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Applications of silica-based nano-particles for multimodal bio-imaging

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Abstract

Multimodal imaging, as an important approach to circumvent the limitations of single imaging modality, has attracted extensive attention in recent years. With the rapid development of nanotechnology and the ongoing efforts to improve their targeting capability and endow multiple imaging ability, nanoprobes are expected to play crucial roles in multimodal imaging through integrating different imaging moieties or molecules into a single nanoparticle, where silica has been used intensively as a carrier or a medium for the construction of the nanoprobes due to its preferable characteristics including good biocompatibility, long blood circulation time, and ease of modification. Based on the types of the silica used for the fabrication of nanoprobes, solid silica-based and mesoporous silica-based nanoparticles were developed for multi-modal imaging. Herein, the newly developed silica-based nanoparticles as multi-modal imaging agents for disease diagnosis and therapy in the last five years were summarized, along with their fabrication process, specific applications and especially the role of the silica.
Key words

multimodal imaging, silica, nanoparticles, spectroscopy, computed tomography, magnetic resonance.
1. Introduction

Nanomaterials have recently become one of the most attractive research frontiers in the biomedical fields with the rapid development of nanoscale science and technology (1, 2). Particularly, when combined with various bioimaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), optical imaging (OI) and ultrasound (US), nanomaterials can be utilized as contrast agents (CAs) to enhance the imaging effects, thus providing useful information for clinical diagnosis (3, 4).

In spite of many advantages of single bioimaging modality, more intrinsic limitations involving imaging targets, spatial resolution, tissue penetration ability and imaging sensitivity become evident when accurate and reliable diagnosis are to be attained. As a result, multi-modal imaging technology combining two or more imaging modalities has emerged to provide more reliable and efficient information about the lesion sites by joining their strengths (5).

Although multimodality nanoprobes seem to be very promising in the imaging diagnosis, the designing and fabrication of appropriate nanoprobes for in vivo imaging is facing great challenges such as good biocompatibility, high sensitivity, good chemical and thermal stability, high spatial resolution, sufficient tissue penetration, and low preparation cost (6).

Among the nanomaterials that are facilely functionalized, silica nanoparticles offer numerous advantages for the construction of in vivo imaging nanoprobes, which manifest in three aspects (7):
(1) Some basic and necessary features including good biocompatibility, chemical inertness, water solubility, optically transparent, ease of preparation and free of interference with magnetic radiation. (2) Silica nanoparticles have tunable structures in term of size and morphology. The surface can be easily modified with aptamers, peptides or other specific receptors to obtain tumor-targeting nanomaterials and maximize their tumor imaging performance. (3) There are two main types of silica nanoparticles: solid silica nanoparticles (SiNPs) and mesoporous silica nanoparticles (MSNs). MSNs possess unique advantages such as high surface area, large pore volume and tunable pore size. Generally, which kind of silica should be used for the fabrication of the multi-modal imaging nanoparticles depends on the different merit they have and the objectives to be accomplished.

Accordingly, many kinds of multi-modal bioimaging probes based on SiNPs and MSNs have been developed in recent years. In this review, we primarily focus on the recent contributions of silica nanoparticles as the carrier for the fabrication of multi-modal imaging nanoprobes, as well as corresponding applications in diagnosis and therapy. To facilitate elucidation, the article is arranged according to the types of the silica used and the various combination of different kinds of bioimaging techniques.

2. Applications of silica-based nanoparticles in bimodal imaging

2.1. Applications of SiNPs-based nanoparticles in bimodal imaging

The way of synthesizing SiNPs mainly includes the Stöber method and the reverse
microemulsion method. The classical Stöber method was developed by Stöber et al. in the late 1960s (8), in which the controlled hydrolysis and the condensation of the silica precursor with ethanol and aqueous ammonia as catalyst were the key factors affecting the formation of silica. The reverse microemulsion method was developed by Arriagada and Osseo-Asare in the early 1990s (9) in which the polymerization of TEOS was catalyzed by ammonia in a reverse microemulsion. In multimodal imaging, SiNPs obtained from either of the aforementioned methods can be used as a shell to form multifunctional probes with core-shell structures, as long as a pre-synthesized core is suspended in the solution (10). It is easy to functionalize SiNPs by the addition of hydrophilic functional molecule, which makes it possible to incorporate various functional moieties into the silica matrix to obtain single NPs for multimodal imaging.

2.1.1. CT-Fluorescence bimodal imaging

Computed Tomography (CT) is one of the most commonly used medical diagnosis instruments with high spatial resolution and no tissue penetrating limit (11). CT can provide accurate anatomical information of organism including the location, size and shape of lesion tissues. However, some soft tissues such as tumors cannot be well identified by CT imaging, which limits its diagnostic sensitivity towards cancer. Fluorescence imaging, in contrast, has poor tissue penetration ability but was extraordinarily sensitive even at cellular or molecular levels (12). So, in order to overcome the intrinsic drawbacks of single-mode bioimaging, CT and fluorescence imaging are combined to simultaneously attain high sensitivity and
three-dimensional structural information of the target, which allows for more accurate early
diagnosis and follow-up therapy of cancer.

Au NPs have been widely used as CT contrast agents. Feng et al. (13) investigated a new silica
hybrid nanocomposite containing Au nanoparticles (NPs) and FITC dyes (FITC-Au@SiO$_2$) as
CT-fluorescence bimodal contrast agent, which emitted strong fluorescence signal around 520
nm under the excitation of 490 nm. After injecting FITC-Au@SiO$_2$-PEG into C57BL/6J mice
through the tail vein, the whole body can be monitored in real time. Within 2 hours, strong
fluorescence can be observed clearly in the area of liver, and after 24 h of injection, the signal
decreased gradually. Moreover, the liver site also showed an apparent CT enhancement and high
CT values within 2 hours, which was well consistent with the fluorescence imaging result.

