer drug delivery

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Abstract

The development of delivery vehicles that would carry therapeutic agents selectively to cancer cells has become an important focus in biomedical research. Nanoparticles have received much attention because the advances made in this field have resulted in multiple biocompatible materials. In particular, mesoporous silica nanoparticles (MSNs) offer a solid framework with porous structure and high surface area that allows for the attachment of different functional groups. In this article we discuss the different surface modifications made to MSNs that have allowed for the construction of targeted nanoparticles to enhance accumulation and uptake in target sites, the incorporation of nanomachines for controlled cargo release and the combination with superparamagnetic metals for MRI cell labeling. We also discuss biocompatibility, biodistribution and drug-delivery efficacy of MSNs. Finally, we mention the construction of multifunctional nanoparticles that combine all of the previously examined nanoparticle modifications.

Nanotechnology can be defined as the engineering of functional systems at the molecular scale. The theoretical basis for this field was suggested by Richard Feynman in the 1960s with his idea that microtechnology could be further reduced in size to the molecular level [1]. In the 1980s, K Eric Drexler established fundamental principles in molecular design, protein engineering, and productive nanosystems [2], which was the beginning of nanotechnology. Since then, major advances in the field have led to the development of nanoscale devices that have the potential to revolutionize medicine. One area in particular where nanotechnology can have a great impact is drug delivery. The limitations of traditional drug therapy have increased the necessity to develop better ways to deliver therapeutics to target sites and to control the release of drugs to increase tumor-killing efficacy. Nanotechnology has provided novel nanomaterials that are capable of encapsulating various molecules and are able to be endocytosed by cells. Nanoscale devices composed of polymers, lipids, iron oxide nanoparticles and **mesoporous silica nanoparticles** (MSNs) and so on, have been shown to be efficient delivery vehicles but improvements can be made to increase nanoparticle uptake and control drug release. Because of their relative stability, which enables a variety of chemical modifications, MSNs have shown particular promise in developing nanoparticles equipped with a variety of **nanomachines**, including nanovalves and nanoimpellers. In addition, studies are being

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carried out to investigate the efficacy of mesoporous silica materials for the oral delivery of small molecules [3 – 6], which would increase the wide variety of applications of the mesoporous silica to medicine. In this article, we will describe recent advances in the development of MSNs for drug delivery by describing some examples and discussing advantages of using these nanomaterials compared with other delivery platforms.

Mesoporous silica nanoparticles

MCM-41 silica is a solid material with a porous structure capable of encapsulating relatively high quantities of bioactive molecules. The synthesis of particles ranging from micrometer to nanometer size has been extensively studied by various groups. These materials are relatively stable to heat, pH and mechanical stress. The nanoparticles can protect the cargo until it gets released inside the cell. Studies regarding the stability of mesoporous silica materials have provided promising results of the thermal, hydrothermal and mechanical stability of these materials [6] but further investigation is needed. The synthesis of these nanoparticles can be modified to control the diameter of the pores from 2 to 6 nm allowing for the loading of different types of cargo [7]. The large surface area and the fact that there are two functional surfaces, the internal (pores) and external surface, allows for differential functionalization with different moieties. MSNs have a honeycomb, 2D hexagonal porous structure with cylindrical channels running from one end of the nanoparticle to the other (Figure 1). The lack of interconnectivity between the channels allows individual pores to serve as independent reservoirs for molecule encapsulation.

Surface modifications

Surface modifications to alter surface charges have been carried out. For example, attachment of methyl phosphonate has been used in a number of cases to reduce nanoparticle aggregation and increase stability in aqueous solution [8]. Unmodified MSNs aggregate due to interparticle hydrogen-bonding interactions between amine groups and the silanols. The addition of the inert functional group methyl phosphonate, causes the ζ potential of nanoparticles to become more highly negative resulting in strong electrostatic repulsion [9].

Active targeting of MSNs to cancer cells can maximize the uptake of particles and decrease the required dosage of the drug-delivery system, but it requires the functionalization of the outer surface of the nanoparticles in order to conjugate different targeting moieties. The surface modification depends on the targeting moiety being used. The successful targeting of MSNs to cancer cells using folate [10, 11], mannose [12], transferrin (Tf) and Argenine-Glycine-Aspartic acid (RGD) peptides [13] has been reported by various groups. The attachment of folate is achieved by forming an amide linkage between the carboxyl group of folate and the amine group of aminopropyltriethoxysilane (APTS) and then grafting this complex onto the surface of the nanoparticles [11]. The overexpression of the folate receptor by cancer cells enhances the uptake of the folate-linked nanoparticles resulting in increased therapeutic delivery. The Tf receptor is also overexpressed by certain cancer cells and the attachment of Tf onto the surface of MSNs can be achieved by first phosphonate coating the particles and then condensing onto the non-phosphonated portions (the pore openings) the linker 3-glysidoxypropyltrimethoxysilane (3-GPTMS) (Figure 2). This linker reacts strongly with amines, allowing for the coupling of Tf.

In some cases the attachment of the whole protein is not necessary as a peptide could be sufficient to target MSNs to cancer cells, for example, the RGD peptide can bind to integrin αvβ3– highly expressed on primary tumors and metastatic cancer cells – with high affinity. Since RGD can be modified synthetically to facilitate coupling onto the MSNs surface, a five amino acid analog of the peptide, cyclo(Arg-Gly-Asp-D-Phe-Cys) (c[RGDfC]), which

has similar binding capabilities to the target receptor can be used [14]. The c[RGDfC] peptide has a free thiol in the cysteine residue that allows for particle attachment via a disulfide bond to 3-mercaptopropyltrimethoxysilane-modified MSNs.

Cancer cells have a high metabolic rate and are constantly taking up nutrients; this makes it possible to increase the uptake of nanoparticles by attaching sugars, such as mannose, onto their surface [12]. The attachment of mannose onto MSNs can be achieved by functionalizing the surface of the nanoparticles with APTS and then reacting this group with ethyl squaratate-functionalized mannose. Using this approach, the uptake of mannosylated MSNs, was shown to be greatly increased in the breast cancer cell lines, MDA-MB 231 and MCF 7, and the colorectal cancer cell line HCT-116, all of which have high expression of the mannose receptor [15].

Cellular uptake & exocytosis

The application of MSNs in medicine is based on the discovery that these nanoparticles are readily endocytosed by cells. Our group was one of the first to show that MSNs enter cells in an energy-dependent manner [16]. The mechanism of cellular uptake appears to be mediated by an active endocytosis pathway, as MSNs endocytosis is inhibited by decrease in temperature to 4°C, incubation with metabolic inhibitors as well as by the disruption of microtubules. Once inside the cells, the MSNs co-localize mainly to the lysosomes as evidenced by fluorescent microscopy detection of fluorescently labeled MSNs and staining of lysosomes with LAMP-1 antibody. However, in some cell types, such as mesenchymal stem cells, a population of MSNs that do not co-localize with lysosomes can be detected in the mitochondria [17].

In the above studies, cytotoxicity was also examined. MSNs ranging from 50 to 300 nm in diameter display no major cytotoxicity [7, 8] at concentrations below 100 μg/ml, making them particularly good candidates for drug delivery. It is important to note that the cellular uptake of the nanoparticles depends on their size and shape as nanoparticles greater than 150 nm are not endocytosed as efficiently as particles with a diameter of 110 nm or lower [18]. A study by Huang *et al.* demonstrated that the shape of the nanoparticles plays an important role in the cellular uptake of the material [19]. Nanoparticles with a long rod shape were internalized by cells more efficiently than short rod-shaped and spherical-shaped MSNs.

The uptake of MSNs is important for cargo delivery to the cells but it is also important to determine what happens to the nanoparticles after cargo release; do they accumulate inside the cells or do they exit the cells? A recent study by Slowing *et al.* found that MSNs can undergo cell-to-cell transfer and be exocytosed by cells. Furthermore, they found that when the nanoparticles exit the cells they are coated by proteins on their surface [20]. We have also observed exocytosis of MSNs from human cancer cell lines. By 24 h, over 80% of the MSNs are exocytosed from a lung cancer cell line [Yanes RE, Tamanoi F, unpublished data]. However, efficacy of exocytosis differs among different cancer cell lines. The cellular mechanism for MSNs exocytosis has not been identified and should be the focus of further investigation.

Biocompatibility & biodistribution

The introduction of inorganic materials into an organism requires extensive studies to determine the **biocompatibility** and **biodistribution** of the material. Of major concern is the safety of these materials. An initial study by Hudson *et al.* suggested that intraperitoneal or intravenous injections were lethal to mice but subcutaneous injections displayed no toxicity [21]. However, it should be noted that these studies used very high dosage (1.2 g/kg) and large particle size. The amount to be administered should be limited to what is needed to

deliver effective concentration of drugs. There should also be careful consideration to surface properties, shape and size of MSNs, as they appear to have impact on their behavior. Here, we discuss some examples addressing these issues.

Our group carried out, for the first time, a dose-escalating study of phosphonate–MSNs (100–130 nm) to examine biocompatibility of the material and it was shown that repeated doses of phosphonated MSNs ranging from 3 to 50 mg/kg in mice did not result in significant toxicity as determined by body weight, histology, serological and hematological studies [22]. A short-term treatment of five injections in 14 days and a long-term treatment of 18 injections in 68 days were performed with no major adverse effects detected. Therefore, phosphonate-modified MSNs appear to be biocompatible when administered at doses at or below 50 mg/kg. The determination of the treatment concentration that results in efficient drug delivery with minimal toxicity will provide the parameters necessary to obtain optimal clinical results with reduced adverse effects. Further investigation is needed to study the effects that the different surface modifications and particle size have on the biocompatibility of the nanoparticles as these modifications could affect the interaction of the nanoparticles with different organs and tissues.

