Silica Nanoparticles as Promising Drug/Gene Delivery Carriers and Fluorescent Nano-Probes: Recent Advances

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Abstract: The application of nanotechnology to biomedical research is expected to have a major impact leading to the development of new types of diagnostic and therapeutic tools. One focus in nanobiotechnology is to develop safe and efficient drug/gene delivery vehicles. Research into the rational delivery and targeting of pharmaceutical, therapeutic and diagnostic agents is at the forefront of projects in nanomedicine. Silica, as a major and natural component of sand and glass, is a versatile material due to the variety of available chemical and physical modifications that are available, and recently have been widely applied in nanobiotechnology as drug/gene carriers or fluorescent nano-probes. The goal of this brief review is to illustrate selected examples of various functionalized silica nanoparticles as drug/gene delivery systems that have been applied to the arenas of human disease therapy or detection (molecular and cellular imaging).

Keywords: Silica, nanoparticles, drug/gene delivery, nano-probes, targeting.

INTRODUCTION

Nanoparticles encompass a variety of colloidal nanosystems, which may be inorganic or organic. Nanoparticle drug/gene delivery systems have been studied for several decades, and many of the features that make them attractive drug/gene carriers are well known [1, 2]. One of the major advantages of nanoparticles is their small size, which allows them to pass through certain biological barriers. The second advantage is that a high density of therapeutic agent can often be encapsulated, dispersed, or dissolved within these nanoparticles to yield different properties and release characteristics for the entrapped agents [3]. The third advantage is that nano-shells can protect some specific therapeutic compounds, for instance, enzymes, which are not degraded.

Drug delivery nano-carriers are fundamentally important in improving the pharmacological profiles of therapeutic molecules. Nano-controlled-release systems are attracting a lot of attention currently owing to their large surface area and their ability to target delivery to specific sites in the human body. In addition, they can penetrate the cell membrane for gene, nucleic acid and bioactive peptide/protein delivery [4-6]. One representative of the nano-delivery system is silica nanoparticles or nanocapsules. Silica is also a relatively benign material due to its biocompatibility and low toxicity. Recently, researchers around the world have pursued more efficient drug/gene delivery vehicles for both basic research and clinical trials, amongst them silica nanoparticles are more attractive and widely used for delivery carriers. There is also a summary of the silica applications in gene therapy [7]. Liz-Marzan and co-workers have been synthesized nanosized Au@silica as 15 nm gold as the core. Gold colloids have homogeneously coated with silica using the silane coupling agent 3-aminopropyltrimethoxysilane as a primer to render the gold surface vitreophilic [8]. After the formation of a thin silica layer in aqueous solution, the particles can be transferred into ethanol for further growth using the Stöber method. Other researchers have encapsulated fluorescent probe (*e.g.* fluorescein isothiocyanate, FITC) into the silica nanobubbles after the gold cores were dissolved by sodium cyanide [9, 10]. The fluorescent intensity of this nanoparticle fluorescent probe was highly luminescent and photostable, so the fluorescent life time was also longer than that free FITC dyes which are not encapsulated. Analogous methods can be applied to be a wide range of other molecular probes.

This mini-review assesses the recent advances and illustrates the emerging applications of silica nanoparticles or nanocapsules in drug delivery systems, gene transfection vehicles and fluorescent nano-probes. Finally, it highlights and outlines the future directions for the developments of multi-biofunctional silica nanoparticles, and bridges the gap from basic silica nanomedicine to clinic applications.

SILICA NANOPARTICLE FORMULATION

For synthesis of silica particles, a classic method was reported by Stöber about 40 years ago [11]. Up to now, many silica nanoparticles were synthesized through modified Stöber method. Recently, Nakamura group in Tokushima University of Japan has developed a series of a new type of silica nanoparticles based on Stöber method [12-14]. In 2007, they synthesized a novel organosilica nanoparticles made of 3-mercaptopropyltrimethoxysilane as the single silica source (referred to MPS NPs) by using one-pot synthesis [12]. These MPS NPs doped the fluorescent dye on the silica network have some unique surface properties, such as thiol residues on the surface and reduced zeta potential compared with the nanoparticles made of tetraethoxysilane. MPS NPs modified with various molecules, including fluorescent dyes and proteins, have the potential to be used in various applications, such as biomedical analysis, chip-based tech-