Hayashi et al. (14) synthesized fluorescent PEGylated clustered Au NPs-silica core-shell NPs
(c-Au@SiO$_2$-PEG NPs) with surface plasmon resonance (SPR) effect as probes for
CT-fluorescence bimodal imaging of tumors, where porphyrin as a fluorophore was doped in
silica NPs. Compared with single Au@SiO$_2$ NPs, c-Au@SiO$_2$ NPs demonstrated an obvious SPR
fluorescence enhancing effect, and the CT value of c-Au@SiO$_2$ NPs was higher than that of
Iopamidol at the same concentration. The nanoprobes were injected intravenously into
tumor-bearing mice. After 24 h injection, the CT values of tumor were 2.6-fold increased than
that before injection. Meanwhile, the fluorescence intensity was also significantly brighter than
those of mice injected with PBS, which could last for 60 hours.
Taking into account the importance of lymphatic system in the diagnosis of diseases, near-infrared (NIR) fluorescent dye TCPP-binding silica-coated Au NPs clusters (Au@SiO$_2$) were synthesized by the same authors (15) to visualize the lymph nodes (LNs) and lymph vessel (LV). Fluorescent images of mice were observed after injecting the probes into the left paw, which demonstrated that the probes gradually spread through the LVs from the left paw to the mandibular LNs via the axillary and cervical LNs with enhanced fluorescence signal emitted from Au@SiO$_2$ (Fig. 1). CT values of the LVs and the cervical LNs at 18 h after injection dramatically increased compared with those before injection. Moreover, the size of cervical LNs was approximately 2 mm by estimating from the CT images, which was consistent with the actual size (2.3 mm).

Compared with organic fluorescent dyes, luminescent quantum dots (QDs) have more competitive advantages, such as controllable and narrow emission spectrum, broad excitation spectrum, excellent optical stability, high quantum yield and large Stokes shifts. Song et al. (16) fabricated a composite structure for contrast-enhanced CT and fluorescence imaging. A layer of fluorescent QDs were adsorbed onto silica coated Au nanospheres (Au@SiO$_2$) and further coated with silica to reduce the defect of the QDs, which displayed good imaging performance in vitro. Xia et al. (17) designed folic acid (FA)-conjugated multifunctional NPs (GNR@SiO$_2$@QDs) by incorporating gold nanorods (GNRs) and CdSe/ZnS QDs into silica for CT and fluorescence targeting imaging in vitro and photothermal therapy (PTT). Confocal fluorescence images
showed that the NPs were selectively targeted to the folate receptors overexpressed on the surfaces of HeLa cells, which displayed strong red fluorescence under excitation at 488 nm. Besides, the NPs also exhibited a strong X-ray attenuation for CT imaging in vitro cell experiments and an excellent PTT performance for cancer.

Au NPs can exhibit not only strong X-ray attenuation, but also strong red fluorescence around 650 nm under the excitation of 530 nm. In this regard, Zhou et al. (18) synthesized FA-conjugated silica coated Au nanoclusters (NCs) nanoprobe (AuNCs@SiO$_2$-FA) with a diameter of ~58 nm for targeted in vivo gastric cancer cells CT and fluorescence imaging. The NPs were injected into the nude mice models via tail vein and the tumor location clearly displayed strong fluorescence at 6 h post-injection. Moreover, the CT values of corresponding tumor site increased greatly from 129.16 before injection to 383.32 after injection, which exhibited an excellent CT performance of the nanoprobe.

Au NPs as CT contrast agents attracted much interest due to their good performance. Nevertheless, to obtain a sufficient CT contrast of the target, a large dose of Au NPs is needed, which thus raised the cost of the Au-based CT contrast agents and made it difficult to be commercialized [11].

Among the currently used CT contrast agents, bismuth (Bi) is less expensive compared with Au, and showed the highest X-ray attenuation due to its largest atomic number. Chen et al. (19) synthesized a multifunctional nanoprobe Bi$_2$S$_3$-QD@SiO$_2$-PEG using a one-pot method with
uniform size, good biocompatibility and effective CT and fluorescence bimodal imaging properties in vitro and in vivo. The HU value of the probes dispersed in deionized water was 2000, which was much higher than that of the QD-iodinated oil nanoemulsion at a concentration of 15 mg mL\(^{-1}\). CT images of serial organs could be observed at different time point after injecting the probes through tail vein into the mice. At the same time, to avoid the limitation brought by the 633 nm emission wavelength of the probes (not in the range of near-infrared window), fluorescence imaging was also carried out after the mice sacrificed.

Lanthanide-doped upconversion nanoparticles (UCNPs) is another type of nanomaterial that can be used for fluorescence imaging. Excited at the wavelength of 980 nm, UCNPs can emit near-infrared (NIR) fluorescence, which endows a distinguished tissue penetration depth while avoiding the autofluorescence interference from the target. Upconversion luminescence (UCL) imaging is not limited by tissue penetration depth in vivo since the excitation wavelength at 980 nm ensures there is no autofluorescence interference.