Biodistribution of MSNs in animals is another important issue that needs to be studied. MSNs demonstrate a preferential accumulation in tumors, which could be attributed to the enhanced permeability retention (EPR) effect. In our study, MSNs accumulated in tumors at concentrations ranging from 45 ng/mg (4 h post-injection) to 110 ng/mg (24 h post-injection) before decreasing to 65 ng/mg (48 h post-injection) (Figure 3) [22]. The organs with the highest accumulation of nanoparticles were the kidney and lungs. The rest of the organs, including liver, heart, intestines and spleen had low accumulation 48 hs post-injection ranging from 3–10 ng/mg. Targeting of the MSNs to cancer cells by attaching folate to their surface resulted in an increase in tumor accumulation. Conversely, Souris *et al.* showed that positively charged nanoparticles are excreted from the liver into the GI track and excreted from the animal in the feces while negatively charged MSNs are sequestered within the liver [23]. Therefore, the charge on the surface of the nanoparticles may play an important role in their biodistribution. Another characteristic that affects the biodistribution of this material is the shape of the nanoparticles. Huang *et al.* showed that MSNs with a short rod-shape accumulate in the liver and long rod-shaped nanoparticles accumulate in the spleen [24].

The excretion of MSNs through the urine and feces has been reported by various groups [22,23,25–28]. In our study using phosphonate–MSNs, up to 94% of the silica was excreted from the mice 4 days after injection with 73% being detected in the urine and 21% in the feces [22]. He *et al.* also reported urinary excretion of 45 nm MSNs modified with OH, COOH or polyethylene glycol (PEG) [26]. For these studies, transmission electron microscopy (TEM) was used to detect the excreted nanoparticles and there was no significant damage to their structure. Tumor accumulation and renal excretion of polyethyleneimine (PEI)-coated MSN-have been reported by Mamaeva *et al.* by detection of silica in the urine of MSNs injected mice using inductively coupled plasma optical emission spectrometry (ICP-OES) [27]. Another study by He *et al.* reported urinary excretion of MSNs and PEG-coated MSNs [28]. In both of these studies, the authors suggest that MSNs are degraded, however, no evidence for degradation was given. Further work is needed to determine whether MSNs detected in the urine are intact and, if so, how the nanoparticles are able to cross the glomerular basement membrane in the kidney, which filters the blood and prevents large molecules from leaving the circulation.

Efficacy to deliver anticancer drugs & inhibit tumor growth

The efficient drug delivery to cells, tolerable biocompatibility and good biodistribution led to *in vivo* studies to determine drug-delivery efficacy to tumors in mice. One of the attractive characteristics of nanoparticle drug delivery is that it makes it possible to deliver hydrophobic drugs that do not exhibit appropriate circulation time or biodistribution due to their insolubility in aqueous solutions. By packaging hydrophobic drugs, such as camptothecin (CPT), inside MSNs, the tumor growth inhibition of the drug can be improved. Our group demonstrated that delivery of CPT to xenografts of human breast cancer cell MCF-7 using MSNs inhibits tumor growth more efficiently than the administration of CPT alone [22]. Studies with other xenograft models have provided similar results [25]; for example, tumor growth inhibition of the pancreatic cancer cells PANC-1 and MiaPaca-2 in nude mice has been observed when delivering CPT with MSNs. Furthermore, the active targeting of these nanoparticles with folate significantly reduces tumor growth. In the case of MiaPaca-2 xenografts, there is dramatic tumor suppression by CPT-loaded folate-bound MSNs, with no visible tumors being detected after 4 sets of injections.

Meng *et al.* also demonstrated efficient delivery of doxorubicin (DOX) using MSNs coated with a PEI–PEG copolymer [29]. They used 50-nm MSNs, which are smaller than the ones used in our studies (100–130 nm), however, when coated with the copolymer they can reach a size of 110 nm. They injected 120 mg/kg of nanoparticles on a weekly basis for 3 weeks to a KB-31 xenograft model. The DOX-loaded nanoparticles demonstrated 85% tumor inhibition compared with 70% inhibition by the free drug.

An alternative treatment method to induce tumor shrinkage is photodynamic therapy (PDT), consisting of the use of a photosensitizer, which, in the presence of oxygen, generates cytotoxic species when irradiated at specific wavelengths and leads to irreversible cell damage. Gary-Bobo *et al.* used mannose-functionalized MSNs encapsulating a photosensitizer to carry out two-photon excitation PDT on mice bearing HCT-116 xenografts [15]. In their study they were able to inhibit tumor growth with the treated mice bearing tumors of 0.40 g 30 days after treatment while the control group had tumors of 1.39 g. Furthermore, the treated mice did not exhibit any tumor metastases to the liver and colon while all the control mice had metastases to the liver and two out of the three mice had metastases to the colon.

Nanomachine-equipped MSNs

One of the excitements in the study of MSNs is the development of nanomachine-equipped MSNs. Nanovalve-equipped MSNs were synthesized by attaching pseudorotaxanes on a silica solid support [30]. Pseudorotaxanes are structures composed of 1,5-bis[2-(2-hydroxyethoxy)ethoxy]naphthalene (BHEEN) stalks threaded through cyclobis(paraquat-*p*-phenylene) (CBPQT4+). Since then, more sophisticated machinery has been attached to MSNs that has allowed for the controlled release of guest molecules upon chemical or biological triggers. This has led to the development of nanovalves, nanopistons, snap-tops and nanoimpellers, which can respond to pH, enzyme activation, light activation and magnetic fields.

Controlled release using internal stimuli pH-operated machines

MSNs are endocytosed by cells in an energy-dependent manner and are known to co-localize to the lysosomes. The lysosomes are degradative compartments with a low pH that contain many proteases and hydrolases [31]. In an attempt to activate cargo release once MSNs are taken up by cells and not while on the extracellular environment, pH nanovalves

that remain closed at neutral pH but open at low pH were synthesized and attached to the pore openings of MSNs. An example is the creation of nanovalves consisting of trisammonium stalks threaded on a cucurbit uril (CB(6)) ring [32]. The stalks contain one anilinium and two – $CH_2NH_2^+CH_2$ – centers, the anilinium is placed far from the pore opening while the – $CH_2NH_2^+CH_2$ – sites are placed close to the opening. At neutral pH, the anilinium nitrogen is deprotonated causing the CB(6) ring to reside on the – $NH_2^+(CH_2)_4NH_2^+$–recognition units through ion–dipole binding interactions. When the pH is lowered, the anilinium nitrogen atom is protonated and since the stability constant for the complexation of CB(6) with $–NH_2^+(CH_2)_6NH_2^+–$ is one order of magnitude greater [33] than that of – $NH_2^+(CH_2)_4NH_2^+–$, the CB(6) ring moves to the distal $–NH_2^+(CH_2)_6NH_2^+–$ site. This results in the unblocking of the pore opening, leading to release of the cargo. The release rate and the pH at which the nanovalves open can be adjusted very precisely by changing the pK_a of the anilinium nitrogen by varying the *para*-substituent on the aryl rings.

Nanovalves with different components have been synthesized and conjugated to MSNs. For example, Meng *et al.* constructed a nanovalve in which the gatekeeping molecule completely dissociates from the stalk was synthesize using β-cyclodextrin (β-CD) as the cap and *N*-menthylbenzimidazole (MBI) as the stalk (Figure 4) [34]. This valve was designed so that the β-CD will interact with the stalk at pH 7.4, but will dissociate from it at pH 6 or lower. This pH range response is optimal for *in vivo* application since the pH of blood is 7.4 but the late endosome/lysosome has a pH lower than 6. This mechanized MSN was shown to efficiently encapsulate and release Hoescht dye in a pH-dependent manner. However, release of cationic drugs, such as DOX, required functionalization of the MSN surface to prevent electrostatic interactions between the drug and the particles. After this surface modification, the nanoparticles were shown to be endocytosed by KB-31 cells, co-localize to the lysosomes, and deliver DOX leading to induction of apoptosis.

In the nanovalves discussed so far, the stalks are immobilized on the surface of the MSNs while the caps are able to move up and down the stalk or completely dissociate from the stalk, depending on the environmental conditions. There are other structures with the opposite design, with the rings immobilized and the stalks movable [35]. These nanopiston structures are composed of β-CD rings, attached to the orifices of the pores, and rhodamine B/benzidine stalks that can move in and out of the ring in response to pH changes. These modifications make it possible to store small cargo molecules, such as 2,6-naphthalenedisulfonic acid disodium, within the nanopores and upon lowering of the pH, the nanopiston dissociates from the β-CD ring allowing the cargo to passage through the cavity of the ring. Furthermore, these nanoparticles can also be used to deliver large cargo because the linkers keeping the β-CD ring attached to the nanoparticle contain cleavable imine double bonds. These bonds are hydrolyzed under acidic conditions causing the dissociation of the ring from the nanoparticle allowing for the large cargo to exit the MSNs. Therefore, these nanoparticles can deliver both small and large molecules and the release of each can be tuned for different pH, which could be important for dual drug therapy.

pH-dependent cargo release can also be achieved using pH-sensitive linkers that connect the cargo with the nanoparticle. Lee *et al.* synthesized a delivery system that linked DOX to the MSN nanochannel surface by hydrazone bonds [36]. This pH-sensitive linker is cleaved at pH values between 6 and 4, so the drug remains attached to the nanoparticle in blood circulation (pH 7.4) but gets released when reaching the endosome/lysosome. The uptake and co-localization of the MSN–hydrazone–DOX in lysosomes of human hepatoma cells (Hep-G2) was demonstrated as well as the cell killing efficacy of this system. This construct can be used to deliver drugs that have functional ketones or aldehydes, such as, cerubidine or idarubicin.