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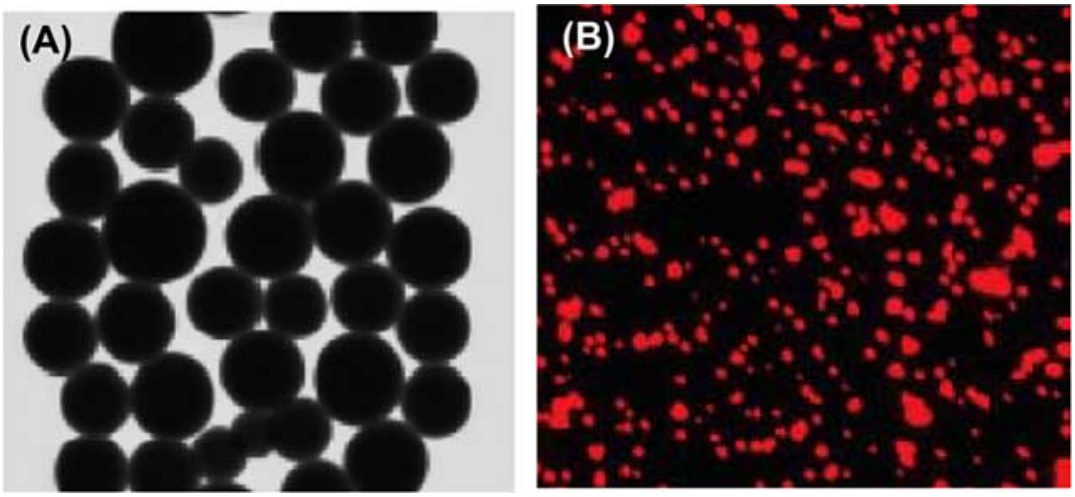

Fig. (1). (**A**) Transmission electron microscopy image of fluorescent silica nanoparticles (MPS-NPs) with diameter about 500 nm, and (**B**) Fluorescence microscopy of silica nanoparticles modified with maleimide-rhodamine red conjugate on the surface. (reproduced from Ref. [14], with permission from ACS Publishing Company).

nology, multi-target detection systems, imaging *in vitro* and *in vivo*, and drug delivery systems (Fig. **1**). Furthermore, other three kinds of thiol-organosilica nanoparticles were also synthesized, and their synthesis conditions and properties were compared [14]. Roy and co-workers [15, 16] reported a hydrated, organically modified silica (referred to ORMOSIL) nanoparticles based on the triethoxyvinylsilane precursor, both empty and loaded with fluorescent dyes. The amino-functionalized nanoparticles were able to electrostatically condense DNA (both plasmid and genomic) and protect it from enzymatic degradation. They also used enhanced green fluorescent protein gene to assess the overall efficiency of *in vivo* transfection.

In our group, we developed several types of hollow mesoporous silica nanoparticles through co-operation with Prof. Miyoshi and Nakamura in recent years as shown in Fig. (**2**) [9, 17-19]. Initially, we synthesized the core-shell nanoparticles of Au@silica with a diameter of approximate 45-60 nm and silica shell thickness in the range of 3-10 nm. Hollow mesoporous silica nanocapsules were obtained by using sodium cyanide to dissolve the gold cores. The mesoporosity on the shells were controlled by 3-aminopropyltrimethoxysilane addition during particle synthesis. Further experiments demonstrated that some light-sensitive molecules could be embedded into the silica shells, and the drugs could be controlled to release from the nanoparticles by irradiation at a specific wavelength. Interestingly, the mesoporous silica shell could also be re-coated as a layer of biodegradable material of poly (D,L-lactide-*co*-glycolide) (PLGA). The encapsulated drug was controlled to release with PLGA degradation, and could sustain for nearly four weeks. Other reports showed that the template core could be replaced by $CaCO_3$ nanoparticles, and the cost would be reduced [20-22].

