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Biocompatibility and biomedical applications of functionalized mesoporous silica nanoparticles

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ABSTRACT

In the past decade, mesoporous silica nanoparticles (MSNs) have attracted great attention for their biomedical applications. In this review, we provide an overview of the recent progress made by different research groups on the synthetic mechanisms used to produce the most common type of MSNs. In addition, two general methods employed to functionalize MSNs' surfaces are reviewed. We further discuss the biocompatibility of MSNs related to the different physico-chemical features of the particles. The review also highlights the recent significant progress in drug delivery using MSNs and their multifunctional counterparts as drug nanocarriers, such as stimuli-responsive drug release and cancer therapy. Finally, we conclude with our opinions and forecast the development trend of MSNs in the field of drug delivery.

Keywords: *mesoporoussilica nanoparticles, functional modification, biocompatibility, biomedical applications.*

1. INTRODUCTION

Since MCM-41-type ordered mesoporous silica nanoparticles (MSNs) as drug delivery system was reported in 2001, there has been a lot of studies on the controlled synthesis and applications of MSNs [1]. Mesoporous silica nanoparticles have exhibited potential biomedical applications due to their high surface area, large pore volume, uniform pore size, biocompatibility and the easily functionalized surface [2]. As silica has good biocompatibility and accepted as "Generally Recognized as Safe (GRAS)" by the U.S. Food and Drug Administration (FDA) [3-5], MSNs has been intensively suggested for use in biomedical imaging/therapy [6-9], biosensors [10] and enzyme supporters [11].

MSNs with different structural features can be synthesized while the loading capacities, binding abilities to special cell and biocompatibility of MSNs can be improved by altering their structures or surface functional groups. In addition, functional mesoporous silica nanoparticles with outstanding magnetic or luminescent properties endow them with unique advantages for disease diagnosis and therapy over traditional drug nanocarriers. Furthermore, we can design a new kind of functional mesoporous silica nanoparticles with unique properties for simultaneous diagnosis and therapy.

In recent years, several investigations have been conducted to understand the possible toxicity of MSNs on biological systems [12, 13]. Many types of MSNs have been shown to have low toxicity in many biological systems if they are prepared with certain optimized structural features and are applied at the right dosages [14]. In addition, the physico-chemical properties of MSNs have been proven to play important roles in the particles' biocompatibility [15]. Therefore, we analyze the relationship between the physico-chemical properties of MSNs and biocompatibility in this article.

2. SYNTHESIS AND FUNCTIONALIZATION OF MSNS

2.1. Synthetic Mechanism of MSNs

At present, recent strategies on the synthesis of ordered MSNs for drug delivery have been reported [16-18]. As a lot of studies on drug delivery applications of MSNs are based on MCM-41, we emphasize the synthetic mechanisms of MCM-41in this review.

The general mechanism involves five steps (Figure 1) [19]:(1) when the concentration is above the critical micelle concentration (CMC), the surfactant would self-aggregate into micelles; (2) organization of the surfactant micelles into cylindrical micelles; (3) formation of a regular array of micelle liquid crystals; (4) the silica precursors condensate at the surface of the surfactant and form silica wall around the micelle liquid crystals; (5) removal of the surfactant micelle templates to obtain MSNs [17,20,21].

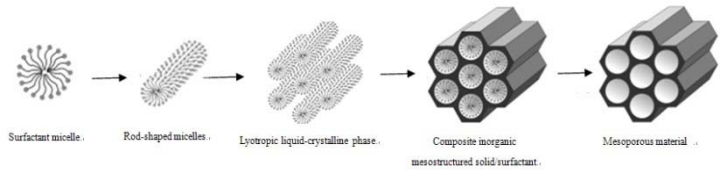

Figure 1. Scheme of synthesis of MCM-41. Reproduced with permission from ref [17]. Copyright 2006, Wiley.

Regardless of the exact mechanism involved during their synthesis, the resulting MSNs have mesoporous structures; uniform pore size (typically between 2 and 15 nm); large surface

areas (typical 1000 m^2/g or even more) [22, 23]. These structural features endow them with unique advantages to load drugs and deliver these agents to the desired location. Therefore, MSNs have been broadly employed as carriers of drug delivery.

For biomedical applications, precise control over particle size, morphology, and pore structure is very important. MSNs with various morphologies and different sizes can be synthesized by changing the reaction conditions. For example, Pang and co-workers synthesized MSNs with different specific surface and pore size using Na_2SiO_3 or TEOS as silica precursors, polyoxyethylenetert-octylphenyl ether (Triton X-100) and CTAB as co-surfactants [24]. When Na_2SiO_3 was used as precursor MSNs have larger pore and higher surface area than that synthesized by TEOS as precursor [24]. They also found that the morphology of MSNs can be changed by controlling the molar ratios between Triton X-100 and CTAB [24].

2.2. Functionalization Strategies OF MSNs

As mentioned above, the physical and chemical properties of MSNs can be tuned by altering their structures or surface functional groups (namely functionalized MSNs), improving the biocompatibility and biomedical applications of MSNs. Functionalized MSNs are widely used in drug delivery because modified MSNs can improve adsorption capacities to drugs and increase binding abilities to specific tissue. The covalent attachment of functional groups either on the external or internal surface, which can be attached using post-synthetic grafting or co-condensation method [25]. We review these two methods that are commonly employed for surface functionalization of MSNs.

The post-synthetic grafting method introduces the functional groups mainly to the surface of MSNs after the nanomaterials are formed. The advantage of post-synthetic grafting method is that the MSNs still remain their mesosporous structure and better porosity within a large extent after the introduction of the functional groups. Alternatively, the grafting of the organic groups can be easily completed on the surface of the MSNs using this synthetic strategy [26].

In the case of the co-condensation synthetic method, organic-functionalized MSNs are prepared using organosilane surface modifiers, the surfactant templates and the silica sources through one step. As the self-assemble and co-condensation processes take place and mesoporous materials are formed, the functional groups are incorporated into the MSNs directly.

Compared with the post-synthetic grafting method, the co-condensation synthetic method offers a more convenient approach to modify MSNs with functional groups. However, the addition of the organosilane surface modifiers into the typical silicate/template solution could affect the self-assembly process and the structure of the final materials, including the mesoporosity and ordered structure of MSNs [26].

By using one of the above methods, multifunctional MSNs can be prepared. Furthermore, the functional groups should be rationally chosen in order to form functionalized MSNs with desired surface properties, morphology, pore size and suitable biocompatibility for biomedical applications [26].