Yb, Tm-codoped LaF\(_3\) UCNPs was coated onto silica and then conjugated with folic acid by Ma et al. (20) to construct a bimodal probe (UCNPs@SiO\(_2\)-FA) with high La contained in a single nanoparticle for simultaneous UCL imaging and X-ray CT imaging. Under continuous-wave excitation at 980 nm, the above nanoprobe displayed a strong NIR emission at around 800 nm. Since the excitation and emission wavelength of this kind of probe are both in the range of NIR window, this NIR-to-NIR fluorescent imaging mode is especially suitable for in
vivo imaging. When the probe was used for bioimaging, a clearly distinguished UCL signal was observed at corresponding lymphatic node after injecting the probes into the right paw of the mice, as well as an enhanced CT contrast compared to other soft tissues. To further demonstrate the targeting feasibility of the probe, it was intravenously injected into gastric cancer-bearing mice, showing a good tumor-targeting capability in either real-time fluorescent or CT imaging.

2.1.2. MR and fluorescence bimodal imaging

Magnetic resonance (MR) is another one of the most powerful and typical non-invasive diagnostic imaging techniques with exceptional spatial and anatomical resolution (21). However, MR has a poor performance in terms of sensitivity just like CT imaging. Accordingly, great efforts were made on the combination of MR and fluorescence imaging, and a series of MR/FL bimodal imaging probes were also explored to make it an efficient imaging tool (22).

Lin et al. (23) reported a new MR/fluorescence multifunctional nanoparticle based on silica-coated CuInS$_2$/ZnS QDs covalently attached with a Gd$^{3+}$-DTPA for cancer cell imaging. The longitudinal relaxivity ($r_1$) of the NPs (8.45 mM$^{-1}$ s$^{-1}$) was much higher than that of Magnevist (5.4 mM$^{-1}$ s$^{-1}$, a clinical Gd-DTPA-based contrast agent) on a 3.0 T scanner. The emission wavelength of the NPs changed with Cu/In ratio, and was located at 647 nm when Cu/In=1/1. The MTT assays indicated negligible cytotoxicity with >80% cell viability after incubation of BXPC-3 cells with the NPs for 3 h. Meanwhile, this kind of core-shell structured MR/FL probe not only emitted clear red fluorescence under 488 nm excitation, but also showed a
significant contrast enhancement in a T<sub>1</sub>-weighted image, demonstrating its great potential for MR/FL bimodal imaging.

Different from the above traditional core-shell structure, Lee et al. (24) synthesized PEGylated dual modality nanoprobes for cancer cell imaging and biodistribution, where silica was used as core, and decorated with Gd<sup>3+</sup> and FITC fluorescent dye for CT and fluorescent imaging, respectively. Similarly, the nanoprobes had a higher relaxivity (r<sub>1</sub>=9.41±0.32 mM<sup>-1</sup> s<sup>-1</sup>) when compared with the clinically used T<sub>1</sub> Gd agent (r<sub>1</sub>=3 mM<sup>-1</sup> s<sup>-1</sup>), and showed good cell staining ability. Further information concerning in vivo distribution indicated that PEGylation of the surface could enhance the circulation time and activity of the NPs in blood vessels.

Although Gd-based contrast agents have a significant impact on the longitudinal relaxivity and have attracted substantial interest, it still suffers from some major drawbacks. The Gd<sup>3+</sup> complexes have insufficiently high thermodynamic stability to prevent the release and final accumulation of the Gd<sup>3+</sup> at cellular level (25). For this reason, novel contrast agents for MRI have been developed and compounds containing gadolinium NPs may be good candidates.

Atabaev et al. (26) synthesized silica-coated Gd<sub>2</sub>O<sub>3</sub> nanoparticles co-doped with Eu<sup>3+</sup> and Tb<sup>3+</sup> as bimodal imaging probe for multicolor fluorescence and MR imaging. L-929 cells viability was not affected by the probe, and blue, green and red fluorescence emission was simultaneously observed when incubated with the NPs for 4 h at a concentration of 50 ppm. In addition, the T<sub>1</sub>-weight images became brighter with the increase of NPs concentration and the r<sub>1</sub> value of
4.73±0.11 on a 1.5 T MR scanner was slightly higher than that of other commercially available Gd-chelates.

Gadolinium carbonate particle is another option for MR imaging. Hu et al. (27) prepared core-shelled nanoparticles of gadolinium carbonate-encapsulated silica spheres embedded with FITC via a two-step wet chemistry method. The fluorescence intensity decreased as the shell thickness increased, but the longitudinal relaxivity \( r_1 \) remained high, which was more than 5.8-times higher than that of Gd-DTPA-BMA on a 0.55 T MR scanner. After incubating with the NPs for 1 hour, the HeLa cells could show clear fluorescence when the NPs were small, suggesting only the NPs with small sizes could enter the cells. After 72 hours’ incubation at a concentration of 50 mg mL\(^{-1}\), the cell viability was still more than 90%, demonstrating low toxicity of the NPs.

As described above, both the Gd\(^{3+}\) complexes and Gd-containing NPs can be used as powerful \( T_1 \)-weighted imaging agents for MR imaging and exhibited better MR imaging ability than clinically used \( T_1 \) MR contrast agent. On the other hand, superparamagnetic iron oxide nanoparticles (SPIONs) have been investigated for \( T_2 \)-weighted MR imaging with outstanding features of biocompatibility, biodegradability and ease of surface modification (28).

