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Recent Development of Silica Nanoparticles as Delivery Vectors for Cancer Imaging and Therapy

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Abstract

In spite of significant advances in early detection and combined treatments, a number of cancers are often diagnosed at advanced stages and thereby carry a poor prognosis. Developing novel prognostic biomarkers and targeted therapies may offer alternatives for cancer diagnosis and treatment. Recent rapid development of nanomaterials, such as silica based nanoparticles (SiNPs), can just render such a promise. In this article, we attempt to summarize the recent progress of SiNPs in tumor research as a novel delivery vector. SiNP-assisted imaging techniques are used in cancer diagnosis both *in vitro* and *in vivo*. Meanwhile, SiNP-mediated drug delivery can efficiently treat tumor by carrying chemotherapeutic agents, photosensitizers, photothermal agents, siRNA, and gene therapeutic agents. Finally, SiNPs that contain at least two different functional agents may be more powerful for both tumor imaging and therapy.

Keywords

silica nanoparticles; tumor diagnosis; tumor therapy; multifunctional

1. Introduction

In the last two decades, applications of nanotechnology in cancer diagnosis and therapy have attracted significant attention.^{1–4} A number of functional nanomaterials have been developed and evaluated for drug delivery,^{5, 6} diagnostic sensors,⁷ imaging agents,⁸ and labeling probes.⁹ Nanomaterials can be organic or inorganic,^{10, 11} with a size from 1 nanometer to a few hundred nanometers,^{12, 13} and surface charges from negative to positive or natural.¹⁴ Gold nanoparticles,¹⁵ quantum dots,¹⁶ carbon nanotubes,¹⁷ magnetic nanoparticles,¹⁸ liposomes,¹⁹ and silica nanoparticles (SiNPs)²⁰ have been developed for imaging as well as drug and gene delivery. Furthermore, multifunctional nanomaterials with two or more different functions have been designed for cancer imaging and therapy both *in vitro* and *in vivo*.^{21–23}

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Among these nanomaterials, SiNPs have gained special attention for tumor imaging and therapy due to their easy synthesis, uniform morphology, adjustable pore volume, controllable diameter, modifiable surface potential, easy functionalization, and significant biocompatibility.^{24–27} There are two main types of SiNPs developed for tumor imaging and therapy: mesoporous silica nanoparticles (MSNs)²⁷ and core/shell silica nanoparticle (C/S-SiNPs).²⁶ MSNs were successfully used for drug delivery and gene delivery because of their unique mesopores and nanochannels, which can render a high payload of the drug²⁸ and easy stimuli-controllable release.²⁹ C/S-SiNPs are useful for imaging agent delivery because imaging agents, such as fluorophores, quantum dots (QDs), and gold nanoparticles, can be easily doped into the nanoparticles. The shell structures can protect imaging agents inside the nanoparticles, and the signal from imaging agents gives the precise location of nanoparticles and tumor tissue.³⁰ SiNPs can accumulate in tumor tissues through passive targeting and active targeting.^{31, 32} More importantly, both MSNs and C/S-SiNPs have shown a significant biocompatibility both *in vitro* and *in vivo*,^{33, 34} a great property for tumor diagnosis and therapy in humans.

In this article, we will briefly discuss the synthetic methods and important characteristics of relatively well-studied SiNPs. Due to the importance of early cancer diagnosis, the applications of SiNPs in *in vitro* and *in vivo* imaging will be discussed as well. By varying imaging agents in SiNPs, the end products can be used in different imaging models, such as magnetic resonance imaging (MRI), fluorescence imaging, positron emission tomography (PET), and X-ray computed tomography (X-ray CT) imaging. Then, the applications of SiNPs in drug and gene delivery for tumor therapy will be summarized. Photosensitizers, photothermal agents and chemotherapeutic agents can be successfully delivered to tumor cells and tissues by SiNPs. The loading and releasing mechanisms of drugs in MSNs and the applications of multifunctional nanomaterials bearing imaging and therapeutic features will be discussed. The different types of SiNPs for tumor imaging and therapy *in vitro* and *in vivo* coupled with their characteristics are summarized in Table 1.

2. Characteristics of silica nanoparticles

2.1 Particle size

As a delivery vector, the particle size and pore dimension of SiNPs are crucial parameters and affected drug loading efficiency, pathway of cell uptake, biodistribution *in vivo* and targeting efficiency in cancer diagnosis and therapy.^{35, 36} Through a reverse microemulsion method, monodispersed C/S-SiNPs can be easily constructed with a controllable size. For instance, Tan *et al.* investigated how the amount of ammonium hydroxide, surfactant, tetraethyl orthosilicate (TEOS), H₂O, and reaction time affect the size of SiNPs.³⁷ They found that the size of the dye-doped SiNPs was reverse-proportional to the concentration of ammonium hydroxide and water to surfactant molar ratio, but direct-proportional to the amount of TEOS and reaction time.³⁷ In order to continuously control the size of the C/S-SiNPs in a more defined range, Zhao *et al.* developed a method by systematically varying the organic solvents used in a reverse microemulsion method.³⁸ Organic solvent may affect the surface area occupied by surfactant molecules, while the diameter of water droplet can increase with the length of the organic molecule. Eventually, through hydrolysis of TEOS on the surface of the droplet, the size of nanoparticles may change by varying the organic molecule. C/S-SiNPs with a tunable size range from 10–100 nm was developed by simply varying the types of organic solvents.

