Chapter 8
Inorganic Nanoparticles for Bioimaging Applications

S. Prabha, D. Durgalakshmi, P. Aruna, and S. Ganesan

Contents

Abstract Nanomaterials for biomedical applications are a more welcoming and positively developing field of research. Based on the material aspects, nanoparticles can be differentiated into organic, inorganic, and polymeric for biomedical applications. Of the various biomedical applications research, nanomaterials designed for bioimaging need more understanding on physics, material science, and biological concepts. Noteworthy, organic fluorescence dyes are widely in use for therapy and bioimaging applications due to its higher quantum efficiency. However during imaging, due to its photobleaching at shorter time of exposure, opens the research possibilities of inorganic nanoparticles with low toxicity and stable used as a sensitive probe for the bioimaging applications. In this chapter we discuss on some of the inorganic nanoparticles such as iron oxide, gold, mesoporous, and quantum dots for bioimaging applications.

Keywords Inorganic nanoparticle · Flouroscence · Bioimaging · Quantum dots · MRI

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8.1 Introduction

Nanoparticles (NPs) and nanostructured materials are an energetic area of research by the reason of adjustable physicochemical characteristics such as electrical, melting point, light absorption, scattering, wettability, and thermal conductivity. In the last 20 years, it has seen large number applications in the field of biomedical. Nanoparticles can be prepared with organic polymers/inorganic elements (Pal et al. 2011; Jeevanandam et al. 2018). Liposomes, carbon nanomaterials, and polymeric micelles are examples of the organic nanoparticle. Liposomes have the bilayer membrane structure similar to the biological membrane. As a result of amphiphilic nature, these transport hydrophilic drugs within their aqueous interior and hydrophobic drugs are dissolved in the membrane. It also has good penetration and diffusion property. Carbon nanotubes are used as biosensor, drug carriers, and tissue repair scaffolds. Quantum dots and magnetic, metallic, and ceramic nanoparticles are the core of inorganic nanoparticles. When the sizes of inorganic materials are reduced to the nanoscale range, they exhibit unexpected chemical, electrical, magnetic, optical, and mechanical properties compared to bulk structures. Quantum dots, i.e., semiconductor nanocrystals, reveal size dependence optical properties when their size is smaller than the Bohr exciton radius. Highly fluorescent quantum dots are used as a fluorescent label due to the unusual optical properties compared to the fluorescent dyes. By varying the composition and size, the emission spectrum shifts from visible to NIR region. Magnetic iron oxide nanoparticles turn to superparamagnetic when their sizes decreased below the critical size where they behave as individual magnetic domains. Graphene exhibits incredible electron mobility, and carbon nanotubes show extraordinary tensile strength. The ultrasmall nanoparticles also play a role in biomedical application because of the majority of their atoms located on their surface. Hence, this increase in surface-to-volume ratio gives some of the enhance properties like iron oxide become paramagnetic and gold as fluorescent (Kim et al. 2013).

Nanoparticles of organic molecules have been widely studied for thermal therapy and imaging. While organic dye molecules with low tissue absorbance exhibit photothermal effects, photobleaching remains one of their major drawbacks. Some common organic dyes used for biological labeling and staining are DAPI Hoechst, MitoTracker, Alexa Fluor, and Allophycocyanin, whereas inorganic nanoparticles have interested attention in the fields of heat-induced cancer therapy and imaging. Hence, they are an attractive alternative for imaging and therapies. In most of the cases, organic dyes were used as a probe because of its high quantum efficiency. However, optically stable sensitive dyes are in need for real-time analysis and detection of biomolecules. However, when using powerful excitation, the dyes undergo photobleaching, which causes drawback in the transport requirement. Hence, the research turns towards the development of inorganic nanomaterial with low toxicity and highly stable and sensitive probes (Liang et al. 2014; Zhang and Ferenz 2018; Le Trequesser et al. 2013).