Wang et al. (29) prepared SPIONs coated with dense-silica and labeled with NIR fluorescence dye 800ZW for MR and NIR fluorescence imaging. Besides, the probes were further labeled with anti-CD146 monoclonal antibody YY146 to specifically target gastric cancer cells.
Fluorescence imaging results indicated that the probes had effectively specific affinity to CD146 in vitro and in vivo. Owing to the strong affinity, the probes could accumulate rapidly in the tumor after being intravenously injected into MKN45 xenograft bearing-mice, and strong fluorescence could be clearly observed at 4 h post injection and peaked at around 24 h post injection. T$_2$-weighted MR imaging showed a significant signal drop in contrast between the time points of 24 h and 48 h and the best MR imaging effect at the time point of 24 h was consistent with the fluorescence imaging.

A new MRI strategy combining T$_1$-positive with T$_2$-negative agents has been proposed to improve the diagnosis accuracy. Shen et al. (30) synthesized silica coated SPIONs/fluorescent CuInS$_2$ (CIS) quantum dots NPs, which enabled further conjugation of a Gd$^{3+}$–DTPA (diethylene-triaminepentaacetic acid) complex and arginine-glycine-aspartic acid (RGD) peptides onto their surfaces after being aminated through silanization to obtain Fe$_3$O$_4$/CIS@SiO$_2$ (Gd–DTPA)–RGD NPs (Fig. 2). Although the values of r$_1$ and r$_2$ of the probes both displayed a certain degree of reduction, it was still strong enough to obtain contrast enhancement in the MR images. The BXPC-3 cells tagged with the NPs emitted clear red emission under a 488 nm excitation after incubation with the NPs for 3 h. The T$_1$ and T$_2$ MR images of the tumor showed significantly brightened and darkened enhancement effect when the NPs were injected into pancreatic adenocarcinoma-bearing mice for 6 h and the signal were further enhanced after 12 h of incubation.
2.1.3. CT, MR and fluorescence trimodal imaging

In order to obtain more accurate information of relevant tissues, more bioimaging modes with complementary functions were integrated and thus a multimodal imaging probe was fabricated, which permits simultaneous attainment of different imaging signal from CT, MR and fluorescence. The currently reported CT/MR/FL trimodal imaging NPs based on silica mainly depended on the special features of UCNPs.

Xia et al. (31) constructed multifunctional nanoparticles with core-shell structure for NIR-to-NIR UCL, CT and T₁-weight MR trimodal imaging. NaLuF₄:Yb³⁺, Tm³⁺ (UCNPs) was the core for UCL and CT imaging, while SiO₂ was the shell layer, and the conjugated DTPA-Gd on the surface was used as MR contrast agent. The above NPs could emit bright fluorescence at 800 nm at the excitation of 980 nm and a strong NIR UCL was observed in the abdomen after intravenous injection of the NPs in a nude mouse for 10 min. As for the MR imaging, the r₁ value of the nanoparticle (6.35 mM⁻¹ s⁻¹) was higher than that of the pure Gd-DTPA complex (4.08 mM⁻¹ s⁻¹) on a 0.5 T MR scanner. Moreover, after intravenous administration of the NPs in the Kunming mouse, the mean intensity of the T₁ MR signal from the liver and spleen increased compared with the original intensities. For the liver, the MR signal increased by 22% after 120 min of injection, while only increased a little for the spleen, indicating a rapid clearance of the NPs by the spleen.

In addition, the CT value of the NPs increased with their concentration and the brightness
was higher than that of Iopromide at the same concentration. During the in vivo CT imaging
process, the CT signal enhanced 6%, 30% and 23% for the liver, stomach and intestines,
respectively. Xing et al. (32) synthesized a sub-50 nm sized multifunctional nanoprobe for
trimodal imaging of tumors via a simple electrostatic adsorption interaction between Gd-rare
earth doped UCNPs and Au NPs. To eliminate the luminescence quenching of Au NPs on the
UCNP, a silica shell was grown around the UCNP, the thickness of which between UCNP and Au
NPs was optimized to be 10-15 nm for minimizing the fluorescence quenching and enhancing
the SPR effect. The cell viability was more than 91.7% after co-incubation with 800 µg/mL NPs
for 6 h, indicating good biocompatibility of the nanoparticles. The confocal luminescence
imaging of MCF-7 cells showed that the NPs could cross the cell membrane and enter the
cytoplasm. The fluorescence emitted from the NPs became stronger with higher concentrations
of NPs and prolonged incubation time. Further in vivo imaging also demonstrated excellent
tri-modal imaging ability of the NPs, where the tumor region emitted strong visible light with
decreased autofluorescence interference under 980 nm laser excitation, the T1-weighted MR
signal sharply increased by 66.6%, and the CT value of the tumor site increased from 40.86 to
102.34 after the administration of the NPs in a tumor-bearing mouse.

Lanthanide doped NaGdF4:Yb3+, Er3+@NaGdF4 UCNP embedded in methylphosphonate
functionalized silica (pSi) nanospheres was transferred into aqueous solution by Liu et al. (33)
through surface coating with Pluronic F127, and the as-prepared single NPs
(pSi@UCNPs@F127) were then used as a probe for trimodal imaging. The MTT assays indicated that the cell viability was more than 90% after incubation with as high as 60 pM NPs for 24 h. After subcutaneous injection of an equivalent amount of pSi@UCNPs@F127 and UCNP@F127 NPs into the thighs of a nude mouse respectively, much brighter UCL was observed from the thigh injected with the pSi@UCNPs@F127 NPs. Furthermore, both the CT and the MR signals increased linearly with the concentration of the NPs. The enhancement of CT signal could be clearly observed in the liver and spleen at a very short time (10 min) after the probes were intravenously administrated in nude mice. Meanwhile, a detectible T₁-weighted MR contrast enhancement was observed in the liver area and the MR signal increased from 7.2% after 20 min injection to 11.8% in 60 min post-injection.