3. BIOCOMPATIBILITY OF MSNS

MSNs have been intensively suggested as drug delivery carriers since they appeared. Compared to the conventional drug carriers, MSNs have higher drug loading capacity, sustained release profile, good thermal stability. However, this development has also led to the growing questions regarding the potential biological effects of MSNs. The biological effects of such materials are complex as they generally rely on a range of nanoscale features of the materials such as particle sizes, shapes, pore structure, etc. Given these different physico-chemical characteristics of MSNs, the overall interaction between these nanomaterials and biological systems are difficult to be fully understood. Thus, further efforts on detail and systematic studies to determine what special structural factors of these materials are responsible for their biological effects under different conditions need to be continued. This section thus reviews recent studies about the effect of size, shape, surface property, and structure on the biocompatibility of MSNs.

3.1. Effect of Particle Size

The size of particles is an important factor to determine the biocompatibility of nanomaterials. A large number of studies have focused on the relationship between the size of MSNs and their biocompatibility.

The effects of size of MSMs on cellular uptake were investigated in detail by Mou and co-workers [27]. They synthesized ordered MSNs with uniform sizes in the range of 30 nm to 280 nm and investigated their internalization by Hela cells. The cellular uptake amount is dependent in the particle size, i.e. 50 nm > 30 nm > 110 nm > 170 nm (Figure 2).These results clear indicate that MSN with the particle size of 50 nm may be most effective in drug delivery from the aspect of cellular uptake. In another study, Vallhov et al. synthesized different types of MSNs with similar surface area and investigated the biological effects of MSNs by incubating the particles with human dendritic cells (DCs).Although MSNs with different sizes entered the DCs through similar mechanisms, MSNs with bigger sizes were found to be ingested more quickly and accumulated more in lysosomes than the MSNs with smaller sizes. It suggests that the cellular uptake amount of MSNs with smaller sizes was less than the MSNs with bigger sizes [28].

To evaluate the effect of size of MSNs on hemolysis, Haynes and co-workers found that MSNs caused size- and concentration- dependent hemolytic effect, with the smallest size nanoparticles exhibiting the highest hemolytic activity [29]. The authors also showed that size-dependent hemolysis was present only when the nanoparticles had long-range ordered porous structure.

Another study showed that MSNs with a bigger size were engulfed more by human red blood cells (RBCs), causing greater membrane distortion in the cell than that of MSNs with a smaller size [30]. When examining the interactions between particles and

RBCs, they found that MSNs with a bigger size were absorbed onto the surface of RBCs, inducing a stronger local membrane deformation and leading to internalization of more particles and ultimately more hemolysis. In contrast, MSNs with a smaller size were absorbed onto the surface of RBCs without disturbing the membrane.

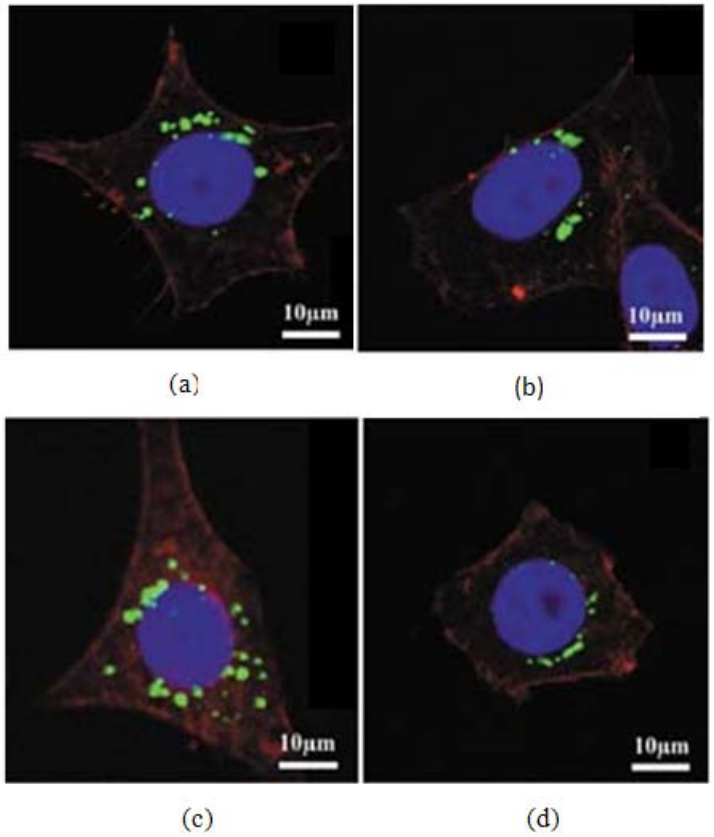

Figure 2. CLSM images of HeLa cells after incubation for 5 h at 37 °C with differently sized MSNs [27]. HeLa cells were treated with 100 μg/mL of (a) 170, (b) 110, (c) 50, or (d) 30 nm in diameter MSNs containing greenfluorescent tags. The cell skeleton was stained with rhodaminephalloidin (red), and the cell nucleus with 4, 6 - diamidino-2-phenylindole (DAPI; blue). Reprinted with permission from ref [27]. Copyright 2009 John Wiley and Sons.

To explain these results, Zhao et al. proposed that the nanoparticles engulfed by RBCs is determined by the combined effect of two competing processes [30]: (1) an exothermic process of covalent bond formation between the surface silanol groups of MSNs and the phosphatidylcholine groups of RBC membranes; When the cells capture the nanopaticles, MSNs with a bigger size and a large external surface area will release more binding energy than MSNs with a smaller size; (2) an endothermic process resulting from the entrapment of MSNs by RBC membranes. In this process, MSNs with a smaller size will require more binding energy than MSNs with a bigger size. The reason for this result is that the cell membrane should undergo more changes in its orientation when accommodating the smaller particles than the accommodation for larger ones.

The size effect of MSNs on their distribution in vivo was investigated in mouse model treated with MSNs having different sizes. Shi and co-workers synthesized spherical MSNs with different diameter (namely 80, 120, 200, and 360 nm, respectively) and traced their distribution in vivo by intravenous administration [31]. In the research, they observed that the particle size is one of the determining factors for distribution in vivo, blood-circulation lifetime and excretion. After intravenous injection, they also found that the particles showed nontoxic effect to the tissues in the mouse but accumulated different in them. MSNs of all sizes were mainly distributed in the liver and spleen, a minority of them in the lung, and a few in the kidney and heart [31]. The distribution in the liver and spleen increased with the increase of particle sizes from 80, 120, to 200 nm at 30 min post-injection, but the particle with size of 360nm exhibited different trend of distribution in spleen [31].