In the structure of lattice, atoms may be arranged in different ways depending on the outward conditions such as pressure and temperature. Therefore, the same elements have different crystal structures. In the case of nanomaterial, the crystal structure difference causes the physicochemical properties, which also affects toxicity. For example, TiO_2 nanomaterial in the form of rutile crystal structure is used in paint and sunscreen, whereas the anatase form is used in photocatalytic application. Not only TiO_2 nanomaterial but also iron oxide, silicon dioxide, and carbon have different crystal structures. Graphite is used in concrete cutting and lubricant due to its hardness and deformability of crystal lattice respectively. Thus, altering the property of crystal structure is an alternative material approach to a broad spectrum of applications.

The metallic semiconductor has covalent bonds between their constituting atoms. The organic semiconductors in the form of molecules are having van der Waals bonding between them. This bond is weaker than the covalent bond. According to the molecular nature of the organic material, the energy level splitting of the molecular orbitals is small so the bands are narrow. In the case of organic material, the valence band is termed as HOMO and the conduction band as LUMO. However, in metal, splitting of energy level is larger, and it causes wide energy bands. In most of the cases, energy band structure must be discussed before the material introduction, because the energy band structure affects the electrical and optical implementation of the material. It is shown in below scheme diagram.

Bioimaging is a diagnostics tool for modern medicine. Nano-assisted bioimaging is used for early detection of cancer. Even very small tumor entities can be detected using these nanoparticle-based targets. There are many techniques available for bioimaging studies, such as in vivo stem cell tracking, in vivo fluorescent imaging, and magnetic resonance imaging. Nanoparticle used as a contrast agent in imaging is chosen by the reason of enhancement of the image. These are offering more advantages compared to conventional chemical agents. The combinations help to the guidance of stem cell therapies, image-guided surgery, pathogen detection, and gene therapy. The detection limit is also improved by these combinations. Even though the inorganic nanoparticle-based imaging techniques give lot of advantages compared to organic fluorophores, there is only limited works available in the former topic. The comparison between inorganic quantum dots, which can be used as fluorophores for bioimaging application with organic fluorophores, is given in Table 8.1.

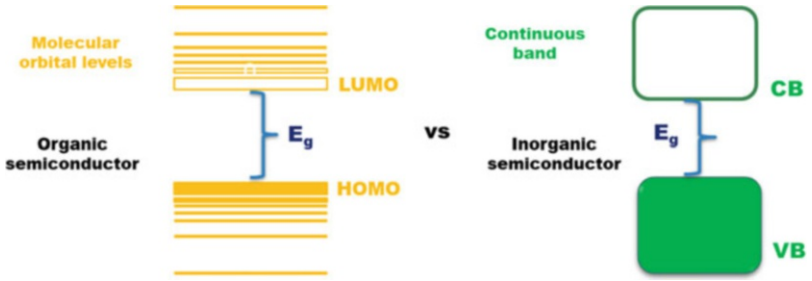
8.2 Functionalization

There are two methods of synthesizing inorganic quantum dots for bioimaging applications: (i) hydrophobic condition and (ii) direct synthesis in an aqueous medium, resulting in hydrophobic and hydrophilic nanoparticle, respectively. Notably, water-soluble functionalized nanomaterial is essential for biomedical application. The coating can aid to convert hydrophobic into hydrophilic water-soluble

Table 8.1 Comparison between inorganic and organic fluorophores for bioimaging applications

Properties	Quantum dots (inorganic nanomaterials)	Organic fluorophores
Photoluminescence (PL) occurrence	Present	Present
Photostability	Superior	Low
Lifetime	10–100 ns	1–10 ns
Quantum yield	High (10–90%)	Lower
Excitation–emission range	Wide (UV–Vis–IR)	Visible
Excitation and emission band	Broad and narrow band	Narrow and broad band
Emission spectra	Size-dependent	Fixed emission wavelength
Size	6–60 nm (hydrodynamic diameter); colloid	~0.5 nm; molecule
Thermal stability	High; depends on shell or ligands	Dependent on dye class; can be critical for NIR-wavelength dyes
Photobleaching	High resistance to photobleaching	Poor resistance to photobleaching

