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Luminescent Silica Nanoparticles for cancer diagnosis

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Abstract

Fluorescence imaging techniques are becoming essential in preclinical investigations, and the research of suitable tools for in vivo measurements is gaining more and more importance and attention. Nanotechnology entered the field to try to find solutions for many limitation at the state of the art, and luminescent nanoparticles (NPs) are one of the most promising materials proposed for future diagnostic implementation. NPs constitute also a versatile platform that can allow facile multi-functionalization to perform multimodal imaging or theranostic (simultaneous diagnosis and therapy).

In this contribution we have focussed our attention only on dye doped silica or silica-based NPs conjugated with targeting moieties to enable specific cancer cells imaging and differentiation, even if also a few non targeted systems have been cited and discussed for completeness. We have summarized common synthetic approaches to these materials and then surveyed the most recent imaging applications of silica-based nanoparticles in cancer. The field of theranostic is so important and stimulating that, even if it is not the central topic of this paper, we have included some significant examples. We have then concluded with short hints on systems already in clinical trials and examples of specific applications in children tumours.

This review tries to describe and discuss, through focussed examples, the great potentialities of these materials in the medical field, with the aim to encourage further research to implement applications that are still rare.

Keywords

Diagnostics; Drug delivery; Fluorescence; Imaging; Luminescence; PEG; Silica Nanoparticles; Theranostics

1. INTRODUCTION

Early diagnosis and treatment of cancer are essential to minimize morbidity and mortality. Accurate identification of even sub-millimetric masses by imaging has important implications for disease staging, prognosis, and clinical outcome. Over the last decade, more than 1400 protein markers have been proposed as potential cancer diagnostics to the FDA, and the FDA has only approved 14 since 2001. This disappointing result is largely explained by the fact that a single molecule is insufficient to account for the complex events occurring during cancer onset and progression. It is evident that we need much more global, integrated information to develop a really effective innovation in cancer diagnostics. The advent of high-throughput technologies is providing complex cancer signatures rather than just single markers. In turn, these signatures can be exploited for the set up of barcode-targeted approaches, in association with state-of-the art delivery and detection systems.

Nanotechnology is now joining medicine to aid to overcome persisting limitations of current cancer treatments, by offering more effective, safer and more affordable diagnostic and therapeutic approaches. Of all the various possibilities, we believe that silica-based luminescent nanoparticles (SNPs) can offer, in this context, very appealing solutions. The choice of dealing with luminescent material is due to the fact that luminescence measurements are usually very sensitive (even single molecule detection is possible), easily performed, and versatile, offering submicron spatial resolution and sub millisecond temporal resolution.¹ This allows, in general, low detection limits (and thus, early diagnosis), and could permit the track down of biological events, opening up the comprehension of the origin and growth of different pathologies, including cancer. Compared to Magnetic Resonance Imaging, optical imaging is much easier to use and cost-effective, and can be applied to cancer diagnosis with high resolution.² This could take to direct visualization of tumour tissues in real-time during the surgical process, providing a direct guidance to surgeons for effective and complete tumour resection.³ Finally, since optical fibres are currently used in the investigation of some specific tumours (as colorectal cancer), NIR imaging can be easily and straight introduced into the clinical practice.⁴

There are different nanocolloids which may, in many cases, offer a valuable alternative to SNPs, such as dye-doped latex nanoparticles⁵⁻⁷ or intrinsically luminescent nanomaterials, such as the so called quantum dots (QDs).^{3,8,9} However, to our opinion, silica is the one capable to offer all the required features for diagnostic and theranostic applications in the most effective way.^{3,8,10-14} Silica is, in fact, intrinsically not toxic, although more in-depth investigations are under way to completely rule out possible hazards related to the tiny dimensions of nanoparticles. Preliminary experiments point in favour of their benign nature even supporting their use for in vivo imaging and therapy.^{3,4,8,11} Silica nanoparticles constitute a robust domain also under different external stimuli, providing to the loaded species increased mechanical strength and chemical stability, protection against enzymatic degradation, an enhanced resistance to photobleaching and an almost constant environment in chemical terms.^{15,16} Since silica is photophysically inert, it is transparent to visible light and it is not involved in energy- and electron-transfer processes that may quench the luminescence of the dyes linked to the matrix. On the other hand, each silica nanoparticle can contain many active species, and this can induce collective effects to be advantageously

used for increasing the brightness and, in general, the signal-to-noise ratio. Moreover, as it will be described below, the great versatility offered by the different synthetic strategies, typically requiring inexpensive reagents and mild conditions, is an extremely valuable characteristic since it opens up the possibility to adapt these materials for a large number of applications.^{4,11}

While some nanomedicine-based drug/dye delivery systems have already been marketed and others are in clinical trial, most still remain in the stage of preclinical development. Mesoporous silica nanoparticles have been highlighted as an interesting drug delivery platform, due to their flexibility and high drug load potential, and have therefore been extensively investigated as delivery systems. In spite of a considerable interest in the biomedical applications of such nanoparticles, the knowledge of their *in vivo* biocompatibility, toxicity and bio-distribution remains limited, which has delayed the onset of human clinical trials.

2. SYNTHESIS OF LUMINESCENT SILICA NANOPARTICLES

Synthetic methods in solution to obtain luminescent silica nanoparticles are characterized by many common characteristics: simplicity, low costs, versatility, and control over the morphology of the resulting materials. They are variation of sol-gel processes that share the common feature of the silica formation by means of the controlled hydrolysis and condensation of tetraethoxysilane (TEOS). This fundamental process can be driven to nanoparticles formation by a proper choice of parameters, that mainly are acid-base catalytic conditions and the nanoparticle nucleation-growth confinement. These main synthetic approaches are the so called Stöber-Van Blaaderen, reverse microemulsions (also called water in oil)^{17,18} and direct micelles assisted methods.^{3,18,19} In most cases they need the use of trialkoxysilane derivatized dyes,^{20,21} to ensures the covalent incorporation of the fluorophores, to avoid dye leaching in the external environment. Every strategy presents its own strengths and limitations, that contribute in a synergistic way to define the overall efficiency of the synthetic method toward a particular range of applications. In Table 1 the principal features of these synthetic strategies are summarized and qualitatively rated.

The overall synthesis efficiency is, in general, evaluated averaging several contributions, that when diagnostic applications are concerned, deal mainly with the brightness of the system (dyes incorporation), the surface modification (targeting, colloidal stability and behaviour in biological environments) and the dimensional range. In complex architectures, in fact, the spatial control over nanoparticle components is often a mandatory prerequisite to perform specific functions.

