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REVIEW ARTICLE

# Mesoporous Silica Nanoparticles for Drug Delivery and Controlled Release

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## ABSTRACT

Drug molecules with lack of specificity and solubility lead patients to take high doses of the drug to achieve sufficient therapeutic effects. This is a leading cause of adverse drug reactions, particularly for drugs with narrow therapeutic window or cytotoxic chemotherapeutics. To address these problems, there are various functional biocompatible drug carriers available in the market, which can deliver therapeutic agents to the target site in a controlled manner. Among the carriers developed thus far, mesoporous materials emerged as a promising candidate that can deliver a variety of drug molecules in a controllable and sustainable manner. In particular, mesoporous silica nanoparticles are widely used as a delivery reagent because silica possesses favorable chemical properties, thermal stability and biocompatibility. Currently, sol-gel-derived mesoporous silica nanoparticles in soft conditions are of main interest due to simplicity in production and modification and the capacity to maintain function of bioactive agents. The unique mesoporous structure of silica facilitates effective loading of drugs and their subsequent controlled release. The properties of mesopores, including pore size and porosity as well as the surface properties, can be altered depending on additives used to fabricate mesoporous silica nanoparticles. Active surface enables functionalization to modify surface properties and link therapeutic molecules. The tunable mesopore structure and modifiable surface of mesoporous silica nanoparticle allow incorporation of various classes of drug molecules and controlled delivery to the target sites. This review aims to present the state of knowledge of currently available drug delivery system and identify properties of an ideal drug carrier for specific application, focusing on mesoporous silica nanoparticles for control drug release and surface functionalization.

## KEYWORDS

Mesoporous Silica Nanoparticle, Targeted Drug Delivery, Controlled Release, Sol-Gel Process, Chemotherapy and Surface Functionalization

## INTRODUCTION

*Nano medicine* is a multidisciplinary field of research where the interest is in using nanomaterial to the advancement of health. One area of Nano medicine is targeted drug delivery where the goal is to deliver medications to the diseased area of the body in a manner that accumulates the drug to that area. The material is processed to different sizes of particles to carry drug molecules. For targeted drug delivery the type of particles are called *nanoparticles*. Nanoparticles are often defined as particles with a diameter less than 100 nm in diameter.[1] The surface of the nanoparticles is modified to increase the circulation time in the body, cell specific targeting and membrane permeability.

During the past several decades, a steadily growing number of drugs have been discovered.

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However, about 40 percent of newly designed drugs, especially those which are based on biomolecules such as peptides, oligonucleotides, proteins and DNA, often exhibit low bioavailability and are rejected by the pharmaceutical industry.[2] Therefore, there is an increasing demand for the development of drug delivery systems to minimize drug degradation, manipulate drug pharmacological profile, diversify drug administration routes, decrease detrimental drug side effects and target specific sites. To achieve these goals, numerous materials have been extensively investigated, such as amphiphilic block copolymers,[3-5] liposomes,[6] dendrimers,[7,8] hydrogels,[9,10] as well as inorganic nanoparticles.[11,12]

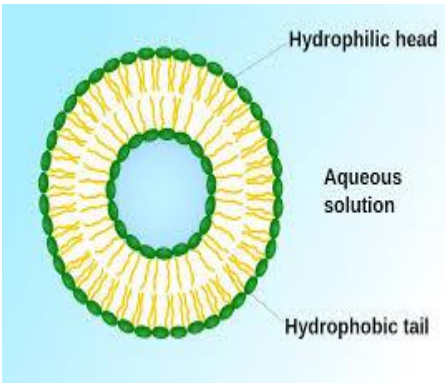
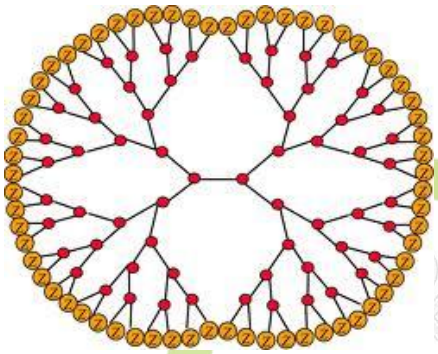
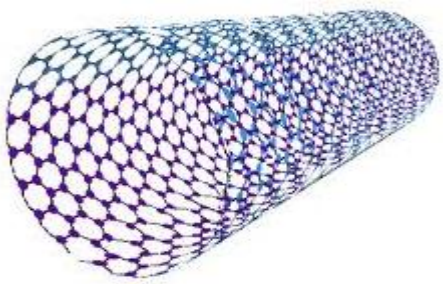
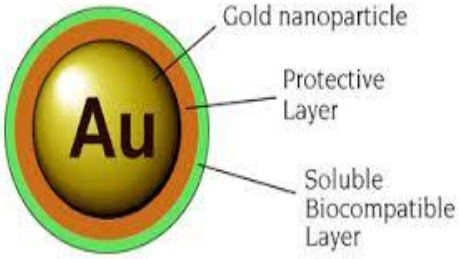
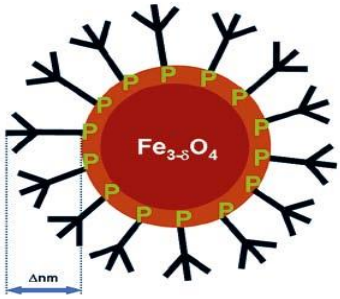
Among numerous drug delivery systems tested, mesoporous silica nanoparticles (MSNs) stand out to be a promising candidate, which have the potential to perform all the above-mentioned functions simultaneously. Typically, MSNs used as drug delivery systems are featured by their ordered arrays of 2D hexagonal micro- or mesopore structure, uniform particle sizes (80-500 nm), large surface areas (>1000 $m^2 g^{-1}$), high pore volumes (0.5-2.5 $cm^3 g^{-1}$), tunable pore diameters (1.3-30 nm), controllable particle morphology and both exterior and interior surfaces that could be independently modified with a variety of functional groups. In contrast to conventional polymer-based drug delivery systems, which usually suffer from problems such as low drug loading capacity and poor drug release control, MSNs-based drug delivery systems successfully avoid these issues. The high surface areas and pore volumes allow for a large payload of drug molecules. The pore environment and surface can be adjusted by functional groups favored by drug molecules in order to further enhance drug loading and releasing ability. The highly stable pore channels prevent encapsulated drug molecules from degradation in harsh environments during drug administration. The tunable particle morphology of MSN materials renders their superb biocompatibility at concentrations adequate for pharmacological applications.

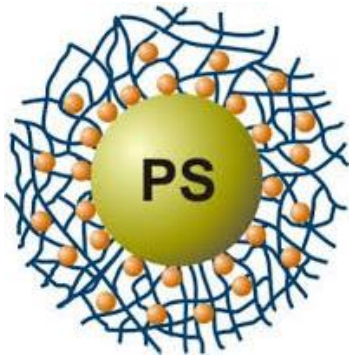
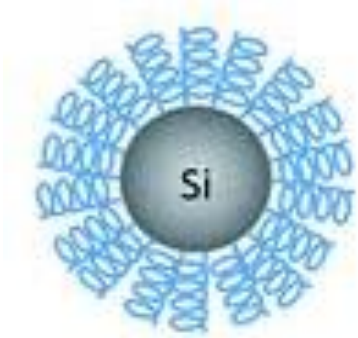
Furthermore, the most remarkable advantage of MSN materials as drug delivery systems is their "zero premature controlled release" property. Namely, drugs are carried with precise control of location and time without leaching prior to reaching the targeted cells or tissues. This technique is realized by encapsulating drug molecules inside the pores of MSN materials followed by blocking the pore entrances with stimuli responsive agents, or so called "caps". Hence, delivery of drug molecules takes place only when these caps leave the MSN assembly when triggered by external or internal stimuli that are manipulated manually at a desired location and time. In addition, it is also possible to deliver guest molecules repeatedly in small portions by reversibly switching the MSNs-based drug delivery system between "on" and "off" status. Thus, delivering biomolecules to the specific site by a controlled drug delivery system is considered as an ideal way to improve quality use of medicine by reducing dose and frequency of drug intake,[8-12] taking into account that effective drug release rates and durations require careful assessment of target site pharmacokinetics, drug delivery vehicle design, the selection of clinically effective drug according to the clinical context, effective dosage and drug release kinetics requirements.[12] As one of the most promising nanocarriers, mesoporous silica nanoparticles (MSNs) are reviewed here focusing on their physicochemical properties and targeting drug delivery applications.