2.1.4. PET-based multimodal imaging

Positron emission tomography (PET) is a powerful diagnostic imaging technique based on the preparation of specific molecular imaging probes labeled with positron-emitting radioisotopes, and showed high sensitivity and specificity (34). As a single bioimaging mode, PET suffers from low spatial resolution for in vivo imaging. So, it is necessary to combine PET with other bioimaging modes to improve its diagnosis efficiency, among which fluorescence imaging mode can be used to realize high resolution and spatial visualization. Monodispersed and size-controllable silica nano-conjugates (NCs) with 20 nm (NC20) or 200 nm (NC200) particle size containing ⁶⁴Cu (for PET imaging) and RITC (for NIR fluorescence imaging) were
synthesized by Tang et al. (35) for lymph nodes (LNs) bimodal imaging. Both NC20 and NC200 emitted strong fluorescence at the wavelength of 802.5 nm and 808.0 nm, respectively.

However, in comparison with NC200, NC20 were prone to stay in the LNs for a longer time and emit stronger fluorescence after being injected into normal mice (Fig. 3). NC20 with superior performance were then functionalized with nucleolin-Apts (NC20-Apt) to realize the targeting imaging of metastatic sentinel LNs. The results showed that a much stronger PET signal was attained on the side injected with NC20-Apt, presumably caused by its higher accumulation (2.3 times higher) than that of NC20-Ctrl (only NC20, without conjugation with aptamer) after their subcutaneous injection into a mouse. The above silica NCs functionalized with aptamers were firstly developed as a FL/PET bimodal probe for active targeting and bioimaging of lymphatic metastases.

Ultrasmall multifunctional NPs based on silica particles synthesized by Philips et al. (36) were firstly used in human clinical trial as a molecular targeting probe for PET and fluorescence imaging of cancer, in which silica particles were labeled by $^{124}$I and encapsulated with cRGDY peptides modified Cy5 fluorescent dye. The above NPs exhibited no significant toxicity when used in a small group of 5 patients for metastatic melanoma diagnosis, and could be excreted completely via kidneys and bladders. Moreover, the NPs were found in the tumor region of some patients through simultaneous fluorescence and PET imaging, indicating its efficiency as a bimodal PET/FL bioimaging probe. However, many more patients are still needed to further
examine the safety and effectiveness of the NPs.

MRI could also be combined with PET to impart exceptional spatial and anatomical resolution. Iron oxide nanorods (NRs) as an efficient MR contrast agent were coated by gallium-68 radio-labeled silica to fabricate a MR/PET bimodal imaging probe with various DO3A (macrocyclic ligand):PEG as passive targeting moiety by Burke et al. (37). To optimize the DO3A to PEG ratio, the influence of the DO3A on the probes was examined, it turned out that different amounts of DO3A in the coatings (0% DO3A, 50% DO3A, and 100% DO3A) brought no significant changes to the radioisotope labeling and serum stability. Therefore, the ratio of DO3A to PEG was chosen as 0:100 to be linked with the probes as passive targeting moiety for the future PET and MR imaging.

Apart from the above FL/PET and MRI/PET bimodal imaging probes, combinations of FL, MRI and PET to construct novel tri-modal nanoprobes for in vivo diagnosis have also been reported. Cobalt ferrite magnetic nanoparticles as MR contrast agent were coated by NIR797 dye-doped silica matrix with $^{68}$Ga labeled on its surface by Kim et al. (38) to obtain a trimodal probe for PET, MR and fluorescence imaging. The as-prepared probe showed satisfactory performance as a $T_2$ contrast agent with a transverse relaxivity coefficient of 297 mM/s on a 1.5 T MR scanner. As for the trimodal bioimaging, the axillary LN showed clear NIR fluorescence and PET signals after subcutaneous administration of the NPs in the left forepaws of the mice for 5 min. Moreover, when the probes with two-fold concentration as that for FL and PET imaging
were used for MR imaging, strong T2-weighted gradient echo signals emitted from axillary LN after administration of the NPs for 30 min, and the locations of LN were consistent with those on PET images.

A similar structure with cancer-targeting ability was further developed as a new tri-modal bioimaging NPs by Kang et al. (39). Compared with the above tri-modal probe, the MR contrast agent and PET label were the same while NIR797 was replaced by rhodamine dye in this structure. In addition, an aptamer targeting uMUC-1 was linked to endow it tumor-targeting property, and this new NPs was designated as MFR-uMUC-1. The in vivo bioimaging results indicated a good tri-modal imaging ability of the new NPs with high specificity to the tumors overexpressed on uMUC-1. After intravenous injection for 2 h, an increased $^{68}$Ga radioactivity from the right thigh was observed compared with the control (same NPs without tumor-targeting moiety). Meanwhile, brightened fluorescence image and darkened T2-weighted MR image were also displayed in the right thigh after the MFR-uMUC-1 was injected for 12 h and 24 h, respectively.

2.2. Applications of MSNs-based nanoparticles in multi-modal imaging

Compared with SiNPs, MSNs exhibited some different properties including high surface areas, tunable pore sizes, rigid frameworks and large pore volumes. The conventional synthetic way of MSNs is just a simple adjustment to the classical Stöber method via a surfactant-templated sol-gel approach (40). The large pores of the MSNs allow them to be loaded with small
molecules, which made them suitable for multimodal imaging and the establishment of drug delivery systems. Similar to SiNPs, the MSNs could also be functionalized by different bioimaging moieties to combine different kinds of nano-particles for multi-modal imaging.