2.1 STÖBER METHOD

With this method the TEOS hydrolysis and nanoparticles formation is promoted by ammonia in an ethanol-water mixture. The accessible dimensional range is quite large (10 to several hundred of nm, with one pot procedures) and can be controlled varying concentrations and ratios of the components in the reaction mixture (TEOS, water and ammonia). An interesting variation of the Stöber method, especially for the development of very tiny nanoparticles ($d \approx 10$ nm), and of multiple-shell monodisperse nanoparticles,

employs amino acids as hydrolytic catalyst and a phase transfer approach to modulate the TEOS precursor supply into the nanoparticles growing medium.²² Generally the obtained nanoparticles have very large negative Z-potential values due to deprotonated Si-OH groups on the surface that strongly stabilized the resulting colloidal suspension by electrostatic repulsion. Further functionalization with additional alkoxysilane components is still possible for many applications, even if with very small particles, aggregation problems and incomplete passivation of the surface can make this process quite tricky. Dyes incorporation is usually obtained by introduction of alkoxysilane derivatized dyes. The number of molecular dyes usable is quite large, even if few limitations can arise from scarce solubility in the reaction milieu. Purification processes may include precipitation-centrifugation, ultrafiltration, dialysis, and more sophisticated instrumental techniques such as FIFFF (Flow Field Flow Fractionation).²³ These last three purification methods stand out for mildness and contribute to preserve the nanoparticles monodispersity.

2.2 REVERSE MICROEMULSION METHOD

The reverse microemulsion (also named water in oil) methods are basically Stöber processes that are carried out in a macroscopically isotropic dispersion of a surfactant and water in an hydrocarbon. The aqueous nuclei of the reverse microemulsion act as nano-vessels where the hydrolysis and condensation processes are confined to form the nanoparticles. Several synthetic protocols are able to give quite simple access to nanoparticles synthesis and surface modification in the dimensional range of about 15–200 nm,^{18,24} that mainly depend upon surfactant type and by the surfactant to water molar ratio.²⁵ This method is particularly versatile in preparing nanoparticles with a layer by layer structure, to develop hybrid structures incorporating different kinds of nanoparticles (metals, QDs, magnetic, etc.) and also for surface modification with chemical functionalities to impart stabilization or to allow bio-molecules targeting insertion. All these modifications can be in fact obtained by subsequent additions of reactants within the microemulsion, without intermediate purification steps. In general the method has a very good applicability toward positively charged water soluble dyes, that in principle can be incorporated and retained in the nanoparticle silica matrix by strong electrostatic interactions, but it has strong limitations in incorporating poorly water soluble fluorophores or negatively charged ones. Final purification steps are always needed to separate nanoparticles from the large amount of surfactant and organic solvent that constitute the reverse microemulsion. These work-up procedures are usually constituted by subsequent centrifugations-sonication steps, that can bring to irreversible aggregation if unsuitable surface modifications or work-up conditions are adopted.

2.3 DIRECT MICELLES ASSISTED METHODS

The possibility to use direct aggregates of surfactants in water is the most recently proposed method between the ones described so far. The strategy is to confine the nanoparticle formation within aggregates or co-aggregate, exploiting the accumulation of silica precursors such as TEOS and organo-alkoxysilane that have a lipophilic nature before and during the first hydrolysis and condensation steps. These methods usually provide very mono-disperse nanoparticles in water, with a dimensional range of 10–60 nm, features that make them versatile and reliable strategies for the development of luminescent colloids for

in-vivo and *in-vitro* applications. The use of water as solvent and of far lower surfactant (co-surfactant) quantities in comparison with the ones necessary in the reverse microemulsions strategies represent the major strengths of this approach. Within the direct micelle assisted synthetic routes, the main differences reside on the molecular weight of the surfactant that is involved in the nanoparticles formation.

Following this approach Prasad and co-workers developed many systems in the 20–30 nm dimensional range based on *ORMOSIL* nanoparticles (*Organic - Modified - Silica*), obtained using oil in water systems usually made by surfactant / 1-butanol / DMSO / water, mixtures. The low molecular weight surfactants normally used are AOT (Bis(2-ethylhexyl) sulfosuccinate sodium salt) or Tween 80, while the lipophilic silica precursor is VTES (triethoxyvinylsilane), which hydrolysis is promoted by addition of APTES (3-aminopropyltriethoxysilane) or ammonia. This kind of nanoparticles have a certain degree of mesoporosity so that covalent condensation of the doping agents is needed to avoid leaching from the particle.²⁶ With this strategy, surface functionalization ($-\text{NH}_2$, $-\text{COOH}$, $-\text{SH}$ also together with PEG chains)²⁷ is ready accessible and allows for conjugation with bioactive molecules.^{28–30} The incorporation of dyes,³¹ PDT agents,^{32,33} and the possibility to obtain hybrid structures containing QDs and Fe_3O_4 nanoparticles³⁴ make the *ORMOSIL* particles as multimodal imaging and therapeutic agents.

Another possibility is to use this general strategy with high molecular weight surfactants to form micellar aggregates. The main consequence is that thanks to entrapment-adsorption phenomena, the surfactant molecules can be irreversibly bound to the silica matrix, becoming part of the whole nanoparticle structure in a one-pot procedure. Suitable surfactants are for example tri-block A-B-A copolymers, such as commercial Pluronic surfactants, bearing a PEG-PPO-PEG (poly(ethylene glycol)-poly(propylene oxide)-poly(ethylene glycol)) structure. Using micelles of Pluronic F127 (MW 12.6 KDa), triethoxysilane-derivatized dyes and TEOS in an acidic environment we have recently proposed a synthetic strategy^{35,36} useful to obtain ordered luminescent core-shell silica-PEG nanoparticles with very high monodispersity and colloidal stability in water, bearing a silica core of about 10 nm and an overall hydrodynamic diameter (PEG shell) of about 25 nm. This last feature, deriving from the entrapped Pluronic F127, together with the non-toxic properties of Pluronic F127 are valuable characteristics able to confer stealth properties in biological environments.³⁷ This synthetic method stands out for the vast variety of trialkoxysilane derivatized dyes that can be included in the silica core, independently by their solubility properties. These core-shell silica-PEG nanoparticles can act as labels, thank to the possibility to modify the Pluronic F127 hydroxy end groups. These modifications are carried out in the pristine Pluronic 127 surfactant, then used in the nanoparticles synthesis in the desired amount, so that no post nanoparticle functionalization is needed to introduce chemical functionalities ($-\text{COOH}$, $-\text{NH}_2$, $-\text{SH}$, $-\text{N}_3$, alkynes, etc.) for targeting and bio-molecules conjugation.^{38–40}

3. IMAGING WITH SILICA-BASED NANOPARTICLES IN CANCER

A plethora of novel silica-based nano-objects for cancer imaging are being developed that combine (i) different detection approaches, e.g. fluorescence, MRI, Raman spectroscopy

and, often, (ii) therapeutic agents, with the possibility of achieving a selective targeting of tumors through the functionalization by tumor-specific ligands, e.g. antibodies, proteins, synthetic peptides.

