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Biomedical Applications of Functionalized Hollow Mesoporous Silica Nanoparticles: Focusing on Molecular Imaging

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Abstract

Hollow mesoporous silica nanoparticles (HMSNs), with a large cavity inside each original mesoporous silica nanoparticle (MSN), have recently gained increasing interest due to their tremendous potential for cancer imaging and therapy. The last several years have witnessed a rapid development in engineering of functionalized HMSNs (i.e. *f*-HMSNs) with various types of inorganic functional nanocrystals integrated into the system for imaging and therapeutic applications. In this review article, we summarize the recent progress in the design and biological applications of *f*-HMSNs, with a special emphasis on molecular imaging. Commonly used synthetic strategies for the generation of high quality HMSNs will be discussed in detail, followed by a systematic review of engineered *f*-HMSNs for optical, positron emission tomography, magnetic resonance, and ultrasound imaging in preclinical studies. Lastly, we also discuss the challenges and future research directions regarding the use of *f*-HMSNs for cancer imaging and therapy.

Keywords

Hollow mesoporous silica nanoparticles (HMSNs); molecular imaging; nanomedicine; theranostics; cancer; optical imaging; positron emission tomography (PET)

Introduction

The last decade has witnessed a rapid development in the design and synthesis of various types of multifunctional nanosystems that can potentially be used for cancer targeted imaging and therapy [1–3]. Since silica is “generally recognized as safe” by the United States Food and Drug Administration (FDA; <http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm084104.htm>), silica-based nanomaterials have been extensively investigated because of the non-toxic nature and facile chemistry for

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surface modification [4, 5]. Recently, dye-doped ultrasmall silica nanoparticles called Cornell dots (C dots [6]) have entered clinical investigation in melanoma patients, which is an important milestone for the use of inorganic nanomaterials in the same fashion as a drug in humans.

In comparison with pure silica nanoparticles, mesoporous silica nanoparticles (MSNs) possess many attractive properties, such as large surface area, high pore volume, uniform and tunable pore size, and low mass density [7]. Although MSNs have been intensively investigated for drug delivery applications since 2000 [8], how to improve the drug loading capacity and in vivo targeting efficiency while minimizing the undesired side-effects to healthy organs remains a major challenge. Hollow mesoporous silica nanoparticles (HMSNs), with a large cavity inside each original MSN, have recently been developed to greatly enhance the drug loading capacity [4]. With the availability of well-established techniques for integrating various types of inorganic functional nanocrystals (e.g. iron oxide nanoparticles, gold nanoparticles, etc.) inside or at the surface of HMSNs, in addition to surface functionalization of HMSNs to confer biocompatibility, imaging capability, specific targeting, etc., such functionalized HMSNs (denoted as *f*-HMSNs, Scheme 1) with a rattle-type (or yolk-shell) structure are highly attractive multifunctional nanoplatforms for future cancer imaging and therapy applications.

In this review article, we summarize the recent progress in the design and biomedical applications of *f*-HMSNs, with a primary focus on molecular imaging since limited progress has been made to date in the use of *f*-HMSNs for cancer therapy in vivo. First, commonly used synthetic strategies for the generation of HMSNs and *f*-HMSNs will be discussed in detail. Next, the progress to date in the engineering of *f*-HMSNs for optical imaging, positron emission tomography (PET), magnetic resonance imaging (MRI), and ultrasound (US) imaging in small animals will be reviewed. Lastly, we also discuss the challenges and future research directions in the use of *f*-HMSNs for cancer imaging and therapy applications.