SILICA NANOPARTICLES AS DRUG/GENE DELIVERY SYSTEMS

Nanomedicine is an emerging new field created by fusion of nanotechnology and medicine. It uses a nanoparticle platform for diagnostic probes and effective targeted therapy [16]. Silica is regarded as a possible option of materials for a drug/gene delivery system. Silica nanoparticles are often used as core particles for biomedical applications because they are highly hydrophilic and easy to prepare and separate, and their surfaces may be modified or labeled. One of the recent interests in the technology of conjugating silica and DNA is focused on the utilization of silicas as nonviral vectors of DNA for gene delivery applications. Generally, pure silica nanoparticles without surface modification do not seem to be able to aid and enhance gene delivery because there are hydroxy groups on the surfaces for most. Thus, silica nanoparticles are firstly required to be modified with functional substituents for binding duplex DNA through electrostatic interaction or surface group reactions. For example, silica nanoparticles modified with aminosilanes were able to condense and deliver DNA, very much like cationic polymers, without the addition of other cationic transfection reagents. Kneuer and co-workers reported that colloidal silica nanoparticles with covalently linked cationic surface modifications could be applied in plasmid DNA (pCMVβ)

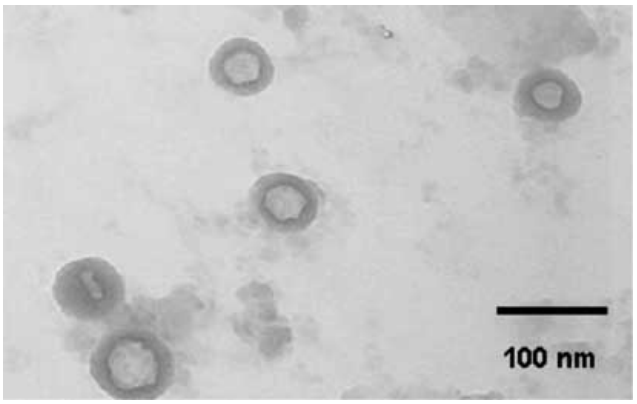

Fig. (2). Transmission electron microscopy image of hollow mesoporous silica nanocapsules with an average diameter of 50 nm. (reproduced from Ref. [9], with permission from Elsevier Publishing Company).

transfection *in vitro*. The nanoparticle-DNA complexes, sized between 10 and 100 nm and with zeta potentials ranging from +7 to +31 mV at pH 7.4, might be utilized as DNA carriers for gene delivery [23, 24]. They also demonstrated that the absorbed DNA was protected from enzymatic degradation by DNase I.

Bharali *et al*. [15] synthesized a kind of highly monodispersed, stable aqueous suspension of ORMOSIL nanoparticles, surface-functionalized with amino groups for binding of DNA, and demonstrated that the ORMOSIL nanoparticles were as a nonviral vector for efficient *in vivo* gene delivery. The stereotaxic injections of nanoparticles, complexed with plasmid DNA encoding for enhanced green fluorescent protein, into the mouse ventral midbrain and into lateral ventricle, allowed to fluorescently visualizing the extensive transfection of neuronal-like cells in substantia nigra and areas surrounding the lateral ventricle. No ORMOSIL-based toxicity was observed four weeks after transfection, and the efficiency of transfection equaled or exceeded that obtained in studies using a viral vector. An *in vivo* optical imaging technique provided an effective means to show the retention of viability of the transfected cells. The ORMOSIL-mediated transfections also were used to manipulate the biology of the neural stem/progenitor cells *in vivo*. Transfection of a plasmid expressing the nucleus-targeting fibroblast growth factor receptor type 1 resulted in significant inhibition of the *in vivo* incorporation of bromodeoxyuridine into the DNA of the cells in the subventricular zone and the adjacent rostral migratory stream. These results of this nanomedicine approach using ORMOSIL nanoparticles as a nonviral gene delivery platform have a promising future direction for effective therapeutic manipulation of the neural stem/progenitor cells as well as *in vivo* targeted brain.

Silica nanoparticles can also be used to delivery antisense oligonucleotides (anti-ODNs), which are able to interfere with gene expression at the mRNA level, and have potential activity in the treatment of various diseases, including cancer [25-29]. It was reported that anti-ODNs carrier based on amino silica nanoparticles was applied in cancer therapy [25]. The amino silica nanoparticles-ODN complexes were formed by electrostatic interaction. It was found that the amino silica nanoparticles were able to protect anti-ODNs from degradation by DNase I. *In vitro* experiments showed that the amino silica nanoparticles could greatly improve the inhibition efficiency of anti-ODNs for the proliferation and survivin expression in HeLa cells and A549 cells. Compared with liposomes, the amino silica nanoparticles presented a better biocompatibility and had almost no cytotoxicity at the concentrations required for efficient transfection.