3.1 UNTARGETED IMAGING

The simplest nanoparticles are for diagnostic use only and non-targeted, being particularly designed to improve their optical/magnetic properties while optimizing their geometry. A huge literature about non-targeted NPs is available and only some representative examples will be discussed in this review while targeted systems will be discussed, in the next section, in a more exhaustive way.^{41–54} In a first example, fluorescent conjugated polymers and superparamagnetic iron oxide nanocrystals have been integrated into silica nanocapsules, to obtain uniform and stable nanosystems with dual-mode (fluorescence and MRI) functionality. Different polymers were investigated, demonstrating the versatility of these materials; the corresponding nanoparticles were investigated *in vitro* for their capability to identify cultured human liver cancer cells in dual mode imaging. The super-paramagnetic behavior of these particles was further exploited for their magnetic guidance into cancer cells.⁵⁵ A similar dual modal nanoprobe, combining optical and MR imaging was produced as a 16 nm-silica carbonate nanoparticle simultaneously doped with a rare earth ion (Terbium) and Gadolinium. *In vitro*, these nanoparticles were efficiently delivered to different cancer cell lines, e.g. SGC7901 gastric cancer cells and NCI-H460 lung cancer cells, without causing any toxic effect at the experimental doses.^{56,57} The application of such nanomaterials to preclinical models is the successive step toward possible clinical translation. A PET/MRI silica nanoprobe with an enhanced near infrared fluorescence signal was obtained by encapsulating a near infrared dye (NIR797) into magnetic silica nanoparticles labeled with Gallium 68 as a radiotracer. When injected into the forepaw of mice, they demonstrated high fluorescent intensity and stability, providing multimodal *in vivo* imaging of sentinel lymph node.⁵⁷

3.2 UNTARGETED THERANOSTIC

Most untargeted systems, however, exhibit a further level of complexity by associating a therapeutic agent (e.g. a chemotherapeutic drug) and/or approach (e.g. a photosensitizer for photothermal therapy) to the imaging probe. Mesoporous silica-coated gold nanorods have recently been developed to combine two-photon imaging of cancer cells with the delivery and laser-triggered release of a chemotherapeutic drug, i.e. doxorubicin (by low power density), associated with laser-induced hyperthermia (by high power density) *in vitro*.⁵⁸ Other high technology-based approaches associate (i) fluorescence-surface-enhanced Raman scattering of SERS tags obtained by coupling silver nanoparticles (as optical enhancers) with 4-mercaptopyridine (a reporter molecule) in the silica shell, with (ii) a fluorescent dye (flavin mononucleotide) and (iii) doxorubicin both loaded into an external coating made of mesoporous titania. The high performance and low toxicity of the deriving nanoparticles were assayed *in vitro* on MCF-7 human breast cancer cells.⁵⁹ Being hydrophilic and therefore easily embeddable in silica-based materials, doxorubicin has been exploited as an exemplary anti-cancer compound for a number of *in vivo* applications as well. Hollow mesoporous silica nanoparticles were multi-functionalized with manganese oxide, a sensor for acidic conditions, to obtain a pH responsive MRI agent that detects and responds to the

acidic environment of cancer tissues. The relaxation rate of such a compound was proven to increase 11-fold compared to the neutral condition, and is almost 2-fold higher than commercial Gd(III)-based agents, this was integrated with the ultrasonographic function deriving on the hollow structures of the nanoparticles. The same mesopores and hollows could also include doxorubicin, which was delivered intracellularly, bypassing the multidrug resistance of cancer cells and therefore attaining strong *in vitro* anti-proliferative efficacy.⁶⁰

The same research group recently published another multimodal nanostructure based on magnetic core/mesoporous silica shell nanoellipsoids coated by a uniform layer of gold nanorods. The latter allows to superimpose to the MRI detection both photo-thermotherapy and optical imaging; furthermore, doxorubicin was electrostatically associated to the negatively charged surface of the nanoellipsoids, in a reversible way, depending on the redox state of the microenvironment. A synergism between photo-thermal and drug therapy was demonstrated at a moderate power intensity at least *in vitro*, paving the way for *in vivo* applications.⁶¹ Other core-shell structured silica-based nanomaterials have been recently described that couple MRI and fluorescence/luminescence imaging with PDT. For these purposes, a photosensitizer (hematoporphyrin and silicon phthalocyanine dihydroxide) was covalently grafted onto a silica shell grown on a 10 nm core of rare-earth oxide (Ytterbium, Gadolinium, Erbium) nanophosphors. Under excitation at 980 nm, this nanomaterial gave luminescence bands at 550 nm and 660 nm: one peak could be used for fluorescence imaging and the other was suitable for the absorption of the photosensitizer to generate singlet oxygen for killing HeLa cancer cells *in vitro*. On the other side, because of the presence of Gadolinium(III), the inner core proved to act as a good contrast agent, with efficiency comparable to clinical commercial compounds.⁶² Photothermal agents have also been employed in the design of nonplasmonic multi-dye silica nanoparticles, in which a modified near infrared fluorescent heptamethine cyanine dye is embedded into a mesoporous silica matrix, and a metallo-naphthalocyanine dye is loaded into the pores of these particles.

The imaging and therapeutic capabilities of these nanoparticles were investigated *in vivo* in a direct tumor injection model. These near infrared fluorescent nanoprobes were extremely brilliant (300-fold enhancement in quantum yield *versus* free dye). Further, exposure to laser excitation of the naphthalocyanine dye led to a temperature increase of the surrounding environment. As a consequence, tumours co-injected with the nanoparticles were easily visualized, and significantly elevated levels of tumour necrosis (95 %) could be induced upon photothermal ablation.⁶³

3.3 TARGETED IMAGING

A different level of complexity, and a net improvement in nanoprobe specificity for the diseased tissues is achieved by surface-functionalization of the cognate nanoparticles by specific targeting moieties. We recently translated a similar approach *in vivo*, in a mouse model of human metastatic colorectal cancer. For this purpose, we designed a high-performance imaging platform based on silica-PEG nanoparticles doped with rhodamine B and cyanine 5, with the possibility of obtaining a dual-color simultaneous detection as a basis for background subtraction and signal amplification, thus providing high-sensitivity

imaging. The PEG tails on the external face of the nanoparticles were functionalized with metastasis specific peptides (i.e., H₂N-CGIYRLRSC-COOH and H₂N-CGVYSLRSC-COOH), identified in our laboratory by phage display screenings on human biopsies of liver metastases.⁶⁴ The deriving nanoprobe selectively homed to and accumulated at target tissues, resulting in specific visualization even of submillimetric metastatic *foci*.⁴⁰ Other examples of silica nanoparticles containing more than one fluorophoric species are present in literature to allow the simultaneous imaging of multiple targets. In particular W. Tan and co-authors⁶⁵ proposed the use of fluorescence resonance energy transfer (FRET) NPs conjugated with aptamers to perform multiplexed cancer cell monitoring. Three different dyes (a fluorescein and two rhodamines) were used to prepare single-, dual- and triple-dye doped silica nanoparticles with different doping ratios, allowing the tuning of the FRET dependent emission signature of these materials that consequently present different colors under the same excitation (λ_{ex} =488 nm). The covalent surface-modification with a polyethylene glycol (PEG) linker terminating with biotin allowed the decoration of the NPs with an external layer of neutravidin. This decreases the inherent nonspecific adsorption onto the cell surface of these materials, and it allows to conveniently immobilize the aptamers of interest by introducing a biotin group in the aptamer sequence. Three specific aptamers for three different cancer cell lines were selected: sgc8 aptamer for CEM cells (which are human acute lymphoblastic leukemia cells); TDO5 aptamer against Ramos cells (which are human Burkitt's lymphoma cells), and T1 aptamer for Toledo cells (which represent a type of human diffuse large cell lymphoma). The simultaneous and sensitive imaging of multiple cancer cell targets was achieved. The same kind of NPs, but singularly doped with FITC or Ru(bpy)₃²⁺, were used to conjugate a series of aptamers specific for CEM, Ramos, HeLa and HL-60 and proved to be able to enhance conventional flow cytometry sensitivity the detection of these cancer cells.⁶⁶