3.2. Effect of Particle Shape

Particle shape is an important structural feature which influences the biocompatibility of MSNs. In order to establish the relationship between particle shape and biocompatibility, the effect of shapes of MSNs on biological systems was investigated in recent years.

To examine the effect of particle shape on cellular endocytosis, Lin and co-workers synthesized MSNs with spherical shape (size distribution from 80-150 nm) and rod shape (400-1000 nm length and 80-150 nm width) and investigated their cellular uptakes by Chinese Hamster Ovarian (CHO) and normal human fibroblast cells [32]. For CHO cells, the uptake rates for above both MSNs were similar and rapid, whereas the spherical shaped MSNs were internalized faster than the rod-like nanoparticles by normal human fibroblast cells [32]. This result clearly demonstrates the effect of shape of MSNs on their biological activities.

In another study, Tang and co-workers investigated the biodistribution and excretion of MSNs with different aspect ratio in mice after injecting the tail veins of mice. They synthesized two rod-like fluorescein-conjugated MSNs with different aspect ratios of 1.5 and 5.In this research, after 2-24 h of administration, the concentration of MSNs with lager aspect ratios remain almost unchanged in bloodstream, whereas the concentration of MSNs with the smaller aspect ratios dramatically decreased [33]. It suggests that the shape of MSNs can influence the particles' circulation times in the bloodstream.

To further confirm the influence of shape of MSNs, the deep research is needed. In a recent study, it was found that the MSNs with tube-shape (54 nm in length and 15 nm in diameter) penetrated tumors 4.1 times faster than the MSNs with spherical shape (35 nm in diameter) [34]. This result shows that MSNs with large aspect ratio and suitable diameter have longer circulation time than the MSNs with spherical shape. Tang and co-workers investigated the interaction between A375 human melanoma cells and MSNs with diameter of about 100 nm and aspect ratio of 1, 2, and 4[35]. They found that the MSNs with a larger aspect ratio may result in the accelerated cellular internalization rate and increased uptake amount [35]. All these findings may provide useful information for the rational design of efficient MSNs in the future.

3.3. Effect of Structure

As structure is another important feature of MSNs and determines their biological activities, so that the development of MSNs with

novel structure for biomedical application has attracted great attention. Alternatively, the pore size and geometry, surface area, matrix with or without porosity will influence the biocompatibility of MSNs. Although a large number of studies have focused on the structure of MSNs and their biocompatibility, it is rather difficult to find a certain relationship between them.

From above presentation, it is known that MSNs can be synthesized with different mesostructures such as hexagonal, cubic, worm-hole, etc. Besides sizes and shapes, many studies have found that the type of mesostructures can influence the MSNs' biocompatibility. Tao and co-worker found that the MCM-41 and SBA-15 MSNs showed different catalytic activities toward the oxidation of epinephrine, which was attributed to their difference in macrostructures [36]. In addition, The MCM-41 MSNs generally produced milder toxicity than SBA-15 MSNs [37]. The different biological effects exhibited by these two types of MSNs can be explained according to their structural differences. The MCM-41 MSNs has larger surface area and smaller pore diameter and these structural features can make these nanoparticles thermodynamically more favorable for cellular internalization [38].

In another study, Tang and co-workers investigated the vivo toxicity and biodistribution of silica nanorattles (SNs) with mesoporous and hollow structure. The lethal dose 50 (LD_{50}) of 110 nm SNs was higher than 1000 mg/kg [39]. They also found that the SNs can be captured by the mononuclear phagocytic cells and excreted from the mice with an entire clearance time over four weeks. These results suggest that SNs with low toxicity can be used in drug delivery.

MSNs with porous structure and large surface area may pose an increased risk with higher reactive and oxidative activity [40]. It has been generally confirmed that the nanoparticles with large surface and abundant silanol groups can generate reactive oxygen species, which play important role for nanomaterial-caused injury [40]. Tao and co-workers found that MCM-41 can inhibit cellular respiration, which is because the nanoparticles limited access to cellular mitochondria [41]. Recently, two other studies also found that MSNs had lower hemolytic activity [29] and cytotoxicity [42] than the non-porous silica nanoparticles. Alternatively, the biodegradability of MSNs has attracted great attention. It has been found that surfactant-extracted MSNs have a remarkably faster degradation rate in simulated body fluid than the calcined MSNs and the amorphous solid silica [43].Therefore, the biodegradability of MSNs with a controllable degradation rate makes it suitable for biomedical application.

3.4. Effect of surface properties

The surface properties are considered to be an important aspect which influences the biocompatibility of MSNs. The surface properties need to be assessed when the biological responses of MSNs are investigated. Tao and co-workers found that quaternary amine-functionalized MSNs tended to be adsorbed onto negatively charge cell membranes of the cells, rather than penetrating the cells and entering the cytoplasm [37]. The action of nanoparticles engulfed by cells can be a complicated process. Thus the MSNs should be well designed to ensure that they can cross cell membranes and finally reach the cellular targets to release the payloads drugs. For example, poly (ethyleneimine) (PEI), polyamidoaminedendrimers and natural chitosan were used to coat to the outer surfaces of MSNs in order to increase cellular uptake of nanoparticles [44]. As PEI contains a high density of amine groups, it can serve as an excellent proton buffering medium at virtually any pH. Therefore, PEI-coated MSNs exhibit an improved ability to delivery drugs to intracellular or even nuclear sites to improve cellular uptake [45]. The modification using polyethylene glycols (PEGs) to improve the biocompatibility of MSNs is other surface modification strategy. PEGs approved by FDA can form a hydrophilic layer around particles with dispersity increase [46, 47]. It was reported that the distribution of mesoporous nanoparticles modified by PEG in reticuloendothelial system (RES) decreases after intravenous administration. Meanwhile, the blood-circulation lifetime is prolonged while excretion rate is also decreased [31, 48]. In addition, properly performed PEGylation could also help the mesoporous structures of MSNs remain intact [49, 50]. Hence, the surface modification of MSNs using PEG or other similar chemicals with functional groups can improve the biocompatibility of MSNs.

Other surface modifications of MSNs have also been researched. It has been suggested that surface-modification of MSNs (fluorescein isothiocyanate (FITC) functionalized) with functional groups, 3-aminopropyl (AP), guanidinopropy (GP), N-(2aminoethyl)-3-aminopropyl (AEAP) and N-folate-3-aminopropyl (FAP) could influence the particles endocytosis by Hela cells (Figure 3a) [51]. MSNs with different surface modification could be endocytosed by Hela cells *via* different endocytosis pathway [51]. FITC- and FAP-MSNs were endocytosed via clathrin-pitted mechanism, FAP-MSNs were endocytosed by folic acid receptors, and AP- and GP-MSNs were endocytosed via caveolae-mediated mechanism (Figure 3b and c) [51].