2.2.1. CT-based multimodal imaging

Unlike SiNPs, to date, relatively few studies on the application of MSNs for CT-fluorescence bimodal imaging have been reported. A PVP-modified probe based on MSNs was synthesized by Song et al. (41), in which Au nanospheres as CT contrast agent were coated with positively charged MSNs by TTA (N-trimethoxysilyl-propyl-N,N,N-trimethylammonium chloride). Besides, negatively charged NIR fluorescence dye IR-783 as FL imaging agent was then absorbed onto the silica coating via electrostatic interactions to finally obtain a NPs (Au@mSiO$_2$-TTA/IR-783/PVP) for CT/FL bimodal imaging. The N$_2$ adsorption–desorption isotherm curve demonstrated that the Au@mSiO$_2$-TTA NPs had high surface area (530 m$^2$/g), large pore volume (0.8 cm$^3$/g), and uniform pore size (2.2 nm), indicating a great potential for effective loading of dyes. After the Au@mSiO$_2$-TTA/IR-783/PVP NPs were injected into nude mice through tail vein, obvious fluorescence and significantly enhanced CT signal was emitted from both liver and spleen. However, the fluorescence from the liver was brighter than that from the spleen, while the CT signal exhibited an opposite effect. No obvious morphological changes in main organs and tissues were observed after the injection of probes into mice for 6 days, which demonstrated a good biocompatibility of the probes.
To the best of our knowledge, clinically used iodinated compounds seldom serve as CT contrast agent for the fabrication of multimodal imaging probes. Xue et al. (42) reported for the first time a novel trimodal imaging probe for the simultaneous CT, MR and fluorescence imaging, in which the Cy5 dye-doped mesoporous silica loaded with iodinated oil served as the core and the superparamagnetic iron oxide as coating. During the in vitro studies, the fluorescence of Cy5 dye got brighter, while the CT and T2-weighted MR images became darker with the increase of probes concentration. After intravenous injection of the probes in nude mice, the fluorescence and T2-weighted MR signal as well as CT contrast enhancement of the liver could be observed at 30 min and the signals became much stronger at 1 h post injection. On the contrary, the fluorescence of the probes was strong only in the initial 30 min and disappeared at 1 h post injection, indicating a more rapid clearance of the probes by the lung than by the liver.

In order to obtain imaging-guided therapy of cancer, Yang et al. (43) synthesized NPs composed of Cy5.5-labeled MSNs with WS2 nanosheets coating as the core and self-assembled iron oxide on the surface as the shell. In this structure, the core exhibited strong NIR emission and X-ray absorption, which could serve as photothermal and CT contrast agent. The MTT assay showed no obvious toxicity of the probe to different types of cells. Significantly enhanced fluorescence, MR and CT signals could be observed after intravenous injection of the probes in tumor-bearing mice. Furthermore, doxorubicin (DOX)-loaded probes were injected into the mice and the tumor growth was almost completely inhibited under a laser irradiation.
To the same end, Yang et al. (44) conjugated CuS NPs with MSNs coated core-shell-shell structured NaGdF₄: Yb, Er@NaGdF₄: Yb@NaNdF₄: Yb for simultaneous multi-modal imaging and therapy. MTT assay showed that the cell viability still remained as high as 90% even when the concentration of incubated probes was as high as 500 µg mL⁻¹, suggesting a fabulous biocompatibility of the probe. The in vitro results showed that after incubation with the probes, bright green luminescence was emitted from the HeLa cells under the excitation of 808 nm laser and the T₁-weighted MR signals also positively increased (the r₁ value increased to 2.239) with the probes concentration. After intra-tumor injection of the NPs in the tumor-bearing mice, the CT value in the tumor increased from 43.8 to 959.5. Moreover, when the probes were loaded with DOX and injected into the tumor-bearing mice, the tumor growth inhibition efficacy reached the best without losing the mice weight under an 808 NIR irradiation as the probe circulation time lasted.

2.2.2. MR- fluorescence bimodal imaging

Compared with other multimodal imaging modes, combining MR with fluorescence to develop MR/FL bimodal imaging technique has become one of the most attractive and widely used strategies in recent years. As a result, more studies on the applications of both SiNPs and MSNs as carrier for MR/FL bimodal imaging were reported.

A simple example was illustrated by Zhu et al. (45) that a MR/FL bimodal imaging probe was fabricated through conjugating NIR fluorescent dye IR-808 as fluorescence contrast agent
and Gd-DTPA as MR contrast agent with highly aminated mesoporous silica NPs via a one-step method. The in vitro experiments showed that the probes exhibited good NIR fluorescence imaging property with an emission at 794 nm and CT enhancement effect with a high $r_1$ value of 14.54 mM$^{-1}$ s$^{-1}$ (3-fold as high as that of Gd-DTPA at the same concentration). On the other hand, the in vivo results also demonstrated the favorable bimodal imaging ability of the probe. After injection of the probes into tumor-bearing mice for 4 h, the anatomic information could be provided by the enhanced MR images and the NIR fluorescence from the tumor region could still be observed even after 48 h injection probably due to the high sensitivity and the spreading of the NPs to the tumor surface.