The same authors have also proposed antibody-conjugated dye doped silica nanoparticles: in this case the derivatization is covalent and directly on the surface of the particles. They have proved to be valuable means to label *in vitro* cultured cancer cell lines through antigen-antibody recognition. In the case of MGC-803 gastric cancer cells a double-labeling process was followed thanks to FITC doped anti-CEA antibody-conjugated and Ru(bpy)₃²⁺ doped anti-CK19 antibody-conjugated fluorescent silica nanoparticles.⁶⁷ *In-vitro* and *ex-vivo* experiments incubating the cells with the mixture of the two NPs were imaged using confocal laser scanning microscopy: the green and red signals were simultaneously obtained without crossreactivity by confocal laser scanning microscopy imaging. On the other side improved class of Cy5 doped core-shell NPs were obtained employing positive biomolecules such as IgG or polysine conjugated Cy5 as the core material and silica coating it. After conjugation with C-erb-B2 antibody they have been successfully applied to recognize the breast cancer cells through confocal microscopy with high photostability.⁶⁸

Other silica NPs obtained with different synthetic methods were proposed for breast cancer cells targeting such as Nile Red doped small (diameter circa 35 nm) ORMOSIL NPs⁶⁹ (see paragraph 2.3), quite large (diameter circa 100 nm) FITC doped mesoporous ones,⁷⁰ and very large (diameter more than 400 nm) Stöber like ruthenium phenanthroline doped NPs⁷¹ or smaller (diameter ~ 45 nm) Ru(bpy)₃²⁺ doped ones.⁷²

All these species were conjugated with bio-molecules such as apo-transferrin or folic acid, as in the first case, or with the monoclonal antibody anti-HER2/neu or the anti-EGFR antibody, as in the last case, in order to specifically target breast cancer cells. Fluorescence microscopy, immunoassay and flow cytometry data evidenced that all the labeling systems, despite their differences (size, morphology, functionalization) were successfully uptaken by the cells and could selectively target cancer ones. Moreover, ORMOSIL NPs due to their small diameter could in principle be use also for *in vivo* imaging, and to this aim, the authors also prepared PEG-transferrin conjugated derivatives suitable for future *in vivo* animal experiments.

Another system based on small (20 nm) ORMOSIL NPs is the one presented by N. Prasad and co-workers to target pancreatic cancer cells (Mia-PaCa cells).²⁸ The particles covalently incorporate rhodamine-B and are externally conjugated with different target molecules such as transferring or monoclonal antibodies (anti-claudin 4 and anti-mesothelin). The surface of the NPs can be easily functionalized with different active moieties (amines, carboxylates, etc.) and the cell uptake was carefully evaluated and compared for all surface variations considered using confocal microscope. It resulted extremely poor in the case of on targeted but generically activated species or for normal healthy cells, while significant for Mia-PaCa cells treated with antibody conjugated NPs, this confirming its receptor-mediated nature. Similar results of specificity were obtained for slightly bigger (diameter 75 nm) still rhodamine-B silica probes but modified with anti-HER2 antibody to target ovarian tumor cells⁷³ or with anti-CEA antibody, a tumor marker now routinely used as part of annual medical checkups,⁷⁴ and also with Ru(bpy)₃²⁺ doped galactose-conjugated silica probes able to specifically identify liver cancer cells from blood cells via confocal microscopy.

In the latter case, cell differentiation was proved also via flow cytometry analysis of a mixed heterogeneous cell system and the identification of even very few target cells was successfully registered.⁷⁵ Ru(bpy)₃²⁺ doped particles and mixed cell systems were also exploited by Tao, L. et al.⁷⁶ to prove the selective detection of colon cancer cells via fluorescent silica nanoparticles conjugated on surface with anti-human epithelial cell adhesion molecule (EpCAM) antibody. Confocal microscopy analysis showed that Three kinds of colo205 target colon cancer cells were distinguished from sw480 (EpCAM-deficient human colon cells) and NCM460 (normal human colon cells). This targeting system also allow the visualization of the distribution and abundance of EpCAM in cells membrane, and it provides a possibility to quantify special membrane proteins on different regions of the surface of the cell. Very similar NPs still Ru(bpy)₃²⁺ doped but anti-IL-6 conjugated were also reported as labels for sensitive immunoassay of protein biomarkers.⁷⁷

The protein microarray format with a fluorescence scanner proved to have a detection limit for IL-6, proposed as a model analyte, down to 0.1 ng/mL and a linear response till 10 ng/mL. The absence of cross reactivity with co-existing proteins, the versatility and photostability of the nanoprobe indicate a good potential of these materials also in this technique for clinical diagnosis applications.

Other tumor-targeting functionalizations are instead obtained by a covalent linking of natural molecules on the surface of the nanoparticles. Among these, a number of research

groups exploited the high affinity of folate for its receptor, a protein upregulated on the surface of different cancer cells. A simple example is provided by silica nanoparticles doped with fluorescein isothiocyanate, which were PEG-conjugated to folate, thus forming folate receptor-targeted fluorescent nanoprobes.

The quantitative analysis of their cellular internalization *in vitro* demonstrated that the delivery efficiency in the KB cell line (human oral carcinoma, folate receptor-positive cells) is >6-fold higher than that of A549 cells (human lung carcinoma, folate receptor-negative cells), and that this delivery is highly specific, being competed by the addition of free folate.⁷⁸

Targeting tumors through the folate/folate receptor pair proved to be efficient also *in vivo*. In this case, a two-photon absorbing and aggregation-enhanced near-infrared emitting pyran derivative was encapsulated in silica nanoparticles, providing a bioimaging that overcomes the fluorescence quenching associated with high chromophore loading.