Wojnarowska-Nowak et al. (2017)



Scheme: Band structure of organic/polymeric and inorganic semiconductors. *VB* valence band; *CB* conduction band

particles. Chemical functionalization onto the particle surface can be done by covalent bonding with water molecules. Another way to change from hydrophobic into hydrophilic surfaces involves (i) ligand exchange by hydrophilic ligands like thiols or another functional groups, (ii) direct encapsulation of the hydrophobic nanoparticles by hydrophilic polymers, and (iii) formation of interdigitated bilayer between the amphiphilic polymer and passivating layer on the surface of the nanoparticle. Water-soluble quantum dots utilize a coating of thiols, polymers, micelles, and silica. Table 8.2 shows the different functionalization and coating material. In the synthesis process of nanoparticles, ligand-bound is used not only to control growth but also to stop aggregation. The ligand chosen is very important because it plays a major role in enhancing the stability of the nanoparticles. The

Table 8.2 Nanoparticles surface functionalization with respective coating materials

Function of modification	Coating material	Description
Increase of suspension molecules stability	Molecules, polymers, charged polymers	One layer mostly applied during production or during processing
Improvement of wettability	Molecules, polymers, inorganic layers	For an easier production of nanoparticle mixtures with water (hydrophilic) or organic solvents and polymers (hydrophobic)
Decreased solubility	Inorganic layers, mostly silica	Smaller particles have an increased solubility. Layer shall retain the chemical properties (ZnO/SiO_2; Ag, Au/ SiO_2)
Improvement of physical and chemical function, improved efficiency	Inorganic layers, mostly silica or ZnO, in combination with SiO_2	Strong increase in fluorescence of quantum dots, if free surface bonds are saturated
Reduction of costs and material	Noble metals, e.g., palladium	Thin, incomplete layers of noble metal as a catalyst on a cheap carrier material
Protection	Organic layers	Protection of particle functionality, e.g., from catalyst poisoning
Biocompatibility and functionality	Biocompatible polymers and inorganic layers, antibodies, and peptides	Biocompatible SiO_2 or polyethylene glycol (PEG) can decrease the corona effect Antibodies used for targeting cells

Gubala et al. (2018)

ligand bound to the nanoparticle can be obtained by electrostatic attraction or hydrophobic interaction at the head of the ligand. However, biomolecules like protein, DNA/RNA, oligonucleotides, fluorescent dyes, antibodies, polymers, and tumor markers can also be functionalized for biological application. In this point of view, polyethylene glycol (PEG) is used as a nanocarrier in the broad area. It has excellent stabilization and biodistribution in vivo and in vitro. Any other molecules bound to the nanoparticle by non-bonding interaction, i.e., steric effect, are prevented by PEG. Thus PEG act more stable in the biological domain in avoiding nonspecific binding to cells and proteins, which is important for in vivo application. For functionalization, the fluorophores are also used to observe the biologically relevant targets. For example, a green fluorescent protein (GFP) detects the protein such as fibrinogen, immunoglobulin G, and human serum albumin in biological elements (serum). Functionalization of nanoparticle with biomolecule faces various obstacles. Even after conjugate with a biomolecule, the stability of the nanoparticle is considered to be an important factor of functionalization process. The standard protocol for functionalization is not yet to be reported. It only depends on the stability, functionalized biomolecule, and environmental factors such as temperature, pH, and solvent. The efficient biomolecule conjugation with the nanoparticle is acheived through optimizing these parameters. Some of the relevant techniques for this can be obtained by functionalization like electrostatic binding, covalent

coupling, and physical adsorption (Erathodiyil and Ying 2011; Selvan et al. 2009; Sperling and Parak 2010; Conde et al. 2014).