## Currently Available Drug Delivery Systems

The high or frequent dosing, systemic absorption in unrelated sites and suboptimal concentration of bioactive agents in target site contribute to the restriction in accessibility of therapeutic agents. By developing drug delivery systems, the function of drugs can be significantly improved which could also render huge economic benefits. For example, Wong et al.[13] estimated that US$8 billion could be saved by only developing more effective drug delivery systems for hydrophobic drugs. Thus, many studies investigated different forms of drug

| Drug delivery system | Structure | Chemical properties | Reference |
|---|---|---|---|
| Liposomes |  | • Consists of hydrophobic tail and hydrophilic head group<br>• Forms closed vesicles with an aqueous core<br>• Internal aqueous domain between the lipid bilayers<br>• Encapsulation of drugs occurs either in the aqueous space or intercalated into the bilayer. | 14-16 |
| Dendrimers |  | • Hyper branched and globular macromolecules<br>• Well defined core, backbone and multivalent periphery<br>• By hydrophobic and electrostatic interactions, incorporate biomolecules<br>• Convergent – endo-receptor<br>Divergent – exo-receptor | 17-19 |
| Carbon nanotubes |  | • Rolling up a single layer of grapheme sheet – single walled<br>• Rolling up many layers to form concentric cylinders – multi-walled | 20-24 |
| Gold nanoparticles |  | • Gold nanoparticle serves as core<br>• Photosensitive | 25,26 |
| Iron oxide nanoparticles |  | • Superparamagnetic particles<br>• Need trigger to release biomolecules, for example, laser irradiation | 25,27 |

| | | | |
|---|---|---|---|
| Titanium dioxide nanoparticles |  | • Self-ordered<br>• Nano-tubular structure<br>• Photodynamic therapy | 28-30 |
| Silica nanoparticles |  | • Mesoporous structure<br>• Honeycomb-like structure<br>• Active surface | 31,32,33 |

delivery vehicles, and the most popular systems are listed in Table 1.

The listed drug carriers have different physicochemical properties which make them suitable for different drugs. The common goal of the carrier is to transport drug molecules to the target site in a controlled manner. Ideally, they should be biocompatible, not cause any immunogenic or cellular reactions and release drug molecule controllably at the target sites without altering its therapeutic effects.[34]

**Synthesis of Mesoporous Silica Nanoparticles**

The family of mesoporous silica materials was independently discovered by Kresge et al.[35] at the Mobil Oil Company and the Kuroda group[36] at Waseda University in the early 1990s. Since then, research in this field has tremendously expanded. Mesoporous silica materials with different mesophases have been synthesized by varying experimental conditions such as pH, temperature, templates and molar ratios.[37,38.] The earliest and most well-known representative is MCM-41, exhibiting a 2D hexagonal mesopore arrangement (Fig. 1).

Another example is SBA-15, sharing a 2D hexagonal structure, but it bears wider, tunable pore size range and greater hydrothermal stability than MCM-41. Both types of MSN materials have found applications as drug delivery devices.[35,36-40]

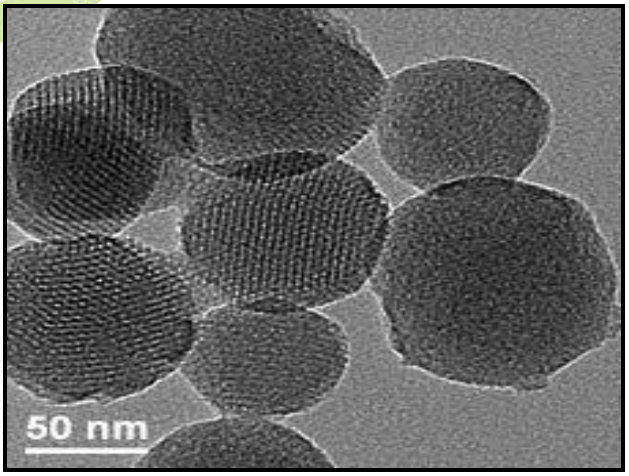



Figure 1: Transmission Electron Micrograph

The key principle for synthesizing MSN materials is the condensation of silica precursors directed by self-assembled liquid-crystal arrays of surfactants. In-depth investigations have led to two proposed mechanisms involved in the

formation of supramolecular aggregates of surfactants and subsequent generation of MSN materials.[41] synthesis process shown in Fig. 2. In the liquid crystal templating mechanism (LCT), surfactants form liquid crystal structures at concentrations above the critical micelle concentration (CMC) of the surfactant and serve as templates, without requiring the addition of silica precursors. While an alternative mechanism was proposed that the final mesopore ordering is a process of cooperative interaction between surfactants and silica precursors. For example, MSN materials could be prepared even at surfactant concentrations far below the CMC, in which case an ordered liquid crystal structure could develop under the second mechanism.[42]

In a typical surfactant-silica precursor interaction, tetramethyl- (TMOS) or tetraethyl-orthosilicate (TEOS) is normally added as silica precursors, and cationic alkyltrimethyl-ammonium salts are used as templates under a basic reaction condition. Further exploration was conducted by the Stucky research group, where they employed a series of block-copolymer surfactants as structure directing agents to synthesize MSNs in acidic environments.[42-45]

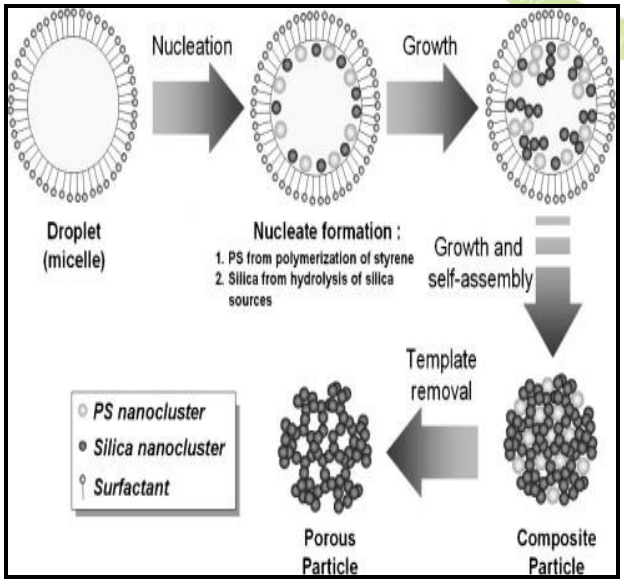



Figure 2: Mechanism for synthesis of MSNs

A variety of strategies have been proposed to attain tunable pore sizes from less than 2 nm up to 30 nm, including the adjustment of the hydrocarbon chain length of small surfactant templates,[45,46] the use of pore swelling agents such as mesitylene,[47] or hydrothermal treatments.[48] The control over the surfactant-silica interaction enables a versatile synthesis condition for MSN materials, and thus allows for the functionalization of other species into the silica framework.