The surface of these nanoparticles, again, was functionalized with folic acid. *In vitro* studies confirmed their uptake by HeLa human cervix cancer cells; most importantly, following intravenous injection they showed specific homing to HeLa tumor xenografts in mice, extravasation from the leaky tumor vessels and internalization by the tumor cells. Two-photon fluorescence microscopy bioimaging provided a 3D tumor imaging up to 350 μm deep.⁷⁹

3.3.1 TARGETED MULTIMODAL IMAGING—Indeed, a selective targeting of malignant cells promises to mitigate side effects of conventional chemotherapy and to enable delivery of the unique drug combinations needed for personalized medicine. Porous silica nanoparticle-supported lipid bilayers were modified with the targeting peptide H₂N-SFSIILTPILPLGGC-COOH (identified via filamentous phage display) that binds to human hepatocellular carcinoma cells with a 10,000-fold greater affinity than for either hepatocytes, endothelial or immune cells. These hybrid liposome-silica nanoparticles were provided as versatile carriers to be loaded with combinations of therapeutic and diagnostic agents and modified to achieve endosomal escape and nuclear accumulation.⁸⁰ Other targeted MRI contrast agents were designed with a core of Fe₃O₄ and a fluorescent SiO₂ shell, obtaining 47.0 ± 4.0 nm nanoparticles that were grafted with hyperbranched polyglycerol and conjugated with folic acid.

Parallel MRI and fluorescence microscopy analyses showed significant preferential uptake of these targeted nanoparticles by human ovarian carcinoma cells (SKOV-3), in comparison with macrophages and fibroblasts, suggesting a potential use for real-time imaging in ovarian cancer resection.⁸¹ Other targeting molecules are presently being explored with various specificity. Mesoporous silica nanospheres for MRI were synthesized by covalently linking Gadolinium(III) chelates via a redox-responsive disulfide moiety. These nanospheres were further functionalized with PEG and an anisamide ligand as a targeting moiety. The effectiveness of the deriving probes was evaluated *in vitro* in human cell lines from colon adenocarcinoma and pancreatic cancer. A 3T scanner was used for preliminary *in vivo* MRI studies, demonstrating that the Gadolinium(III) chelate is rapidly cleaved by the blood pool

thiols and eliminated through the renal excretion pathway. Therefore, for translation into clinical imaging, an improved tuning of the chelate release kinetics will be needed to use the tumor-targeted nanoparticles as new MRI contrast agents.⁸² Antibodies against transmembrane receptors overexpressed in cancer cells are also proving to be useful targeting moieties. In a first example, magneto-fluorescent silica nanoparticles, based on a ferrite core covered by silica-PEG/rhodamine B, were conjugated with the clinical antibody cetuximab, for the targeting and imaging of colon cancers that overexpress the epidermal growth factor receptor. *In vivo*, the cetuximab-conjugated magneto-fluorescent nanoparticles specifically targeted HCT-116 human colon carcinoma cell subcutaneous xenografts, allowing a detection of specific fluorescence. In parallel, significant MRI signal changes were observed in the same mouse model.⁸³ In a second example, nanoprobe constituted by a core of Fe₃O₄ (for MRI) and by multiple silica layers embedding visible-fluorescent quantum dots (600 nm emission) and near infrared-fluorescent quantum dots (780 nm emission), were surface-conjugated with an anti-HER2 (human epidermal growth factor receptor 2) antibody. *In vitro*, these multimodal nanoparticles bound with high specificity to KPL-4 human breast cancer cells. *In vivo*, the near-infrared fluorescence imaging and the T(2)-weighted magnetic resonance of KPL-4 xenografts in mice were explored, demonstrating that breast tumors can be successfully identified by fluorescence.⁸⁴ For similar applications, Raman-silica-gold-nanoparticles were designed, and the acute toxicity and biodistribution of their cognate PEGylated forms was evaluated following different routes of administration in mice. After intravenous administration, these nanoparticles were removed from the circulation by macrophages in the liver and spleen (that is, the reticulo-endothelial system). At 24 hours, they elicited a mild inflammatory response and an increase in oxidative stress in the liver, which subsided by 2 weeks after administration. No evidence of significant toxicity was observed by measuring clinical, histological, biochemical, or cardiovascular parameters for 2 weeks. With the aim of designing targeted nanoparticles, e.g. labeled with an antibody that binds specifically to the epidermal growth factor receptor, to detect colorectal cancer, their local toxicity was tested as well, confirming no significant bowel or systemic toxicity, and no systemic detection.⁸⁵ Using a different approach, a smart targeting sensor for the tumor microenvironment was designed to be activatable in the presence of tumor metalloproteinase 2, through a specific enzyme substrate bioconjugated with cyanine 5.5 as the external functionalization of iron oxide nanoparticles with thin silica-PEG coating. In this structure, the surface fluorescence was quenched with an efficiency of 97.2%. The presence of metalloproteinase 2 in the medium caused a specific cleavage of the dye-conjugated peptide, thus switching the fluorescence on. *In vivo*, tumor xenografts obtained by subcutaneous injection of SCC7 human squamous cell carcinoma cells were successfully imaged by both MRI and near infrared fluorescence, following intravenous injection of the targeted nanoparticles. Both signals gradually increased up to 12 h post injection; at this time, the intensity of tumor fluorescence was 3–4 times higher than that in normal tissue, and the maximum difference in the magnetic resonance was 34% between tumor tissue and healthy muscle. The addition of a metalloproteinase inhibitor caused a significant lowering of the fluorescence signals, demonstrating the specificity of this system.⁸⁶

H. Y. Tsai et al.⁸⁷ developed a sandwich immunoassay detection system based on labeled nanoparticles. To demonstrate the validity of their approach this methodology was applied to carcinoembryogenic antigen (CEA) detection in serum. The immunoassay consists of magnetic NPs (80 nm) and silica rhodamine 6G (R6G) doped NPs (180 nm) both labelled with anti-CEA, to obtain a colloidal assembly mediated by the anti-CEA/CEA/anti-CEA interactions. This methodology was faster respect to related ELISA protocols, and thanks to the huge NPs surface available for the CEA recognition processes together with the enhanced fluorescent signal and the separation-purification capabilities induced by the magnetic NPs allowed a CEA detection limit of 1.8 pg/mL, with an very extended linear range (18 ng/mL to 1.8 pg/mL).

Wan, J. et al.⁸⁸ developed nanoprobe with a magnetic core and silica shell with fluorescent and optical contrast capabilities. This system was conjugated to the NPs surface with chlorotoxin, a targeting ligand for the specific targeting of human U251-MG glioma cells via receptor-mediated endocytosis. This recognition process was monitored by MRI and also by confocal microscopy. Yang, H. et al.⁸⁹ synthesized bimodal nano-probes (MRI, fluorescent) made by amino functionalized silica-coated manganese oxide NPs (d = 35 nm), that were then conjugate to rhodamine B isothiocyanate dye and targeted to folate moieties.

Several techniques (flow cytometry, confocal microscopy, and magnetic resonance imaging) showed that this nanocomposites can specifically target cancer cells over expressing folate receptors.