Varying the amount of ammonium hydroxide was also useful for synthesizing MSNs with different sizes. Mou *et al.* synthesized MSNs with sizes from 30 nm to 280 nm using this strategy.³⁵ It was also found that the uptake of MSNs by cells was size-dependent and 50 nm was the preferable diameter for cell uptake. Pan *et al.* developed a tunable particle size

of MSNs from 25 nm to 105 nm, and size effect on nuclear targeting was investigated.³⁹ They found that only MSNs of 50 nm or smaller can effectively target nucleus with the help of a TAT peptide. Using the Stöber method, large nanoparticles from 60 nm to 880 nm were synthesized by the Rosenzweig group by changing the amount of ammonium hydroxide.⁴⁰ The toxicity of SiNPs was also associated with their diameters. Hoet *et al.* developed a method to obtain amorphous spherical SiNPs of different sizes ranged from 13.8 nm to 335.0 nm and investigated their cytotoxicity to human endothelial cells. It was found that the smaller the particles, the higher the toxicity to cells.⁴¹

2.2 Surface modification

The surface of SiNPs is usually negatively charged without further modification because of the presence of the hydroxyl group after hydrolysis of TEOS. However, it is convenient to modify the SiNPs' surface through the silane chemistry. Polyethylene glycol (PEG), amine, carboxyl, and phosphate groups could be easily conjugated to hydroxyl SiNPs by hydrolysis of the corresponding silanes. For example, He *et al.* synthesized three types of SiNPs with different surface charges, including OH-SiNPs, COOH-SiNPs, and PEG-SiNPs, and found that the biodistribution and excretion of the SiNPs were dependent on surface modifications. Neutrally-charged SiNPs (PEG-SiNPs) exhibited relatively longer blood circulation and lower uptake by the reticuloendothelial system (RES) organs than the other two.³³ As a result, these PEG-modified SiNPs showed better passive targeting effects to the tumor site when they were used for the delivery of drugs and imaging agents.^{42, 43}

Furthermore, as an effective delivery vector, active targeting is another strategy for better targeting efficiency. After surface modification with different functional groups, targeting molecules, including antibodies,⁴⁴ peptides,^{45, 46} folic acid,⁴⁷ and aptamers,⁴⁸ can be easily conjugated to the surface of SiNPs for active targeting tumor tissues.⁴⁹ For example, Lu *et al.* modified SiNPs with folic acids through EDC-NHS conjugation.³² Tan *et al.* reported modification of COOH-SiNPs with aptamers through EDC-NHS conjugation reaction and used the aptamer-modified dye-doped SiNPs for the detection of cancer cells.³¹ By using these strategies, the surface of SiNPs is easily modified with recognizing molecules to enhance the ability of the SiNPs to recognize cancer markers. Thus, the efficiency of SiNPs for cancer imaging and therapy is improved.

3. Imaging applications of silica nanoparticles

With tunable particle sizes, easy surface modification, and good biocompatibility, SiNPs have been widely used for imaging of cancer cells or tissues both *in vitro* and *in vivo*. Imaging agents can be doped into or modified on the surface of SiNPs. In the next section, we will review the latest progress in SiNPs-mediated tumor imaging with fluorescence and MRI techniques.

3.1 Fluorescence imaging

Fluorescence technology possesses high resolution compared to other imaging strategies, and the sensitivity of the fluorescence detection on cancer cells is promising.^{50, 51} Traditionally, fluorescent dyes suffer from the disadvantages of low brightness, photobleaching, and non-specific targeting.⁵² However, fluorescent SiNPs may overcome these shortcomings and provide highly sensitive and selective tumor imaging. By doping thousands of fluorescent dye molecules into one SiNP, the brightness of SiNP was much higher than a single dye molecule.⁵³ For example, Herr *et al.* compared the fluorescence intensity between dye and dye-doped SiNPs.³¹ Their results showed that an aptamer modified dye-doped SiNP was brighter and more stable in fluorescence imaging than a dye-aptamer alone. Flow cytometry demonstrated that the signal from the SiNPs-aptamer was

100 times higher than that of the dye-aptamer. Therefore, these dye-doped SiNPs with high fluorescence intensity may be useful for creating highly sensitive biosensors.⁵³ Another important advantage of dye-doped SiNPs compared to free dye molecules is that silica matrix can protect the dye from photobleaching, allowing long-duration imaging of tumor cells and tissues.⁵⁴ As shown in Figure 1, with continual irradiation under a confocal microscope, the fluorescence of cells stained by pure FITC quenched almost completely within 20 min.⁵⁵ However, 60% of fluorescence in the FITC-doped SiNPs retained for imaging after 20 min, and 30% of the fluorescence intensity retained even after 1 h irradiation. This indicates that fluorescent SiNPs may have better photostability than free dyes and thus were more powerful as fluorescent markers for long-time imaging of living cells and tissues.

Moreover, the facileness to modify SiNPs makes the modification of targeting ligands convenient and effective. Targeting ligands, such as aptamers,^{56, 57} antibodies,^{58, 59} peptides,⁶⁰ and folic acid,^{61, 62} can be attached on SiNPs. Aptamer is a short fragment of DNA or RNA which can selectively bind to certain targets, including ions, small biomolecules, peptides, and cells.⁶³ In 2011, Tan *et al.* developed an aptamer-conjugated nanoparticle for fluorescence imaging and extraction of tumor cells.⁶⁴ In this assay, silica-coated magnetic and fluorophore-doped SiNPs modified with aptamers were used to recognize and isolate cancer cells from mixed cell populations using an external magnetic field.

The excellent optical characteristics of the dye-doped SiNPs ensure their usage as fluorescence agents for both tumor cells *in vitro* and *in vivo* imaging. Furthermore, the high biocompatibility and controllable size of SiNPs make *in vivo* tumor imaging possible.^{65, 66} The feasibility of SiNPs for *in vivo* imaging has been confirmed by many research groups. For instance, He *et al.* developed methylene blue-encapsulated phosphonate-terminated silica nanoparticles (MB-encapsulated PSiNPs) for *in vivo* imaging.³⁰ They found that the MB-encapsulated PSiNPs could be used as an effective NIR fluorescence imaging agent in mice using three different injection routes.