Drug-doped silica nanoparticles are an effective drug-carrier system for the therapy and treatment of cancer and other diseases. In 2003, Roy *et al.* [30] synthesized the photosensitizer-doped organically modified silica-based nanoparticles (diameter~30 nm), which entrapped water-insoluble photosensitizing anticancer drug 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH) (Fig. **3**). The synthesized doped particles were shown to be spherical and highly monodispersed. Irradiation of the photosensitizing drug entrapped in nanoparticles with light of suitable wavelength results in efficient generation of singlet oxygen, which is made possible by the inherent porosity of the nanoparticles. The doped nanoparticles were actively taken up by tumor cells, and irradiation with visible light has resulted in irreversible destruction of such impregnated cells, which suggested the potential of the silica particles as carriers for photodynamic drugs in cancer treatment (Fig. **4**). In further experiments, they also reported another silica nanoparticles, which photosensitizer molecules were covalently incorporated, and demonstrated that the covalently incorporated photosensitizer molecules retained their spectroscopic and functional properties and could robustly generate cytotoxic singlet oxygen molecules upon photoirradiation [31].

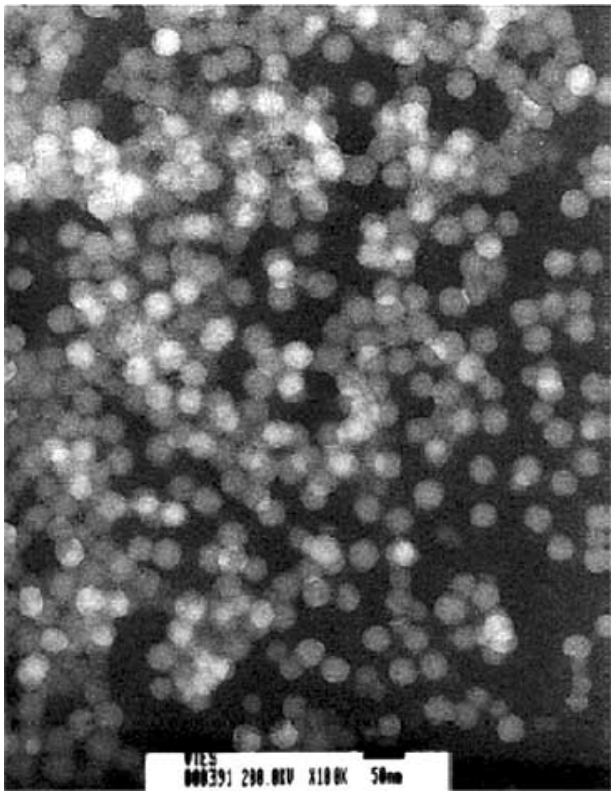

Fig. (3). Transmission electron microscopy picture of HPPH-doped silica-based nanoparticles showing highly monodispersed particles with an average diameter of 30 nm. (reproduced from Ref. [30], with permission from ACS Publishing Company).

Recently, mesoporous silica nanoparticles (MSNs), in the form of stable aqueous dispersion, are emerging as an ideal agent for biomedical applications. Many investigations showed that mesoporous silica nanoparticles are promising as controlled release drug delivery and carriers [20, 32-34]. It was reported that a stimuli responsive, controlled release drug delivery system was designed by using these biocompatible MSNs as carriers. In contrast to many current polymer-based delivery systems, the molecules of interest were encapsulated inside the porous framework of the MSN not by adsorption or sol-gel types of entrapment, but by covalently capping the openings of the mesoporous channels with size-defined "caps", such as dendrimers, proteins, and semiconductor nanocrystals (quantum dots), to physically block the drugs from leaching out [35-38]. Drug molecules loaded into the pores were released by the introduction of