Hwang, D. W. et al.⁹⁰ developed a multimodal cancer-targeted imaging system for in vivo fluorescence, radionuclide and MR imaging. This nanocomposite was developed starting from a commercial nanoparticles (Biterials - Seoul, Korea, d = 50 nm) comprising a cobalt-ferrite core surrounded by a rhodamine doped carboxy-functionalized silica shell. Conjugation was carried with AS1411 aptamer (MF-AS1411), having targeting capabilities toward nucleolin (a cellular membrane protein highly expressed in cancer), and finally with 2-(p-isothio-cyanatobenzyl)-1,4,7-triazacyclonane-1,4,7-triacetic acid (p-SCN-bn-NOTA) a chelating agent able to bind ⁶⁷Ga-citrate. Confocal microscopy analysis showed how these NPs can give specific fluorescence signals in nucleolin-expressing C6 cells. Radionuclide imaging was used for tumor imaging in vivo after systemic injection on mice together with fluorescence confocal microscopy. This last technique however revealed severe nonspecific liver accumulation by the mononuclear phagocytic system, even if these nanoparticles were endowed with PEG moieties on their surface to confer stealth properties and longer circulation times.

A multiply-engineered structure, endowed with magnetic, luminescent and recognition properties was developed by Mi Y. et al.⁹¹ for applications related to immunomagnetic methods or microfluidic devices for CTC (circulating tumor cells) sorting and detection. These mesoporous silica NPs loaded with polyhedral oligomeric silsesquioxanes (POSS) scaffold of hydrophobic conjugated fluorescent oligomers (COs) were then decorated on their surface by Iron oxides (IOs) nanoparticles.

The nanohybrid was finally functionalized through adsorption with the targeting ligand herceptin (Trastuzumab), able to recognize the HER2 receptor (Human Epidermal growth factor Receptor 2) overexpressed in the 25–30% of the invasive breast cancer cells. With an overall diameter of 100 nm and a Z-potential value of 12 mV, this nanosystems showed very low cytotoxicity (< 20% mortality) towards SK-BR-3 cancer cells and NIH/3T3 fibroblast cells, with an essential contribute of herceptin toward cellular uptake and selective detection.

Tan and coworkers⁹² used a novel two-nanoparticle assay to develop a method for the rapid collection and detection of leukemia cells. In order to achieve specific recognition these authors functionalized the NPs with an aptamer sequence selected using a cell-based SELEX strategy. This approach allowed specific recognition of CCRF-CEM acute leukemia cells both from complex mixtures and whole blood samples. Magnetic nanoparticles, modified with the aptamer, were used for target cell extraction, while aptamer-modified fluorescent nanoparticles were simultaneously added for sensitive cell detection. Thanks to this combination rapid, selective, and sensitive detection became possible. As demonstrated by fluorescent imaging and flow cytometry a single aptamer binding event, in fact is amplified thanks to the high brightness of the fluorescent NPs with respect to the labelling with individual dye-labeled probes. Functionalized magnetic NPs, on the other hand, allow for rapid extraction of target cells not possible with other separation methods. The same approach was followed for the detection and separation of multiple cancer cells⁹³ in combinations of CCRF-CEM cells (CCL-119 T-cell, human acute lymphoblastic leukemia), Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma), and Toledo cells (CRL-2631, non-Hodgkin's B cell lymphoma).

3.4 TARGETED THERANOSTIC

As maximum complexity level, several theranostic nanoparticles have been very recently designed that combine imaging, therapy, and targeting. In another example, mesoporous silica nanoparticles loaded with doxorubicin hydrochloride were surface-functionalized with hyaluronic acid, a specific ligand for cells overexpressing the tumor biomarker CD44 and tested *in vitro*. In this setting, specific uptake of CD44-targeted fluorescent nanoparticles by HCT-116 human colorectal cancer cells is coupled to enhanced doxorubicin-mediated cytotoxicity.⁹⁴ Similarly, carbon and Si nanocrystals, respectively encapsulated in the mesopores and within the framework of mesoporous silica nanoparticles, were bio-conjugated with a PEGylated phospholipid compound and hyaluronic acid. Such nanoparticles further embed high payload of a typical anti-cancer drug, camptothecin. The formed crystals allow unique near infrared-to-visible luminescence imaging feature, while the chemotherapeutic drug is specifically targeted to MCF-7 human breast cancer cells *in vitro*.⁹⁵ A number of reports describe nanotheranostic particles in which the targeting moiety is, again, the folate/folate receptor pair. In a first setting, a mixed layer of PEG-conjugated phospholipids is formed on the silica nanoparticle surface and folic acid is surface-linked for targeting. These nanoparticles are covalently bound to the chemotherapeutic drug docetaxel, and embed quantum dots as fluorescence imaging agents. The *in vitro* characterization was performed on MCF-7 human breast cancer cells, confirming specific fluorescence uptake and drug-mediated toxicity.⁹¹ Translation *in vivo* of such targeted nanotheranostics represents the most important preclinical step toward the clinical application, and has been

explored for a number of particle variants. Among the most recent and better characterized, mesoporous-silica-coated upconversion fluorescent nanoparticles were used as a carrier of two photosensitizers (for photodynamic therapy) and of a nanotransducer (to convert deeply penetrating near-infrared light to visible wavelengths). The multicolor-emission properties of such nanoparticles, at a single excitation wavelength for a simultaneous activation of the two photosensitizers, provides an improved photodynamic therapy. *In vivo*, this system is highly efficient when injected intratumorally in a mouse model of human melanoma, or when a folate acid-functionalized counterpart is administered intravenously into tumor-bearing mice. This is the first demonstration of a validated multimodal, targeted nanoparticle, for innovative combination of phototherapy and optical detection, serving as a platform for future noninvasive deep-cancer therapy.⁹⁶

An interesting multimodal system with multiple core-shell architecture was developed by Wang, F. et al.⁹⁷. Following a reverse microemulsion strategy a 6 nm diameter Fe₃O₄ core was coated with a FITC-APTES doped silica shell of about 17 nm. The deposition of a 9 nm second mesoporous silica layer covalently doped with the PDT agent aluminum phthalocyanine (AlC4Pc), was obtained through a CTAB modified Stöber method. These NPs were addressed as specific PDT using folic acid targeting ligands conjugated to the mesoporous silica shell, this ligand is able to recognize the over-expressed α -folate receptor in many cancer cells.

The system take advantage of the MRI and optical contrast properties endowed by the magnetic core, and of the sensitivity of fluorescence imaging, while folate receptors increase the directing specificity of the system to cancer cells, reducing the effect of PDT processes on normal tissues. The cellular uptake and the contrast effect of these NPs was evaluated on human hepatoma cells (QGY-7703) and the relative cell viabilities was measured on human hepatocyte cells (QSG-7701). The efficiency of the PDT was evaluated finally on QGY-7703 cells, and the obtained results indicated the key role of light in killing tumor cells *in vitro*.

A preferential uptake of folate targeted NPs compared to untargeted ones was measured on Human cervical carcinoma HeLa cells, and thank to this increased affinity the first system was more efficient in promoting Hela cells death by PDT.