## Properties of Mesoporous Silica Nanoparticles

Different parameters of MSN fabrication contribute to different delivering mechanisms of active agents. The parameters that control the kinetics of drug release from MSN are outlined in Table2.

Summary of different factors that regulate controlled release of MSN.[49]

### *Textual Properties*

The size of the drug delivery carrier is an important determinant which can be divided into three scales: macro, micro and nano. 'Macro'-sized delivering agents are used to transport biomolecules to organs, whereas 'micro'-scaled carriers target tissue delivery. With respect to intracellular drug delivery, the large-sized carrier is limited, as it cannot be engulfed by mammalian cells via endocytosis, which may ultimately cause accumulation of drug vehicle. Also, it was shown that larger sized materials are more likely to trigger an acute immune response in vivo, as it is within the size window of bacteria.[49] Therefore, particle size in micrometer range is unfavourable in drug delivery.[50] In biomedical applications, 'nano'-scaled delivery carriers should be employed in order to deliver therapeutic agents at a cellular level such as in the cell membrane, cytoplasm or nucleus by facile endocytosis.[42,46,66]

As particle size increases, the efficiency of uptake by the cell decreases.[68] The diameter of MSN can be tuned controllably in the range 20–500 nm. It was stated that particle size between 50 and 300 nm can be engulfed by living animal cells without causing any cytotoxicity, while MSN of diameter <300 nm is desirable from a

| | | | Adsorption | Release |
|---|---|---|---|---|
| Host–guest interactions and controlled adsorption and release kinetics | Textural properties | Mesopore diameter<br>Surface area<br>Mesopores volume | Size selectivity<br>Enhanced adsorption | Rate modulator |
| | Chemical properties | Surface functionalisation | Higher drug loading<br>Allow loading<br>Increase loading | Slow down |

biomedical point of view.[43,47] Slowing et al.'s65 study conceded that particle size around 200 nm or smaller will have highest efficiency and particle size larger than 1000 nm will cause little uptake. Similarly, another study confirmed that nanoparticles below 200 nm will induce endocytosis,[48] whereas nanoparticles with larger size may be internalised by phagocytosis or not internalised at all.[49] The term 'mesoporous' refers to the sizes between 2 and 50 nm.[50] The pore size can be tuned selectively with a narrow distribution[51] between 2 and 6 nm in diameter with pore volume of around 1 cm3/g, depending on the type of drug molecules that will be incorporated.[52,56] Larger pore size is suitable to load a high dose of drug molecules.[51] In addition, large surface area allows the high adsorption of therapeutic agents on the surface of MSN, which is related to high-loading dose of therapeutic agents.[57,61,88]

### *Internal Structure*

The internal mesopore structure of MSNs is the most relevant and fascinating property. Mesopores are not randomly distributed, but rather specifically aligned and structured presenting honeycomb-like structures with hundreds of empty channels. The channels are considered individual reservoir of drugs without interconnections between channels.[58] The internal structure of the MSNs, including size, volume and aligned structure of mesopores, can be controlled by the initial reagents or the surfactant. Several reviews have discussed this aspect of MSNs in detail.[58,61,72] Apart from this, the most commonly used MSNs (MCM- 41, MCM-48, Santa Barbara–type mesoporous particle-15 (SBA-15), SBA-16).

### *Structural Differences*

Mesoporous silicon/silica-based materials provide a possibility to tailor the carrier structure and the surface composition according to the different needs. The pore size can be modified to fit the size of the drug molecule that will be loaded into the porous material, as well as to achieve the aimed release profile. The release profile can be controlled also via different surface treatments of the materials, leading to desired interactions between the porous carrier and the loaded substance. The surface treatment can also affect the loading of the molecules into the pores via hydrophobic-hydrophilic interactions.[59]

### *Pore Morphology*

The fabrication method used affects the structural order of the pores in each material. Silica gel is formed via condensation polymerization and the material is composed of nonordered silica network resembling a sponge-like structure. The porosity can be slightly modified by adapting the synthesis conditions; however the outcome is amorphous, irregular PSiO2 with variable pore size and shape. The pore structure of Psi depends on the fabrication conditions including, for example, the type of the silicon source, current density and HF concentration used. Generally, one can conclude that with lower current densities the pores are more tortuous, fir tree or sponge-like, and with increasing current densities the pores become wider and more linear. A schematic example of one possible mesopore structure of PSi is shown in Figure 3A.

The pore diameter of the particle is one important factor affecting the release rate of the

loaded substance. The pore diameters of PSi can vary from few nanometers to micrometers, however in drug delivery the mesoporous silicon is the most studied addition, many parameters affect the pore diameter, which in turn can be adjusted to optimize the pore properties. The pore size distribution of PSi is usually wider than that of mesoporous silicas, whose pore sizes mainly depend on the utilized template materials. The definite advantage of mesoporous silicas is their uniform pore sizes which can be tailored by selecting an appropriate template system. The most studied mesoporous silica in drug delivery applications are MCM-41 and SBA-15 materials. They exhibit highly ordered two-dimensional tube-like pore structures (Figure 3B). MCM-41 pore diameter lies typically between 1.5 and 10 nm, whereas polymer-templated SBA-15 has wider pores of 4.6-30 nm. The initial publications of ordered mesoporous silica presented these two-dimensional hexagonal structures. The versatile templating systems enable various silica network assemblies, such as cubic or three-dimensional hexagonal ordered PSiO2 structures; also several variable ordered silica materials have been reported.[60-65]

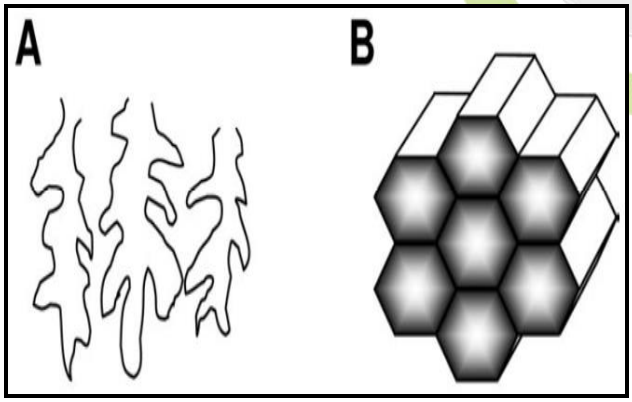



Figure 3: Schematic representations of the mesopores of PSi (A) and ordered mesoporous SiO2 (B). Adapted from (Kresge et al., 1992; Lehmann et al., 2000)

***Surface Chemistry***

Surface interactions between the porous particle and the loaded substance are critical in adjusting the pharmaceutical functions of the material. The surface areas of freshly-made mesoporous silicon, silica gel and ordered silica are about 300 m2/g, 10-1000 m2/g and >700 m2/g, respectively. The as-anodized surface of PSi contains hydrides (Si-H, Si-H2 and Si-H3), which are prone to spontaneous oxidation in ambient air. The freshly made surface can be stabilized by converting the reactive groups into more stable oxidized, hydrosilylated or (hydro)carbonized forms (Figure 4).