For *in vivo* imaging, there are two different pathways to target tumors with nanoparticles. The first one is passive targeting by enhanced permeability and retention (EPR) effect. In this case, certain sizes of particles would accumulate at the tumor sites rather than normal tissues because the newly formed blood vessels of tumor tissues were abnormal.⁶⁷ The small nanoparticles, such as gold nanoparticles,⁶⁸ gold nanoclusters,⁶⁹ quantum dots,⁷⁰ upconventional nanoparticles,^{71, 72} have been used to prove this theory for tumor targeting *in vivo*. The second pathway is active targeting by modifying target ligands on the surface of nanoparticles. In 2010, Lu *et al.* investigated the biodistribution of fluorescent SiNPs in mice with xenograft tumors.⁶⁵ They found that both fluorescent silica nanoparticle (FMSNs) and folic acid modified fluorescent silica nanoparticles (F-FMSNs) could preferentially accumulate in tumor tissues after injecting to mice through the tail vein injection. The tissue section of different organs also confirmed the results from the *in vivo* fluorescence imaging. In these two different types of nanoparticles, FMSNs targeted tumor through EPR effect, while the F-FMSNs targeted tumor through the folate receptor on the surface of tumor cells. Meanwhile, Qian *et al.* reported PEG-modified NIR SiNPs for mouse tumor imaging due to its deep penetration ability and low autofluorescence in NIR region.⁶⁶ The NIR SiNPs accumulated in the tumor tissues through EPR effects, also these NIR SiNPs exhibited bright and stable NIR fluorescence that could be used for long-term *in vivo* imaging. It was noteworthy that SiNPs could be cleared from the mice through the hepatobiliary excretion, indicating their relatively low toxicity and perhaps high applicability in humans.

In order to enhance the performance of fluorescence imaging, other fluorescent nanomaterials have been coupled to SiNPs for cancer imaging. For instance, quantum dots, which possess brighter fluorescence, narrower photoluminescence spectra and better photostability than traditional dyes, were embedded into SiNPs for tumor fluorescence imaging. In 2012, Jun *et al.* reported an ultrasensitive QDs-embedded SiNP for tumor fluorescence imaging (Figure 2a).⁷³ Compared to a single QD, the QDs-embedded SiNPs showed brighter fluorescence intensity and increased uptake by HeLa cells (Figure 2b). For *in vivo* fluorescence cell tracking, single QD labeled- or QDs-embedded SiNPs labeled-HeLa cells were injected into mice for long-term fluorescence imaging, and the results showed that even after 10 days, the QDs-embedded SiNPs emitted strong fluorescence for *in vivo* imaging (Figure 2c). Therefore, the QDs-embedded SiNPs may have the potential for long-term fluorescence imaging *in vivo*.

3.2 Magnetic resonance imaging (MRI)

Compared to fluorescence imaging, MRI has better penetration ability in tissues. As a result, MRI is routinely used as a primary diagnosis method for cancer in hospitals. However, most of the currently-used MRI contrast agents are small molecules such as gadolinium chelates, which cannot provide a high contrast image for early diagnosis of cancer because of its low sensitivity and poor selectivity. By applying SiNPs, thousands of MRI agents like gadolinium chelates may be loaded into one nanoparticle. This high loading efficiency in SiNPs enhances the sensitivity of the MRI agent. Taylor *et al.* synthesized a highly efficient MSN-based MRI contrast agent for *in vitro* and *in vivo* MRI imaging.⁷⁴ This MSN-based MRI contrast agent was an ideal platform for MRI benefitting from its large payload of Gd centers and enhanced water accessibility of the Gd chelates. This is the key parameter for the design of highly efficient MRI contrast agents. Lin *et al.* modified the Gd chelates onto SiNPs using layer-by-layer self-assembly coupled with doping a fluorescence contrast agent during the synthesis.⁷⁵ With the modification of a targeting peptide, the K7RGD-SiNPs had the ability to detect certain tumor cells using MRI and fluorescence imaging. Also, Gd chelates could be coated onto SiNPs through lipid coating.⁷⁶ Coupled with quantum dots doping in the same SiNPs, the imaging nanocomposite had the abilities of fluorescence and MRI imaging for tumor cells.

In addition, super paramagnetic iron oxide (SPIO) nanoparticles are another type of useful MRI contrast agents for tumor imaging *in vitro* and *in vivo*. In 2008, Kim *et al.* doped single magnetic iron oxide nanoparticle in mesoporous silica nanoparticles ($\text{Fe}_3\text{O}_4@\text{mSiO}_2$), followed by doping the fluorescent dye in the silica shell to form multifunctional nanoparticles for tumor imaging and therapy (Figure 3A).⁷⁷ PEG modification on SiNPs increased the biocompatibility and targeting efficiency to tumor sites through EPR effects. After intravenous injection of the $\text{Fe}_3\text{O}_4@\text{mSiO}_2$ into the mice implanted with MCF-7 cells, researchers took the T2-weighted MRI imaging and observed that the T2 signal intensity in the tumor site decreased, indicating the accumulation of $\text{Fe}_3\text{O}_4@\text{mSiO}_2$ in the tumor site (Figure 3B). Meanwhile, the fluorescence of the $\text{Fe}_3\text{O}_4@\text{mSiO}_2$ in the tumor site was captured to verify the results from the T2-weighted MR imaging. Furthermore, the nanoparticles coupled with MRI agents and drugs were developed for simultaneous tumor imaging and therapy. For instance, Liong *et al.* enclosed multiple magnetites in the core of SiNPs, and then doped fluorescent dye or drug in SiNPs to form a multifunctional delivery vehicle for tumor imaging and therapy. With the modification of folic acids on the surface of SiNPs, these multifunctional SiNPs showed the potential for active targeting tumor sites for diagnosis and therapy.⁷⁸ Lee *et al.* developed a novel silica nanovector for tumor MRI, fluorescence imaging and drug delivery. The fluorescent dyes were doped into SiNPs, whereas multiple iron oxide nanoparticles were decorated on the surface of the SiNPs.⁷⁹ In

addition to tumor imaging, iron oxide nanoparticles embedded SiNPs were widely used for MRI imaging of stem cells *in vitro* and *in vivo* by varying the targeting ligands.^{80, 81}

4. Drug and gene delivery using silica nanoparticles

4.1 Drug delivery

SiNPs have been widely used for tumor therapy *in vitro* and *in vivo* because of their unique properties, such as large surface area and pore volume, easy functionalization, great biocompatibility, high stability, and controllable particle sizes. In the following section, we will discuss applications of SiNPs for drug delivery into tumor cells or tissues.