Lu and coworkers prepared multifunctional biocompatible and water-soluble magnetic NPs for the detection of Human hepatoma 7402 and H446 lung cancer cell lines.⁹⁸ These NPs have a mesoporous core-shell structure and bear a rhodamine containing polymer chain as a labelling segment while folic acid is used as the cancer targeting moiety. Moreover the porous silica oxide structure and long molecular chains of polymethacrylic acid are suitable to efficiently host drug molecules allowing to use the nanocomposites for directional release. The loading, on the other hand, did not affect the magnetic properties of the carrier and the release of the loaded drug was observed over 100 h under *in vitro* conditions. The authors also reported that, their NPs penetrate into the cell membranes and release the anticancer drug into the cytoplasm and that they effectively target the tumor cells.

A similar multifunctional approach was followed by Lo and coworkers who reported the synthesis and application of tri-functionalized mesoporous silica NPs for use as theranostic compounds able to combine imaging, target and therapy.⁹⁹ These NPs were functionalized in a first steps with a NIR emitting dye, ATTO647N, that enable optical detection of particle targeting. was chosen in order to exploit. In a second step, an oxygen-sensing, palladium-porphyrin based photosensitizer (Pd-porphyrin; PdTPP) for photodynamic therapy was incorporated into the nanochannels of the mesoporous NPs. Finally cRGDyK peptides, were used for targeting the overexpressed $\alpha_v\beta_3$ integrins of cancer cells, and to ensure the internalization of the photosensitizer PdTPP. In vitro application of this theranostic platform was demonstrated for MCF-7 human breast cancer cells and U87-MG human glioblastoma cells.

4. SILICA-BASED NANOPARTICLES IN CLINICAL TRIALS

To be clinically successful, the next generation of nanoparticle agents should exhibit favorable targeting and clearance profiles, and be nontoxic. Developing probes that meet these criteria is challenging and requires extensive *in vivo* evaluations. Only a relatively small percentage of new imaging agents undergoing comprehensive preclinical testing potentially satisfy diagnostic criteria for translation. Indeed, although a number of fluorescent particle platforms have been investigated, only Cornell dots (C dots) have received the first FDA-approved investigational new drug approval for a first-in-human clinical trial (Jan 2011). Bradbury and coworkers¹⁰⁰ report that these dye-encapsulating ~7-nm particles, surface functionalized with the $\alpha_v\beta_3$ integrin-targeting cyclic arginine-glycine-aspartic acid (cRGDY) peptides and radioiodine, exhibit high-affinity/avidity binding, favorable tumor-to-blood residence time ratios, and enhanced tumor-selective accumulation in melanoma xenografts in mice. They further exploited the sensitive, real-time imaging of lymphatic drainage and nodal metastases obtained with this multimodal platform for staging metastatic disease in the clinical setting. The feasibility of performing intraoperative image-guided metastatic disease detection, localization, and staging was evaluated in a larger-animal (miniswine) spontaneous melanoma model, that simulates more accurately the application of sentinel lymph node biopsy procedures in humans. Mapping of sentinel lymph nodes was achieved by using portable, real-time optical camera systems in an intra-operative setting for direct visualization of the draining tumor lymphatics and fluorescent nanoparticles. This work demonstrates that C dots combine the benefits of PET (tissue penetration, quantification) with those of IR/NIR optical imaging (sensitivity/contrast, multispectral capabilities) for the development of surgically-based or interventionally-driven therapies.^{55,101} Discussions have begun with the FDA to start clinical trials using carbon nanotubes to improve colorectal cancer imaging. This imaging agent and associated is being developed by the Center for Cancer Nanotechnology Excellence Focused on Therapy Response (Stanford University CCNE). (Source: NCI Alliance for Nanotechnology in Cancer)

5. SILICA-BASED NANOPARTICLES IN CHILDREN TUMORS

An extremely small number of reports have been produced describing the application of silica-based nanotheranostics to the diagnosis and therapy of children tumors. There is

therefore an urgent medical need for innovative, nanomedicine approaches to be translated into the clinics, especially for the poor-prognosis tumors neuroblastoma and glioblastoma, and for retinoblastoma.

High-risk neuroblastoma is associated with the worst outcome in pediatric oncology, because the initial remission in response to chemotherapy is followed by a recurrence that has become refractory to further treatment. Recently, silica nanoparticle-based targeted delivery of a tumor suppressive, proapoptotic microRNA, miR-34a, to neuroblastoma tumors in a murine orthotopic xenograft model has been described. Nanoparticles were functionalized with an antibody recognizing the cancer-specific cell surface antigen disialoganglioside GD2, providing tumor-specific delivery. The specific targeting of these modular nanoparticles to tumor sites in a mouse model was demonstrated, which paralleled with significantly decreased tumor growth, increased apoptosis and a reduction in vascularisation.¹⁰²

Another silica-based multimodal nanosystem has been developed that combines imaging with photodynamic therapy of glioblastoma. To promote the vascular effects commonly associated to photodynamic therapy, nanoparticles were functionalized with a specific peptide ligand for the tumor angiogenic marker neuropilin-1. For the MRI imaging, these polysiloxane nanoparticles were further surface-bound with gadolinium chelated by DOTA derivatives, while chlorin was used as photosensitizer. *In vivo*, i.e. after intravenous injection in rats bearing intracranial U87-derived glioblastoma xenografts, a positive MRI contrast enhancement was specifically observed in tumor tissue.¹⁰³ Alternative systems for glioblastoma imaging by MRI have been developed that allow for dual-contrast T(1)- and T(2)-weighted targeted MRI. These silica-coated iron oxide core-shell nanoparticles have a diameter of ~21 nm, and exhibit a surface functionalization with amine (for the covalent conjugation of a paramagnetic gadolinium complex with DTPA) and, again, with $\alpha_v\beta_3$ integrin-targeting RGD peptides. Validation was performed both *in vitro* and *in vivo*, in mouse models obtained by subcutaneous implant of U87 human glioblastoma cells, demonstrating a specific visualization of $\alpha_v\beta_3$ integrin-expressing tumor mass by dual-contrast imaging.¹⁰⁴

Photodynamic therapy has been successfully associated to chemotherapy in an *in vitro* model of retinoblastoma, a rare disease of childhood and a most common intraocular pediatric tumor. Mesoporous silica nanoparticles were designed, conjugating the presence of a therapeutic agent (camptothecin) with either fluorescein (for confocal microscopy evaluation of cell uptake) or a porphyrin-based photosensitizer (for one- or two-photon excited photodynamic therapy).

In this case, a specific targeting of cancer cells was achieved by covering the nanoparticle surfaces with a mannose derivative that binds the lectins overexpressed by cancer cells. This targeted bi-therapy was efficient in inducing retinoblastoma cancer cell death *in vitro*, as a basis for future preclinical applications.¹⁰⁵

6. CONCLUSIONS

Fluorescence imaging techniques are becoming essential in biomedical applications and the research of suitable tools for *in vitro* and *in vivo* measurements is gaining more and more importance and attention. Nanotechnology is supplying many solutions for present limitations of the field and, in particular, luminescent nanoparticles (NPs) are one of the most promising materials proposed for future diagnostic implementations.