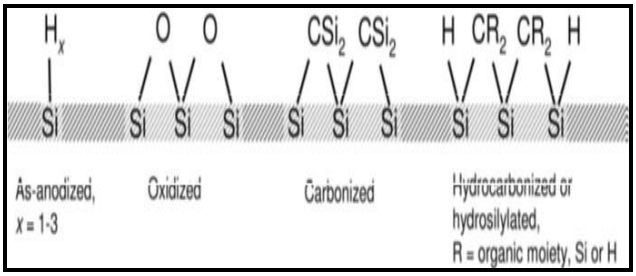



Figure 4: Surface chemistry of mesoporous silicon after anodization and various surface treatments

Thermal oxidation is one of the simplest ways to oxidize silicon surfaces; the product is called thermally oxidized PSi (TOPSi). Other methods include, for example, chemical and anodic oxidation. The Si-C bond is kinetically more stable than the oxidized Si-O bond. The stabilization can be achieved by a catalyzed reaction of terminal alkenes or alkynes with the hydrides of silicon, called hydrosilylation. The hydrosilylation method can also be used to functionalize the surface of PSi for different purposes by using selected organic moieties in the other end of the alkene or alkyne.[68]

The surface of amorphous silica is covered with silanol (≡Si-OH) and siloxane (≡Si-OSi≡) groups. In addition, there are structurally bound water molecules inside the silica network, called internal silanol groups. The silanols can exist in three different forms: isolated, vicinal and geminal as shown in Figure 5. Isolated and germinal silanol groups can be used as grafting templates for, *e.g.* amino or dendrimer functionalized silica. The functionalization can also be performed during synthesis of the material as a co-condensation process. Silanol and siloxane groups can also interact as such with the loaded substances to form hydrogen bonds.

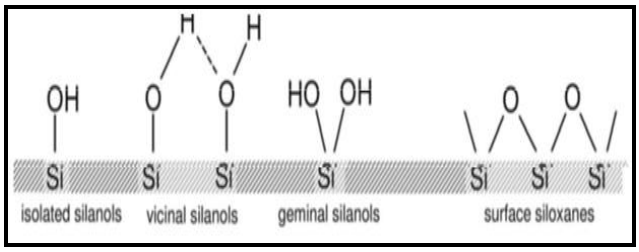



Figure 5: Different forms of silanols

Silanol number, αOH, is used to describe the number of OH groups per unit surface area of the silica materials. The value varies between different silica materials and is also affected by the post-synthetic treatments, such as the calcination time of the material. This makes the comparison of various results challenging, but generally, αOH is the highest in amorphous silica gel (4.2-5.7), with decreasing values from SBA-15 (2.8-5.3) to MCM-41 (1.4-3) materials.

**Characteristics of Silica Nanoparticles**

Many research efforts have focused on (i) controlling the particle morphology and (ii) preparing the organic/inorganic hybrids through functionalization of the exterior and/or interior surfaces. The particle morphology and degree of functionalization were dictated by the concentration, molecular size, and hydrophilicity/hydrophobicity of the organoalkoxysilane precursors.

(i) Controlling the particle morphology

- Tunable particle size: 50 to 300 nm
- Stable and rigid framework
- Uniform and tunable pore size: 2 and 6 nm
- High surface area and large pore volume: (> 900 $m^2$ /g) and (> 0.9 $cm^3$ /g)
- Unique porous structure

(ii) Functionalization of silica nanoparticles.

***Three Dimensional Structures of Mesoporous Silica Nanoparticles (MSN)***

MSN have an internal surface (i.e., cylindrical pores) and an external surface (i.e. exterior particle surface). This characteristic allows the selective functionalization of the internal and/or external surfaces of MSN with different moieties. The keystone in the development of silica mesoporous materials as DDSs is the modification or functionalization of the surface through organic groups. The conventional formulations of CyA (Sandimmun) caused marked intra- and inter-individual variation in drug pharmacokinetics. Neoral, another formulation, is a microemulsion of pre-concentrated CyA designed to provide better consistent absorption of the drug. After oral administration this compound is absorbed only incompletely and variably, leading to a relative bioavailability of less than 50%. In contrast to most peptides, it is particularly lipophilic. It is practically insoluble in water and is soluble in alcohol. These characteristics are favorable for encapsulation in particles. The nanoparticles formulation had a notably increased bioavailability compared with that of the commercial formulation.

***Biological Safety of Mesoporous Silica Nanoparticles***

Silicon is one of the most abundant chemical elements on the Earth's crust, usually present as silicon dioxide, silica. The exact role of silicon in human biology is still under investigation. It is absorbed daily from food in the form of orthosilicic acid [Si(OH)4], and its positive role in the health of connective tissues and bone has been recognized. Silicon does not accumulate in the body; instead, it is readily excreted into urine as orthosilicic acid.

An unavoidable thought when it comes to dosing of silicon/silica-based materials to humans is silicosis – a respiratory disease derived from breathing of crystalline silica dust. Other diseases, such as lung cancer and rheumatoid arthritis, have also been connected to crystalline silica exposure. It is important to recognize the difference between crystalline and amorphous silica. The International Agency for Research of Cancer (IARC) has classified inhaled crystalline silica as carcinogenic to humans, but amorphous silica is not classifiable as to its carcinogenicity to humans.

Silicon dioxides are generally recognized as safe food substances and listed in the FDA (U.S.

Food and Drug Administration) inactive ingredients database as used in, *e.g.*, oral and topical drug products (FDA, 1979, 2011). Probably due to the history of crystalline silica, human safety studies with amorphous silica have also mainly focused on pulmonary exposure. Some reversible lung symptoms have been reported, but no evidence of chronic diseases has been proven. On the other hand, chronic pulmonary effects have not been definitely excluded. The mechanism behind the better lung tolerability towards amorphous silica could be its higher surface area, which leads to faster dissolution and removal from the alveoli as compared to crystalline silica. However, the mechanism remains unclear and is not fully understood yet. The emergence of mesoporous silicon/silica-based materials as drug delivery systems has promoted the safety studies in the field.

In addition, bio-safety of MSN can be associated with different routes of administration. In Fu et al.'s133 study, despite the fact that silica nanoparticles could cross different biological barriers into the liver, low absorption rate of silica nanoparticles were observed when they were administrated by the intramuscular injection. In contrast, silica nanoparticles were well absorbed into the intestinal tract and persisted in the liver when it administered through oral route. Additionally, silica nanoparticles were mainly present in the liver and spleen when they were injected intravenously. In contrast to other studies, this study found that most of silica nanoparticles were excreted through urine and faeces after different routes of administration, which indicates that silica nanoparticle is reasonably biocompatible and can be used for different biomedical applications.[60] Further studies need to be conducted to justify bio-safety of silica nanoparticles and to discover MSNs with maximal bio-safety.

### *Surface Functionalization of Mesoporous Silica Nanoparticles*

MSNs possess well-defined structure and high density of surface silanol groups, which can be modified with a wide range of organic functional group.[61] The surface functional groups can play several roles in biomedical applications of MSNs: (a) to control the surface charge of MSNs, (b) to chemically link with functional molecules inside or outside the pores and (c) to control the size of pore entrance for entrapping molecules in the nanopores. There are three methods of surface functionalization for MSNs: co-condensation, post-synthesis grafting and surfactant displacement methods. Two popular pathways are available for surface functionalization (Fig. 6). One is to introduce organosilanes simultaneously with silica precursors during the synthesis of MSN materials ("co-condensation") (Fig. 6a). The other pathway is to prepare unfunctionalized MSN materials and subsequently modify their surfaces with organosilanes ("grafting") (Fig. 6b).