Ru(bpy)$^{3+}$ as fluorescence imaging agent was loaded on the Gd$^{3+}$ and Al$^{3+}$ co-doped MSNs to build a MR/FL bimodal imaging probe by Zhang et al. (46). Co-doping with Al$^{3+}$ was used to increase Gd loading on the surface of MSNs to enhance the MR contrast efficiency. The probes exhibited a higher $r_1$ value of 19.2 mM$^{-1}$ s$^{-1}$ than that of Gd-DTPA ($r_1$=4.19 mM$^{-1}$ s$^{-1}$) and emitted a bright orange-red fluorescence at the wavelength of 621 nm under 475 nm excitation. Moreover, the fluorescence intensity of the probes was also much higher than that of other SiNPs-based probes at the same concentration. Strong fluorescence was observed from the abdomen after the probes were injected into the mice for 30 min which mainly concentrated in the liver and the spleen. Then, the probes were excreted through renal routes. The enhanced MR signal in the liver was also observed after intravenous administration of the probes in Kunming
mice. Besides, the same research group further verified the bimodal imaging efficiency of the NPs with similar structure (Gd-Al@MSNs-Cy5), and the results were reasonable (47).

To obtain the bimodal imaging probes with targeting capability, Hu et al. (48) synthesized RGD-labeled multifunctional nanoprobes to target integrin \( \alpha_\text{v} \beta_3 \) receptors based on MSNs containing FITC and Gd-DOTAGA, which exhibited strong green fluorescence under excitation of 365 nm. The in vitro cell experiments indicated a favorable stability and biocompatibility of the probe and both fluorescence and MR cell imaging confirmed the superior integrin \( \alpha_\text{v} \beta_3 \)-specific binding capability of the probe. After the tumor-bearing mice with U87MG tumor on the left shoulder (\( \alpha_\text{v} \beta_3 \) overexpressed tumor) and the A431 tumor on the right shoulder (as control) were injected with the probes via tail vein for 1 h and 6 h, the probes were observed to rapidly accumulate in the tumors together with a 2.3-fold signal-to-noise enhancement of the \( T_1 \)-MR image as that in A431 tumors. After 24 h, the signal enhancement decreased possibly due to the biodegradation and clearance of the probes.

Fan et al. (49) designed a sub-50 nm nuclear-targeting rattle-structured upconversion core/mesoporous silica multifunctional nanoprobe (RUMSNs-TAT-MMC), in which TAT peptides were used as nuclear localization signal ligands and Mitomycin C (MMC) as radio-sensitizing drug to achieve synergetically enhanced chemo-/radiotherapy and simultaneous MR and fluorescence bimodal imaging. The cell viability showed a negligible change after incubation with RUMSNs-TAT at a high concentration up to 1 mg mL\(^{-1}\) for 24 h and the
RUMSNs-TAT could enter the cell nuclei and emitted yellow luminescence under a NIR excitation. The MR intensity of the tumor was enhanced with the increase of incubation time. After the probes of the same concentration were injected for the same time period, the MR signals of the NPs increased by about 31% which is higher than that of non-targeting NPs (13%). The tumor growth could be effectively inhibited and a remarkable regression (about 60%) was achieved through the combination of radiotherapy with chemotherapy for half a month.

On the other hand, Liu et al. (50) constructed a hybrid nanostructure to obtain T2-weighted MR and fluorescence bimodal imaging. NaYF4:Yb, Er UCNPs was coated by mesoporous silica which were then modified with superparamagnetic iron oxide NPs to get NaYF4: Yb, Er@mSiO2@Fe3O4-PEG nanoparticles (MFNPs). In order to minimize the quenching effect of Fe3O4 NPs on the UCNPs, the amount of Fe3O4 NPs in the MFNPs was optimized to attain the best fluorescence and MR signals. MTT assays showed that the MFNPs with concentration as high as 500 µg mL⁻¹ did not exert obvious influence on the viability of HeLa cells after incubation for 24 h. The UCL signal was observed to be weak after the MFNPs were incubated with HeLa cells for 30 min and gradually became stronger with increasing incubation time. Furthermore, as the MFNPs concentration increased, the T2-weighted MR images of MFNPs darkened with an r2 value of 28.7 mM⁻¹ s⁻¹. DOX was further encapsulated in MFNPs as MFNPs-DOX NPs for chemotherapy. After the MFNPs-DOX NPs was intravenously injected into the tumor-bearing mice, the tumor exhibited the lowest growth rate compared with other
treatment, which indicated that the MFNPs-DOX should be an efficient agent for in vivo anti-tumor diagnosis and therapy.

2.2.3. PET- fluorescence bimodal imaging

For the MSNs-based PET and fluorescence bimodal imaging NPs, much attention was paid to improving the loading capacities of the PET labels and fluorescence imaging agents via the special pore structure of the mesoporous silica NPs to achieve an outstanding bimodal imaging performance.

Chen et al. (51) reported the successful conjugation of a PET tracer $^{64}$Cu, a NIR fluorescence dye 800CW and a vascular targeting moiety TRC105 to the surface of MSNs, and the resultant multifunctional probe ($^{64}$Cu-MSN-800CW-TRC105) was used for in vivo tumor vasculature imaging. To investigate the tumor targeting ability, the above as-synthesized probes and $^{64}$Cu-MSN-800CW (without targeting group) were intravenously injected into 4T1 murine breast tumor-bearing mice at the same time. The accumulation of the probes in the 4T1 tumor was readily observed in PET imaging with $5.4 \pm 0.2\%$ ID/g at 4 h post injection, which was 2 times higher than that without targeting group. Similarly, significantly stronger fluorescence signal in the tumors was also observed at the corresponding time of PET imaging compared with that without targeting group.