Enzyme activation—A different drug-release system was created using snap-top-covered silica nanocontainers. One type of snap-top system constructed consists of [2] rotaxanes composed of tri(ethylene glycol) chains threaded by α-CD tori that are held in place by cleavable stoppers [37]. The stoppers are made of ester-linked adamantyl and are tethered to the surface of MSNs. The ester link can be cleaved by esterase enzymes that are present inside cells. Upon cleavage of the ester link, the stoppers are released resulting in the dissociation of the cap and allowing the guest molecules to exit the nanopores.

A different approach was used to develop a matrix metalloproteinase (MMP)-responsive system using hybrid nanoparticles [38]. MMPs are enzymes capable of degrading the extracellular matrix and are upregulated in tumor environments. The MSNs were coated with polyethylene glycol diacrylate (PEGDA)–peptide macromer, which has MMP substrate polypeptides. They delivered DOX using this system to 3T3-J2 fibroblasts and found that nanoparticles coated with the highly degradable MMP sequence induced apoptosis of the cells faster than PEG-coated nanoparticles. Blocking secretion of MMPs resulted in a decrease in chemotoxicity of the nanoparticles confirming that MMP is required for release of the drug.

Redox activation—Using a design similar to the enzymatic activation snap-top system, a redox-sensitive [2] rotaxane machine was designed by Ambrogio *et al.* and conjugated to MSNs [39]. The rotaxanes in this system contain disulfide bonds that can be reduced, leading to the breakdown of the stalk and release of the gatekeeping molecule [2]. The use of disulfide bonds is important because they are stable outside the cells, however, once reaching the intracellular environment they can be reduced by glutathione [40]. This allows the nanomachine to operate autonomously utilizing the biochemical processes already taking place within the cell to induce drug release. This system has been shown to work properly in aqueous solution with cargo release upon exposure to reducing agents, such as dithiothreitol or 2-mercaptoethanol. Current work is focused on showing its operation in cells. A different redox-driven system was developed in which cysteine is linked via disulfide bonds to the inner particle core and reduction of these bonds leads to its release [41]. In this study, it was demonstrated that promoting endosomal escape of the nanoparticles by photochemically opening the endosomes was required for delivery of cysteine to the cytosol.

Controlled release using external stimuli Light activation

The nanomachines described so far are activated autonomously upon reaching a specific environment, either the low pH in lysosomes, reducing environment or in the presence of a specific enzyme. There is a class of mesoporous silica nanomachines that can be activated externally allowing for on-demand activation. Lu *et al.* developed the nanoimpeller, which has azobenzene attached to the inner surface of the nanopores(Figure 5) [42]. The design of this nanomachine is different from the ones previously discussed in that there is no gatekeeping molecule at the pore orifice; instead, the inner surface of the channel is conjugated to azobenzene derivatives that can have a *cis* or *trans* conformation. The azobenzene derivatives block the diffusion of the cargo out of the nanoparticle. Upon photo excitation the azobenzene fluctuates between cis and trans conformations causing a mixing of the cargo within the pores and the cargo closest to the pore opening exits the nanoparticles. This nanomachine has been shown to release dyes and anticancer drugs in a time- and light intensity-dependent manner. Furthermore, the light intensity needed to activate the impellers does not damage the cells, so apoptosis is only induced in the cells that have taken the drug-loaded nanoparticles and that receive enough light to activate the nanomachine. This development could lead to a system for drug delivery with both spatial and temporal external control.

A similar light-dependent nanomachine was constructed with β-CD and azobenzene derivatives but the design of this system is different. In this system, the β-CD serves as the cap for the pore while the azobenzene is part of the stalk [43]. The binding affinity of β-CD to *trans* azobenzene is high in aqueous solution while the binding affinity to *cis*-azobenzene is low [44, 45]. This differential binding affinity allows for controlled release of the β-CD ring, uncapping the nanopore and allowing the guest molecules to exit the nanoparticles. The nanomachines were constructed with *trans*-azobenzene on the stalks and loaded with Rhodamine B dye. Irradiation with λ=351 nm led to the *trans*-to-*cis* isomerization of azobenzene, the dissociation of β-CD and the release of Rhodamine B in aqueous solution. This nanomachine design is promising for on-demand drug release in cells but experiments in a biological setting have not been reported.

Knezevic *et al*. developed a light-responsive controlled-release delivery system consisting of mercaptopropyl-functionalized MSNs and the coordination compound $[Ru(bpy)_2(PPh_3)\ Cl]\ Cl$ [46]. This compound forms coordination bonds with the mercaptopropyl moieties in the inner surface of the nanopores blocking the release of cargo. Upon irradiation with visible light, the coordination bonds are cleaved resulting in the release of $[Ru(bpy)_2(PPh_3)Cl]\ Cl$ allowing the cargo to exit the pores. This group also constructed a system that releases DOX in response to light and pH [47]. This construct consists of MSNs functionalized with nitroveratryl carbamate protected aminopropyl (NVCAP). When this system is irradiated with UV light (350 nm), the carbamate linkage is degraded leaving propylammonium ions attached on the MSN surface. Since DOX attaches to the silica surface via hydrogen bonds and charge interactions with silanols, the propylammonium ions create electrostatic repulsion with DOX, inducing its release from the silica surface. This system also releases DOX in response to pH because at pH 6.4 the amine groups from DOX are fully protonated and the NVCAP–MSNs have a higher positive charge inducing release of the drug. In contrast, at pH 7.4 there is less electrostatic repulsion between DOX and NVCAP–MSNs resulting in reduced drug release.

Magnetic field activation—The combination of superparamagnetic (SPM) metals with silica nanoparticles is of particular importance because of the ability of altering the nanoparticle behavior in biological settings using a magnetic field. A system combining mechanized MSNs with zinc-doped iron oxide nanocrystals (ZnNCs) resulted in a magnetically activated release system [48]. The nanovalve used for this nanomachine is thermally responsive and application of an alternating magnetic field to the nanoparticles causes the nanocrystals to generate local internal heating resulting in the disassembly of the molecular machines. These mechanized nanoparticles were shown to be efficient nanocarriers of DOX and to induce apoptosis of MDA-MB 231 breast cancer cells in a magnetic field-dependent manner.

The combination of MSNs, iron oxide SPM nanocrystals and DNA was explored by Ruiz-Hernandez *et al.* resulting in the development of DNA–magnetic nanoparticle gates [49]. These structures consist of single DNA strands conjugated to the surface of MSNs and the complementary strand covalently bound to the surface of iron oxide nanocrystals. When the MSNs and the iron oxide particles are mixed, the DNA strands hybridize, resulting in the capping of the MSNs pores with the iron oxide particles. The MSNs used in this study have iron oxide particles incorporated in their structure, so that when an alternating magnetic field is applied to them they generate heat. The heat generated by this system results in melting of the DNA double strand and the iron oxide nanocrystals blocking the pore dissociate from the MSN allowing for release of the cargo.

Hybrid materials

Modifications to the mesoporous silica surface can lead to the interaction with different molecules and this has led to the development of hybrid nanoparticles. MCM-41 can be combined with polymers [50, 51], lipids or metals to create nanoparticles with different characteristics. For example, the sequential deposition of poly(vinylpyrrolidone) and thiolated poly(methacrylic acid) on MSNs forms a polyelectrolyte multilayer on the surface of the nanoparticles [52]. The polyelectrolyte multilayer can be reduced by dithiotheritol causing the release of the cargo. This hybrid system was used to deliver DOX to HeLa and QGY7703 cells with optimal results. Similarly, the combination of MSNs and lipids has produced hybrid nanoparticles that exhibit superior suspendability in solution and reduction of non-specific binding of proteins. For example, MSNs functionalized with 13-(chlorodimethylsilylmethyl)heptacosane allowed for the coating of the nanoparticles with PEGylated phospholipids [53]. Targeting of this hybrid system was achieved by functionalizing the phospholipid and attaching folate as the ligand resulting in increased uptake by HeLa cells. Combination of MSNs with polymers can enhance the tumor accumulation of these systems and improve biodistribution of these systems. In the study by Meng *et al.*, the coating of MSNs with PEG improved the circulation time of the material and decreases its liver accumulation [29]. By coating the nanoparticles with a co-polymer consisting of PEI and PEG, the accumulation in the tumor was increased from 3% (PEG–MSN) to 12% at 72 h post-injection. Furthermore, this hybrid system delivered the anticancer drug DOX and significantly inhibited tumor growth.

Multifunctional nanoparticles

The application of MSNs in medicine is not limited to drug delivery. This material can be combined with different types of nanostructured materials to aid in the imaging of diseased tissue or to label cells and track their movement inside an animal. Mesoporous silica has been successfully combined with gadolinium, iron oxide nanocrystals, quantum dots and manganese oxide among others. The imaging agents are encapsulated within the mesoporous framework and their original physical properties are retained. Furthermore, since the surface of the encapsulated materials is surrounded by silica, it lowers their cytotoxicity and increases their biocompatibility. This is important for the use of quantum dots because their advantages in optical and chemical properties [54] are outweighed by their insolubility in aqueous environments [55] and their toxicity [56]. These obstacles can be overcome by encapsulating them within MSNs and then coating this system with PEGylated liposomes [57].