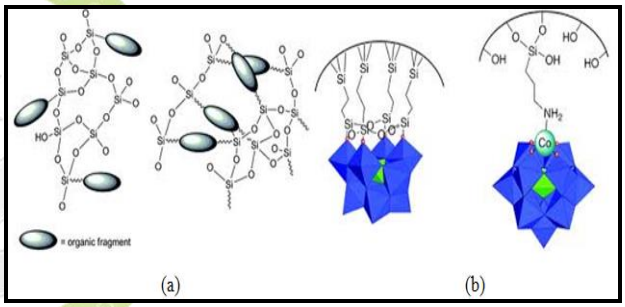



Figure 6: Surface functionalization of mesoporous silica nanoparticles by the (a) co-condensation method and (b) post-synthesis grafting method

*Surfaces Functionalization by Co-condensation Method*

Co-condensation is a direct synthesis method where organosilanes are condensed along with the silica precursors in the presence of surfactant templates. As a result, the organic groups are homogeneously distributed within the mesoporous structure. Also, it is possible to control mesoporous silica nanoparticle morphology by the introduction of different organosilanes. Lin and coworkers proposed that interactions between organosilanes and surfactant molecules, such as electrostatic interaction, hydrophobic interaction or hydrogen bonding could contribute to the variation in

particle morphology.[62] They demonstrated that organosilanes with hydrophobic groups tend to intercalate their organic groups into the surfactant micelles and interact with the hydrophobic tails of surfactants, thus stabilizing the formation of long cylindrical micelles and giving rise to rod-shaped MSN materials. On the contrary, hydrophilic organosilanes would inhibit micelle growth and yield spherical particles with randomly oriented pore structures. As a result, by employing two organosilanes with opposite head group properties and varying their ratios, the surface functionality and particle morphology of MSN materials can be fine tuned with the co-condensation method.[63]In order to preserve the pore structure and long-range pore ordering of MSNs, the amount of functional groups incorporated by the co-condensation method does not normally exceed 25 percent of surface coverage due to the difference in condensation rates between organosilanes and silica precursors. The efficiency of loading depends on the nature of organic functional groups.

*Surface Functionalization by Post-synthesis Grafting Method*

In the grafting method, major functionalization reactions take place between organic precursors and free silanol groups at the exterior surface and at the opening of the pores of MSNs. Compared to the co-condensed material, organosilane grafted MSN materials have better retained pore structures and are more thermally stable. However, in most cases, the degree of functionalization by the grafting method is lower than that of co-condensation method, owing to the limited number of free surface silanol groups. As opposed to the homogeneous functional groups coverage obtained by the co-condensation method, it has been reported that most functional groups are preferentially attached to the external surface or the pore openings, since the silanol groups are more easily accessible there than the interior pore surface which suffer from lower diffusion rates of organic precursors.32 In certain situations such as when organic precursors are too big for the pores or they are unfavorable for the pore environment, their penetration to the inner sites of the pores is extremely impaired, leading to an unmodified internal surface. Taking advantage of this feature, it is feasible to selectively functionalize the external and internal surfaces of MSN materials with different functional groups.

*Multi-functionalization*

To satisfy the need for constructing more complex MSN based drug delivery systems, it is desirable to be able to incorporate more than one type of functional group with the MSN. In a recent paper reported by Lo and coworkers,[64] a trifunctionalized MSN material was synthesized containing three distinct domains: the silica framework, the hexagonal pores and the outermost surfaces which were independently functionalized with contrast agents for imaging, drug payloads for cancer therapy and biomolecular ligands for cancer cell targeting, respectively. A near-infrared fluorescent contrast agent was co-condensed with TEOS for the optical tracking of MSN materials. The surfactant templates were then removed, followed by the grafting of nanochannels with a palladium-porphyrin based photosensitizer, which was exploited in photodynamic therapy. The third functionalization reaction occurred on the external surfaces with cRGDyK peptides that specifically bind to overexpressed integrins of cancer cells.

***Mesoporous Silica Nanoparticles for Drug Controlled Release***

As mentioned in the first section, the large drug loading capability, the flexible surface modification, the rigid porous structures and the excellent biocompatibility of MSNs are ideal for drug delivery applications. The first example of using biocompatible MSNs as carriers and inorganic nanoparticles as caps to effectively deliver drug molecules into animal cells with zero premature release was developed in the Lin research group[65]. They prepared spherical mesoporous silica nanoparticles with a uniform particle diameter of 200 nm and pore size of 2.3 nm, and then functionalized them with 2-(propyldisulfanyl) ethylamine groups.

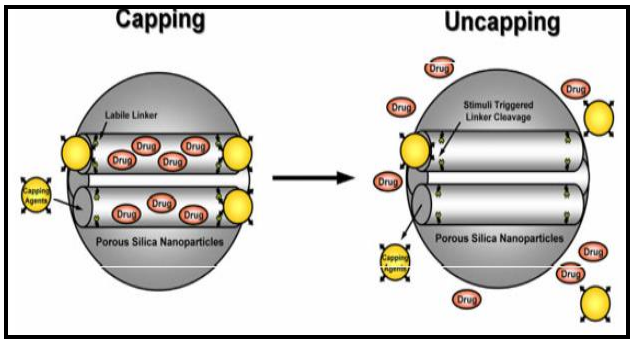



Figure 7: Design principle of mesoporous silica nanoparticles for controlled drug release

Water-soluble cadmium sulfide (CdS) nanocrystals with mercaptoacetic acid groups were then added to react with the terminal amino groups on the surface of the MSNs. Thus, the CdS nanocrystals were covalently bonded to the MSNs and blocked the pore entrances. The capping agents block pore entrances and trap drug molecules by labile linkers which later respond to specific stimuli, triggering a drug release.

Over the past decade, many research groups have made great endeavors in the development of MSNs based drug delivery systems with stimuli-responsive triggered release property (Fig. 7). A number of regulating mechanisms have been proposed and confirmed their feasibility for intracellular drug delivery with precise control of location and timing. The triggers or stimuli can be internal, meaning that they are already present in the living organism, or external, which requires a simple and convenient pathway for application. Internal stimuli are often unique to the targeted pathology, which enables drug delivery systems to respond specifically to the desired location and release drugs in a self-regulated fashion. External controls are mostly noninvasive and easy to manipulate, so that they could assist to localize the drug release and optimize the degree of the drug delivery process. Examples of these triggers include pH, light, redox potential, temperature, enzymes, etc.

*pH - Triggered Release*

A series of pH-responsive linkers have been exploited for controlled release applications by taking advantage of the acidic environment at tumor or inflammatory sites (pH ~ 6.8), endosomal or lysosomal compartments of cells (pH ~ 5-6) as well as the stomach (pH ~ 1.5-3.5). These pH-responsive linkers feature an inert respond to physiological pH and a robust release at low pH environment.

An early example of a pH-responsive release system was reported by Casacus et al., where they created a pH and anion controlled drug delivery system56. The MSN materials were prepared by the co-condensation of mercaptopropyltriethoxysilane with TEOS. A second grafting reaction was carried out with N-(3-triethoxysilylpropyl-2-aminoethyl)-ethylenediamine to get a preferential anchoring of amino groups on the external surfaces.

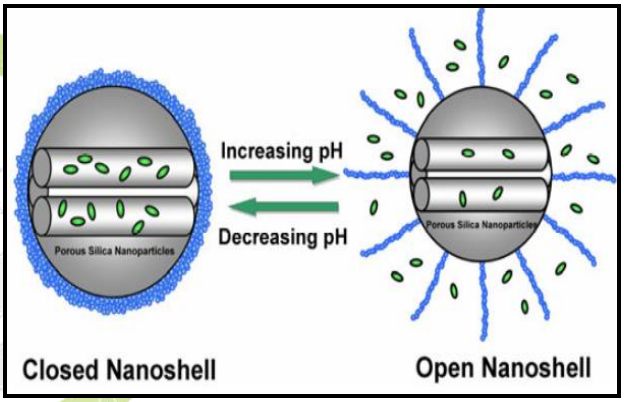



Figure 8: Schematic graph of opening and closing of the core-shell structured nanoparticle triggered by pH

At high pH values, the amines were deprotonated and were tightly packed through hydrogen bonding interactions so that the delivery system was at its "open gate" state. However, when the amines were protonated at low pH conditions, they repelled each other and covered the pore openings due to the coulombic repulsion effect between positively charged amine groups and the delivery system was monitored to its "close gate" state. In addition, a significant synergic effect was observed in the presence of anions, which could intercalate into the open-chain polyamines and seal the pore openings. This effect is clearly associated with the anion size and the strength of the polyamine-anion electrostatic interaction. Employing this

pH-responsive drug delivery system, they have successfully demonstrated pH-controlled release of squaraine and vitamin B2.[65]

*Light-Triggered Release*

Light irradiation is a convenient remote control approach for site-specific drug delivery. The uptake and release of guest molecules can be rapidly induced upon exposure to light with certain wavelengths. After Tanaka and coworkers first demonstration of a coumarin functionalized MSN material to manipulate drug release as previously discussed, photochemical responsive linkers such as azobenzene, *o*-nitrobenzyl ester and thymine bases are incorporated onto the surface of MSNs to render them photochemically susceptible for light controlled release.