Templated methods for the synthesis of HMSNs and *f*-HMSNs

Generally speaking, soft- and hard-templating are two of the most popular methods used for the synthesis of HMSNs. Soft-templating method uses certain surfactants (e.g. tetrapropylammonium hydroxide) and co-structure-directing agents (e.g. tetraethyl orthosilicate [TEOS] or 3-aminopropyl-triethoxysilane [APTES]) to form interior hollow structures and mesoporous silica shell simultaneously [9, 10]. For example, a hollow-structured aluminosilicate with highly ordered 3-dimensional mesoporous shell and significantly improved hydrothermal stability was generated using this technique (Figure 1A) [9]. In another report, HMSNs with improved controllability in both morphology and size were synthesized by employing micelle and emulsion as dual soft templates [10]. However, reducing the aggregation of nanoparticles was a major challenge. Besides HMSNs, modified soft-templating method could also be used to prepare yolk/SiO₂ shell structures with different cores inside, such as Au and Fe₂O₃ (Figure 1B) [11–14]. One major limitation of soft-templating methods is that due to the difficulty in controlling the droplet size, it is quite challenging to obtain monodisperse HMSNs with well-controlled size, morphology, and reproducibility.

Hard-templating method has recently been demonstrated to be a better strategy for the synthesis of high quality HMSNs (or rattle-type *f*-HMSNs) with good monodispersity and reproducibility, thanks to the improved control over the synthesis of templates and well-established techniques for selective etching [4, 15]. The basis of such improved method relies on the compositional or structural difference between the core and shell of the

nanoparticles, where typically the inner core could be selectively etched away to leave a large cavity inside the thin porous shell to form HMSN. The selection of suitable hard templates and etchants is critical, which is also highly dependent on the different etching mechanisms [9, 16–19]. To date, various materials including polystyrene sphere, dense silica, iron oxide nanoparticles, etc. have been used as hard templates, whereas agents such as sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrofluoric acid (HF), hydrochloric acid (HCl), and even hot water have been successfully employed as the etchant [16, 19–22]. For example, a flexible, scalable, and robust method to synthesize tailored silica nanorattle structures was developed using organic-inorganic hybrid solid silica sphere as the template and HF as the etchant, which have small silica core inside each volume-tunable hollow cavity with a mesoporous shell (Figure 1C) [16]. Similar etching mechanisms, such as structural difference-based selective etching (Figure 1D) and cationic surfactant assisted self-templating method, have also been utilized to synthesize high quality HMSNs (or rattle-type HMSNs) with Na_2CO_3 as the etchant at elevated temperature [19, 23].

Besides the abovementioned selective etching of core/shell structured nanoparticles to form HMSNs, surface-protected etching using NaOH as the etchant is another means to prepare HMSNs [22, 24, 25], for which poly(vinylpyrrolidone) (PVP) and poly-(dimethyldiallylammonium chloride) (PDDA) have been demonstrated to be good surface protectors. In order to apply this strategy for the synthesis of silica-based HMSNs with particle sizes less than 100 nm, a milder etching strategy using hot water has also been developed [17, 20, 26], which have provided researchers with alternative techniques for the design and generation of HMSNs with suitable sizes. Overall, using the hard-templating method, the cavity volume, size, and shell thickness of HMSNs could be readily controlled by selecting suitable templates and etching strategies [4]. More importantly, inorganic functional nanocrystals such as Au nanoparticles, Fe_3O_4 , MnO_x , and upconversion nanoparticles (UCNPs) could also be encapsulated inside the HMSNs to fabricate $\mathcal{F}$ HMSNs with various molecular imaging capabilities [17, 27–32]. The following section will be focused on the engineering of $\mathcal{F}$ HMSNs for in vivo imaging applications using different modalities.

Engineering of $\mathcal{F}$ HMSNs for molecular imaging

Molecular imaging, defined as the “visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems” [33], has greatly facilitated the investigation of complex biological events in both the preclinical and clinical setting [34]. Although clinical translation of novel imaging agents has so far been rather slow, molecular imaging does hold great potential in multiple aspects such as drug development, disease diagnosis, monitoring therapeutic responses, and the understanding of complex interactions between nanomedicine and living biological systems [35]. Over the last decade, engineered $\mathcal{F}$ HMSNs have been investigated with various imaging techniques such as optical, PET, MRI, and US.