4.1.1 Chemotherapeutic agents—Chemotherapy is a mainstream treatment of cancer. However, there are some limitations of existing chemotherapeutics, such as the hydrophobicity, low targeting efficiency, side toxic effects, and unsatisfactory therapeutic efficacy. To overcome these limitations, biocompatible SiNPs have been employed as nano-vehicles for tumor targeting imaging and therapy. Camptothecin (CPT) and its derivatives are one of the most promising anticancer drugs; however, their hydrophobic property limits their clinical applications. In order to transfer CPT efficiently into tumor tissues without modification of the molecule, Lu *et al.* reported a method for loading CPT in MSNs (MSNs/CPT) and delivering it to cancer cells to induce apoptosis.⁸² Furthermore, they investigated anticancer effects of the MSNs/CPT *in vivo*.⁶⁵ Folic acid was modified onto MSNs for active targeting cancer cells (Figure 4A). As shown in Figure 4B, folic acid-modified MSNs/CPT resulted in a higher therapeutic efficiency to SK-BR-3 cells than that to MCF-7 cells because of the high level expression of folate receptor on SK-BR-3 cells. In contrast, the MSNs/CPT without folic acid modification demonstrated similar cytotoxicity to these two human cancer cells. This indicated that the folic acid-mediated active targeting to folate receptor positive tumor cells by MSNs showed efficacy in tumor treatment. Having achieved the exciting results from cancer cell treatment of MSNs/CPT, researchers investigated the *in vivo* tumor-suppressing effect of MSNs/CPT. As shown in Figure 4C and D, nude mice with xenografts of human breast cancer cell MCF-7 received different therapeutic agents. The MSNs/CPT and folic acid modified MSNs/CPT showed faster tumor-shrinking effects in mice than that of the CPT alone group, and no obvious subcutaneous tumors were found in these two testing groups at the end points. In contrast, saline solution and MSNs did not inhibit the tumor growth in nude mice. In the work of Tsai *et al.*, a non-cytotoxic anionic surfactant was coupled to the control releasing of a hydrophobic drug, resveratrol, loaded in the MSNs to kill HeLa cells.⁸³ The amount of resveratrol-loaded MSNs uptaken by tumor cells increased by almost 4-fold compared to calcined MSNs due to the targeting delivery of surfactant. In another work, TAT peptide-modified MSNs were used for the delivery of doxorubicin (DOX) to nuclei of cancer cells.³⁹ With the modification of a TAT peptide on the surface of these MSNs, it was found that 50 nm or smaller TAT-MSNs can efficiently target the nucleus of HeLa cells. DOX in TAT-MSNs was released into the nucleus to kill cancer cells with enhanced efficiencies compared to MSNs. In addition, other targeting ligands such as aptamer have also been used for drug delivery using SiNPs.⁸⁴

In order to control the release of drugs loaded in SiNPs, stimuli-responsive released systems based on SiNPs were developed. MSNs have size tunable pores which allow adjusting the loading of different drug molecules and triggering the drug release from MSNs. Therefore, we will focus on the applications of stimuli-responsive release MSNs. These MSNs-based stimuli-responsive systems were originally developed by Lin's group.⁸⁵ The principle of these systems is shown in Figure 5.²⁵ Drugs were loaded into MSNs through diffusion and chemical binding. Then, a gatekeeper, such as nanoparticles, organic molecules, and supermolecular assemblies, was used to regulate the encapsulation and release of drugs by

certain stimulus conditions in tumor cells and tissues. Through this controllable loading and releasing manners in MSNs, the treatment efficacy for cancer increased markedly.

Using a self-complementary duplex DNA as the gatekeeper to control the release of drugs in certain regions, Chen *et al.* developed a MSNs-based drug delivery system for cancer therapy.⁸⁶ The drugs were trapped within the porous channels of MSNs, and then the self-complementary duplex DNA was modified on the surface of MSNs to regulate the release of drugs. When the duplex DNA was denatured by heating or hydrolyzed by endonucleases in cancer cells, drugs in MSNs were released to kill cancer cells. Singh *et al.* coated MSNs with a shell of polymer, which was then triggered by the temperature and proteases presented in tumor cells.²⁹ Afterwards, DOX that was doped in the core and shell was released into tumor cells to induce apoptosis. pH can also be used as another triggering factor for drug release from MSNs for tumor therapy. Meng *et al.* developed β -cyclodextrin (β -CD)-coated MSNs for DOX delivery into cancer cells.⁸⁷ The β -CD-based nanovalves were responsive to the endosomal acidification conditions in cancer cell lines. Upon the triggering by the acid condition, the DOX in the MSNs could be released into nuclei of cancer cells. Furthermore, Gao *et al.* investigated the effect of pore sizes and pH for drug release rates in MSNs-based drug delivery systems.²⁸ With increasing pore sizes, drug release rates also increased. In addition, acid conditions could trigger the release of the drug into tumor cells.

Nanoparticles were another kind of gatekeeper for the controllable MSNs-based drug delivery system. Quantum dots,⁸⁵ gold nanoparticles,⁸⁸ and iron oxide nanoparticles⁷⁹ have been investigated for controllable drug release systems. Utilizing stimuli-responsive drug delivery systems based on MSNs, drugs for cancer therapy could be efficiently and specifically delivered to cancer cells and tissues *in vitro* and *in vivo*. Therefore, more effective therapy and fewer side effects of the chemotherapeutic drugs could be obtained by the stimuli-responsive MSNs.

4.1.2 Photodynamic therapy agents—Photodynamic therapy (PDT) of cancers is a relatively new treatment compared to chemotherapy. Photosensitizer, oxygen, and irradiation constitute necessary factors for PDT. Upon the irradiation of light, photosensitizers absorb photons and transfer the energy to surrounding oxygen to generate singlet oxygen that can irreversibly oxidize molecules, such as proteins and nucleic acids of tumor cells, resulting in cell killing.

Several photosensitizers have been developed for PDT. However, there are some limitations for clinical applications of these photosensitizers, such as hydrophobic property, low target efficiency to tumor sites, instability in biological environments, and side effects to normal cells. In order to overcome these limitations, SiNPs were used to deliver the photosensitizers to tumor cells with high targeting efficiency and low side effects. In 2003, Roy *et al.* entrapped a hydrophobic photosensitizer, 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), in SiNPs for tumor therapy.⁸⁹ With the irradiation of visible light, irreversible destruction of tumor cells was induced by singlet oxygen generated by the photodynamic process. To increase the penetration depth of light, Kim *et al.* used a two-photo absorbing fluorescent dye co-encapsulating with HPPH in SiNPs to form a photodynamic therapy system to effectively kill tumor cells.⁹⁰ In these two examples, the photosensitizers were encapsulated in the SiNPs through a physical process.