8.3 Inorganic Nanoparticles for Bioimaging

8.3.1 Gold nanoparticles

The properties of gold nanoparticles (Au NPs) are different from its bulk form. There is notable advantageous shift in their physicochemical properties like surface plasmon resonance, redox behavior, conductivity, and high surface area. The colour of the Au NPs in the solution is depend on the size of the particles; for example the bulk form of Au NPs is yellow in colour, and thus the colours vary depending on the particle size. The sizes are ranging from 1 nm to 8 μm. The shape of the Au NPs will also enhance the property, resulting in various applications. Au nanoparticles can also be synthesized in different shapes such as in spherical, nanotriangle, nanorod, decahedral, octahedral, nanoprism, multiple twined, and irregular. Among all shapes, triangle shape has comparatively amplified the optical properties compared to rest of the shapes. The fluorescent Au NPs possess highly biocompatible property and hence been used in cell labeling, imaging, and therapy. This particle also has better enhancement and tunable emission in both visible and NIR regions and opens its application possibilities in biosensor and photothermal therapy for cancer. The biosynthesis of Au NPs, i.e., synthesized from plant extracts, is also suggested for various biomedical applications. Due to their versatile shape and size, biocompatibility and ease to functionalized with biomolecules, they are travel very easily to target the cell for drug delivery applications. Due to its low toxicity, it binds with various organic molecules as a biomarker in biomedical application. They can also be readily integrated with antibodies or oligonucleotides for the detection of biomolecules. As a result of its optical properties, it has been used in cell imaging in different techniques (Yeh et al. 2012; Khan et al. 2014).

There are various methods for prepared gold nanoparticle such as physical method, chemical method, and green method (Guo et al. 2017). Colloidal gold nanoparticles were first prepared by Faraday et al. in 1857 (Faraday 1857). In this work, the gold chloride was reduced by phosphorous and stabilized by carbon disulfide. In 1951, Turkevich et al. (1951) synthesized the colloidal gold nanoparticle using trisodium citrate as a reducing agent and tetrachloroauric acid as precursor. Later Frens et al. modified the same procedure for improved formation of particles. In the resent years, different shapes and sizes of gold nanoparticles were synthesized by numerous chemical methods. Depending on the properties, the applications were also well progressed. Sandhya Clement et al. synthesized gold nanoparticle conjugated with verteporfin (VP) and compared the photodynamic therapy effectivity of the photosensitizer by using deeply penetrating X-rays administered in standard radiotherapy doses using red light. The results show that the Au NPs enhance the interaction of ionizing radiation with a photosensitizer. Both VP

and gold nanoparticle conjugated with VP were tested in pancreatic cancer cells and the nuclei stained with Hoechst 33345 (blue) using laser scanning confocal microscopy. Both show bright fluorescence in 690 nm. Hence, they conclude that this material can be used as a dual-mode PDT treatment for deep tissue tumors and also for bioimaging. Alexander Nazirov et al. (2016) prepared gold nanoparticles and water-soluble luminescent with an average size of 2.3 nm by green method. In this method, chitosan derivative nontoxic N-(4-imidazolyl) methyl chitosan acts as both reducing and stabilizing agent. By this method, catalytic activity was high compared to other Au nanoparticle with the luminescence at 375 nm. The imidazoyl shows good binding capability with both the proteins and drug results in enhanced biological activity, and it suggested for bioimaging, drug delivery, and catalysis. Sang Bong Lee et al. (2016) reported the radionuclide embedded gold nanoparticle as an optical imaging agent for dendritic cell (DC)-based immunotherapy and tracking of DC migration to lymph nodes. It was prepared by the simple strategy of DNA-based radiolabeling chemistry in addition to gold shell formation. It has longtime monitoring of DC migration and also can image through PET owing to strong and stable radio sensitivity. Further, it is also applicable for CLI-based optical imaging due to their sensitivity and penetration depth. Hence, it poses the possibility in new work for multimodal imaging for optical and nuclear imaging applications. Shengnan Huang et al. (2017) designed the gold nanocage with hollow and porous structure. Doxorubicin was loaded into the system consists of hyaluronic acid grafted and A54 peptide targeted PEGylated gold nanocage for liver cancer drug delivery. The cell uptake was demonstrated by the BEL-7402 cells incubated with HA-grafted PEGylated gold nanocage (HPAuNCs) and HA-grafted A54 peptide-targeted PEGylated gold nanocage (HTPAuNCs) for different time period and investigated by the fluorescence microscopy. The cells treated with HTPAuNCs show strong fluorescence intensity than the HPAuNCs. Hence the smart delivery system could increase the cellular uptake of AuNCs. Thus, they conclude, this system enhanced the therapeutic effect with limited toxicity. Yong Wang et al. (2013) proposed the nanocomposite of transferrin functionalized gold nanoclusters with graphene oxide as NIR fluorescent probe for bioimaging. These composites have good water solubility and biocompatibility with negligible cytotoxicity. The fluorescence bioimaging of cancer cell in mice at varied time period is shown in Fig. 8.1.