Paramagnetic materials, such as Gadolinium (Gd^{3+}), iron oxide (Fe_3O_4) and manganese oxide (MnO) serve as contrast agents for MRI. MRI is one of the most important diagnostic tools used in the medical field because of its non-invasive nature, unlimited tissue penetration and zero ionizing radiation [58, 59]. In order to improve the images obtained by MRI, contrast agents that either have high *T*2 relaxivities [60, 61] or that shorten the longitudinal relaxation time (*T*1) [62] are used. These contrast agents must have good colloidal stability and biocompatibility. The incorporation of gadolinium to MSNs has been reported by various groups [63,64] with enhancement of both *T*1-weighted and *T*2-weighted signals at doses much lower than what is currently being used [65]. MSNs with Fe_3O_4 at their core have also been shown to be efficient MRI contrast agents with low cytotoxicity and efficient cellular uptake [11, 66]. Additionally, the incorporation of MnO into MSNs has proven to be an efficient positive *T*1 contrast agent that can be used for MRI tracking of mesenchymal stem cells [67].

The individual modifications to MSNs discussed so far give specific advantages over traditional drug therapy but the combination of these functionalizations creates unparalleled benefits with cell-specific drug delivery, controlled release and imaging capabilities. The development of multifunctional systems based on MSNs is at an early stage with some groups being able to combine drug delivery, targeting and imaging successfully. For example, a multifunctional system consisting of an supermagnetite iron oxide nanocrystal core encapsulated in a mesoporous silica shell with folate acid attached to the surface was created with successful enhancement of *T*2-weighted MRI images and increased uptake by cells over expressing the folate receptor [11]. These nanoparticles were able to carry camptothecin or paclitaxel in their pores and deliver it to the pancreatic cancer cell lines PANC-1 and BxPC-3 to inhibit their proliferation. Another group showed that similar nanoparticles, but without the targeting moiety, were able to deliver DOX to cancer cells and accumulate in tumors [68]. These nanocomposite nanoparticles were fluorescently labeled with Rhodamine B isothiocyanate and were able to provide both MRI and optical images of subcutaneously injected cancer cells in mice. This multimodal imaging capability is particularly useful for guidance to surgical treatment and noninvasive diagnosis [69].

To date, there hasn't been a multifunctional system that combines targeting, imaging and nanomachines. This would be the next frontier so that, in addition to enhancing the nanoparticle uptake by cancer cells and being able to locate them, the release of the drug can be controlled either internally or externally. Nanomachines that respond to internal stimuli will reduce leakage of the drug outside the target cells so that when the nanoparticles are internalized they will be able to release sufficient drugs to induce cell death. Nanomachines that respond to external stimuli will allow physicians to control the drug release rate considering that a slow continuous release may be more efficient at inducing tumor regression.

Comparison with other delivery platforms

Nanoparticles have been synthesized using a variety of materials and each material provides specific advantages over traditional drug therapy. All of them can increase drug delivery to cells, improve the drug's circulation time and achieve tissue-specific delivery. Liposomes and polymeric micelles are biodegradable organic nanoparticles with good biocompatibility but premature release of the cargo is always a concern with these types of systems. Inorganic nanoparticles composed of SPM materials, gold and nanodiamonds can also be used as delivery vehicles and advances in this field have greatly improved drug delivery using these different platforms.

Liposomes are artificial vesicles consisting of one or more lipid bilayers encapsulating an equal number of internal aqueous compartments [70]. Liposomal vesicles between 50 and 500 nm in diameter are the preferred liposome delivery system for drugs because of their ability to achieve favorable drug:lipid ratios and their more predictable drug release kinetics. The therapeutic delivery efficacy of the liposome depends on the physical and biochemical properties including stability, size, charge, hydrophobicity, interaction with serum proteins and interaction with non-target cell surfaces. One way to maximize localization and accumulation of liposomes in tumors is to actively target breast tumors by functionalizing with anti-HER2 scFv F5 antibody [71]. Intravenous injections of these constructs encapsulating topotecan, at a dose of 5 mg/kg, results in a twofold enhancement in anti-tumor activity when compared with non-targeted liposomes.

Polymer-based nanoparticles composed of different polymers have been shown to be efficient drug carriers. This is a very dynamic field with new nanoparticle formulations being created constantly. There are polymer nanoparticles composed of poly(lactic-co-

glycolic acid) [72], poly(D,L-lactic acid) [73], chitosan [74], gelatin [75] and poly-ε-caprolactone [76] among others. Polymer micelles ranging from 10 to 100 nm in diameter composed of amphiphilic copolymers can self-assemble in aqueous environments [77]. Some polymer micelle formulations have undergone various phases of clinical trials and have shown promising results. For example, NC-6004, a cisplatin-incorporating polymeric micelle, was examined in a Phase 1 clinical trial and was well tolerated in 17 patients, seven of which had stable disease [78]. Genexol PM, a paclitaxel-containing PEG–PLA micelle, was examined in a Phase II clinical trial with 41 breast cancer patients [79]. The patients had an overall response rate of 58.5% with a treatment of 300 mg/m^2 every 3 weeks.

Liposomes and polymeric micelles are considered ‘soft’ nanoparticles because of their relative instability and biodegradability. Nanoparticles composed of ‘hard’ materials, such as ferromagnetic materials, gold, nanodiamonds and mesoporous silica have been shown to be efficient nanocarriers. These ‘hard’ materials provide a solid structure upon which different molecules can be conjugated. SPM nanoparticles can be used as targeted probes for diagnostics or for therapeutic applications. Therapeutic drugs are usually coupled to the surface of SPM nanoparticles via covalent bonds and the release can occur through enzymatic activity, change in pH or temperature, depending on the nature of the bond [80]. An advantage of using SPM nanoparticles is that due to their magnetic properties they can be tracked in biological systems by MRI so the pathway of the drug can be monitored. The movement of the nanoparticles in an organism can also be controlled using an external magnetic field that can guide the nanoparticles. Additionally, by exposing the nanoparticles to an alternate magnetic field, the temperature of the nanoparticles can be elevated to induce drug release.

Gold is another material used to produce nanoparticles with the capacity of producing heat through the plasmon resonance effect. This heat generation can induce cell death, which, in combination with targeting to cancer cells, provides a feasible therapy candidate. Targeting of gold nanoparticles to cancer cells has been achieved using anti-EGFR [81] and anti-Her2 [82] antibodies. Targeting of these systems increases nanoparticle uptake by cells expressing the target receptors and, in the case of EGFR, lowers the light energy required to kill EGFR expressing cells when compared with non-tumorigenic skin cells [83].

Diamond nanoparticles smaller than 10 nm, also known as nanodiamonds (NDs), can be generated using the detonation technique [84]. This material is chemically inert and has displayed low toxicity in several cell lines [85]. NDs have been shown to be efficient carriers of DOX that facilitate cellular uptake of the drug [86]. An Initial study investigating the biodistribution of NDs was carried out by Rojas *et al.* with the discovery that most of the nanoparticles accumulated in the lung, spleen and the liver, and were excreted in the urine [87].

MSNs have advantages over other delivery platforms. Liposomes and polymeric micelles can encapsulate therapeutic agents and are readily taken up by cells but their instability can lead to premature leakage of the cargo before reaching the target site leading to reduced tumor killing efficacy. SPM, ND and gold nanoparticles have a more stable structure but the cargo is covalently attached to their surface so the drugs that can be carried by these particles are limited to drugs that can be chemically conjugated to the particles. In contrast, nanoparticles composed of MCM-41 mesoporous silica provide a solid structure in which cargo is encapsulated within the pores of the particles with no covalent attachment necessary, leaving the outer surface of the particles available for modifications that allow for targeting. Furthermore, the use of gatekeeping molecules on the opening of the pores makes it possible to achieve controlled drug release either by internal or external stimuli.

Future perspective

In the last decade there has been dramatic progress in nanotechnology that has brought us closer to implementation of nanoparticles in the medical field, however, there are still many challenges that need to be overcome in order to make this a reality. One of the biggest challenges in the development of MSNs is biosafety and, even though there have been encouraging results in studies with mice, biosafety and biodistribution studies need to be carried out in higher mammalian species so that eventually clinical trials in humans can be performed. It is very important to determine the toxicity and biodistribution of this material in humans to evaluate the prospect for biomedical applications.

If clinical trials give encouraging results, we can envision an era where targeted drug delivery using mechanized nanoparticles will be an important weapon against cancer. The advent of personalized medicine will aid greatly in the implementation of MSNs in medicine. For example, when a patient is diagnosed with cancer, studies can be done to determine the molecular markers of the tumor and find out what receptors are overexpressed in the cancer cells. Since multiple-targeting moieties can be attached to the surface of MSNs (i. e. proteins, peptides, small molecules), nanoparticles with a specific ligand for the overexpressed receptor can be used to deliver drugs to the tumor. Furthermore, if a specific enzyme is found to be highly expressed in the tumor, an enzyme activated snap-top machine could be used for the controlled release of therapeutics inside the cancer cells. Alternatively, pH-operated nanovalves or nanopistons could be used for the controlled release of drugs. In instances where there is no overexpression of a specific enzyme, nanomachines that respond to external stimuli would give physicians the ability to activate drug release when appropriate, either by using light or magnetism. The efficacy of the treatment can be monitored by MRI thanks to the imaging capabilities of hybrid MSNs with SPM metals. The heterogeneity of cancer cells requires various approaches for treating different tumors and this can be achieved by the variety of mechanized MSNs that have been developed.