Drug leakage during systemic circulation is one of the disadvantages of these systems. Ohulchanskyy *et al.* covalently incorporated a photosensitizer to SiNPs and investigated singlet oxygen generation efficiency upon photo-irradiation.⁹¹ Tumor cells incubated with these SiNPs demonstrated low survival rates than those of the control group. Zhang *et al.*

described a versatile photosensitizer delivery system based on SiNPs for tumor therapy.⁹² In their design, an upconverting nanoparticle was doped in SiNPs, and the photosensitizers were encapsulated within the silica shell. The surface of the silica shell was modified with antibodies that were specific for antigens of tumor cells. Upon the NIR irradiation, the upconverting nanoparticles absorbed the NIR light and transferred the energy to photosensitizers in the shell to generate singlet oxygen, leading to oxidative damage to tumor cells nearby the nanoparticles. An *in vivo* PDT based on SiNPs was investigated by He *et al.* in 2009.³⁰ Methylene blue (MB), a NIR photosensitizer, was doped in phosphate-modified SiNPs. It showed that these MB-SiNPs not only caused the death of tumor cells *in vitro*, but also induced damage on mouse tumor tissues due to the NIR irradiation (Figure 6). Meanwhile, fluorescence *in vivo* imaging was obtained by the fluorescence emission of MB in the SiNPs. Also, Gary-Bobo *et al.* combined a chemotherapeutic agent and photosensitizer into SiNPs for both chemotherapy and PDT to further enhance tumor killing.⁹³ Zhu *et al.* encapsulated the photosensitizers in small MSNs with a high loading capacity.⁹⁴ The resulting delivery system increased the amount of photosensitizers in cancer cells by two orders of magnitude and the therapy efficiency was enhanced by more than four-folds.

4.2 Gene therapy using SiNPs-based vectors

The development of gene therapy technologies has generated great interest in cancer treatments about two decades ago. However, cancer gene therapy lacks specific delivery systems for carrying therapeutic genes to target tumor cells. Since He *et al.* reported that SiNPs could protect DNA from the cleavage by nucleases in 2003, these nanomaterials might have the potential for gene delivery to tumor cells.⁹⁵ Antisense oligonucleotide-loaded SiNPs were also developed for targeting HeLa cells by the same group.⁹⁶ It was found that these nanoparticles efficiently inhibited the proliferation and survival of HeLa cells. Xia *et al.* applied DNA or siRNA attached and polyethyleneimine (PEI) modified MSNs for gene therapy.¹¹ To improve this technology, a GFP-containing siRNA was used as a model for siRNA delivery to kill tumor cells. Flow cytometry (Figure 7A) and fluorescence confocal microscopy (Figure 7B) were used to evaluate the transfection efficiency of the siRNA-loaded MSNs. The results showed that the MSNs coated with 10 kD PEI had the best transfection efficiency in transducing the GFP-siRNA into tumor cells. The fluorescence intensity of GFP in these cells was the lowest compared to the control groups due to the death of targeted cells. Meanwhile, a GFP-expressing plasmid was also delivered by MSNs into tumor cells to test the feasibility of the SiNPs for DNA delivery.

Overexpression of drug efflux transporters is one of the issues for cancer therapy. For example, P-glycoprotein (Pgp) is one of the well-known multiple drug resistance proteins. For efficient treatment of tumor cells with chemotherapeutics, siRNA used to silence the expression of Pgp was a new approach for the drug resistant tumor cells. Meng *et al.* designed MSNs to deliver DOX and Pgp for tumor therapy.⁹⁷ This group found that the fluorescence intensity of DOX in tumor cells increased dramatically when the Pgp siRNA was co-delivered with DOX using MSNs, indicating that the Pgp expression was silenced by a Pgp siRNA on the MSNs. By staining with Annexin V-SYTOX, an apoptotic indicator, the tumor cells treated with the siRNA and DOX co-delivered MSNs showed significantly increased apoptosis compared to those treated with free DOX or DOX delivered by non-siRNA MSNs. Pgp is a pump resistant protein for multi-drug resistance, while for the nonpump resistance of multi-drug resistance, activation of cellular anti-apoptotic defense triggered by Bcl-2 protein is the main mechanism. To deliver a Bcl-2 siRNA into tumor cells, Chen *et al.* loaded Bcl-2 siRNA and DOX in MSNs to enhance chemotherapy.⁹⁸ DOX and Bcl-2 siRNA were loaded in the pore channel of MSNs (Figure 8). When the particles were taken up by tumor cells, the gatekeeper, G2 PAMAM, was opened by the acid pH in

the endosome. Subsequently DOX was released into cells to induce apoptosis, and Bcl-2 siRNA was released to silence Bcl-2 protein to increase apoptosis. Owing to the Bcl-2 siRNA directed delivery, the chemotherapeutic efficacy of DOX was enhanced in tumor cells.

5. Multifunctional silica nanoparticles

To increase the therapy efficiency, not only is the therapy process important, but also diagnosis, especially at early stages, is crucial for patient survival. Theranostics has emerged as an inter-disciplinary field by integrating imaging and therapy. Therefore, the development of novel multifunctional platforms for both imaging and therapy is one of the challenges in cancer treatments.⁹⁹ The success of SiNPs in delivery of imaging agents and drugs attests the potential of SiNPs for theranostics of tumor using multifunctional nanomaterial entities.¹⁰⁰ The capability of these multifunctional nanomaterials in imaging provides high quality images for guiding therapeutic agents.¹⁰¹ During and after the therapy, the images could be used for monitoring the effects of therapy. These properties may improve drug delivery efficacy and survival rates, and minimize side effects of drugs to normal tissues.