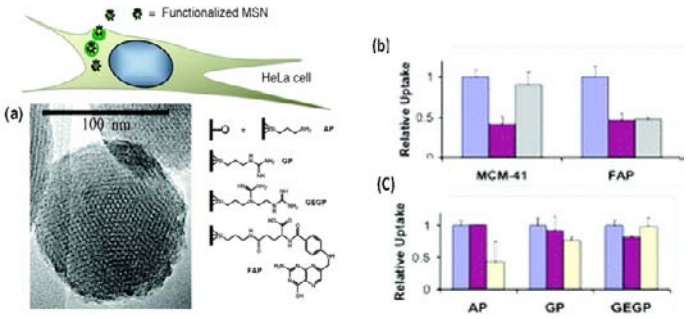

Figure 3. Effect of surface functionalization of MSNs on endocytosis by Hela cells. (a) Schematic representation of the endocytosis of organically functionalized MSNs with 3-amino- propyl (AP), guanidinopropyl (GP), N-(2-aminoethyl)-3-aminopropyl (AEAP), and N-folate-3-aminopropyl (FAP). (b) and (c) Uptake of the materials in absence (blue bars) and presence of a series of inhibitors: 450 mM sucrose (prune); 1 mM folic acid (gray); 200 mMgenistein (cream). Reproduced with permission from ref. [51]. Copyright 2006, American Chemical Society.

As exocytosis of nanoparticles is an inevitable step and the surface property of nanomaterials can influence the final fate of the nanomaterials, we should investigate the relationship between

them. Exocytosis can be influenced by many physicochemical factors associated with the nanomaterials such as size, shape, surface property, etc. For example, the effect of surface charge on secretion of nanomaterials was investigated for MSNs. The results showed that the surface charge on MSNs plays an important role in regulating the cellular excretion of MSNs [52, 53].Compared with the negative charged MSNs, the positive charged counterparts at physiological pHs were more absorbed by serum protein and being more rapidly transported into the gastrointestinal tract and finally eliminated with feces [52].Another study has also found that the positively charged MSNs were effectively eliminated via hepatobiliary pathway [53]. These results clearly indicate that the negatively charged MSNs had a tendency to remain in cells. As a result, negatively charged MSNs could be a potential threat to hepatic health.

3.5. Effect of Cell Type

The exocytosis of MSNs could be influence by the type of cells. To properly evaluate the biocompatibility of MSNs, an appropriate biological system needs to be chosen. Different *in vivo* and *in vitro* studies on the interactions between various nanomaterials (mesoporous silica nanoparticles, quantum dots, etc) and diverse biological entities (cells, microorganisms, tumor, etc) have been widely documented in the literature [10, 13, 54, 55]. A few studies have been conducted on exocytosis by different cell lines, Slowing and co-workers studied the exocytosis of MSNs by normal and cancerous cells. The exocytosis of MSNs was found to be much more efficient in healthy cells than in malignant HeLa cells [56]. Chang and co-workers found that the cytotoxicity of the silica nanoparticles was correlated with the type of cells [57]. Tao and co-workers also found that the nanoparticles exhibited different cytotoxicities on different cell types [37]. In contrast to human T-cell lymphoma cells, adherent SK-N-SH cells (derived from human neuroblastoma) showed more drug resistance when treated using all the three types of nanomaterials (MCM-41, SBA-15 and solid spherical silica nanoparticles) [37]. Therefore, the biocompatibility of nanomatericals is depended on the type of cell. For this reason, one may have to also expect that nanomatericals administrated as medicines could interact differently with different targets, such as tumors with different origins, size and biochemical markers.

4. Biomedical Applications

4.1 Stimuli-Responsive Drug Delivery and Release

A series of studies have provided a large amount of evidences that MSNs are highly biocompatible in vivo at the desirable dosage [26]. Based on the excellent biocompatibility and unique structural characteristics for drug delivery, MSNs are expected to be the best candidates for loading various guest cargos by adsorbing them onto the particles. With the special mesoporous structure and high special surface area, MSNs have a high capacity to accommodate guest molecules and release the loaded molecular in physiological condition. Furthermore, most of the pharmaceutical drugs have severe toxicity to normal cells. It is not desired that the delivered drugs would be released from drug delivery system before reaching the targeted tissues. As the stimuli-responsive drug delivery nanoparticles can release the cargos in the targeted tissues triggered by outer environmental changes such as pH [58-63], temperature [64], enzymes [65-69], photo [70-73] etc, the controlled drug release can be achieved when they circulate in the blood stream.

In 2003, Lin and co-workers used surface-derivatized cadmium sulfide (CdS) nanocrystals as chemically removable caps to encapsulate the drug molecules in the mesopore of MSNs via disulfide linkages between nanocrystals and MSNs (Figure 4) [74]. With stimuli molecules of disulfide bond-reducing molecules as trigger, the encapsulated drug molecules can be released from the mesopores.

Up to now, most of these studies were performed*in vitro*. Very few studies have used the stimuli-responsive drug delivery systems (SRDDSs) for *in vivo* application. How to realize the *in vivo* stimuli-responsive drug release is crucial for further clinical applications. Zhao and co-workers synthesized rotaxane-functionalized MSNs (RFMSNs) with photothermal-responsive behaviors [75].They demonstrated the *in vivo* remote-controlled drug release on wild, optically transparent zebrafish larvae (Figure 5).

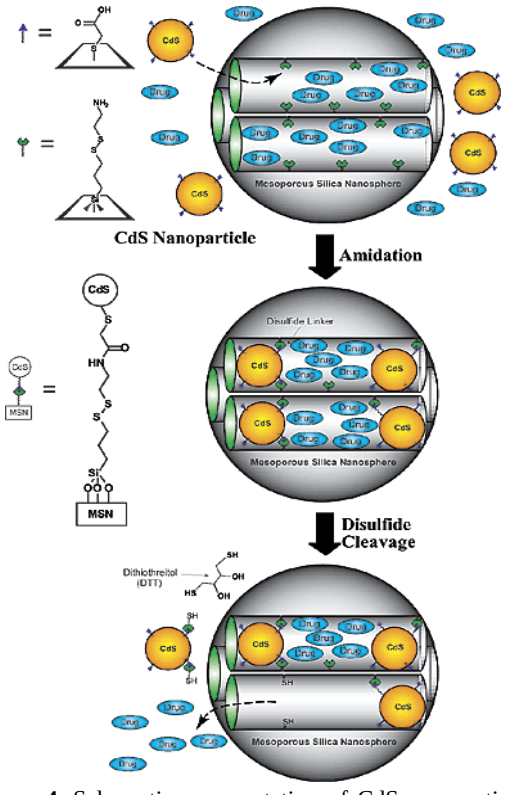

Figure 4. Schematic representation of CdS nanoparticle-capped MSN-based drug delivery system. The controlled release mechanism of the system is based on chemical reduction of the disulfide linkage between the CdS caps and the MSN hosts. Reproduced with permission from ref. [74]. Copyright 2003, American Chemical Society.