8.3.2 Iron Oxide Nanoparticles

In the last decades, magnetic nanoparticle shows potential interest in the research field, and these applications are in the broad area due to their unique properties of low Curie temperature, high magnetic susceptibility, low toxicity, and higher surface-to-volume ratio. The applications of iron oxide nanoparticles in the biomedical field include detection and bioseparation of a cell, enzyme, protein, bacteria, etc. and further applications in magnetic resonance imaging and in magnetic-targeted drug delivery process (Ali et al. 2016).

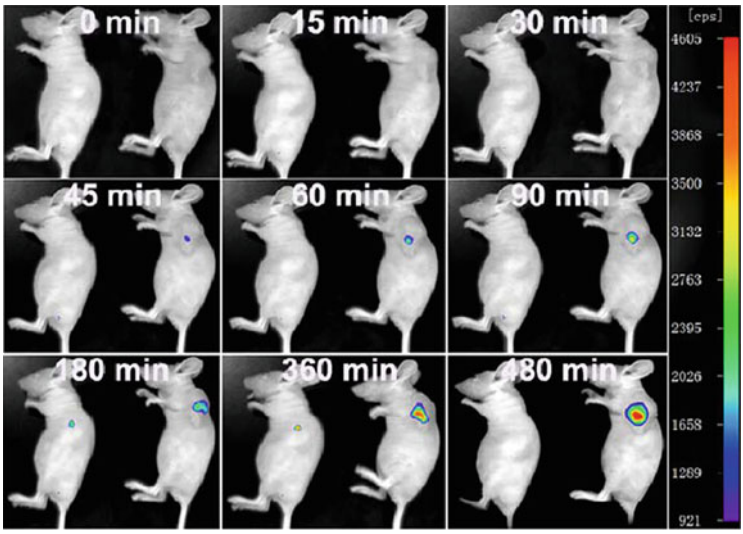


Fig. 8.1 Time-dependent fluorescence imaging of HeLa tumor-bearing mice and normal nude mice. The fluorescent probe was intratumorally injected into the tumor-bearing mice (right) and subcutaneously injected into the left forelimb region of the normal nude mice (left) (Wang et al. 2013)

These nanoparticles do not only apply in the biomedical field but also extend its application in commercial and technological applications such as data storage, magnetic fluids, and catalysis. Iron oxide is magnetically soft with high magnetic moment density, and they are inexpensive. However, when the size is less than 20 nm, these materials acquire superparamagnetic properties. They are widely used in biomedical field by the reason of innocuous toxicity. Functionalization of these nanoparticles is also possible with small molecules, nanostructures, and coating agents such as fatty acids, PVA, and PEG. As of now, eight types of iron oxides were found; among them, magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (α-Fe_2O_3) are admirable for various applications, and their crystal structure is shown in Fig. 8.2 (Wu et al. 2015).