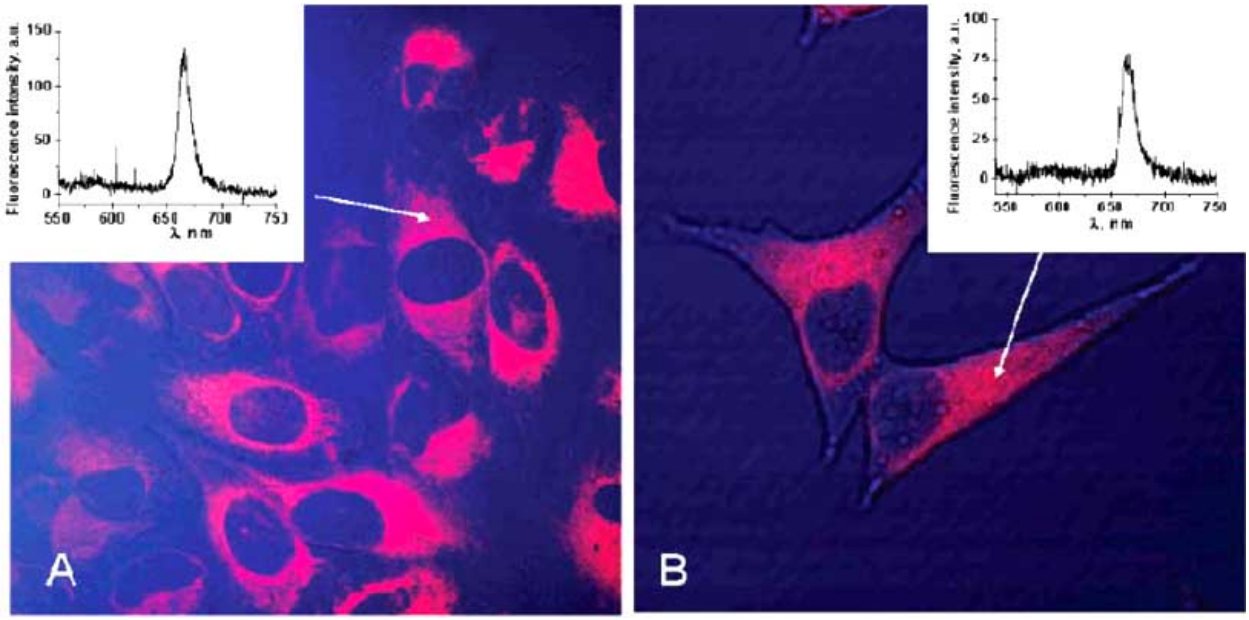

Fig. (4). Confocal fluorescence image of tumor cells (**A**) HeLa and (**B**) UCI-107 treated with HPPH-doped nanoparticles. Transmission (blue) and fluorescence (red) channels are shown. (Inset) Localized fluorescence spectra from the cytoplasm of the treated cell. Excitation is at 532 nm. (reproduced from Ref. [30], with permission from ACS Publishing Company).

"uncapping triggers" (molecules that can cleave the chemical linkers connecting the caps to the mesoporous surface). The rate of release was controlled by the concentration of the trigger molecules. Prior to uncapping, the capped MSN system exhibited negligible release of drug molecules.

To improve the drug delivery efficacy and specificity to targeted cells or tissues, targeted silica nanoparticles to mediate drug intracellular delivery received more attention. Rosenholm *et al.* [39, 40] developed a hybrid silica nanoparticle carrier system, which exhibited both cancer cell-targeting ability and capacity to retain a hydrophobic agent with subsequent specific release into the endosomal compartment. The incorporated drug was shown to be able to escape from the endosomes into the cytoplasm, making the silica nanoparticles promising candidates as carriers for targeted drug delivery for cancer treatment. Except for molecular target and particle size, there is another factor of particle shape, which will affect the drug delivery efficiency. It was documented that different shapes of silica nanoparticles were readily internalized in human melanoma cells by nonspecific cellular uptake [41]. Nanoparticles with larger aspect ratios were taken up in larger amounts and had faster internalization rates. Likewise, nanoparticles with larger aspect ratios had a greater impact on different aspects of cellular function including cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration.

SILICA NANOPARTICLES AS FLUORESCENT NANO-PROBES

The application of nanomaterials in medical and biological fields has drawn great research interest in recent years. Currently, nanoparticles have become more and more prevalent in reports of novel contrast agents, especially for molecular imaging, the detection of cellular processes. The advantages of nanoparticles include their potency to generate contrast, the ease of integrating multiple properties, lengthy circulation times, and the possibility to include high payloads [42]. For this potential to be realized, the ability to target nanoparticles to specific tissues and cell types is important. Presently, there are several types of nanoparticles used in molecular imaging in diagnosis, such as liposomes, dye-molecule-doped silica nanoparticles, quantum dots, gold nanoparticles, immunotargeted nanoshells, perfluorocarbon nanoparticles, and magnetic nanocrystals.