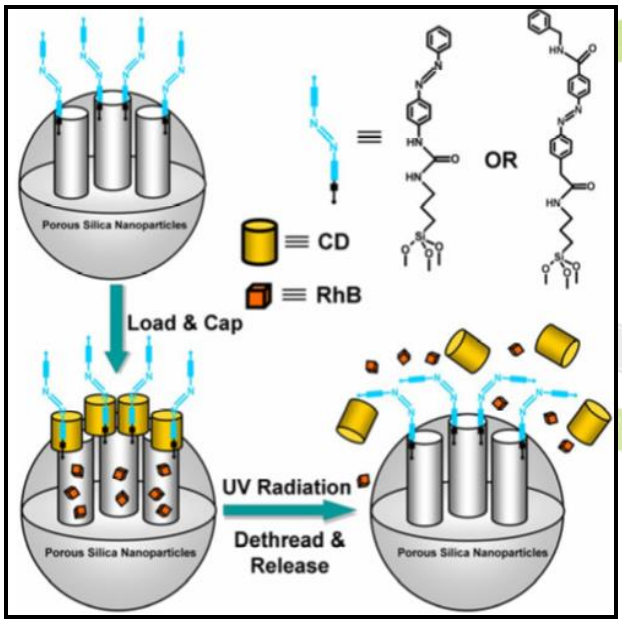



Figure 9: Schematic representation illustrating the capping of MSNs by Py-β-CD based pseudorotaxane, and the dethreading of pseudorotaxane upon UV radiation

By varying the stalk and ring components, the pseudorotaxanes approach was also applied to control drug release with light (Fig. 9). Zink, Stoddart and coworkers constituted a photosensitive pseudorotaxane of azobenzene derivative (AB) and β-cyclodextrin (β-CD).[63] Unfunctionalized MSNs were grafted with 4-(3-triethoxylsilyl18 propylureido) azobenzene (TSUA) groups or more water-soluble (E)-4-((4-(benzylcarbamoyl) phenyl)diazenyl)benzoic acid groups, as the pseudorotaxane stalks. Upon irradiation to 351 nm light, both azobenzene derivatives isomerized from the more stable *trans* form to a less stable *cis* configuration. β-CD or fluorescently labeled pyrene-β-cyclodextrin (Py-β-CD) were then introduced. The high binding affinity between *trans*-AB and β-CD locked the β-CD rings at the orifice. On the other hand, owing to the weak binding between *cis*-AB and β-CD, the isomerization of *trans*- to *cis*- AB stalks led to the dissociation of pseudorotaxanes, thus permitting the release of cargo molecules. Experimental data confirmed that the AB stalks and Py-β-CD assembly was stable without 351 nm UV radiation, whereas a complete Py-β-CD dissociation was determined when the sample was exposed to a 351 nm excitation beam for 400 minutes.

Likewise, results from RhB loaded samples revealed that more than 90 percent of RhB was released from the laser light exposed sample, while less than 30 percent was released from the unexposed one, over a period of 7 hours. They concluded that their material was applicable to light-operated intracellular drug delivery systems. Cyclodextrin was also employed by the Kim group to cover the porous reservoirs.[66] Gold nanoparticles, for the demonstrated excellent biocompatibility, were used as pore-blocking caps in a research conducted by Lin and coworkers (Fig. 10).[67] A photoresponsive linker, thioundecyl-tetraethyleneglycolester-*o*-nitrobenzylethyldimethyl ammonium bromide, was immobilized onto the surface of Au nanoparticles (PR-AuNPs). These positively charged species then attached on the negatively charged MSN materials through the electrostatic interaction to produce a PR-AuNP capped MSNs system, the structure of which was later confirmed by transmission electron microscopy (TEM). A good capping efficiency was verified by the fact that no release of cargo was found even after 80 hours in the dark. Photoirradiation at 365 nm resulted in the cleavage of the *o*-nitrobenzyl ester containing linker, forming the negatively charged, thioundecyltetra-ethyleneglycolcarboxylate functionalized Au

nanoparticles. Hence, the charge repulsion between Au nanoparticles and MSNs uncovered the pores and allowed the diffusion of guest molecules. In addition, intracellular studies were executed using an anticancer drug as cargo for the controlled release in human liver and fibroblast cells.

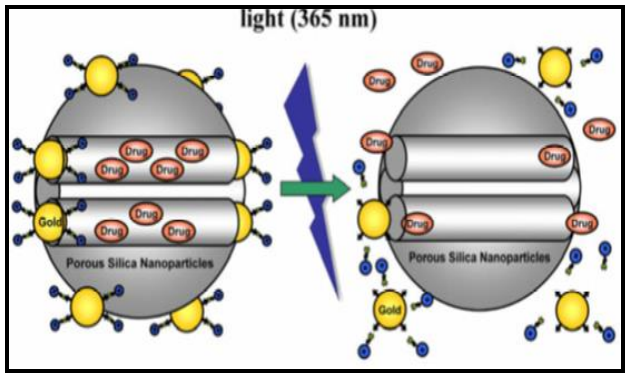



Figure10: Schematic illustration of photo-induced controlled drug release of PR-AuNPs capped MSNs

*Redox Potential-triggered Release*

The designs of redox responsive linkers are mainly based on the disulfide bond. It draws much interest in these systems because of its relative stability in plasma and is reversible. Intracellular antioxidant species levels are 100 to 1000 fold higher than that in the extracellular space, resulting in a high redox potential difference between the oxidizing extracellular space and the reducing intracellular space.[68] This difference is more significant in cancer cells than that in healthy cells, which renders the disulfide linkage more vulnerable in cancer cells leading to potentially higher drug concentrations at tumor sites. Thus, the demand for both outstanding delivery efficiency and minimized cytotoxicity can be realized by MSN based drug delivery systems.

The feasibility of the redox-responsive linkage has been well established and reported in a number of recent publications. In addition to the original CdS-capped MSN materials, Lin and coworkers refined the system by replacing the CdS caps to more biocompatible superparamagnetic iron oxide (Fe3O4) nanoparticles.[69]

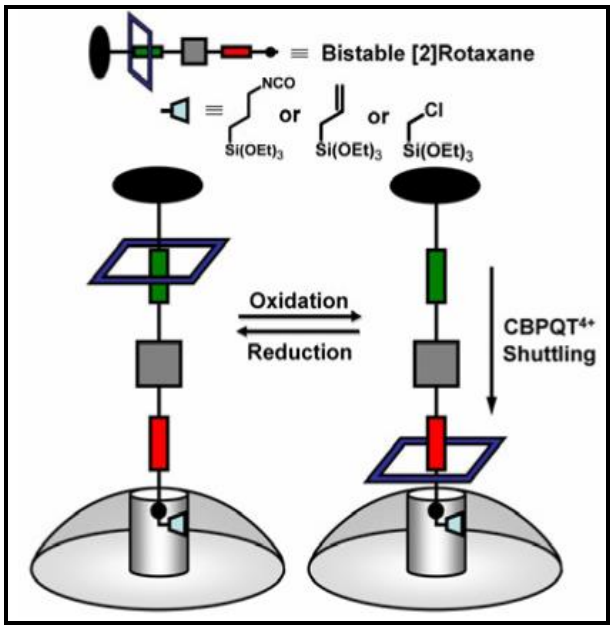



Figure 11: Graphical representations of the assembly of the bistable [2] rotaxanes to form nanovalves and the possible positions (IN and OUT) regulated by the oxidation state. The cycle can be repeated several times

A series of pseudorotaxanes have been developed in the Zink research group (Fig. 11).[70] In their first attempt, 1,5-dioxynaphthalene containing derivatives (DNPD) were tethered to the surface of the MSNs, acting as the pseudorotaxane rod, followed by the assembly of cyclobis (paraquat-*p*-phenylene) (CBPQT4+), which noncovalently complexed with DNPD. The bulky CBPQT4+ tetracations thus obstruct the pore openings.