Optical imaging

Generally speaking, optical imaging is inexpensive, widely available, easy-to-handle, and highly sensitive, which has been extensively used for monitoring various molecular/biological events in cells and small animal models [35–37]. Although various strategies (e.g. organic dye doping, fluorescent nanoparticle encapsulation, etc.) have been developed to engineer MSNs for optical imaging applications in vitro and in vivo [38, 39], the design and synthesis of $\mathcal{F}$ HMSNs for in vivo optical imaging is still in its infancy and needs much more investigation in the future [40–42].

In one study, fluorescein isothiocyanate (FITC) and doxorubicin (DOX) were co-loaded into multi-shelled HMSNs by sequentially mixing HMSNs with aqueous solutions of FITC and DOX [40]. Although green fluorescence signal from FITC could be detected in mice after intraperitoneal injection of FITC-loaded HMSNs at various dosages (Figure 2A, B), other dyes with near-infrared (NIR, 700–900 nm) excitation and emission will be more desirable for future studies because of much better tissue penetration of light and significantly lower tissue autofluorescence in the NIR window [43, 44].

Besides dye-doped HMSNs, integration of fluorescent nanoparticles such as quantum dots (QDs) or UCNPs inside or at the surface of HMSNs can also be highly desirable for in vivo optical imaging applications. Although polyethylene glycol (PEG) modified and liposome-coated QD@MSN core/shell nanoparticles have been investigated for imaging studies in cell culture [45], the combination of QDs with HMSNs has not been reported to date, possibly due to the difficulty in trapping single QD inside HMSNs and toxicity-related concerns for cadmium-based QDs [46, 47]. When compared to conventional organic dyes and QDs, UCNPs can avoid the potential ultraviolet photo-damage of tissue (since the excitation wavelength of UCNPs is in the NIR window, typically 980 nm) and exhibit good biocompatibility in living systems [48]. Such upconversion luminescence (UCL) typically occurs when low-energy light (e.g. NIR) is converted to higher energy light through the sequential absorption of multiple photons or energy transfer. In addition, UCNPs have many other attractive UCL features such as sharp emission lines [49], superb photostability [50], high detection sensitivity [51], deep tissue penetration depth [52], and extremely low autofluorescence [53], which make them one of the best classes of nanoparticles for optical imaging applications.

In a recent study, an ion-exchange process was utilized to fabricate nanorattles, each of which consists of a UCNP (i.e. $\text{-NaYF}_4\text{:Er/Yb}$)-containing shell and a loose magnetic nanoparticle core (Figure 2C) [42]. It was demonstrated that the multifunctional nanorattles could be directed by an external magnetic field for in vivo tumor targeting, which can also be non-invasively imaged using the visible luminescence (i.e. green and red emissions) under 980 nm excitation (Figure 2D). Instead of coating UCNPs at the surface of HMSNs, a different strategy was used in another report [41]. Core/shell structured $\text{NaYF}_4\text{:Er/Yb@NaGdF}_4$ UCNP was first encapsulated inside a dense silica shell, part of which was then selectively etched away via a protective etching strategy to form a multifunctional rattle-structured UCNP@HMSN nanosystem (Figure 2E) [41]. With the presence of $\text{NaYF}_4\text{:Er/Yb@NaGdF}_4$ UCNP inside the nanosystem, in vivo UCL imaging after intratumoral injection into HeLa tumor-bearing mice was achieved (Figure 2F). In addition, with the doping of Gd^{3+} ions into the UCNP surface/matrix, this multifunctional nanosystem could also be detected by T_1 -weighted MRI (Figure 2G). However, neither in vivo optical nor MRI imaging after intravenous injection of the UCNP@HMSN nanosystem was reported, possibly due to very low tumor accumulation of the nanoparticle based on passive targeting alone.