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References

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Feynman, R. Miniaturization. Gilbert, HD., editor. Reinhold, N.Y.: 1961. p. 282-296.
2. Drexler KE. Molecular engineering: an approach to the development of general capabilities for molecular manipulation. Proc Natl Acad Sci USA. 1981; 78(9):5275–5278. [PubMed: 16593078]
3▪. Manzano M, Collila M, Vallet-Regi M. Drug delivery from ordered mesoporous matrices. Expert Opin Drug Deliv. 2009; 6:1383–1400. Reviews advances made in the drug-delivery field using silica-based ordered mesoporous materials. [PubMed: 19941412]
4. Vialpando M, Aerts A, Persoons J, Martens J, Van den Mooter G. Evaluation of ordered mesoporous silica as a carrier for poorly soluble drugs: influence of pressure on the structure and drug release. J Pharm Sci. 2011; 100:3411–3420. [PubMed: 21387318]
5. Qian KK, Bogner RH. Application of mesoporous silicon dioxide and silicate in oral amorphous drug delivery systems. J Pharm Sci. 2011; 101(2):444–463. [PubMed: 21976048]

6▪. Vialpando M, Martens JA, Van den Mooter G. Potential of ordered mesoporous silica for oral delivery of poorly soluble drugs. Therapeutic Delivery. 2011; 2:1079–1091. Discusses significant developments and challenges for efficient drug loading and rapid release of drugs onto ordered mesoporous silica materials and their prospect for oral delivery.

7. Slowing II, Vivero-Escoto JL, Wu CW, Lin VS. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Deliv Rev. 2008; 60:1278–1288. [PubMed: 18514969]

8▪▪. Lu J, Liong M, Zink JI, Tamanoi F. Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs. Small. 2007; 3:1341–1346. One of the first studies to demonstrate that mesoporous silica nanoparticles (MSNs) can be used to deliver hydrophobic drugs to cells. [PubMed: 17566138]

9. Bagwe RP, Hilliard LR, Tan W. Surface modifications of silica nanoparticles to reduce aggregation and nonspecific binding. Langmuir. 2006; 22:4357–4362. [PubMed: 16618187]

10. Slowing I, Trewyn BG, Lin VS. Effect of surface functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells. J Am Chem Soc. 2006; 12:8, 14792–14793.

11▪▪. Liong M, Lu J, Kovochich M, et al. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. ACS Nano. 2008; 2:889–896. One of the first studies to develop multifunctional nanoparticles by adding folate-targeting and iron oxide cores, for improved MRI contrast, to MSNs. [PubMed: 19206485]

12. Brevet D, Gary-Bobo M, Raehm L, et al. Mannose targeted mesoporous silica nanoparticles for photodynamic therapy. Chem Commun. 2009; 12:1475–1477.

13. Ferris DP, Lu J, Gothard C, et al. Synthesis of biomolecule-modified mesoporous silica nanoparticles for targeted hydrophobic drug delivery to cancer cells. Small. 2011; 7:1816–1826. [PubMed: 21595023]

14. Aumailley M, Gurrath M, Muller G, Calvete J, Timpl R, Kessler H. Arg–Gly–Asp constrained within cyclic pentapeptides. Strong and selective inhibitors of cell adhesion to vitronectin and laminin fragment P1. FEBS Lett. 1991; 21(1990):50–54. [PubMed: 1718779]

15. Gary-Bobo M, Mir Y, Rouxel C, et al. Mannose-functionalized mesoporous silica nanoparticles for efficient two-photon photodynamic therapy of solid tumors. Angew Chem Int Ed. 2011; 50:1–6.

16. Lu J, Liong M, Sherman S, et al. Mesoporous silica nanoparticles for cancer therapy: energy-dependent cellular uptake and delivery of paclitaxel to cancer cells. Nanobiotechnology. 2007; 3:89–95. [PubMed: 19936038]

17. Huang DM, Hung Y, Ko BS, et al. Highly efficient celular labeling of mesoporous nanoparticles in human mesenchymal stem cells: implication for stem cell tracking. FASEB J. 2005; 19:2014–2016. [PubMed: 16230334]

18. Lu F, Wu SH, Hung Y, Mou CY. Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles. Small. 2009; 5:1408–1413. [PubMed: 19296554]

19. Huang X, Teng X, Chen D, Tang F, He J. The effect of mesoporous silica nanoparticles on cellular uptake and cell function. Biomaterials. 2010; 31:438–448. [PubMed: 19800115]

20. Slowing II, Vivero-Escoto JL, Zhao Y, et al. Exocytosis of mesoporous silica nanoparticles from mammalian cells: from asymmetric cell-to-cell transfer to protein harvesting. Small. 2011; 7:1526–1532. [PubMed: 21520497]

21. Hudson SP, Padera RF, Langer R, Kohane DS. The biocompatibility of mesoporous silicates. Biomaterials. 2008; 29:4045–4055. [PubMed: 18675454]

22▪▪. Lu J, Liong M, Li Z, Zink JI, Tamanoi F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles, for cancer therapy in animals. Small. 2010; 6(16): 1794–1805. Important *in vivo* study determining MSNs biodistribution and the dosage that gives efficient tumor drug delivery and is tolerated by mice. [PubMed: 20623530]

23. Souris JS, Lee CH, Cheng SH, et al. Surface charge-mediated rapid hepatobiliary excretion of mesoporous silica nanoparticles. Biomaterials. 2010; 31:5564–5574. [PubMed: 20417962]

24. Huang X, Li L, Liu T, et al. The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility *in vivo*. ACS Nano. 2011; 5:5390–5399. [PubMed: 21634407]

25. Lu J, Li Z, Zink JI, Tamanoi F. *In vivo* tumor suppression efficacy of mesoporous silica nanoparticles-based drug-delivery system: enhanced efficacy by folate modification. Nanomedicine. 2012; 8(2):212–220. [PubMed: 21703996]
26. He X, Nie H, Wang K, Tan W, Wu X, Zhang P. *In vivo* study of biodistribution and urinary excretion of surface-modified silica nanoparticles. Anal Chem. 2008; 80:9597–9603. [PubMed: 19007246]
27. Mamaeva V, Rosenholm JM, Bate-Eya LT, et al. Mesoporous silica nanoparticles as drug delivery systems for targeted inhibition of notch signaling in cancer. Mol Ther. 2011; 19:1538–1546. [PubMed: 21629222]
28. He Q, Zhang Z, Gao F, Li Y, Shi J. *In vivo* biodistribution and urinary excretion of mesoporous silica nanoparticles: effects of particle size and PEGylation. Small. 2011; 7:271–280. [PubMed: 21213393]
29. Meng H, Xue M, Xia T, et al. Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model. ACS Nano. 2011; 5:4131–4144. [PubMed: 21524062]
30▪. Ambrogio MW, Thomas CR, Zhao YL, Zink JI, Stoddart JF. Mechanized silica nanoparticles: a new frontier in theranostics nanomedicine. Acc Chem Res. 2011; 44:903–913. Comprehensive review of the developments in nanomachine design. [PubMed: 21675720]
31. Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. Nat Rev Mol Cell Biol. 2007; 8:622–632. [PubMed: 17637737]
32. Angelos S, Khashab NM, Yang YW, et al. pH clock-operated mechanized nanoparticles. J Am Chem Soc. 2009; 131:12912–12914. [PubMed: 19705840]
33. Mock WL, Pierpont J. A curcubituril based molecular switch. J Chem Soc Chem Commun. 1990; 1990(2):1509–1511.
34. Meng H, Xue M, Xia T, et al. Autonomous in vitro anticancer drug release from mesoporous silica nanoparticles by pH-sensing nanovalves. J Am Chem Soc. 2010; 132(36):12690–12697. [PubMed: 20718462]
35. Zhao Y, Li Z, Kabehie S, Bostros YY, Stoddart JF, Zink JI. pH-operated nanopistons on the surfaces of mesoporous silica nanoparticles. J Am Chem Soc. 2010; 132:13016–13025. [PubMed: 20799689]
36. Lee CH, Cheng SH, Huang IP, et al. Intracellular pH-responsive mesoporous silica nanoparticles for the controlled release of anticancer chemotherapeutics. Angew Chem Int Ed. 2010; 49:8214–8219.
37. Patel K, Angelos S, Dichtel WR, et al. Enzyme-responsive snap-top covered silica nanocontainers. J Am Chem Soc. 2008; 130:2382–2383. [PubMed: 18232687]
38. Singh N, Karambelkar A, Gu L, et al. Bioresponsive mesoporous silica nanoparticles for triggered drug release. J Am Chem Soc. 2011; 133:19582–19585. [PubMed: 21981330]
39. Ambrogio MW, Pecorelli TA, Patel K, et al. Snap-top nanocarriers. Org Lett. 2010; 12:3304–3307. [PubMed: 20608669]
40. Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983; 52:711–760. [PubMed: 6137189]
41. Sauer AM, Schlossbauer A, Ruthardt N, Cauda V, Bein T, Brauchle C. Role of endosomal escape for disulfide-based drug delivery from colloidal mesoporous silica evaluated by live-cell imaging. Nano Lett. 2010; 10:3684–3691. [PubMed: 20677799]
42▪▪. Lu J, Choi E, Tamanoi F, Zink JI. Light-activated nanoimpeller-controlled drug release in cancer cells. Small. 2008; 4:421–426. Development of light-dependent nanomachine for on-demand drug release. [PubMed: 18383576]
43. Ferris DP, Zhao YL, Khashab NM, Khatib HA, Stoddart JF, Zink JI. Light-operated mechanized nanoparticles. J Am Chem Soc. 2009; 131:1686–1688. [PubMed: 19159224]
44. Sanchez AM, de Rossi RH. Effect of β-cyclodextrin on the thermal cis-trans isomerization of azobenzenes. J Org Chem. 1996; 61:3446–3451.
45. Zhao YL, Stoddart JF. Azobenzene-based light-responsive hydrogel system. Langmuir. 2009; 25:8442–8446. [PubMed: 20050041]

46. Knezevic NZ, Trewyn BG, Lin VS. Functionalized mesoporous silica nanoparticles-based visible light responsive controlled release delivery system. Chem Commun. 2011; 47:2817–2819.