In this research, RFMSNs were used to deliver curcumin to zebrafish larvae for the treatment of heart failure. The remote-controlled drug release is based on the back and forth movement of the α-CD rings owing to photothermal-induced reversible trans–cis isomerization of the azobenzene axle. Upon irradiation with 365 nm UV light, the trans-to-cisphotoisomerization of the azobenzene

unit caused a shift of the α-CD ring from the azobenzene unit to the triazole/ethylene glycol position, thus the drugs could be absorbed within the mesopores.

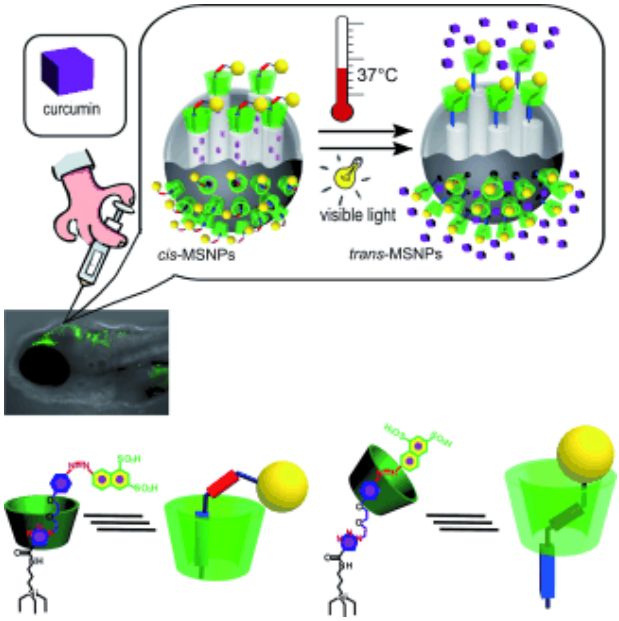

Figure 5. The schematic presentation of the administration of drug-loaded MSNs into zebrafish larvae for *in vivo* controlled drug release, triggered by either heating or visible light irradiation. Reproduced with permission from ref. [75] Copyright 2012, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

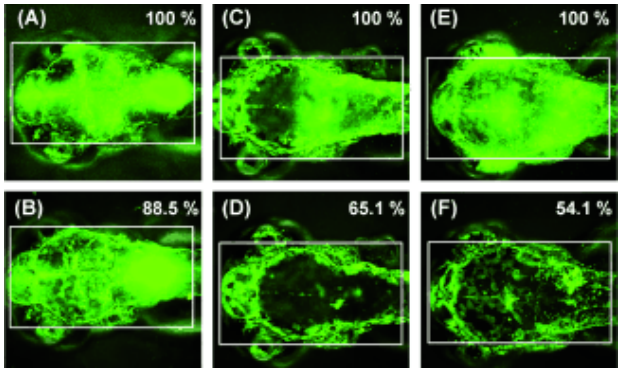

Figure 6. Fluorescence intensities of zebrafish brains microinjected with curcumin-loaded photo thermal MSNs. Fluorescent images were acquired immediately after injection (A) and after 1 h in the dark at 24 °C (B); immediately after injection in the dark (C) and after 1 h with continuous visible light illumination at 24 °C (D), and immediately after injection in the dark at 24°C (E) and after 1 h in the dark at 37 °C (F). Initial mean fluorescence intensity (A,C,E) was normalized to 100% and the intensity after 1 h (B,D,F) was calculated as a percentage of the initial signal (using Image J software) Reproduced with permission from ref. [75] Copyright 2012, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

The cis-to-trans isomerization of the azobenzene unit upon exposure to visible light or heating causes the α-CD ring to move back to the trans-azobenzene position, resulting in the opening of nanopores for the controlled release of drugs. In vivo evaluation using optically transparent zebrafish larvae showed that 34.9 % (Figure 6C and D) and 45.9 % (Figure 6E and F) reduction in the mean fluorescence intensity of curcumin in 1 h post-treatment under visible light and thermal treatment (37 °C), while only 11.5 % (Figure 6A and B) reduction of curcumin in zebrafish was found without the photothermal treatment[75]. The decrease of fluorescence intensity was due to the in vivo metabolism of the released curcumin. This result strongly demonstrates that the curcumin-loaded MSN can serve as an efficient technique for in vivo drug delivery [75].

4.2. Cancer Target Therapy

Cancer has been the leading cause of death in developed countries and the second leading cause of death in developing countries. There exists an enormous challenge for curing cancer at present. Recent advances in nanotechnology have offered new opportunities for cancer treatment [76]. Tremendous efforts have been made using MSNs as drug delivery system for cancer-targeted therapy. Here is an example.

To decrease RES uptake and maximize the ERP effect, Nel, Zink and co-workers coated 50 nm MSNs with PEI-PEG copolymer to reach a high passive accumulation of about 12 % at tumor site, compared with 1 % of 100 nm phosphonate-coated MSNs and 3 % of 50 nm PEGylated MSNs (Figure 7A). The increased tumor accumulation leads to an enhanced tumor inhibition rate (Figure 7B) [77]. However, the tumor passive targeting efficiency in mice is lower than 10 % [78]. It is still a challenge to further increase the in vivo circulation time and the passive targeting efficiency for MSN-based drug delivery systems.

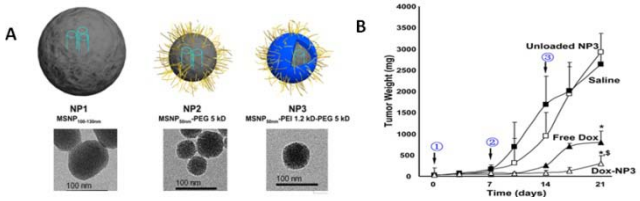

Figure 7. (A) Graphic representation and TEM image of modification of mesoporous silica particle. (B) Comparison of the tumor inhibition effect of doxorubicin-loaded NP3 (Dox-NP3) *versus* free drug (free Dox), empty particles, and saline in the KB-31 xenograft model. Reproduced with permission from ref. [77]. Copyright 2011, American Chemical Society.