Although UCNP-functionalized HMSNs possess attractive UCL features for in vivo optical imaging, to date only HMSNs functionalized with UCNPs emitting in the visible range have been reported [41, 42]. With the rapid development in optimizing $\text{Tm}^{3+}/\text{Yb}^{3+}$ co-doped -NaYF_4 UCNP with strong NIR-to-NIR emission intensity [54, 55], $\text{-NaYF}_4\text{:Tm/Yb}$ UCNP-functionalized HMSNs will likely become a vibrant research area in the near future. One major limitation of optical imaging, even in the NIR range, is limited tissue penetration depth which typically cannot reach beyond a few cm. In addition, optical imaging is qualitative in nature and semi-quantitative at the best. Therefore, the use of quantitative imaging techniques capable of deep tissue penetration is highly desirable for interrogating the fate of HMSNs in vivo. PET is sensitive, quantitative, clinically relevant, and has

excellent tissue penetration [56–60], which makes it a highly desirable imaging technique for in vivo investigation of f-HMSNs.

Positron emission tomography

Because of the several abovementioned advantages of PET over other imaging modalities (e.g. optical and MRI), labeling nanoparticles with positron-emitting radionuclides has been generally recognized as the most accurate means for non-invasive evaluation of their biodistribution and pharmacokinetics [61, 62]. Since radiolabeled nanoparticles (especially with PET isotopes) represent a newly emerged research area over the last decade, to the best of our knowledge, no reports on radiolabeled HMSNs exist in the literature to date.

We have successfully developed novel antibody-conjugated and ^{64}Cu ($t_{1/2} = 12.7$ h) labeled HMSNs, denoted as ^{64}Cu -NOTA-HMSN-TRC105 (Figure 3A, B), and demonstrated the proof-of-principle for in vivo active tumor vasculature targeting which can be monitored with non-invasive serial PET imaging (Figure 3C). Since many nanoparticles suffer from poor extravasation in the tumor tissue [61, 62], tumor vasculature targeting was adopted where extravasation is not required to observe the tumor signal [63–65]. CD105 (also called endoglin) was chosen as the vascular target, which is almost exclusively expressed on proliferating tumor endothelial cells [66, 67], and TRC105 (a human/murine chimeric IgG1 monoclonal antibody that binds to both human and murine CD105 [68]) was used as the targeting ligand. Significantly higher uptake in 4T1 murine breast tumors (which express high level of CD105 on the tumor vasculature [69, 70]) was observed for ^{64}Cu -labeled, TRC105-conjugated HMSNs, which is about two fold of that for the non-targeted HMSNs (Figure 3C). With demonstrated active tumor targeting efficiency in vivo and high drug loading capacity, ^{64}Cu -NOTA-HMSN-TRC105 is a promising platform for future image-guided, tumor-targeted drug delivery and more efficacious cancer therapy.

Although PET imaging is highly sensitive and quantitative for in vivo applications, its spatial resolution (mm level) is significantly lower than that of MRI (typically $< 500\ \mu\text{m}$). With exquisite soft tissue contrast, MRI has been a widely used imaging modality in the clinic because of its high spatial resolution and lack of ionizing radiation [71–73]. By integrating HMSNs with Gd^{3+} -complex or various nanoparticles (e.g. Fe_3O_4 , Gd^{3+} -doped UCNPs, MnO_x , etc.) [27, 28, 41, 74], the resulting f-HMSNs could become detectable with MRI while maintaining the capability for drug loading and delivery.