There are at least three different models for multifunctional SiNPs including multimodel imaging, multimodel therapy and multimodel theronostics. For the multimodel imaging system, the designed SiNPs possess different kind of imaging strategies, such as optical imaging, MRI, PET, CT, ultrasonic, *etc.* This multimodel imaging system can greatly increase the diagnosis accuracy and sensitivity.^{101, 102} For example, Huang *et al.* developed a multifunctional MSN that incubated fluorescence imaging, MRI and PET for tumor imaging and probe localization.¹⁰³ The multimodel therapy system contains the SiNPs that incubated different therapeutic strategies. For example, chemotherapy, gene therapy, thermotherapy, PDT and photothermal therapy (PTT) can be combined for the multimodel therapy of tumor.^{104, 105} The multimodel therapy system can enhance the therapeutic efficiency. Furthermore, the most important multifunctional SiNPs possess multimodel imaging abilities and multimodel therapeutic abilities for tumor diagnosis and therapy,^{106–109} which is illustrated as following.

Liong *et al.* proposed the use of multifunctional MSNs for imaging, targeting, and drug delivery to tumors.⁷⁸ The iron oxide nanocrystal-doped in MSNs and fluorescein on the silica shell provided the feasibility of MRI and fluorescence imaging for tumors. Folic acid was used to target tumor cells and tissues due to the high expression of folate receptor on tumor cells. A drug was loaded in the pore channel of MSNs for killing tumor cells. Meanwhile, the iron oxide nanocrystal could be used for magnetic manipulation to modulate the platform to a desired location. Therefore, the multifunctional MSNs boasted five functions together for tumor imaging and therapy. Chen *et al.* developed similar multifunctional SiNPs for simultaneous cell imaging and drug delivery.¹¹⁰ Iron oxide nanocrystal or gold nanoparticles were doped in SiNPs, and then co-loaded with a chemotherapeutic agent. *In vitro* and *in vivo* MRI and therapeutic functionalities of the platform were shown to be promising for tumor imaging and therapy in mice.

In 2011, Huang *et al.* constructed silica-modified gold nanorods for X-ray/CT imaging and photothermal therapy for tumor.¹¹¹ As shown in Figure 9A, gold nanorods were encapsulated in the silica nanoshell, which was modified with folic acids for active targeting tumor cells. Upon the irradiation of light, the tumor cells that underwent apoptosis were increased as stained by PI (Figure 9B). Furthermore, *in vivo* X-ray imaging showed that tumor tissue displayed a sharp contrast compared to normal tissues (Figure 9C). To explore the thermal effect of gold nanoparticles, Huschka *et al.* investigated the thermal effect of gold nanoshell and gold nanorod for DNA release, which might be useful for tumor

targeting gene delivery and therapy.¹¹² With a similar design, Zhang *et al.* developed mesoporous silica-coated gold nanorods (Au@SiO₂) for cancer treatment.¹¹³ The Au@SiO₂ was monitored using two-photon imaging. Doxorubicine was also doped into the nanocomposite which could be released by the low power density laser for chemotherapy. Meanwhile, with high power density laser, the gold nanorods induced photothermal effect could be used for the hyperthermia. Similarly, He *et al.* developed a MB-doped SiNPs for simultaneous fluorescent imaging and PDT of tumor cells *in vitro* and tissues *in vivo*.³⁰ Zhao *et al.* doped upconversion nanoparticles into SiNPs, which were then coupled with photosensitizer for NIR imaging and PDT.¹¹⁴ Interestingly, this nanomaterial also demonstrated good MR contrast in tumor cells, and thereby could be used for MRI. In a recent review, Bardhan *et al.* summarized the design and synthesis of nanoshell-based theranostic agents using enhanced near-infrared fluorescence or magnetic resonance imaging guided photothermal ablation in both cellular models and animal models.¹¹⁵

Collectively, the goals of theranostics may be achieved by combining imaging agents and therapy agents into appropriate SiNPs for tumor imaging and therapy. More important, these multifunctional nanoparticles may provide combined imaging strategies, such as fluorescence and MR imaging, fluorescence and X-ray imaging, fluorescence and Raman imaging, and MR and X-ray imaging, for precise guidance of drug selection and monitoring the efficacy during tumor therapy.

6. Biocompatibility of silica nanoparticles

With the development of SiNPs for cancer diagnosis and therapy, the safety issues have been gradually recognized and need serious and full further investigation. The biocompatibility of SiNPs at different levels, including cells, tissues and animals, were demonstrated by several groups. Because of the chemical inert of silica, the SiNPs showed great biocompatibility at different levels. However, the physiochemical properties, such as size, surface charge and modification, can all play important roles on the toxicity and pharmacokinetics of SiNPs in the cells and animals. Meanwhile, because of the various synthesis methods and treated bio-matrixes, it is hard to draw a general conclusion about the toxicity of the SiNPs on cells and animals. Herein, we briefly reviewed the recent developments of the investigations of the *in vitro* and *in vivo* toxicity of SiNPs regarding the size and surface modification of the nanoparticles.

The toxicity of SiNPs was affected by several factors, including the cell lines, concentrations, particle sizes, surface charges, particle shapes and structures of the SiNPs. Mou *et al.* investigated the cellular uptake amount of different sized MSNs using Hela cells.³⁵ They found that the MSNs of 50 nm diameter showed the highest uptake efficiency. Jin *et al.* synthesized different sizes core/shell SiNPs and investigated their cytotoxicity.³⁸ It was found that the smaller SiNPs (23 ± 3 nm) showed higher cytotoxicity than that of the larger SiNPs (85 ± 5 nm). At the animals level, He *et al.* investigated the biodistribution and urinary excretion of MSNs with different particle size from 80 nm to 360 nm.¹¹⁶ They found that the accumulation of MSNs in the liver and spleen and the excretion efficiency increased with the increase of particle size from 80, 120, to 200 nm after intravenous injection. However, within one month, neither MSNs nor PEGylated MSNs showed toxicity to the treated mice.

The surface modification of SiNPs was another factor that could affect the toxicity on the cells. The SiNPs with cationic charge surface had strong interaction with the cell membrane because of the electrostatic interaction, which would induce more immune response and cytotoxicity compared with the neutral and anion SiNPs. For the unmodified SiNPs, the surface was covered with silanol groups which induced a negative charge of the SiNPs.