The sample was loaded with a fluorescent dye, tris (2,2'-phenylpyridyl)iridium(III), to investigate the release behavior of this complex system. Upon addition of reducing agent, the pseudorotaxanes on the MSNs surface immediately dethreaded and opened up the pores. A fast increase in luminescence intensity was observed, indicating a rapid release of entrapped molecules.

*Temperature-Triggered Release*

The local temperature difference between tumor sites and non-tumor sites has been shown to be useful as an internal trigger for the control release of drugs. It is desirable to design a temperature-responsive drug carrier that only

releases drugs at temperatures above 37 °C, but keeps drugs encapsulated while in circulation. Poly(*N*-isopropylacrylamide) (PNiPAm), a popular thermal-sensitive polymer, has been functionalized onto MSNs to modulate the transport of guest molecules.70-74 It holds a low critical solution temperature (LCST) close to 37 °C,75 below which the PNiPAm is hydrated and swollen so that it covers the cavities of MSNs. While at temperatures above LCST, the PNiPAm undergoes a conformation change to a hydrophobic, shrunken state in aqueous solution, and thus opening up the pore entrance.[71, 74]

*Enzyme-Triggered Release*

The development of smart, controlled-release delivery systems triggered by biomolecules such as enzymes is a very promising research direction, owing to their excellent biocompatibilities and their rapid and specific biological activities. An enzyme-responsive cyclodextrin containing rotaxane was reported by Patel et al (Fig. 12).[72] MSNs were functionalized with monoazide-terminated triethyleneglycol by a two-step grafting process.

After soaking the MSN materials in a solution of RhB, α-CD was threaded onto the triethyleneglycol chain and effectively blocked the pores. A bulky adamantyl ester-linked stopper group was tethered to the terminal azide groups by Huisgen cycloaddition, and hence interlocked α-CD to the rotaxane stalk. An enzyme triggered release was verified by the addition of porcine liver esterase which broke the ester bonds on the stopper groups and enabled α-CD rings to escape from the triethyleneglycol thread, therefore permitting the diffusion of cargo molecules. It is interesting to note that when the ester bond of the stopper group was replaced by amide bond, it was no longer activated by esterase cleavage, demonstrating a high selectivity of the enzyme.

An extensively applied linker system is the biotin-avidin linker first prepared by Bein and coworkers, synthesizing biotinylated MSNs by a reaction of thiol-functionalized MSNs and biotin-maleimide.[73] The avidin caps were then strongly complexed to the biotin terminals on the MSNs, forming a tight closure of the pores. The avidin-biotin linked MSNs exhibited zero release until the addition of protease trypsin, which digested avidin by the tryptic hydrolysis process and led to the release of guest molecules. Release reached completion after 140 minutes following the treatment of trypsin. The biotin-avidin linker was also useful for the targeted drug delivery.

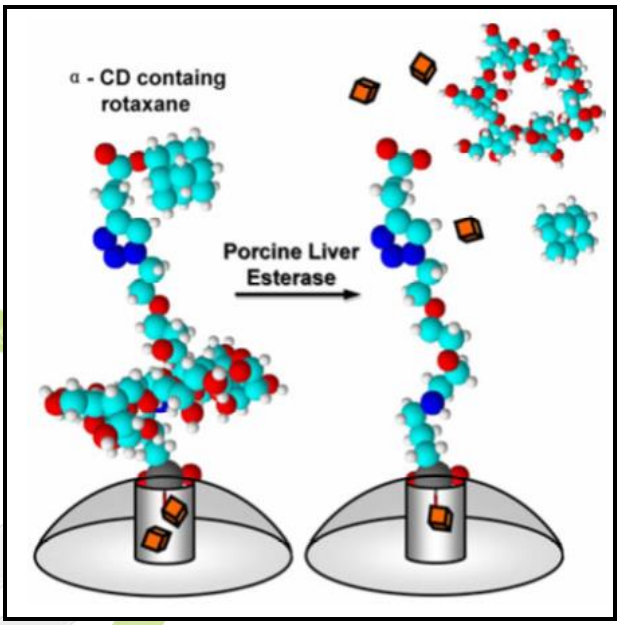



Figure 12: Esterase activated pore opening of α-CD containing rotaxane capped MSNs

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*Other Stimuli*

A novel biocompatible surfactant-assisted controlled release system was proposed by Tsai

and Trewyn et al.[74] The high cytotoxicity has always been a defect of the traditional cetyltrim ethylammonium pore template, therefore the surfactant molecules have to be adequately removed before administration. To avoid this problem, a non-cytotoxic anionic surfactant, undec-1-en-11-yltetra (ethylene glycol) phosphate monoester (PMES), was developed to function as a structure directing agent. The presence of hydrophobic tails on the surfactants makes this material especially effective for the uptake of hydrophobic molecules. The MSNs with PMES (PMES-MSNs) sample possess a fourfold greater loading ability for hydrophobic molecules in comparison to the calcined sample.

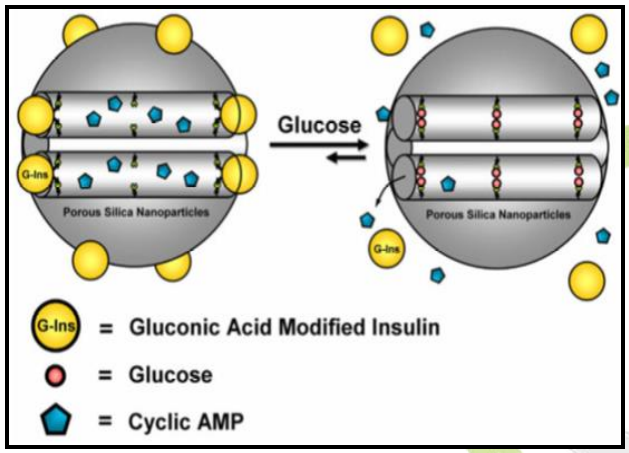


Figure 13: Schematic illustration of the glucose-responsive MSN based delivery system for controlled release of bioactive G-Ins and cAMP

A glucose-responsive delivery system was fabricated by Lin and coworkers consisting of phenylboronic acid modified MSNs (BA-MSNs) and gluconic acid modified insulin (G-Ins) (Fig. 13).[75] The G-Ins serving as caps were attached to BA-MSNs through reversible covalent bonding between the vicinal diols of G-Ins and the phenylboronic acid groups on BA-MSNs. Release of guest molecules can be triggered by introducing saccharides such as glucose, which forms much more stable cyclic esters with phenylboronic acid than the acyclic diols and hence substitutes G-Ins moieties. This system is especially promising for the treatment of diabetes because it responds only at diabetic glucose levels while remains intact at normal conditions. Moreover, cyclic adenosine monophosphate (cAMP), known as an insulin secretion stimulating agent, can be encapsulated inside the pores and released subsequent to G-Ins diffusion to achieve a synergic effect for the regulation of blood glucose levels.[76]