Magnetic resonance imaging

Iron oxide (Fe_3O_4) nanoparticle is among the most widely used contrast agents for T_2 -weighted MRI. Although Fe_3O_4 @MSN core@shell nanostructures have been documented in the literature as useful tools for tracking various types of cells with MRI [75–78], the development of Fe_3O_4 @HMSN is very slow and severely understudied. In the only available report to date, a relatively complex strategy was utilized to synthesize Fe_3O_4 @HMSNs [27]. Ellipsoidal Fe_2O_3 nanocrystals were first coated with layers of dense and mesoporous silica sequentially, which were then sealed in autoclave for selective etching with ammonia at $150\ ^\circ\text{C}$ to form Fe_2O_3 -silica nanocapsules without mesopores. In order to generate Fe_3O_4 @HMSNs with high surface area and pore volume (Figure 4A), two more steps (i.e. calcination and hydrogen/argon reduction) were needed to first burn out the surfactants (i.e. octadecyltrimethoxysilane, C_{18}TMS) and subsequently reduce the Fe_2O_3 core to Fe_3O_4 . The resulting Fe_3O_4 @HMSNs exhibited low cytotoxicity to various cell lines, as well as low hemolyticity against human red blood cells. With the presence of large interior cavities and magnetic Fe_3O_4 nanoparticles, the potential of Fe_3O_4 @HMSNs as a drug delivery nanopatform and T_2 MRI contrast agent was convincingly demonstrated (Figure 4B).

By substituting the Fe_3O_4 core with other inorganic functional nanoparticles (e.g. Au, QDs, etc.), this strategy may also be extended to the design of other multifunctional nanosystems for image-guided drug delivery and cancer therapy. However, one of the biggest drawbacks of this strategy is the inevitable aggregation of final nanocapsules caused by calcination at high temperature (i.e. 550°C for 10 h), which appeared to be the only known method to remove the surfactant (i.e. C_{18}TMS) from the silica network. Future research effort in this area should be directed towards optimizing the stability of Fe_3O_4 @HMSNs in biological systems and demonstrating their potential for tumor targeted image-guided drug delivery and cancer therapy.

Besides encapsulating Fe_3O_4 nanoparticles into HMSNs for T_2 -weighted MRI, alternative strategies for securely trapping manganese (II) into the mesoporous silica shell can lead to other interesting nanostructures with both MRI and drug delivery capabilities. Manganese (II) is a well-known T_1 MRI contrast agent, due to its low toxicity and long electronic relaxation time of five unpaired electrons [79, 80]. For example, MSN coated hollow manganese oxide (i.e. MnO_x) nanoparticles were developed in one report, which were investigated for serial and long term monitoring of adipose-derived mesenchymal stem cells with MRI [81].

In a few other studies, MnO_x -based HMSNs (denoted as HMSN@MnO_x , Figure 4C) were reported for MRI and drug delivery, where an in situ redox reaction between oxidants (e.g. potassium permanganate) and surfactants (e.g. cetyltrimethyl ammoniumbromide, C_{16}TAB) was used to generate ultrasmall MnO_x nanoparticles within the mesoporous network [28, 74]. By decreasing the valence of Mn after treatment with H_2/Ar , significantly increased r_1 relaxivity was observed for HMSN@MnO_x (from 0.57 to $1.84\text{ mM}^{-1}\text{s}^{-1}$), which was substantially higher than that of the previously reported MnO_x @MSN nanostructures [81, 82]. It was suggested that such phenomenon could be attributed to the higher dispersity of Mn(II) paramagnetic centers and faster diffusion of water molecules in HMSN@MnO_x [28]. The potential of HMSN@MnO_x as a pH-sensitive MRI contrast agent was also demonstrated [74]. Interestingly, it was found that treating HMSN@MnO_x with weak acid could induce the dissolution of MnO_x , leading to the release of Mn^{2+} ions and consequently a significant increase of r_1 value (up to $8.81\text{ mM}^{-1}\text{s}^{-1}$, Figure 4D). Such pH-responsive MRI performance was suggested to be potentially useful for tumor detection, where the acidic tumor microenvironment may trigger the release of Mn^{2+} ions to enhance the MRI contrast. However, in vivo MRI of tumor-bearing rats at various time points post-injection only revealed slight difference in image contrast without significant enhancement (Figure 4E).