47▪. Knezevic NZ, Trewyn BG, Lin VS. Light-and pH-responsive release of doxorubicin from a mesoporous silica-based nanocarrier. Chem Eur J. 2011; 17:3338–3342. Development of MSNs that respond to UV light or pH by releasing doxorubicin. [PubMed: 21337435]

48. Thomas CR, Ferris DP, Lee JH, et al. Noninvasive remote-controlled release of drug molecules in vitro using magnetic actuation of mechanized nanoparticles. J Am Chem Soc. 2010; 132:10623–10625. [PubMed: 20681678]

49. Ruiz-Hernandez E, Baeza A, Vallet-Regi M. Smart drug delivery through DNA/magnetic nanoparticle gates. ACS Nano. 2011; 5:1259–1266. [PubMed: 21250653]

50. Liu R, Zhao X, Wu T, Feng P. Tunable redox-responsive hybrid nanogated ensembles. J Am Chem Soc. 2008; 130:14418–14419. [PubMed: 18841893]

51. Liu R, Zhang Y, Feng P. Multiresponsive supramolecular nanogated ensembles. J Am Chem Soc. 2009; 131:15128–15129. [PubMed: 19746981]

52. Zhu CL, Song XY, Zhou WH, Yang HH, Wen YH, Wang XR. An efficient cell-targeting and intracellular controlled-release drug delivery system based on MSN-PEM-aptamer conjugates. J Mater Chem. 2009; 19:7765–7770.

53. Wang LS, Wu LC, Lu SY, et al. Biofunctionalized phospholipid-capped mesoporous silica nanoshuttles for targeted drug delivery: improved water suspensibility and decreased nonspecific protein binding. ACS Nano. 2010; 4:4371–4379. [PubMed: 20731423]

54. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labeling and sensing. Nat Mater. 2005; 4:435–446. [PubMed: 15928695]

55. Alivisatos AP, Gu WW, Larabell C. Quantum dots as celular probes. Annu Rev Biomed Eng. 2005; 7:55–76. [PubMed: 16004566]

56. Celik A, Comelekoglu U, Yalin S. A study on the investigation of cadmium chloride genotoxicity in rat bone marrow using micronucleus test and chromosome aberration analysis. Toxicol Ind Health. 2005; 21:243–248. [PubMed: 16463956]

57. Pan J, Wan D, Gong J. PEGylated liposome coated QDs/mesoporous silica core-shell nanoparticles for molecular imaging. Chem Commun. 2011; 47:3442–3444.

58. Caravan P. Strategies for increasing the sensitivity of Gadolinium based MRI contrast agents. Chem Soc Rev. 2006; 35:512–523. [PubMed: 16729145]

59. Sitharaman B, Kissell KR, Hartman KB, et al. Superparamagnetic gadonanotubes are high-performance MRI contrast agents. Chem Commun. 2005; 31:3915–3917.

60. Zhang CF, Jugold M, Woenne EC, et al. Specific targeting of tumor angiogenesis by RGD-conjugated ultrasmall superparamagnetic iron oxide particles using clinical 1.5T magnetic resonance scanner. Cancer Res. 2007; 67:1555–1562. [PubMed: 17308094]

61. Kircher MF, Mahmood U, King RS, Weissleder R, Josephson L. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. Cancer Res. 2003; 63:8122–8125. [PubMed: 14678964]

62. Na HB, An K, Park Y, et al. Development of a T1 contrast agent for magnetic resonance imaging using MnO nanoparticles. Angew Chem Int Ed. 2007; 46:5397–5401.

63. Carniato F, Tei L, Cossi M, Marchese L, Botta M. A chemical strategy for the relaxitivity enhancement of Gd(III) chelates anchored on mesoporous silica nanoparticles. Chemistry. 2010; 16:10727–10734. [PubMed: 20669190]

64. Hsiao JK, Tsai CP, Chung TH, et al. Mesoporous silica nanoparticles as a delivery system of gadolinium for effective human stem cell tracking. Small. 2008; 4:1445–1452. [PubMed: 18680095]

65. Taylor KML, Kim JS, Rieter WJ, An H, Lin W, Lin W. Mesoporou silica nanospheres as highly efficient MRI contrast agents. J Am Chem Soc. 2008; 130:2154–2155. [PubMed: 18217764]

66. Lee JE, Lee N, Kim H, et al. Uniform mesoporous dye-doped silica nanoparticles decorated with multiple magnetite nanocrystals for simultaneous enhanced magnetic resonance imaging, fluorescence imaging, and drug delivery. J Am Chem Soc. 2010; 132:552–557. [PubMed: 20017538]

67. Kim T, Momin E, Choi J, et al. Mesoporous silica-coated hollow manganese oxide nanoparticles as positive T1 contrast agents for labeling and MRI tracking of adipose-derived mesenchymal stem cells. J Am Chem Soc. 2011; 133:2955–2961. [PubMed: 21314118]
68. Kim S, Kim H, Lee N, et al. Multifunctional uniform nanoparticles composed of a magnetite nanocrystal core and a mesoporous silica shell for magnetic resonace and fluorescence imaging and for drug delivery. Angew Chem Int Ed. 2008; 47:8438–8441.
69. Kim S, Lim YT, Soltesz EG, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol. 2003; 22:93–97. [PubMed: 14661026]
70. MacLachlan, I. Liposomal formulations for nucleic acid delivery. In: Crooke, ST., editor. Antisense Drug Technology: Principles, Strategies, and Applications. CRC Press; Boca Raton, FL, USA: 2008. p. 237-270.
71. Drummond DC, Noble CO, Guo Z, et al. Development of a highly stable and targetable nanoliposomal formulation of topotecan. J Control Release. 2010; 141:13–21. [PubMed: 19686789]
72. Jain AK, Das M, Swarnakar NK, Jain S. Engineered PLGA nanoparticles: an emerging delivery tool in cancer therapeutics. Crit Rev Ther Drug Carrier Syst. 2011; 28(1):1–45. [PubMed: 21395514]
73. Leroux JC, Allemann E, De Jaghere F, Doelker E, Gurny R. Biodegradable nanoparticles: from sustained release formulations to improved site specific drug delivery. J Control Release. 1996; 39:339.
74. Tiyaboonchai W. Chitosan nanoparticles: a promising system for drug delivery. Naresuan Univ J. 2003; 11:51.
75. Oppenhiem RC. Paclitaxel loaded gelatin nanoparticles for intravesical bladder cancer therapy. Int J Pharm. 1981; 8:217.
76. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surfaces B: Biointerfaces. 2010; 75:1–18.
77. Katakoa K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev. 2001; 47:113–131. [PubMed: 11251249]
78. Matsumura Y. Polymeric micellar delivery systems in oncology. Jap J Clin Oncol. 2008; 38:793–802. [PubMed: 18988667]
79. Lee KS, Chung HC, Im SA, et al. Multicenter Phase II trial of Genexol-PM, a cremophorfree, polymeric micelle formulation of paclitaxel in patients with metastatic breast cancer. Breast Cancer Res Treat. 2008; 108:241–250. [PubMed: 17476588]
80. Xu C, Sun S. Superparamagnetic nanoparticles as targeted probes for diagnostic and therapeutic applications. Dalton Trans. 2009; 7:5583–5591. [PubMed: 20449070]
81. Sokolov K, Follen M, Aaron J, et al. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. Cancer Res. 2003; 63:1999–2004. [PubMed: 12727808]
82. Loo C, Lowery A, Halas N, West J, Drezek R. Immunotargeted nanoshells for integrated cancer imaging and therapy. Nano Lett. 2005; 5:709–711. [PubMed: 15826113]
83. El-Sayed IH, Huang X, El-Sayed MA. Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. Cancer Lett. 2006; 239:129–135. [PubMed: 16198049]
84. Greiner NR, Phillips DS, Johnson JD, Volk F. Diamonds in detonation soot. Nature. 1988; 333:440–442.
85. Li J, Zhu Y, Li W, Zhang S, Peng Y, Huang Q. Nanodiamonds as intracellular transporters of chemotherapeutic drug. Biomaterials. 2010; 31:8410–8418. [PubMed: 20692696]
86. Huang H, Pierstorff E, Osawa E, Ho D. Active nanodiamond hydrogels for chemotherapeutic delivery. Nano Lett. 2007; 7:3305–3314. [PubMed: 17918903]
87. Rojas S, Gispert JD, Martin R, et al. Biodistribution of amino-functionalized diamond nanoparticles In vivo studies based on 18F radionuclide emission. ACS Nano. 2011; 5(7):5552–5559. [PubMed: 21657210]

Key Terms

Mesoporous silica nanoparticles	Particles in the nanoscale composed of MCM-41 or SBA-15 mesoporous silica with pores running through the nanoparticle where molecules can be encapsulated
Nanomachine	A device in the nanoscale consisting of fixed and moving parts that responds to specific stimuli
Surface modification	The addition of molecules not present during the synthesis process to the surface of nanoparticles
Biocompatibility	Study of the interaction of biomaterials with the body of experimental animals to evaluate possible toxicity effects
Biodistribution	Tracking the localization of compounds of interest in the organs and tissues of experimental animals to determine the accumulation in different parts of the body

Executive summary

Mesoporous silica nanoparticles

- The synthesis of mesoporous silica nanoparticles (MSNs) has been well established by various groups and they provide a solid framework capable of encapsulating biomolecules.