However, other functional groups, such as amino group, carboxyl group, phosphonate group and PEG were easily modified on the surface of the SiNPs with the silanol chemistry. Among these groups, PEG was by far the best way to increase the dispersity and increase the biocompatibility of the SiNPs. With the modification of PEG, the blood half-life in small animals would significantly increase, and the accumulation in RES tissues would greatly decrease, which was enhancing the tumor targeting efficiency and biocompatibility. With PEGylation, the endocytosis of the SiNPs may be diminished, which could reduce the hemolytic activity and cytotoxicity.^{117, 118} After intravenous injection of different modified SiNPs, He *et al.* found that all the SiNPs accumulated in the liver and spleen with the increase of time.³³ However, the PEGylated SiNPs showed longer blood circulation times and lower uptake by the RES tissues than that of the hydroxyl modified SiNPs and carboxyl modified SiNPs. Meanwhile, all the SiNPs were partly excreted through the renal excretion route, which was confirmed by the fluorescence in the bladder and urine, and TEM images of SiNPs in the urine. The excretion process might greatly reduce the long-term toxicity of SiNPs *in vivo*.

7. Conclusions

In conclusion, the applications of SiNPs in *in vitro* and *in vivo* tumor imaging demonstrated the potential for further developing the SiNPs into clinically-useful reagents. Fluorescence and MRI imaging are currently the main strategies for the applications of SiNPs in cancer imaging. X-ray/CT may also be used along with SiNPs as imaging agents. As biocompatible nanomaterials, SiNPs can be used for drug delivery, especially for chemotherapeutic agents, photosensitizers and photothermal agents. Stimuli-responsive MSNs for drug delivery and release have improved therapeutic efficacy and decreased side effects. Furthermore, the multifunctional SiNPs that combined imaging and therapy functionalization provided the most promising tool for tumor diagnostics and treatment.

However, there are some challenges that need to be resolved before successful applications of SiNPs in clinics. First of all, the delivery efficiency to the targeting tumor cells and tissues should be improved. Even several targeting ligands, including antibodies, aptamers and peptides, have been tested for the active targeting for tumor diagnosis and therapy; most of them have great absorption in the liver and spleen. This might induce side effects to normal tissues and organs. Therefore, the excellent targeting efficiency to tumor is of great promise. Secondly, multifunctional SiNPs is in the early stage for tumor diagnosis and therapy. With different functions, the multifunctional SiNPs can accurately detect/arrive tumor sites, and then kill the tumor cells. Importantly, the therapy efficiency can also be monitored. By carefully designing of the multifunctional SiNPs, it will be of significance to accomplish all the necessary function in one SiNP composite. Thirdly, the biocompatibility and toxicity of the SiNPs to normal tissues of rodents and primates need extensive investigation. Several investigations in SiNP toxicity have been recently carried out by different groups; however, it is premature to make a general conclusion on the toxicity of the SiNPs in bio-systems. Much research needs to be done before the realization of the great potential of novel SiNPs in bio-medical applications. For example, a systemic investigation for the toxicity *in vitro* and *in vivo* should be performed in small animal and primates. We look forward to witnessing more promising discoveries for tumor diagnosis and therapy using novel silica nanomaterials.

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Abbreviations

SiNPs	silica nanoparticles
MSNs	mesoporous silica nanoparticles
C/S SiNPS	core/shell silica nanoparticle
QDs	quantum dots
MRI	magnetic resonance imaging
PET	positron emission tomography
X-ray CT	X-ray computed tomography
TEOS	tetraethyl orthosilicate
PEG	polyethylene glycol
RES	reticuloendothelial system
EPR	enhanced permeability and retention
FMSNs	fluorescent silica nanoparticle
F-FMSNs	folic acid modified fluorescent silica nanoparticles
SPIO	super paramagnetic iron oxide
CPT	camptothecin
DOX	doxorubicin
PDT	photodynamic therapy
HPPH	2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide
MB	methylene blue
PEI	polyethyleneimine
Pgp	P-glycoprotein
PTT	photothermal therapy

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This review summarized the development of the silica nanoparticles for tumor imaging and therapy. With the great biocompatibility, easy synthesis and modification, SiNPs were designed to carry out different functions in the treatment of cancer. The imaging ability can be used for the diagnosis of cancer, and the therapy ability can be used for the treatment of cancer. By combining these two functions, SiNPs are promising for the diagnosis and therapy of cancer in the future.

Figure 1. Confocal micrographs of continued irradiation of cells leading to photobleaching over a 1 h time scale. A) FITC loaded HUVEC cells. B) 30 nm fluorescent silica loaded cells. The cells are incubated with 100 µg/mL of nanomaterials for 3 h and are washed and subjected to continued irradiation for a period of 1 h. Images are taken every 20 min and the photobleaching of pure FITC and FITC loaded nanomaterials are studied. Reprinted from ref. 55 with permission by the SpringerLink.

Figure 2.

Cellular uptake *in vivo* test of single QDs and Si@QDs@Si NPs. a) Illustration of sQD or Si@QDs@Si-NP labeling to HeLa cells. b) Fluorescence images of sQD- and Si@QDs@Si-NP-uptaken cells (Red color: QD, blue color: DAPI). c) Maestro *in vivo* fluorescence image of sQD-labeled (left leg) and Si@QDs@Si-NP-labeled (right leg) cell-transplanted mouse. Reprinted from ref. 73 with permission by Wiley-VCH.

Figure 3.

A) Schematic illustration of the synthetic procedure for magnetite nanocrystal /mesoporous silica core-shell NPs. B) fluorescence images of subcutaneously injected MCF-7 cells labeled with Fe₃O₄@mSiO₂(R) (10 μg Fe mL⁻¹) and control MCF-7 cells without labeling into each dorsal shoulder of a nude mouse. Reprinted from ref. 77 with permission by Wiley-VCH.

Figure 4.

A) Schematic illustration of FMSNs modified with folic-acid-targeting ligands on the surface. B) Cell-proliferation assay with FMSNs and F-FMSNs in MCF10F (M) and SK-BR-3(SK) cells. The cells were treated for 48 h with 20 $\mu\text{g mL}^{-1}$ of nanoparticles only (FMSN), CPT-loaded nanoparticles (FMSN/CPT), CPT-loaded F-FMSNs, or the same concentration of CPT dissolved in DMSO. The cells were then washed and stained with 10% WST-8 solution from the cell-counting kit (DojindoCo.) for 2 h. The absorbances were measured at 450 nm with a plate reader. The percentages of each sample relative to control cells are presented as mean values $\pm$ SD. C) The average tumor volumes are shown as means $\pm$ SD. * = $p < 0.05$; ** = $p < 0.01$. D) Representative images of mice from different groups. Red arrows indicate the location of subcutaneous tumors. Reprinted from ref. 65 with permission by Wiley-VCH.