Ultrasound imaging

When compared with other techniques, US imaging has several advantages such as significantly wider availability and relatively high spatial resolution that can be comparable to MRI [83]. Gold nanoparticles and perfluorohexane (PFH)-encapsulated HMSNs have been used for both contrast-enhanced ultrasound imaging and high intensity focused ultrasound (HIFU) surgical therapy [31, 84, 85], demonstrating that HMSN s can serve as novel theranostic agents for both imaging and therapy under ultrasound guidance.

To address the drawbacks of microbubbles and liposomes as enhancement agents (EAs) in HIFU therapy, such as the relatively large size, low strength, and heat-resistance, a novel HMSN -based inorganic EA was recently developed [84]. To enhance the US imaging efficiency, temperature-sensitive PFH (well recognized as being highly hydrophobic and biocompatible) was selected and loaded into $\sim 300\text{ nm}$ sized HMSNs as bubble generator to form HMSN(PFH) for US imaging and enhanced HIFU therapy (Figure 5A, B). Pronounced difference between HMSN and HMSN(PFH) in US image contrast was observed at a high HIFU power output ($\sim 120\text{ W}$, Figure 5C), clearly demonstrating the feasibility of

HMSN(PFH) as an ultrasound EA. Considering the high hydrophobicity of PFH and no purification step was involved during the synthesis of HMSN(PFH), more detailed investigation on the PFH loading capacity as well as releasing profile is needed in the future.

In a follow-up study, improved nanoparticle stability, US imaging capability, as well as HIFU therapy was achieved by decorating HMSNs with gold nanoparticles and subsequent coating with a layer of PEG [31]. Such improvement was attributed to the anchored gold nanoparticles, which can alter the responsive amplitude and simultaneously absorb US energy when exposed to appropriate ultrasonic frequency and power, thereby intensifying the acoustic echo signals and enhancing thermal response [86]. As a proof-of-concept, US triggered drug release was performed by using a hydrophobic dye (i.e. Pyrene) as a model drug in this study [31]. Although HMSN and HMSN@Au themselves could show slight enhancement in US imaging, loading PFH into HMSNs was suggested to be the key for significantly improved performance in both US imaging and HIFU therapy [31, 84].

Engineering of $\mathcal{F}$ -HMSNs for multimodal imaging

$\mathcal{F}$ -HMSNs with a single modality detection capability, as discussed in detail above, have both advantages and limitations for cancer imaging applications. For example, it is difficult to accurately quantify fluorescence signal in living subjects with dye-doped HMSNs, particularly in deep tissues [40]. $\mathcal{F}$ -HMSNs that can be imaged with MRI could give high resolution image with excellent soft-tissue contrast, yet the sensitivity is very low and it lacks quantitative information [27, 41]. Radionuclide-based imaging techniques, especially PET, are very sensitive and quantitative but they have relatively poor spatial resolution and can also be quite costly. US imaging is fast, painless, and low-risk with high spatial resolution and acceptable sensitivity, however it is impossible to use PFH-loaded HMSNs for whole-body imaging [31, 84]. Therefore, rational integration of multiple imaging modalities into a single HMSN-based nanosystem for the specific application may be able to provide complementary features which could not be achieved with any single modality alone.

For example, encapsulating Gd^{3+} -doped UCNPs into HMSN represents one of the best examples in integration of UCL and MRI, which offers high sensitivity of luminescence and high spatial resolution of MRI [41]. Another fascinating choice is the combination of ultrasound and MRI, where MRI could potentially be employed as the pre-surgical evaluation tool while ultrasound can be exploited for real-time guidance during HIFU therapy. Integration of PET imaging capability into HMSN-based multimodal nanosystems will be highly attractive for potential clinical translation, since it is the ideal imaging technique for non-invasive, sensitive, and quantitative evaluation of the pharmacokinetics and tumor-targeting efficacy of these intriguing nanosystems, both in preclinical animal models and potential clinical setting. Currently, engineering of $\mathcal{F}$ -HMSNs for multimodal molecular imaging is at a very early stage, which we believe will certainly become a highly dynamic research area over the next decade.