Silica is a useful material for biological applications in particular, because it provides a biocompatible host for the dye molecules, which has been shown to aid in the photostability of the dye, and also serves as a convenient surface for bioconjugation. Luminescent, photostable, and easily functionalized silica nanoparticles have been widely used for biochemical sensing, time-resolved fluoroimmunoassay, *in vivo* imaging of blood vessels, and bioanalytical assays [43]. In 1999, Makarova *et al.* successfully synthesized fluorescein isothiocyanate (FITC)-doped silica nanoparticles. This nanoparticle probe has a high potential in biological labeling [10]. Tan and coworkers in the University of Florida has synthesized a new form of highly luminescent and photostable nanoparticles, which were generated by doping a fluorescent dye (Rubpy) inside silica nanoparticles [44]. Because thousands of fluorescent dye molecules are encapsulated in silica matrix that protects Rubpy from photodamaging oxidation, the Rubpy-doped nanoparticles are extremely bright and photostable. They also used these nanoparticles successfully in various fluorescence labeling techniques, including fluorescence-linked immunosorbent assay, immunocytochemistry, immunohistochemistry, DNA microarray analysis, and protein microarray assay. Lin *et al.* demonstrated that functionalized nanosized mesoporous silica nanoparticles embedded fluorophores can be used as a fluo-

rescence cell tag [45], and found that the embedded fluorophores are stable against enzymatic digestion and thus good for long time cell tracking (Fig. **5**). By combining the high-intensity luminescent nanoparticles with the specificity of antibody-mediated recognition, ultrasensitive target detection has been achieved. This technique can be also used to detect tumor antigen markers and provide some helpful information in early cancerization detection.

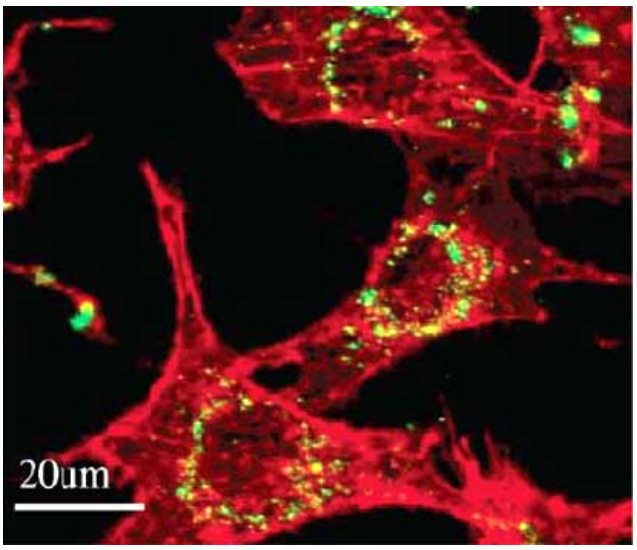

Fig. (5). Confocal images of FITC-mesoporous silica nanoparticles (green) in 3T3-L1 cells; cell skeleton was stained with rhodamine phalloidin (red). Cells were incubated with FITC-mesoporous silica nanoparticles for 1 h, washed, and further incubated in particle-free medium overnight. (reproduced from Ref. [45], with permission from ACS Publishing Company).

In 2006, Wang and Tan have prepared silica nanoparticles encapsulated with three organic dyes using a modified Stöber synthesis method [46]. By varying the doping ratio of the three tandem dyes, fluorescence resonance energy transfer-mediated emission signatures can be tuned to make the nanoparticles to exhibit multiple colors under one single wavelength excitation (Fig. **6**). These nanoparticles are intensely fluorescent, highly photostable, uniform in size, and biocompatible. This study provides the information of highly fluorescent and photostable barcoding nanoparticles that permit simultaneous and sensitive detection of multiple targets, as well as it opens up a new perspective in the design of multifunctional structures based on silica nanoparticles, which will have a variety of interesting applications in the future.