By bioconjugating MSNs with specific targeting ligands, active targeting to cancer cells has been realized. The targeting ligands now used for active targeting of MSN-based drug delivery systems include antibody for targeting Her-2 receptor overexpressed in breast or lung cancer[79], transferrin for targeting transferrin receptor [80], aptemer sgc8 for targeting human protein tyrosine kinase-7 overexpressed in colon carcinomas[81] etc..

5. CONCLUSION AND OUTLOOK

In this review, we first introduced the general mechanism to explain the formation of MSNs, we then discussed the advanced strategies being developed to functionalize different types of MSNs. The chemical modification strategy can tailor the particle size, shape or surface property of MSNs, which has influence the biological activities of the nanomaterials.

A broad evaluation at the interface between the nanomaterials properties and their biological surrounding is crucial

to determine whether a given material is biocompatible. Many studies being conducted by various researchers to fully understand these structure-related biological effects of MSNs inducing their possible negative impacts on human health. According to the studies, the main structural properties of MSN such as size, shape, surface property can strongly affect the biological activities of these nanomaterials. The size of MSNs may play a superior role in causing toxicity in vivo, allowing or disallowing their further penetration into different organs to take part in some biological processes. Thus, the particle shape could also influence the internalization of MSNs into mammalian cells. Alternatively, different surface properties regulate the particle circulation in the bloodstream. The mesoporous structure is also considered to be the major reason for the different in vivo toxicity and biodistribution of nanoparticles. Such structure-related features of MSNs have been discussed in this review.

This review also highlighted the biomedical applications in drug delivery depends on the well-controlled fabrication of MSNs. Many intelligent drug delivery systems can be delivered into targeted organs or cells and release drugs in some controlled fashion by various external triggers, such as pH, magnetic, light irradiation, specific antibodies, etc. However, there are still many challenges, especially the in vivo applicable stimuli-responsive mechanisms, which need to be investigated more comprehensively. The multifunctionalization of MSNs by integrating the imaging diagnostic function with the drug delivery is currently under extensive investigation, which is desired to realize synchronous diagnosis and therapy.

For cancer therapy, MSNs show obvious advantages over other nanoparticulate drug delivery systems because of their extraordinarily high drug loading ability, controlled drug release behavior, and co-delivering ability. With the diversity and multifunctionality of MSN-based nano-composites, it is a promising way to develop cooperative therapies such as utility of the synergistic effect of photothermal therapy and chemotherapy. In addition, by bioconjugating MSNs with specific targeting ligands, the drug delivery systems can release the drugs at specific sites. Although the passive and active targeting have been studied for a long time, targeting efficiency is not ideal. Improvement of the knowledge of cancer physiopathology, discovery of new targets and development of new targeting ligands are most significant challenges for improving MSN-based cancer target therapy.

6. REFERENCES

[1] Vallet-Regi M., Ramila A., Del Real R. P., et al. A new property of MCM-41: drug delivery system. *Chemistry of Materials*, 13, 2, 308-311, **2001**.

[2] Barbe C., Bartlett J., Kong L., et al. Silica particles: anovel drug-delivery system . *Advanced Materials*, 16, 21, 1959-1966, **2004**.

[3] Rosenholm J. M., Mamaeva V., Sahlgren C., et al. Nanoparticles in targeted cancer therapy: mesoporous silica nanoparticles entering preclinical development stage. *Nanomedicine*, 7, 1, 111-120, **2012**.

[4] Garcia-Bennett A. E. Synthesis, toxicology and potential of ordered mesoporous materials in nanomedicine. *Nanomedicine*, 6, 5, 867-877, **2011**.

[5] Rigby S. P., Fairhead M., van der Walle C. F. Engineering silica particles as oral drug delivery vehicles. *Current Pharmaceutical Design*, 14, 18, 1821-1831, **2008**.

[6] Caruso F., Caruso R. A., Möhwald H. Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. *Science*, 282,5391, 1111-1114, **1998**.

[7] Lou X. W. D., Archer L. A., Yang Z. Hollow micro-/nanostructures: Synthesis and applications. *Advanced Materials*, 20, 21, 3987-4019, **2008**.

[8] Zhu Y., Shi J., Shen W., et al. Stimuli-responsive controlled drug release from a hollow mesoporoussilica sphere/polyelectrolyte multilayer core–shell structure. *Angewandte Chemie*, 117, 32, 5213-5217, **2005**.

[9] He Q., Shi J., Chen F., et al. An anticancer drug delivery system based on surfactant-templatedmesoporous silica nanoparticles. *Biomaterials*, 31, 12, 3335-3346, **2010**.

[10] Trewyn B. G., Giri S., Slowing I. I., et al. Mesoporous silica nanoparticle based controlled release, drug delivery, and biosensor systems. *Chemical Communications*, 31, 3236-3245, **2007**.

[11] Kao K. C., Lee C. H., Lin T. S., et al. Cytochrome c covalently immobilized on mesoporoussilicas as a peroxidase: Orientation effect. *Journal of Materials Chemistry*, 20, 22, 4653-4662, **2010**.

[12] Benezra M., Penate-Medina O., Zanzonico P. B., et al. Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. *The Journal of Clinical Investigation*, 121, 7, 2768, **2011**.

[13] Slowing I. I., Vivero-Escoto J. L., Wu C. W., et al. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers[J]. *Advanced Drug Delivery Reviews*, 60, 11, 1278-1288, **2008**.

[14] Vivero-Escoto J. L., Slowing I. I., Trewyn B. G., et al. Mesoporous silica nanoparticles for intracellular controlled drug delivery. *Small*, 6, 18, 1952-1967, **2010**.

[15] Tang F., Li L., Chen D. Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery. *Advanced Materials*, 24, 12, 1504-1534, **2012**.

[16] Monnier A., Schüth F., Huo Q., et al. Cooperative formation of inorganic-organic interfaces in the synthesis of silicate mesostructures. *Science*, 261, 5126, 1299-1303, **1993**.

[17] Hoffmann F., Cornelius M., Morell J., et al. Silica-based mesoporousorganic–inorganic hybrid materials. *Angewandte Chemie International Edition*, 45, 20, 3216-3251, **2006**.

[18] Slowing I. I., Vivero-Escoto J. L., Trewyn B. G., et al. Mesoporous silica nanoparticles: structural design and applications. *Journal of Materials Chemistry*, 20, 37, 7924-7937, **2010**.

[19] Beck J. S., Vartuli J. C., Roth W. J., et al. A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society*, 114, 27, 10834-10843, **1992**.