Conclusion and future perspective

In this review, we summarized the current status of engineering $\mathcal{F}$ -HMSNs for biological applications with a primary focus on nanoparticle synthesis and the applications in molecular imaging. In most cases, the synthesis of high quality HMSNs with tunable particle size and cavity volume was achieved through either soft- or hard-templating method. We then gave a comprehensive summary of various types of $\mathcal{F}$ -HMSNs for optical, PET, MRI, and US imaging studies in small animal models. Besides imaging applications, further functionalization of HMSNs by adding therapeutic agents for cancer therapy is also under

active development [41, 42, 87–89], which is not within the main focus of this review. With the presence of large cavities for loading small anti-cancer agents, and more importantly, the capability to integrate various types of inorganic functional nanocrystals inside or at the surface of HMSNs, *f*HMSNs are one of the most promising nanoplatforms for future image-guided drug delivery and cancer therapy, as evidenced by the rapidly growing literature reports over the last 5 years.

Long-term safety of HMSNs is an important issue that needs to be thoroughly addressed before potential clinical translation. Generally regarded as safe, silica-based nanoparticles are clearly more favorable in this regard than other inorganic nanoparticles that are based on heavy metals. However, much more research effort will be needed in the following areas that are related to molecular imaging/therapy with *f*HMSNs. First, although far from ideal, optical imaging of *f*HMSNs will remain one of the most convenient ways in the near future to understand the behavior of HMSNs in vitro and in vivo due to virtually ubiquitous availability. Stable and efficient functionalization of HMSNs with NIR optical agents (e.g. NIR emitting dyes, QDs, or UCNPs) will offer significantly improved image contrast with much deeper tissue penetration depth of signal than those using visible dyes (e.g. FITC). Second, when compared to most optical imaging techniques that are qualitative in nature, quantitative PET imaging of radiolabeled HMSNs will be a better choice for investigating in vivo tumor targeting efficacy, biodistribution, and clearance of HMSNs in preclinical models as well as potentially in cancer patients. Third, engineering of HMSNs to integrate two or more synergistic imaging modalities (e.g. PET/MRI or PET/optical) into one nanosystem is highly useful for providing researchers with complementary and more accurate information, which has been a general trend for many other nanoparticle-based agents in the cancer nanotechnology research community. Last but not least, in vivo targeted imaging of *f*HMSNs has not been reported to date, and most of the existing reports have been focused on the synthesis of *f*HMSNs without examining their potential for actively targeted cancer imaging and/or therapy after systemic intravenous administration. With the tremendous progresses made over the last several years regarding controlled synthesis of various *f*HMSNs, as summarized above in this review, the next decade will surely witness a rapid development in the synthesis, surface modification, and applications of these intriguing nanoparticles for molecularly targeted tumor imaging and therapy, ushering in the new era of cancer theranostics.

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Executive summary

Functionalized hollow mesoporous silica nanoparticles (f-HMSNs) as new nanoplatforms

- HMSNs hold great potential for significantly enhanced drug loading capacity and therapeutic efficacy.
- Various techniques for integrating different types of inorganic functional nanocrystals inside or at the surface of HMSNs can make f-HMSNs attractive nanoplatforms for image-guided drug delivery and cancer therapy.

Templated methods for the synthesis of HMSNs and f-HMSNs

- Soft- and hard-templating are two commonly used methods for the synthesis of HMSNs, with the latter being superior for generating high quality HMSNs with enhanced monodispersity and reproducibility.
- Structural difference-based selective etching, cationic surfactant assisted self-templating, and surface-protected etching methods are three major categories of hard-templating synthesis of HMSNs.