Recently, colloidal nanomaterials have been synthesized with complicated structure and composition, designed to exhibit multiple functionality, for example, nanoparticles that are both fluoresce and respond to magnetic fields. The biofunctional contrast agents with both optical and magnetic contrast were demonstrated to serve as good molecular imaging probes both *in vitro* and *in vivo* [47-50]. Thus, dual-modality detections could be simultaneously achieved using a single material. Lu *et al*. [49] used silica for surface coating of SPIO particles because fluorescent dye molecules, for instance, FITC, can be easily incorporated into a silica shell, and silica is quite biocompatible and resistant to biodegradation in the biological environments. Moreover, silica can be easily surface functionalized for bioconjugation and targeting for various applications in biological systems. Lu and coworkers combined the merits of superparamagnetic iron oxide (SPIO) particles and silica, and engineered a new type of biofunctional core-shell nanoparticles of SPIO@SiO_2(FITC) with diameters of 50 nm (Fig. **7**). This nano-probes can efficiently label human mesenchymal stem cells, and can be applied in monitoring cell trafficking *in vivo* and distinguish whether cellular regeneration originated from the exogenous stem cells. Another report [51] showed that multifunctional colloidal core-shell nanoparticles of magnetic nanocrystals or gold nanorods could be encapsulated in silica shells doped with the fluorescent dye of Rubpy. These colloidal heterostructures have the potential to be used as dual-purpose-tags, exhibiting a fluorescent signal that could be combined with either dark-field optical contrast (in the case of the gold nanorods), or enhanced contrast in magnetic resonance images (in the case of magnetic nanocrystal cores).

SURFACE MODIFICATION AND TARGETING

The ability to target pharmacologically active molecules to specific sites in the body has been actively pursued for many decades. Interest in this concept has increased significantly in recent years with the advent of new technology and better understanding of the processes involved in drug/gene

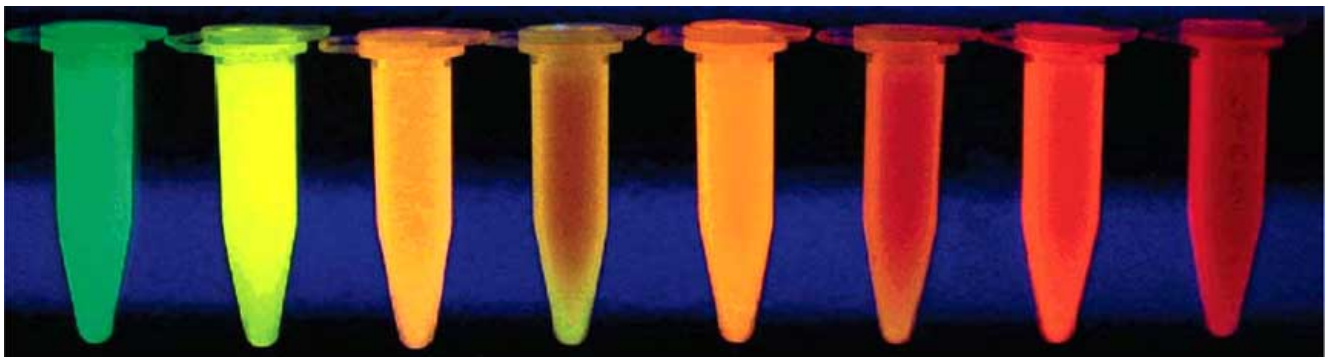

Fig. (6). Silica nanoparticles with different doping dye combinations under 300-nm UV illumination. Dye doping ratio (in order): 1:0:0, 0:1:0, 1:0:1, 4:1.5:3, 0.5:0.5:0.5, 2:2:2, 0:1:1, 0.5:0.5:4. (reproduced from Ref. [46], with permission from ACS Publishing Company).

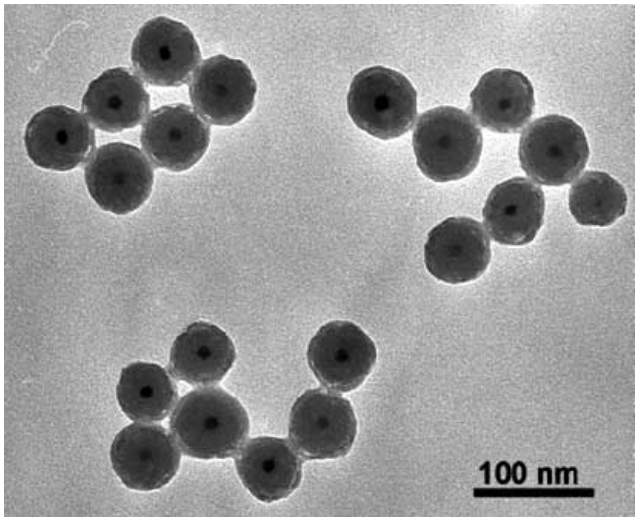

Fig. (7). Transmission electron microscopy image of SPIO@SiO2(FITC) with an average overall size of 50 nm and 10 nm SPIO core. (reproduced from Ref. [49], with permission from ACS Publishing Company).