Hematite (α-Fe_2O_3) is an n-type semiconductor with higher stability compared to other iron oxides. Hematite is used in gas sensors and pigments due to its high resistance to corrosion and bandgap of 2.3 eV. The energy diagram of this compound consists of empty d orbitals of Fe^{3+} in the conduction band and occupied 3d orbitals of Fe^{3+} with the addition of 2p nonbonding orbitals of O (Zhang et al. 1993). Another iron oxide crystal, magnetite (Fe_3O_4), has FCC spinel structure. It is unique among all other iron oxides, as it consists of both divalent and trivalent irons. Hence it can act as both n and p-type semiconductor with a bandgap of 0.1 eV. Maghemite (γ-Fe_2O_3) is a cubic structure and considered as fully oxidized magnetite. It is an n-type semiconductor with the bandgap of 2 eV (Boxall et al. 1996). Till this date, there are several methods for the synthesis of iron oxide nanoparticle which include

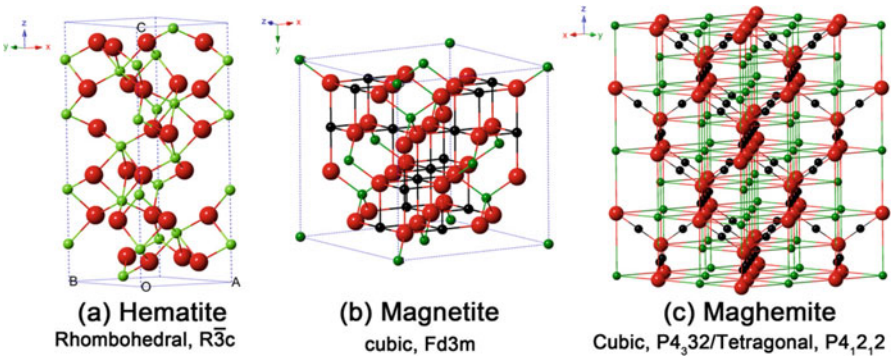


Fig. 8.2 Crystal structure and crystallographic data of the hematite, magnetite, and maghemite (the black ball is Fe^{2+}, the green ball is Fe^{3+}, and the red ball is O^{2-}) (Wu et al. 2015)

both chemical and physical methods, viz., electrochemical method, aerosol/vapor method, sol–gel method, microemulsion, hydrothermal, co-precipitation, biological synthesis, and thermal decomposition

Seda Demirel Topel et al. (2015) reported uniform PEG-coated magnetic Fe_3O_4 by a co-precipitation method, and Bodipy-5 was conjugated by DCC/DMAP coupling reaction. The imaging ability of this sample was found in the presence of A549 cancer cells. From the in vitro experiment, the fluorescence images show that the nanoparticles could be easily penetrated into the cytoplasm. Hence, it is suitable for MRI contrast agent. Kamil R. Wierzbinski et al. (2018) reported DMSA-coated superparamagnetic iron oxide by the thermal decomposition and ligand exchange reaction as an agent for direct cell labeling for stem cell imaging. Heng Li Chee et al. (2018) reported on the ultrasmall superparamagnetic iron oxide nanoparticles (of USPIONs) and the short peptides and ligands on the surface of USPIONs. The bisphosphorylated peptide has enhanced magnetic resonance property compared with the commercially MRI contrast agents. The peptide-coated USPIONs were functionalized with a biomarker for tracking the breast cancer tumors. Hence it is used as a cancer diagnosis with a high-resolution contrast-enhanced MRI. Yuran Huang et al. (2014) were the first to report on the rattle-type metal nanostructure for theranostics application. In this work, multifunctional metal nanocarrier was designed by choosing porous gold shell to carry superparamagnetic iron oxide nanoparticle. The intermediate layer of porous silica was coated by condensation of TEOS. And also functionalized was done with an amino group of APTES. This multifunctional material was used as MRI contrast agent-guided therapy for cancer. Carbon quantum dots doped with SPIONS (FeCD) by hydrothermal method was reported by Bodhisatwa Das et al. (2019). The results show that it has well cytocompatible and also hemocompatible property. It is used for fluorescence as well as MR imaging. It was extended for tissue engineering as a 3D printed composite. To understand the histological study, MR imaging of the kidney and liver of animals treated with the functionalized nanoparticles was examined and shown in Fig. 8.3.