Engineering of f-HMSNs for molecular imaging

- Design and synthesis of f-HMSNs for in vivo optical imaging is under active development. UCNP-functionalized HMSNs possess attractive features for in vivo applications.
- No study on radiolabeled HMSNs has been reported to date, which deserves significant research effort in the near future.
- Upon integration or encapsulation of Gd^{3+} -complex or magnetic nanoparticles (e.g. Fe_3O_4 , Gd^{3+} -doped UCNPs, MnO_x , etc.), f-HMSNs can be used for MRI as well as drug delivery.
- Perfluorohexane-encapsulated HMSNs are attractive enhancers for both ultrasound imaging and high intensity focused ultrasound therapy.
- Engineering of HMSNs to integrate two or more imaging modalities into one nanosystem is highly useful for providing researchers with complementary and more accurate information about the pharmacokinetics, biodistribution, clearance, etc. of HMSNs in vivo, which is an exciting new area of cancer theranostics.

Figure 1. Representative TEM images of HMSNs synthesized via soft- (A,B) or hard-templating methods (C,D)

(A) A high-resolution TEM image of hollow spherical cubic mesoporous aluminosilicate.

(B) Yolk/shell structure with spindle-like Fe₂O₃ particles inside the cavity (scale bar: 200 nm).

(C) Silica-based nanorattles and (D) HMSNs synthesized by selective etching using HF and Na₂CO₃ as the etchant, respectively. Reproduced with permission from [9, 11, 16, 19].

Figure 2. In vivo optical imaging of *f*-HMSNs

(A) A TEM image of hollow double-shelled silica nanoparticles. (B) Optical imaging of FITC-loaded hollow double-shelled silica nanoparticles at 55 minutes post-injection into mice. (C) A TEM image of $\text{Fe}_3\text{O}_4@\text{hollow}@-\text{NaYF}_4:\text{Er}/\text{Yb}$ nanoparticles. Inset: a higher-magnification TEM image (upper left) and a selected area electron diffraction of $-\text{NaYF}_4:\text{Er}/\text{Yb}$ shell (lower left). (D) In vivo UCL imaging of H22 xenograft tumor-bearing mice after intravenous injection of DOX-loaded $\text{Fe}_3\text{O}_4@\text{hollow}@-\text{NaYF}_4:\text{Er}/\text{Yb}$ nanoparticles with (right) or without (left) application of magnetic field (MF) for 1 h. (E) A TEM image of rattle-structured UCNPs@HMSNs. Inset shows the schematic illustration. (F)

UCL imaging and (G) in vivo T₁-weighted MRI of UCNP@HMSNs after intratumoral injection into mice. Reproduced with permission from [40–42].

Figure 3. In vivo PET imaging of radiolabeled HMSN
 (A) A TEM image of HMSN. (B) A schematic illustration of ^{64}Cu -NOTA-HMSN-TRC105.
 (C) Serial coronal PET images of 4T1 breast tumor-bearing mice at different time points post-injection of ^{64}Cu -NOTA-HMSN-TRC105 or ^{64}Cu -NOTA-HMSN. Tumors were indicated by arrowheads.

Figure 4. In vivo MRI with *f*-HMSNs

(A) A TEM image of Fe₃O₄@HMSNs. (B) In vivo MRI of Fe₃O₄@HMSNs in melanoma tumor-bearing C57/BL6 mice after intratumoral injection. (C) A TEM image of HMSN@MnO_x. (D) The change of r_1 values of HMSN@MnO_x in solutions with different pH values. (E) In vivo T₁-weighted MRI of Walker 256 tumor-bearing rat after intravenous injection of HMSN@MnO_x. Reproduced with permission from [27, 74].

Figure 5. In vivo ultrasound imaging with *f*-HMSNs

(A) A schematic illustration of the synthetic route for HMSN(PFH). (B) A TEM image of representative HMSNs. (C) Representative B-mode ultrasound images before (left) and after (right) HIFU exposure on degassed bovine liver at 120 W for 5 s. Reproduced with permission from [84].

Scheme 1.

A schematic illustration of functionalized hollow mesoporous silica nanoparticles.