[20] Kresge C. T., Leonowicz M. E., Roth W. J., et al. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism[J]. *Nature*, 359, 6397, 710-712, **1992**.

[21] Yanagisawa T., Shimizu T., Kuroda K., et al. The preparation of alkyltrimethylammonium-kanemite complexes and their conversion to microporous materials. *Bull. Chem. Soc. Jpn*, 63, 4, 988-992, **1990**.

[22] Wan Y., Zhao D. On the controllable soft-templating approach to mesoporous silicates. *Chemical Reviews*, 107, 7, 2821-2860, **2007**.

[23] Naik S. P., Elangovan S. P., Okubo T., et al. Morphology control of mesoporous silica particles. *The Journal of Physical Chemistry C*, 111, 30, 11168-11173, **2007**.

[24] Pang X., Tang F. Morphological control of mesoporous materials using inexpensive silica sources. *Microporous and Mesoporous Materials*, 85, 1, 1-6, **2005**.

[25] Lim M. H., Stein A. Comparative studies of grafting and direct syntheses of inorganic-organic hybrid mesoporous materials. *Chemistry of Materials*, 11, 11, 3285-3295, **1999**.

[26] Asefa T., Tao Z. Biocompatibility of mesoporous silica nanoparticles[J]. *Chemical Research in Toxicology*, 25, 11, 2265-2284, **2012**.

[27] Lu F., Wu S. H., Hung Y., et al. Size effect on cell uptake in well-suspended, uniform mesoporoussilica nanoparticles. *Small*, 5, 12, 1408-1413, **2009**.
[28] Vallhov H., Gabrielsson S., Strømme M., et al. Mesoporous silica particles induce size dependent effects on human dendritic cells. *Nano Letters*, 7, 12, 3576-3582, **2007**.
[29] Lin Y. S., Haynes C. L. Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *Journal of the American Chemical Society*, 132, 13, 4834-4842, **2010**.
[30] Zhao Y., Sun X., Zhang G., et al. Interaction of mesoporous silica nanoparticles with human red blood cell membranes: size and surface effects. *ACS Nano*, 5, 2, 1366-1375, **2011**.
[31] He Q., Zhang Z., Gao F., et al. In vivo biodistribution and urinary excretion of mesoporous silica nanoparticles: effects of particle size and PEGylation. *Small*, 7, 2, 271-280, **2011**.
[32] Trewyn B. G., Nieweg J. A., Zhao Y., et al. Biocompatible mesoporous silica nanoparticles with different morphologies for animal cell membrane penetration. *Chemical Engineering Journal*, 137, 1, 23-29, **2008**.
[33] Huang X., Li L., Liu T., et al. The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo. *ACS Nano*, 5, 7, 5390-5399, **2011**.
[34] Chauhan V. P., Popović Z., Chen O., et al. Fluorescent nanorodsand nanospheresfor real-time in vivo probing of nanoparticle shape-dependent tumor penetration. *Angewandte Chemie*, 123, 48, 11619-11622, **2011**.
[35] Huang X., Teng X., Chen D., et al. The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. *Biomaterials*, 31, 3, 438-448, **2010**.
[36] Tao Z., Wang G., Goodisman J., et al. Accelerated oxidation of epinephrine by silica nanoparticles. *Langmuir*, 25, 17, 10183-10188, **2009**.
[37] Tao Z., Toms B.B., Goodisman J., et al. Mesoporosity and functional group dependent endocytosis and cytotoxicity of silica nanomaterials[J]. *Chemical Research in Toxicology*, 22, 11, 1869-1880, **2009**.
[38] Heikkilä T., Santos H. A., Kumar N., et al. Cytotoxicity study of ordered mesoporous silica MCM-41 and SBA-15 microparticles on Caco-2 cells. *European Journal of Pharmaceutics and Biopharmaceutics*, 74, 3, 483-494, **2010**.
[39] Liu T., Li L., Teng X., et al. Single and repeated dose toxicity of mesoporous hollow silica nanoparticles in intravenously exposed mice. *Biomaterials*, 32, 6, 1657-1668, **2011**.
[40] Nel A., Xia T., Mädler L., et al. Toxic potential of materials at the nanolevel. *Science*, 311, 5761, 622-627, **2006**.
[41] Tao Z., Morrow M. P., Asefa T., et al. Mesoporous silica nanoparticles inhibit cellular respiration. *Nano Letters*, 8, 5, 1517-1526, **2008**.
[42] He Q., Shi J., Zhu M., et al. The three-stage *in vitro* degradation behavior of mesoporous silica in simulated body fluid. *Microporous and Mesoporous Materials*, 131, 1, 314-320, **2010**.
[43] Maurer-Jones M. A., Lin Y. S., Haynes C. L. Functional assessment of metal oxide nanoparticle toxicity in immune cells. *ACS Nano*, 4, 6, 3363-3373, **2010**.
[44] Serda R. E., Mack A., van de Ven A. L., et al. Logic-embedded vectors for intracellular partitioning, endosomalescape, and exocytosis of nanoparticles. *Small*, 6, 23, 2691-2700, **2010**.
[45] Boussif O., Lezoualc'h F., Zanta M. A., et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proceedings of the National Academy of Sciences*, 92, 16, 7297-7301, **1995**.
[46] Cauda V., Argyo C., Bein T. Impact of different PEGylation patterns on the long-term bio-stability of colloidal mesoporous silica nanoparticles. *Journal of Materials Chemistry*, 20, 39, 8693-8699, **2010**.
[47] Veronese F. M., Pasut G. PEGylation, successful approach to drug delivery. *Drug Discovery Today*, 10, 21, 1451-1458, **2005**.
[48] Zahr A. S., Davis C. A., Pishko M. V. Macrophage uptake of core-shell nanoparticles surface modified with poly (ethylene glycol). *Langmuir*, 22, 19, 8178-8185, **2006**.
[49] Lin Y. S., Haynes C. L. Synthesis and characterization of biocompatible and size-tunable multifunctional porous silica nanoparticles. *Chemistry of Materials*, 21, 17, 3979-3986, **2009**.
[50] He Q., Zhang J., Shi J., et al. The effect of PEGylation of mesoporous silica nanoparticles on nonspecific binding of serum proteins and cellular responses. *Biomaterials*, 31, 6, 1085-1092, **2010**.
[51] Slowing I., Trewyn B. G., Lin V. S. Y. Effect of surface functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells. *Journal of the American Chemical Society*, 128, 46, 14792-14793, **2006**.
[52] Souris J. S., Lee C. H., Cheng S. H., et al. Surface charge-mediated rapid hepatobiliary excretion of mesoporous silica nanoparticles[J]. *Biomaterials*, 31, 21, 5564-5574, **2010**.
[53] Cheng S. H., Li F. C., Souris J. S., et al. Visualizing dynamics of sub-hepatic distribution of nanoparticles using intravitalmultiphoton fluorescence microscopy. *ACS Nano*, 6, 5, 4122-4131, **2012**.
[54] Michalet X., Pinaud F. F., Bentolila L. A., et al. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science*, 307, 5709, 538-544, **2005**.
[55] Medintz I. L., Uyeda H. T., Goldman E. R., et al. Quantum dot bioconjugates for imaging, labelling and sensing. *Nature Materials*, 4, 6, 435-446, **2005**.
[56] Slowing I. I., Vivero-Escoto J. L., Zhao Y., et al. Exocytosis of Mesoporous Silica Nanoparticles from Mammalian Cells: From Asymmetric Cell-to-Cell Transfer to Protein Harvesting. *Small*, 7, 11, 1526-1532, **2011**.
[57] Chang J S, Chang K L B, Hwang D F, et al. In vitro cytotoxicitiy of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environmental Science & Technology*, 41, 6, 2064-2068, **2007**.
[58] Yang Q, Wang S, Fan P, et al. pH-responsive carrier system based on carboxylic acid modified mesoporous silica and polyelectrolyte for drug delivery. *Chemistry of Materials*, 17, 24, 5999-6003, **2005**.
[59] Park C, Oh K, Lee S C, et al. Controlled release of guest molecules from mesoporoussilica particles based on a pH-responsive polypseudorotaxanemotif[J]. *Angewandte Chemie International Edition*, 46, 9, 1455-1457, **2007**.
[60] Sun J T, Hong C Y, Pan C Y. Fabrication of PDEAEMA-coated mesoporous silica nanoparticles and pH-responsive controlled release. *The Journal of Physical Chemistry C*, 114, 29, 12481-12486, **2010**.
[61] Lee C H, Cheng S H, Huang I, et al. Intracellular pH-responsive mesoporoussilica nanoparticles for the controlled release of anticancer chemotherapeutics[J]. *Angewandte Chemie*, 122, 44, 8390-8395, **2010**.
[62] Chen C, Pu F, Huang Z, et al. Stimuli-responsive controlled-release system using quadruplex DNA-capped silica nanocontainers. *Nucleic Acids Research*, 39, 4, 1638-1644, **2011**.
[63] Gao Q, Xu Y, Wu D, et al. pH-responsive drug release from polymer-coated mesoporous silica spheres. *The Journal of Physical Chemistry C*, 113, 29, 12753-12758, **2009**.
[64] Liu C, Guo J, Yang W, et al. Magnetic mesoporous silica microspheres with thermo-sensitive polymer shell for controlled drug release. *Journal of Materials Chemistry*, 19, 27, 4764-4770, **2009**.
[65] Bernardos A, Aznar E, Marcos M D, et al. Enzyme-Responsive Controlled Release Using Mesoporous Silica Supports Capped with Lactose. *Angewandte Chemie*, 121, 32, 5998-6001, **2009**.
[66] Bernardos A, Mondragon L, Aznar E, et al. Enzyme-responsive intracellular controlled release using nanometric silica mesoporous supports capped with “saccharides”[J]. *ACS Nano*, 4, 11, 6353-6368, **2010**.
[67] Coll C, Mondragón L, Martínez-Máñez R, et al. Enzyme-mediated controlled release systems by anchoring peptide sequences on mesoporoussilica supports[J]. *Angewandte Chemie International Edition*, 50, 9, 2138-2140, **2011**.
[68] Patel K, Angelos S, Dichtel W R, et al. Enzyme-responsive snap-top covered silica nanocontainers[J]. *Journal of the American Chemical Society*, 130, 8, 2382-2383, **2008**.
[69] Park C, Kim H, Kim S, et al. Enzyme responsive nanocontainers with cyclodextrin gatekeepers and synergistic effects in release of guests. *Journal of the American Chemical Society*, 131, 46, 16614-16615, **2009**.