Bio-conjugated fluorescent silica NPs have been extensively studied in the last decades and literature reports clear advantages of these materials in comparison with single fluorophore labelling in diagnostics, and lately also in theranostic. In this contribution we have focussed our attention only dye doped silica or silica-based NPs conjugated with targeting moiety to enable specific cancer cells imaging. The results presented demonstrate that they are versatile and convenient platforms for targeted labelling presenting higher solubility and lower cytotoxicity in comparison with the majority of dyes in solution. Most interestingly they also offer higher brightness and photostability, two key points in fluorescent imaging. On the other hand, their drawbacks have been mostly addressed and now synthetic reproducibility can be successfully obtained, so as very small dimensions (< 30 nm) to allow facile cellular uptake.

It is important to underline that one of the most valuable characteristics of silica nanoparticles is the possibility to use them to merge different materials, to combine various functionalities, that is to say to act as a basis to obtain multifunctional medicine dedicated nanoplatforms enabling multimodal imaging and simultaneous diagnosis and therapy.

However, the development of nanostructured targeted labels in general, and for medical applications in particular, already is a very hard and interdisciplinary task, but the translation to clinics is even harder. It also depends, in fact, on safety, costs and conditions of production processes that are stringent and the benefits that the use of fluorescent silica nanoparticles can offer must greatly counterbalance any possible.

The results presented in the examples selected in this review, in our opinion, point out the specific, rare and many advantages that these unique materials may introduce in clinical applications like early diagnosis, functional imaging, guided surgery, theranostic. All this make us envisage new clinical uses of fluorescent multifunctional silica-based medicine dedicated nanoplatforms in the next future since now applications are unfortunately still rare.

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Figure 1.

a) Schematic representation of the structures and structure-related theranostic functions of the mesoporous particles; b) TEM image of NPs (inset: the STEM image with scale bar = 100 nm); c-f) Element mapping of Si (c), O (d) and Mn (e) in NPs (f: color merged image of c, d and e). Adapted from ref. [60].

Figure 2.

Reaction scheme for the synthesis of MDT-NPs. (Left) Hexagonal array of C16TAB cylindrical micelles. (Center) NIRF silica nanoparticles formed by co-condensation of modified IR780-APTES dye conjugate with TEOS. (Right) MDT-NPs synthesized by encapsulation of Si-naphthalocyanine dye within the pores of mesoporous NIRF particles. Abbreviations: APTES, 3-aminopropyltriethoxysilane; C16TAB, cetyltrimethylammonium bromide; DMF, *N, N*-dimethylformamide; IR780, IR780 iodide; MDT-NPs, multi-dye theranostic silica nanoparticles; NIRF, near-infrared fluorescence; TEOS, tetraethyl orthosilicate. Adapted from ref. [63].

Figure 3.

Fluorescent dye-doped silica NPs: scheme illustrating the surface modification of the fluorescent silica NPs with PEG and neutravidin. Adapted from ref. [66].

Figure 4.
 Schematic illustration of routes for the bio-conjugation of ORMOSIL nanoparticles.
 Adapted from ref. [69].

Figure 5.
Scheme of conjugation of Dye@MSNs with Herceptin through multistep modification and MSNs. TEM images: (left) Dye@MSN-SH; (right) Her-Dye@MSN. Adapted from ref. [70].

Figure 6. Schematic representation of antibody immobilization process onto functionalized fluorescent core-shell nanoparticles and TEM image of TRITC fluorescent nanoparticles. Adapted from ref. [73].

Figure 7.
Schematic illustration of the synthetic procedure and TEM image of Rubpy doped silica (RuDS). Adapted from ref. [77].

Figure 8.

Schematic diagram of the preparation of nanoparticles-(fluorescein isothiocyanate)-polyethylene glycol-Folate (NPs[FITC]-PEG-Folate) particles. (A) The maleimide end of maleimide-FITC (Mal-FITC) reacts with thiol groups of 3-mercaptopropyltrimethoxysilane (MPS) to form stable thioether linkages. (B) Folate was activated with ethyl(dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide and reacted with NH_2 -PEG-maleimide to form a reactive intermediate (maleimide-PEG-Folate), then the NPs(FITC) particles react with maleimide-PEG-Folate to produce NPs(FITC)-PEG-Folate particles. Abbreviations: C_3F_8 , octafluoropropane; DMSO, dimethyl sulfoxide. Adapted from ref. [78].

Figure 9.

Molecular structure of DFP (left), the fluorescence emission of DFP ($5 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$) in THF and the THF/water mixture (90% volume fraction of water, ca. 20 nm nanoparticle suspension) under 500 nm laser excitation (middle), and the relative fluorescence quantum yields (Φ_f) of DFP ($5 \times 10^{-6} \text{ mol}\cdot\text{L}^{-1}$) vs water fraction in THF (right). Adapted from ref. [79].

Figure 10.

Synthetic route of folic acid-conjugated HPG-grafted $\text{Fe}_3\text{O}_4@\text{SiO}_2$ fluorescent nanoparticles: (I) overview of the synthetic strategy and (II) chemical synthesis. Adapted from ref. [81].

Figure 11.
Schematic illustration of the formation of MnO@SiO₂ (RBITC)-FA nanocomposites.
Adapted from ref. [89].

Figure 12.

(left) polyhedral oligomeric silsesquioxanes (POSS) scaffold of hydrophobic conjugated oligomers (COs) loaded into silica nanoparticles; Iron oxides (IOs) nanoparticles conjugated to the silica surface; herceptin coating through adsorption. (A) Field emission scanning electron microscope (FESEM) image and (B) transmission electron microscope (TEM) image of the POSS-COs loaded silica NPs with no IO layered on the nanoparticle surface. (C) and (D) TEM images of the POSS-COs loaded silica NPs of the Fe₃O₄ IO surface layer. Adapted from ref. [91].

Figure 13. Synthetic procedure of Fe₃O₄@SiO₂(F)@meso-SiO₂(P)-Folate nanoparticles and TEM images of Fe₃O₄ (left) Fe₃O₄@SiO₂(F) (centre) and Fe₃O₄@SiO₂(F)@meso-SiO₂(P) (right). Adapted from ref. [98].

Figure 14.

Structures of **MSN-FITC**, **MSN-PS**, **MSN-2hv**, **MSN-FITC-man**, **MSN-PS-man** and **MSN-2hv-man** nanoparticles. The carbohydrate moiety (α -d-mannose, **man**) was covalently anchored on the surface of the MSN. Adapted from ref. [107].

Table 1

Main fluorescent silica nanoparticles synthetic methods: versatility

Silica NPs Synthetic Method	Dimensional Range	Pristine Colloidal Stability	Dyes Incorporation	Surface Modification	Accessibility to Hybrid architectures	Overall Efficiency
<i>Stöber</i>	+++	+++	++	+	++	+
<i>Reverse Microemulsion (W/O)</i>	++	++	+	+++	+++	++
<i>Direct Micelles</i>	+	+++	+++	+++	++	+++