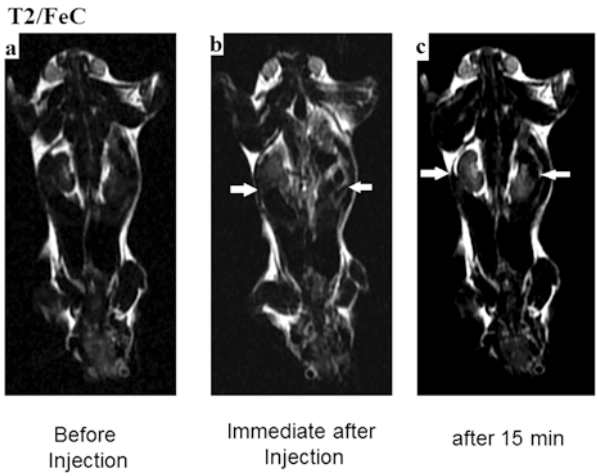


Fig. 8.3 In vivo rodent model MR imaging at the T2 mode: (**a**) before injection; (**b**) immediately after injection; (**c**) 15 min after injection (Das et al. 2019)

8.3.3 Mesoporous Silica Nanoparticles

Mesoporous silica material discovered in 1992 in the labs of the Mobil Oil Corporation was in the M41S phase having pore diameters from approximately 2 to 10 nm (Hoffmann et al. 2006). As per IUPAC (International Union of Pure and Applied Chemistry), the porous materials classified according to their pore size were tabulated (Barrabino 2011). (Table 8.3).

Mobil silica crystalline materials were developed as an inorganic drug delivery agent. The materials are often referred to MCM materials, which stands for Mobil Composition of Matter (Caras 2011), and most popular MCM materials are MCM-41 (Mobile Crystalline Material) and MCM-48 in which MCM-41 material is a 2D hexagonal arrangement of pores, whereas MCM-48 is a 3D cubic pore system. Others are SBA 15 (Santa Barbara Amorphous), TUD, MCM 50, HMS, TMS, etc. It has received considerable attention due to their superior textual properties such as high surface area and large pore volume, tunable particle size (10–1000 nm) and pore diameter (2–30 nm), flexible morphology, effortless surface functionalization and uniform mesoporosity, tunable and narrow pore size distribution, and excellent biocompatibility and biodegradation. MSNs were developed as an inorganic drug delivery agent. The application of MSNPs has controlled drug delivery system, bioimaging and biotherapeutic agent delivery, and tissue regeneration owing to its surface functionalization and drug loading capacity. In imaging, it gives high spatial resolution with enhancing tissue penetration and imaging accuracy with sensitivity. Moreover, fluorescent MSNPs also play a role in multi-labeling in theranostics applications due to its targeting ligands with surface modification and photostability (Wang et al. 2015; Pratiwi et al. 2018).

Tatsuya Nakamura et al. (2015) developed the ^{19}F MRI contrast agent inside the mesoporous silica nanoparticles. For cellular imaging and drug delivery, it was conjugated with folic acid, and also the anticancer drug doxorubicin was loaded into the pores of folate conjugates mesoporous silica. Because of the integrated

Table 8.3 Classification of porous material

Types of porous material	The diameter of pores (nm)
Microporous	Diameter < 2
Mesoporous	2 < diameter < 50
Macroporous	Diameter > 50

Hoffmann et al. (2006)