Figure 5. Representation of MSNs loaded with guest molecules and end-capped with a general gatekeeper. Reprinted from ref. 25 with permission by Elsevier.

Figure 6.

In vivo imaging and PDT of subcutaneous-HeLa-tumor-xenografted mice after different treatment: (A) 100 μ l 44 mg/ml MB-encapsulated PSiNPs injection and 5 min light exposure with power intensity of 500 mw/cm^2 , the red circle indicates the region injected the MB-encapsulated PSiNPs and with light exposure; (B) 100 μ l 44 mg/ml MB-encapsulated PSiNPs injection; and (C) 5 min light exposure with power intensity of 500 mw/cm^2 . Reprinted from ref. 30 with permission by Elsevier.

Figure 7.

GFP knockdown by siRNA in stable transfected GFP-HEPA cells. HEPA-1 cells with stable GFP expression were used for siRNA knockdown assays. MSNP coated with different size PEI polymers were used to transfect GFP-specific or scrambled siRNA and the results compared with LipofectAmine 2000 as transfection agent. (A) GFP knockdown was assessed by flow cytometry in which GFP MFI was normalized to the value of control untransduced cells (100%). (B) Confocal pictures were taken showing GFP knockdown in GFP-HEPA cells. TEX 615-labeled siRNA was used to show the cellular localization of the nucleic acid bound particles (red dots). X, scrambled siRNA. The experiment was

reproduced three times. Reprinted from ref. 13 with permission by the American Chemical Society.

Figure 8. Schematic diagram of a co-delivery system based on MSNs to deliver Dox and Bcl-2-targeted siRNA simultaneously to A2780/AD human ovarian cancer cells for enhanced chemotherapy efficacy. Reprinted from ref. 98 with permission by Wiley-VCH.

Figure 9.

A) Synthetic procedure of GNR-SiO₂-FA. B) Photo-thermal therapy effects on GC803 cells incubated with 12.5 mM of GNR-SiO₂-FA for 24 h at 37°C in the dark prior to irradiation for 3 min with 808 nm laser. (a and b) MGC803 cells on the laser spot center, (d and d) GC803 cells on the boundary of laser spot. (a and c) Bright field, (b and d) Fluorescence field. C) Real-time *in vivo* X-ray images after intravenous injection of GNR-SiO₂-FA in nude mice at different time points. (a) The photograph of the tumor tissue; (b) the X-ray image at 0 h; (c) the X-ray image at 0 h (in color); (d) the X-ray image at 12 h, (e) the X-ray image at 12 h (in color); (f) the X-ray image at 24 h (in color). Reprinted from ref. 111 with permission by Elsevier.

Table 1

The SiNPs for tumor imaging and therapy *in vitro* and *in vivo*.

Type	Diameter (nm)	Surface modification	targets	Targeting pathway	Imaging mode	Therapeutic mode	Ref.
Core/shell SiNPs	20	monoclonal antibodies	cells	Active	Fluorescence (rhodamine-B)		59
	30	Folic acid	cells	Active	Fluorescence (DBF [*])		61
		Aptamer	cells	Active	Fluorescence (FITC [†] , RuBpy [‡] , TMR [§] and Cy5)		64
	31	RGD ^{//}	cells	Active	Fluorescence (QDs [¶]) and MRI (Gd)		78
	25	-NH ₂	cells	Cell uptake	Fluorescence (rhodamine 6G)	Antisense oligonucleotides	99
	105 ± 6.8	-PO ₄ ³⁻	Xenografts		Fluorescence (MB [#])	PDT ^{**} (MB)	67
	25 – 42	-NH ₂ and PEG ^{††}	Xenografts	Passive	Fluorescence (IR820)	PDT (PpIX ^{‡‡})	74
	120		Xenografts	Cell uptake	Fluorescence (QDs)		75
		Folic acid	Cells and xenografts	Active	Dark-field scattering and CT/X-ray imaging (AuNR)	PTT ^{§§} (AuNR ^{//})	103
	150		Cells	Cell uptake		Chemo (DOX ^{¶¶}) and PTT (Pd@Ag)	3
MSNs	50	TAT peptide	Cells	Active	Fluorescence (DOX)	Chemo (DOX)	39
	110–130	Polyethyleneimine	Cells	Cell uptake	Fluorescence (FITC)	siRNA and DNA	13
	~220		Cells	Cell uptake	Fluorescence (FITC)	Chemo (DOX)	28
	~110	RGD	Cells	Active	Fluorescence (FITC)	Chemo (CPT ^{##})	46
	245	Galactose	Cells	Active	Fluorescence (FITC)	Chemo (CPT) and PDT (porphyrin)	96
	200		Cells	Cell uptake	Fluorescence (DOX)	siRNA and Chemo (DOX)	101
	100 – 130	Folic acid	Xenografts	Active	Fluorescence (FITC)	Chemo (CPT)	65
	< 100	PEG	Xenografts	Passive	MRI (Fe ₃ O ₄)		79
	70 ± 6	PEG	Xenografts	Passive	MRI (Fe ₃ O ₄)	Chemo (DOX)	81
	100–130	folic acid	Xenografts	Active		Chemo (CPT)	32
Silica Nanorattle	125	PEG	Xenografts	Passive		Chemo (Dox ^{***})	43

Footnotes:

^{*} 4,4'-(1E,1'E)-2,2'-(9,9-didecyl-9H-fluorene-2,7-diyl)bis(ethene-2,1-diyl)bis(N,N-dibutylaniline); Fluorescein isothiocyanate;

[†] Tris(bipyridine)ruthenium(II) chloride;

[§] tetramethylrhodamine;

// Arg-Gly-Asp;
 ¶ quantum dots;
 # methylene blue;
 ** photodynamic therapy;
 †† polyethylene glycol;
 ‡‡ protoporphyrin IX;
 §§ photothermal therapy;
 // gold nanorod;
 ¶¶ doxorubicin;
 ## camptothecin;
 *** docetaxel.