[70] Mal N K, Fujiwara M, Tanaka Y. Photocontrolled reversible release of guest molecules from coumarin-modified mesoporous silica[J]. *Nature* 421, 6921, 350-353, **2003**.
[71] Vivero-Escoto J L, Slowing I I, Wu C W, et al. Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere[J]. *Journal of the American Chemical Society*, 131, 10, 3462-3463, **2009**.
[72] Lai J, Mu X, Xu Y, et al. Light-responsive nanogated ensemble based on polymer grafted mesoporous silica hybrid nanoparticles[J]. *Chemical Communications*, 46, 39, 7370-7372, **2010**.
[73] Ferris D P, Zhao Y L, Khashab N M, et al. Light-operated mechanized nanoparticles[J]. *Journal of the American Chemical Society*, 131, 5, 1686-1688, **2009**.
[74] Lai C Y, Trewyn B G, Jeftinija D M, et al. A mesoporous silica nanosphere-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules. *Journal of the American Chemical Society*, 125, 15, 4451-4459, **2003**.
[75] Yan H, Teh C, Sreejith S, et al. Functional mesoporoussilica nanoparticles for photothermal-controlled drug delivery in vivo[J]. *Angewandte Chemie International Edition*, 51, 33, 8373-8377, **2012**.
[76] Davis M E. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nature Reviews Drug Discovery*, 7, 9, 771-782, **2008**.
[77] 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*, 5, 5, 4131-4144, **2011**.
[78] De Wolf H K, Snel C J, Verbaan F J, et al. Effect of cationic carriers on the pharmacokinetics and tumor localization of nucleic acids after intravenous administration[J]. *International Journal of Pharmaceutics*, 331, 2, 167-175, **2007**.
[79] Tsai C P, Chen C Y, Hung Y, et al. Monoclonal antibody-functionalized mesoporous silica nanoparticles , MSN) for selective targeting breast cancer cells. *Journal of Materials Chemistry*, 19, 32, 5737-5743, **2009**.
[80] Ferris D P, Lu J, Gothard C, et al. Synthesis of biomolecule-modified mesoporous silica nanoparticles for targeted hydrophobic drug delivery to cancer cells. *Small*, 7, 13, 1816-1826, **2011**.
[81] Zhu C L, Song X Y, Zhou W H, et al. An efficient cell-targeting and intracellular controlled-release drug delivery system based on MSN-PEM-aptamer conjugates. *Journal of Materials Chemistry*, 19, 41, 7765-7770, **2009**.

7. ACKNOWLEDGEMENTS

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