delivery, both at cellular and sub-cellular levels [52]. Surface modification of colloidal carrier systems allows them to selectively extravasate in pathological sites, such as tumors or inflamed regions with a leaky vasculature. As mentioned above, inorganic nanoparticles (e.g., silica nanoparticles) provide a new and promising aspects as drug/gene transfer vehicles. They have the potential to overcome systemic barriers to build drug/gene through appropriate surface modification, and in thin way the drug/gene targeting becomes possible. Silica nanoparticles can be tailored with a variety of surface modifiers, allowing to adjust properties like zeta potential and surface reactivity. On the other hand, the aggregation and non-specific binding could be also reduced through surface modification of silica nanoparticles [53]. Thus, additional surface modifications greatly extend the utility of silica nanoparticles in drug/gene or nano-probe delivery. There are many methods for coupling ligands to silica nanoparticles. The most widely used surface modification is to couple ligands to silica nanoparticles by polyethylene glycol as spacer. Targeting ligands include any molecule that recognizes and binds to target antigens or receptors overexpressed or selectively expressed by particular cells or tissue components. Except for surface-modified silica nanoparticles as the targeted drug/gene delivery system in disease therapy, there is another promising and significant application for molecular detection or imaging in the recent years [54, 55]. It was reported that fluorescent silica core-shell nanoparticles modified with anti-HER2 antibody using the bifunctional cross-linker glutaraldehyde targeted the corresponding tumor antigen in the cell surface of the SKOV3 ovarian cancer cells [56]. The high binding efficiency between surface-functionalized nanoparticles and cells suggested that it was a good alternative method for the detection of human ovarian tumor. Folic acid (folate) can also be covalently attached to the amine-modified fluorescent silica nanoparticles by a carbodiimide coupling reaction [57]. This nanoparticle would target to bind the folate receptors, and could detect certain malignancies (metastatic adenocarcinoma, pituitary adenoma and others), which over-expressed folate receptors. Summarily, these studies demonstrated that surface functionalization and incorporation of a variety of bio-targeting molecules, combined with their observed non-cytotoxicity, make these fluorescent silica nanoparticles potential candidates as efficient probes for optical bio-imaging, both *in vitro* and *in vivo*.

CONCLUDING REMARKS

Nanotechnology is expected to contribute to molecular strategies in the diagnosis and treatment of various human diseases. Of particular, interests are bioengineered nanoparticles, which can be utilized as transport vehicles of diagnostic or therapeutic agents. However, further development is required before nanotechnology can be applied clinically. The challenge has been on three fronts: finding the proper target for a particular disease state; finding a drug that effectively treats this disease; and finding a means of carrying the drug or dye molecules in a stable form to specific sites, while avoiding the immunogenic and nonspecific interactions that efficiently clear foreign material from the body [3].

Although many of the well-established degradable and nondegradable controlled-release vehicles are being investigated for their processing into nanocarriers, multifunctional silica nanoparticles as a new emerging candidate is being studied for their controlled-release properties and effective labeling. On the other hand, there is still a limitation that silica nanoparticles are not degradable compared with other nanomaterials, for instance, PLGA. Thus, the metabolic processes and uptake mechanisms of multifunctional silica nanoparticles are still poor understood *in vivo,* although some primary investigations demonstrated that silica nanoparticles could be eliminated by macrophages. Even then, integration of the silica nano-controlled-release systems ("magic bullets") and specific nano-probes with other desirable functions to create new, cross-discipline applications can be realized in the future.

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ABBREVIATIONS

FITC = fluorescein isothiocyanate

MPS NPs = organosilica nanoparticles made of 3-mercaptopropyltrimethoxysilane

ORMOSIL = organically modified silica

PLGA = poly (D,L-lactide-*co*-glycolide)

anti-ODNs = antisense oligonucleotides

HPPH = 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide

MSNs = mesoporous silica nanoparticles

SPIO = superparamagnetic iron oxide

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