Surface modifications

- The attachment of targeting moieties such as folate, mannose, transferrin and argenine–glycine–aspartic acid peptides onto the surface of MSNs has demonstrated enhanced drug delivery to cancer cells.

Cellular uptake & exocytosis

- MSNs are readily taken up by cells in an energy-dependent manner through active endocytosis but the size and shape of the nanoparticles determines the efficacy of cellular uptake.
- MSNs are exocytosed from cells and the efficiency of exocytosis differs among different cell lines.

Biocompatability & biodistribution

- MSNs are biocompatible at doses up to 50 mg/kg with no major adverse effects detected.
- The biodistribution of MSNs depends on the size, shape and charge of the nanoparticles.

Efficacy to deliver anticancer drugs

- Experiments in mice have demonstrated a significant improvement in tumor growth inhibition when drugs are delivered with MSNs.

Nanomachine-equipped MSNs

- Nanomachines that can respond to intracellular conditions or to external stimuli have been designed and incorporated to MSNs providing a controlled-release capability.
- Controlled release using internal stimuli
  - pH-operated machines: mechanized MSNs that remain close at biological pH but open at low pH have been designed to induce drug release when the nanoparticles are inside the endo/lysosomes of the cell.
  - Enzyme activation: MSNs that cargo is encapsulated with enzyme cleavable stoppers have been designed to induce release upon contact with specific enzymes.
  - Redox activation: the reducing environment within cells, due to the presence of glutathione, lead to the development of redox-sensitive systems that have shown promising in vitro results.
- Controlled release using external stimuli
  - Light activation: externally controlled nanomachines that respond to light have been constructed by various groups to provide on-demand cargo release.

- Magnetic field activation: the combination of MSNs with magnetic nanomaterials has resulted in the development of magnetically activated release systems that generate heat upon exposure to an alternating magnetic field resulting in cargo release.

Hybrid materials

- The combination of MSNs with polymers, lipids or metals has resulted in systems with improved suspendability in solution, biodistribution and tumor accumulation.

Multifunctional nanoparticles

- The combination of drug delivery, targeting and imaging using one type of nanoparticle provides unparalleled advantages that will greatly improve cancer therapy. The next frontier is to incorporate nanomachines to these systems.

Comparison with other delivery platforms

- Nanoparticles composed of different materials have been synthesized with promising drug-delivery capabilities but limitations in either stability (soft nanoparticles) or drug encapsulation (hard nanoparticles) make MSNs one of the attractive options for multifunctional development.

Future perspective

- Biocompatibility and biodistribution studies must be done in higher mammals in order to move towards clinical trials in humans and determine the toxicity and biodistribution of MSNs.
- The application of multifunctional MSNs in the medical field will provide multiple approaches to the treatment of cancer.

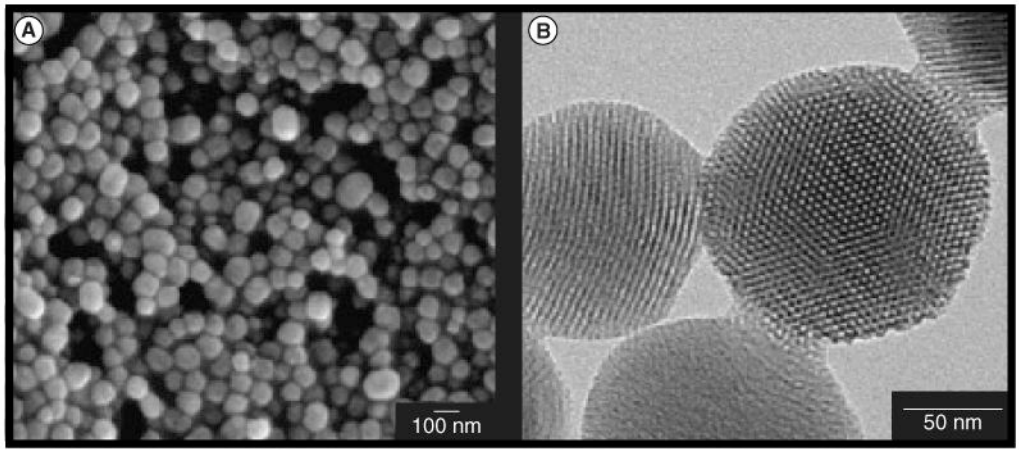

Figure 1. Characterization of mesoporous silica nanoparticles

(A) Scanning electron microscopy and **(B)** transmission electron microscopy images of fluorescent mesoporous silica nanoparticles.

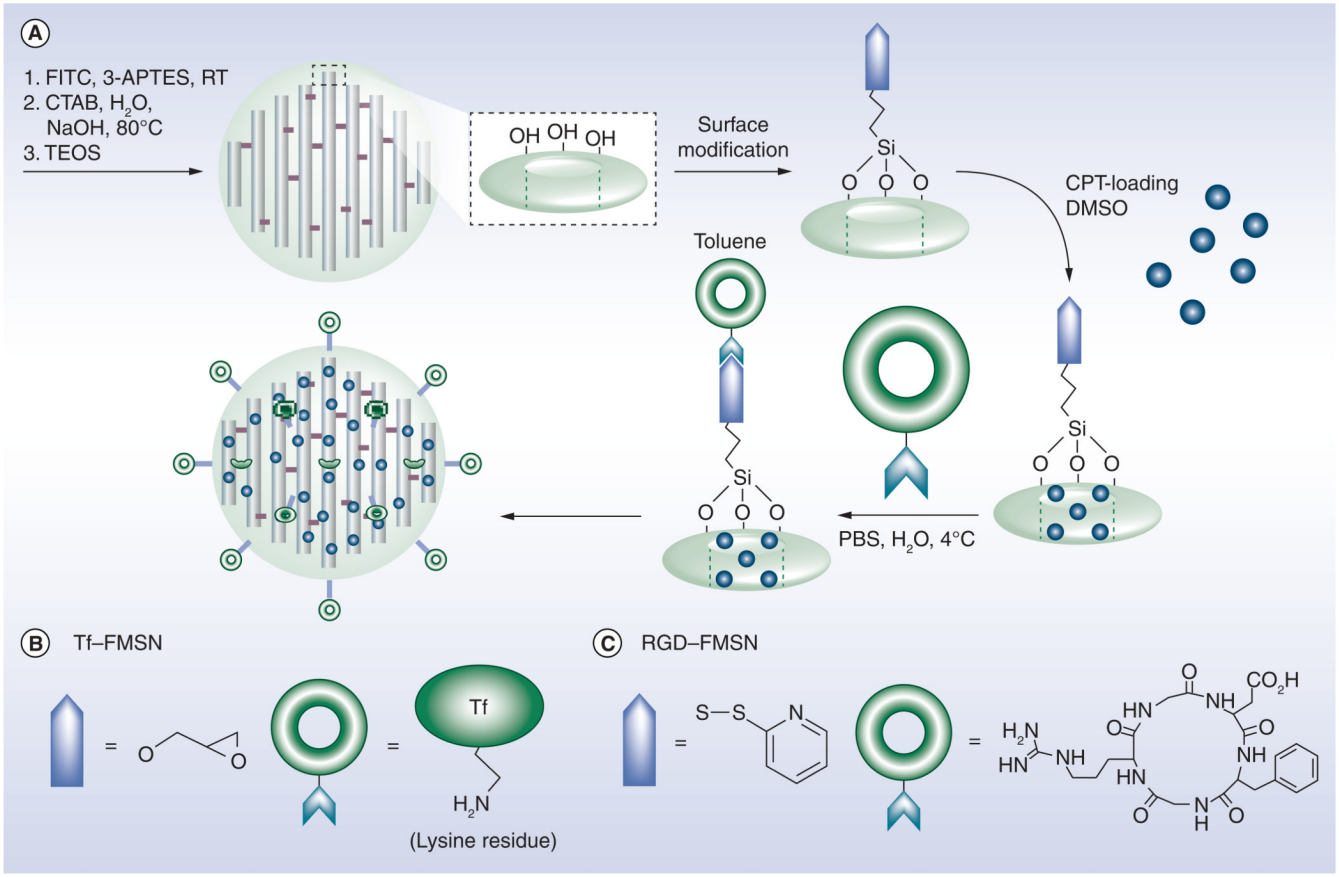

Figure 2. Methods for particle drug loading and attachment of the protein or peptide to the particles

(A) A general overview for each major step in the synthetic scheme is displayed. **(B)** Specifically, to attach the protein transferrin, the mesoporous silica particle is first modified with 3-glysidoxypropyltriethoxysilane, loaded with CPT in anhydrous DMF and then reacted with the transferrin to provide the particle cell signaling and uptake enhancement. **(C)** To attach the RGD cyclic peptide, the surface was thiol modified with 3-mercaptopropyltrimethoxysilane, reacted with 2, 2′-DTP, CPT loaded in DMF and then allowed to react with the peptide to bind it to the particle covalently.