*Multiple Stimuli Triggered Release*

Along with the extensive research conducted on single stimulus triggered drug delivery, multi-responsive controlled release systems have been developed to achieve complex release behaviors in either an independent or a synergistic fashion. A dual pH and light controlled release system based on the combination of pH-sensitive pseudorotaxane and photo-sensitive nanoimpeller azobenzene was designed by Angelos et al.[77]. They illustrated that functionalized silica material could function as AND logic gates, such that cargo delivery occurred only when it was triggered by both stimuli. They envisioned that it is possible to manually regulate delivery dosage with this system. Another pH and photo-switch release approach was demonstrated by Aznar et al.[78]

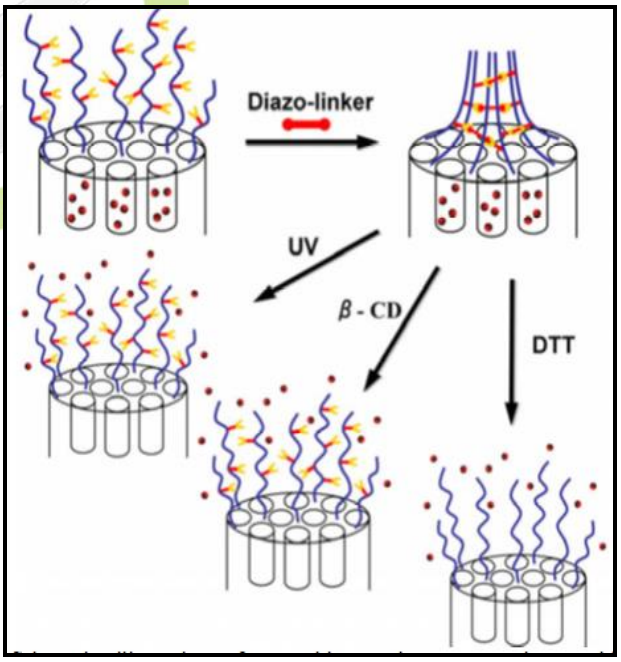


Figure 14: Schematic illustration of a multiresponsive nanogated ensemble based on supramolecular polymeric network-capped mesoporous silica nanoparticle

A tri-stimuli responsive delivery system was developed by Feng and coworkers involving the

construction of a pyridyldithio-containing polymer functionalized MSNs that were disulfide bond linked to thiol-modified β-CD (Fig. 14).[79] The β-CD moieties were further cross-linked by diazo-linkers to inhibit the release of entrapped molecules.

Their assembly was expected to respond to UV light irradiation, as well as the addition of DTT or α-CD. Under a 365 nm UV light, the *trans*-configured diazo-linkers would transform into a *cis*-azobenzene and thus lose their high affinity to β-CD molecules. Addition of DTT would cleave the disulfide linkage between β-CD and MSNs. The introduction of excess α-CD would result in the formation of a more stable α-CD-diazo cross-linkage and displace β-CD. In all three scenarios, the pore-blocking polymeric network was opened, leading to tri-stimuli triggered release. The fact that magnetic nanocrystals are able to generate heat energy under high-frequency alternating magnetic field was applied in the design of temperature-responsive delivery systems by the Vallet-Regí group.[80] Magnetic iron oxide nanocrystals were embedded inside the silica matrix of MSNs and the surface of MSNs was decorated with a thermosensitive copolymer of poly(ethyleneimine)-*b*-poly- (*N*-isopropyl-acrylamide) (PEI/NIPAM). They demonstrated that this device could deliver proteins with preserved activity, triggered by a temperature increase, as well as an alternating magnetic field that heat up the local environment through encapsulated iron oxide nanocrystals.

*Targeted Drug Delivery of Mesoporous Silica Nanoparticles*

Cell-specific targeting is highly attractive as an approach to spontaneously distinguish the site of disease diagnosis, and as a result, this technique reduces drug administration dosage and diminishes toxic side-effects of drugs during circulation. Both passive strategies and active surface decoration methods have been applied to the fabrication of novel MSNs based drug delivery systems for targeted release.

*Passive Routes*

Passive accumulation of MSNs in tumor tissue can be realized by the enhanced permeability and retention (EPR) effect, a theory first postulated by Matsumura and Maeda in 1986.94 They hypothesized that the differential localization of macromolecules as well as particles of certain sizes is attributed to the tumor microenvironment, the relative slow elimination rate and poor lymphatic drainage. Effectiveness of the EPR effect can be mediated by the particle size, surface charge or hydrophobicity. Tamanoi and coworkers demonstrated a preferential accumulation of fluorescently labeled MSNs (100-130 nm in diameter) in tumors of mice, within 4 hours of an intravenous injection. The fluorescent signal then gradually decreased to the same level as the whole body after 48 hr.[81] Similar phenomenon was also reported by the Hyeon group that MSNs less than 200 nm accumulated in tumor 24 hours after administration.[82,83]

*Surface Decoration with Targeting Ligands*

Efforts have been made to functionalize the surfaces of MSNs with cancer-specific targeting ligands for an enhanced MSNs uptake by cancer cells compared to noncancerous cells. One such ligand is folic acid,[84] as folate receptors are known to be overexpressed in several types of human cancer, including ovarian, endometrial, colorectal, breast and lung.[85] Using folic acid-conjugated MSNs (FA-MSNs), Sahlgren, Linden and coworkers observed that the total number of particles internalized by the HeLa cancer cells was about one order of magnitude higher than that of FA-MSNs internalized by noncancerous cells, although noncancerous cells normally do express folate receptors.[86-90] Besides folic acid, other small cell nutrient molecules such as mannose,[87] was also shown to selectively improve the uptake of MSNs by breast cancer cells. Another group of targeting ligands is the RGD peptide, abbreviation for arginine-glycine-aspartic acid, which interacts with the highly overexpressed αvβ3 integrin

 

receptor in metastatic cancers. Lo and coworkers verified an integrin-dependent endocytosis process of cyclic RGD (cRGD)-anchored MSNs by U87-MG cells.[88]

In conclusion, silica particles offer an interesting alternative to organic drug delivery system. Their intrinsic hydrophilicity and biocampatability, as well as the excellent protection they provide for internal payload, makes them perfect candidates for controlled drug delivery applications. Drug delivery system is significant to maximize therapeutic efficacy and minimize side effects of many bioactive molecules. It can address the problems with poor solubility, stability and lack of specificity of drug molecules. This review presented a great potential of MSN in drug delivery. Compared to the other drug delivery systems,[89] MSN offers some advantages such as biocompatibility, ease in modifying structure through active silanol group surface, controlled release and simple synthesis procedure called sol-gel process, which can bring economical benefits as well. Depending on what chemical substituents are used in sol-gel procedure,[90] different morphology of MSN can be produced.[91] Distinctive mesoporous structure and active surface of MSN can incorporate various therapeutic agents and deliver them without altering their therapeutic effectiveness. As MSN is in its developmental stage, there are high expectations on its usage and function in drug delivery.[92]

The emergence of MSN materials has been appreciated by the biomedical field for the design of smart drug delivery devices. The unique characteristics of MSNs have created a fast growing number of stimuli-responsive release systems. A high degree of specificity and control in drug delivery is achieved by versatile uncapping mechanisms and new approaches for MSN synthesis. However, despite the encouraging progress, these systems are mostly investigated outside the biological system, and have not yet been proven for *in vivo* biomedical applications.[93] Although ingenious work is required to conquer remaining challenges, it is reasonable to believe that these multifunctional MSNs drug delivery systems will promote the development in clinical and other biotechnological fields.[94]

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