The same research group further investigated the advantages of hollow MSN (HMSN) as nanocarrier with higher loading capacity compared with MSN (52). They reported a similar
structure NPs $^{64}$Cu-HMSN-ZW800-TRC105 for PET and fluorescence targeting tumor imaging and drug delivery. Under the same conditions, the accumulation of these NPs in tumor was up to 9.9 ±0.9 ID/g at 4 h post injection, which was almost 2-fold of those MSN-based probes. Moreover, due to the presence of hollow cavity in HMSN, the DOX loading capacity of HMSN was also significantly improved up to 1129.2 mg/g, which is much higher than that of MSN (71.6-481.6 mg/g).

**2.2.4. US-based multimodal imaging**

Among the current imaging techniques, ultrasound (US) as a real time imaging modality is noninvasive, inexpensive, fast and widely used clinically, but it still suffers from poor tissue discrimination ability (53). Thus, the combination of US with MR is an excellent choice to improve the accuracy and precision of diagnosis.

Liu et al. (54) designed a multifunctional nanoprobe comprising several kinds of materials including mSiO$_2$ as the carrier, Mn as the MR contrast element, hydrophobic perfluorooctyl bromide (PFOB) as US contrast agent and HA as the targeted matrix for lymph-targeting US and MR bimodal imaging. The in vitro MR imaging of the probes showed that the $T_1$-weighted MR signal was significantly enhanced with the increase of probe concentration and reached an $r_1$ value of 13.6 mM$^{-1}$ s$^{-1}$. In addition, the probes also exhibited a stronger US signal in comparison with the H$_2$O medium. After the rabbits were injected with the probes for 15 min, the US imaging of LNs could be observed clearly and the brightest image appeared at 35 min post
injection. The MR enhanced images of the LNs remained clearly visible, which was consistent with the result of US imaging.

An “all in one” trimodal probe (Au@HMSN/Au&MnO) was reported by Zhang et al. (55), which considerably maximized the probe’s abilities for US, MR and CT imaging. It was worth noting that silica was used not only as a matrix, but also as a US contrast agent unit. Such a “core satellite” structure was useful for intensifying the US imaging signals since the MnO nanoparticle in shell could help HMSNs to enhance the efficiency of US imaging. The uniformly distributed MnO nanoparticles enabled the NPs to harvest much better T1 MR imaging performance with an r1 value of 1.5 mM⁻¹ s⁻¹ compared with conventionally used MnO nanoparticles (r1=0.61 mM⁻¹ s⁻¹). CT images indicated that the NPs acquired brighter contrast and larger HU value than Au@HMSN at the same concentration. PEI (nonviral gene vectors) was modified onto the surface of the NPs by electrostatic adsorption to improve the imaging performance. The accumulation of the probes (6.39%) in VX2 tumor was more than 2 times higher than that of NPs without PEI (2.52%) in vivo experiments. Furthermore, it could be observed that the MR, CT and US signals of the VX2 tumor were evidently higher than that of the normal liver after the administration of the targeting probes in rabbit (Fig. 4).

3. Conclusions

In conclusion, a large amount of examples confirmed that the silica-based nanoparticles are excellent platforms for the applications of multimodal bioimaging and theranostic. SiNPs and
MSNs possess different functions in the process of integrating several imaging moieties into a single particle and MSNs are more likely to be used in drug delivery to achieve therapeutic effects. These materials offer a lot of possibilities for the new development of theranostic nanomedicine. However, it is worthwhile to note that the above probes based on silica and MSNs are still in the preliminary stage of multi-modal bioimaging. The real clinical applications of these nanoprobes for muti-modal bioimaging would put forward some more requirements such as better biocompatibility, negligible toxicity, long circulation time, reasonable particle size and so on. Besides, the bioimaging reproducibility of these nanoprobes and the binding affinity between different imaging moieties still need to be further investigated and optimized. Therefore, much more efforts should be devoted to addressing these fundamental challenges to prompt these silica-based platforms to become real clinical imaging and theranostic agents.

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Fig. 1. In vivo fluorescence images of a mouse injected with Au@SiO$_2$ nanoparticles intradermally into the left paw: A) pre-injection, B) 1 min post-injection, C) 30 min post-injection, D) 1 h post-injection, E) 4 h post-injection, and F) 18 h post-injection. G) Fluorescence image of a dissected mouse 18 h after injection: (inset) isolated LNs; scale bar: 1 mm. H) Fluorescence image of LVs (taken from reference (15)).
Fig. 2. Schematic illustration of the formation of Fe$_3$O$_4$/CIS@SiO$_2$ (Gd–DTPA)–RGD NPs (taken from reference (30)).
Fig. 3 a) Dual-labeled NC20 (left) and NC200 (right) were administered to normal C57BL/6 mice by hock injection; the same amount of radioactivity was injected on each side. b) In vivo whole-body dynamic PET/CT imaging of mice was performed to assess the accumulation of the silica NCs in the P-LNs (yellow arrows) (taken from reference (35)).
Fig. 4. In vivo evaluations of US, CT, and MR trimodal imaging for New Zealand white rabbit bearing VX2 tumor using Au@HMSN/Au&MnO-PEI as CAs. (a) Ultrasonic images using Au@HMSN/Au&MnO-PEI as UCAs under B fundamental imaging (BFI). (b) T₁-weight MR contrast images and yellow arrow indicating VX2 tumor (dose: 3 mg Mn/Kg). (c) CT contrast images and yellow arrow and zone circled by yellow dotted ellipse indicating VX2 tumor (dose: 19.2 mg Au/Kg) (taken from reference (55)).
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