APTES: (3-aminopropyl) triethoxysilane; CPT: Camptothecin; CTAB: Cetyltrimethylammonium bromide; DMF: Dimethylformamide; DMSO: Dimethyl sulfoxide; DTP: Dithiopyridine; FITC: Fluorescein isothiocyanate; FMSN: Fluorescent mesoporous silica nanoparticle; PBS: Phosphate buffer saline; RGD: Argenine–glycine–glutamic acid; RT: Room temperature; TEOS: Tetraethyl orthosilicate; Tf: Transferrin.

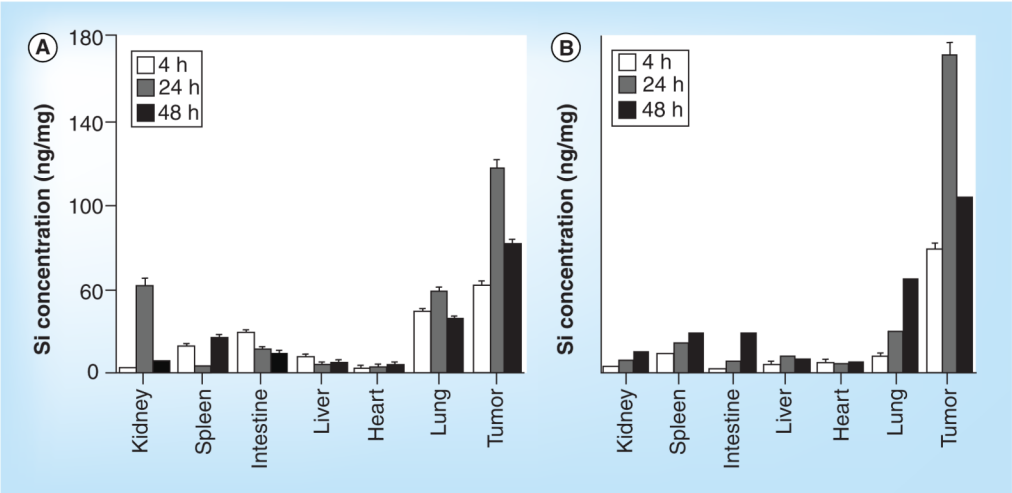

Figure 3. Inductively coupled plasma MS quantitative measurement of Si concentrations in each organ of the mice with xenograft tumors

The mice bearing subcutaneous human breast tumors were injected with (A) fluorescent mesoporous silica nanoparticles or (B) folate-fluorescent mesoporous silica nanoparticles. Mice organs were collected and Si concentrations were measured with inductively coupled plasma MS. The average Si concentrations of each organ of the mice are shown as nanogram of Si per milligram of tissue. The results are shown as mean values ± standard deviation.

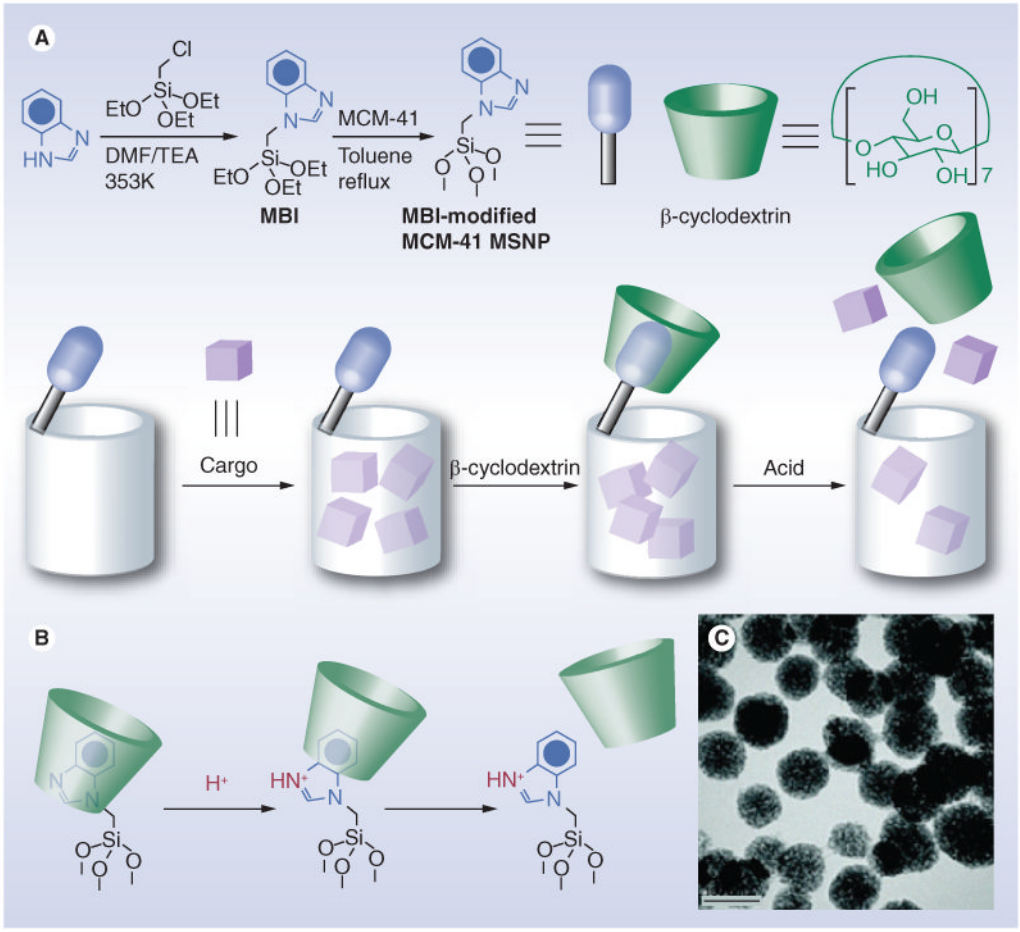

Figure 4. A graphical representation of the pH-responsive mesoporous silica nanoparticle nanovalve

(A) Synthesis of the stalk, loading of the cargo, capping of the pore and release of the cap under acidic conditions. Based on calculations by Meng *et al.*, the maximum number of stalks per nanopore is six and the maximum number of fully assembled nanovalves per nanopore is four [34]. The average nanopore diameter of the MSNP is approximately 2.2 nm and the periphery diameter of the secondary side of β-cyclodextrin is approximtaley 1.5 nm. Thus, for a cargo with diameter >0.7 nm, a single nanovalve should be adequate to achieve effective pH-modulated release. **(B)** Details of the protonation of the stalk and release of the β-cyclodextrin. **(C)** Transmission electron microscopy image of capped MSNP (scale bar = 100 nm).

DMF: Dimethylformamide; MBI: *N*-menthylbenzimidazole; MSNP: Mesoporous silica nanoparticle.

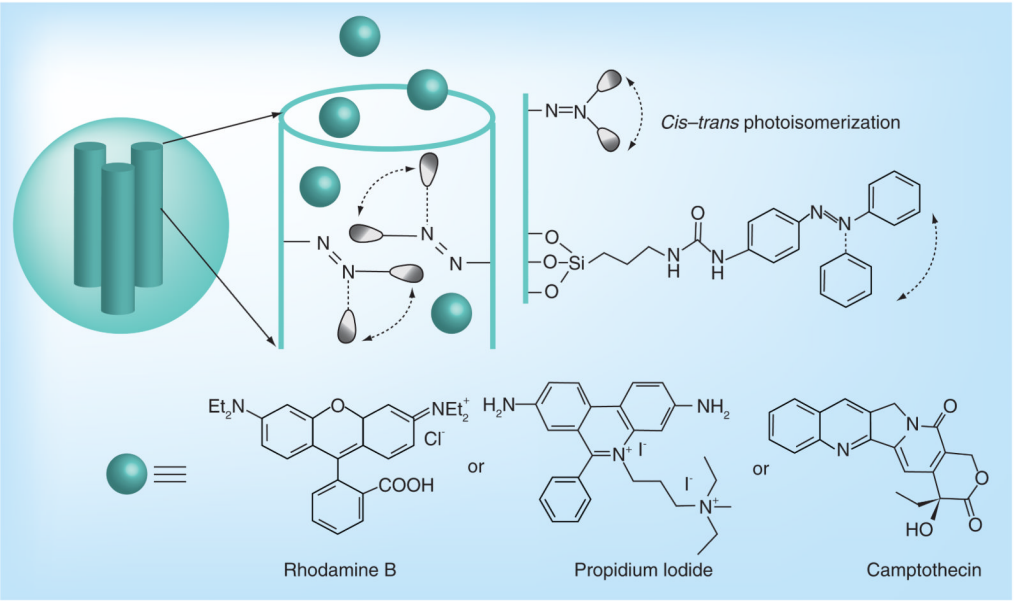

Figure 5. Designed pore interiors of the light-activated mesostructured silica nanoparticles functionalized with azobenzene derivatives

Continuous illumination at 413 nm causes a constant *trans–cis* photoisomerization around the N=N bond, causing dynamic wagging motion of the azobenzene derivatives and results in the release of the molecules through and out of the mesopores.