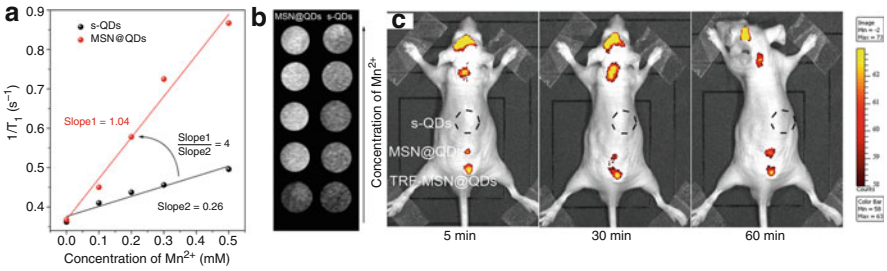


Fig. 8.4 MRI enhancement performance of the MSN@QDs NPs over s-QDs: (**a**) T1 relaxivity curves and (**b**) T1-weighted MR images. (**c**) In vivo fluorescence images of nude mouse after injection with s-QDs, MSN@QDs, and TRF-MSN@QDs (Zhou et al. 2018)

system, it was used as a fluorescence imaging with the drug delivery vehicle tested in tumor-bearing mice. Ronghui Zhou et al. (2018) studied Mn-doped ZnS quantum dots with mesoporous silica for dual model imaging. Mn^{2+} itself maximizes the fluorescence and at the same time shows possibility for MR imaging. Thus, synthesized Mn-doped ZnSe quantum dots were loaded into the large pores of mesoporous silica. Before loading the mesoporous silica functionalized with amine group, it shows enhanced fluorescence brightness and also magnetic signal with higher biocompatibility. For increasing the cell affinity, transferrin is used as tumor-targeting ligand. It has high luminescent and also shows paramagnetism property, so it was used for optical and MRI dual model imaging depicted in Fig. 8.4.

Elisabete Oliveira et al. (2019) reported quantum dots coated with mesoporous silica CdTe@MNs. The luminescent of CdTe helps in imaging of cell internalization and visualized doxorubicin drug release. The ability of the nanocarrier was examined in HeLa cells by laser scanning microscopy, where the nanocarrier acts as a protein scavenger. The protein identification was not found in a sample in the absence of nanoparticle. Qinfu Zhao et al. (2017) presented carbon dots capped with hollow mesoporous silica nanoparticles (HMSN) for bioimaging as well as drug delivery. The carbon dots act as a gatekeeper and imaging agent, grafted on the HMSN opening pores via disulfide bonds. The hyaluronic acid was graft onto the HMSN for drug delivery application. This sample has good biocompatibility with fluorescent properties. The cellular uptake was examined using A549 cells and NIH-3 T3 by laser scanning microscopy. The doxorubicin-loaded material has higher anticancer activity. Thus, this material is used for real-time imaging and drug delivery. Min Sil Kang et al. (2017) prepared carbon dot with mesoporous hollow organosilica nanoparticle for imaging and therapy. In vivo, it showed the

strong optical signal and the stability over a week. In case of drug influence, doxorubicin significantly suppressed the tumor growth with apoptotic function.

8.3.4 Quantum Dots (CdSe, CdTe, and ZnS)

The bandgap of semiconductor (II–VI) has a significant role in most of the electrical and optical applications of materials. It is one of the most attracted areas of all experimental and theoretical researchers. Among all those semiconductor, cadmium telluride (CdTe) has tunable emission possibilities. However, cytotoxicity is the major challenge of this material. Until now, a lot of research is happening to minimize the toxicity of cadmium-based quantum dots, which is a big challenge. The toxic can be reduced by coating silica and zinc-based biocompatible shell with core cadmium nanoparticle. Compared to organic dyes for fluorescence imaging, by using these kinds of inorganic nanoparticles, photobleaching can be greatly reduced. However, cytotoxicity and instability of these materials limit its application in bioimaging. Shaohuazhang et al. (2016) reported watermelon-like structure mesoporous silica core and CdTe quantum dots which was protected by silica for improving the stability. The resultant particle analyzed with A549 cells by confocal laser scanning microscopy and in vivo fluorescence imaging shows high fluorescent image in mice at different times with the injection of material. Sander F. Wuister et al. (2003) reported on colloidal water-soluble CdTe quantum dots. The quantum yields of this material are up to 60% at room temperature. D. Saikia et al. (2017) studied highly stable CdTe@ZnScore@shell quantum dots capped with mercaptosuccinic acid. The stability was excellent after 100 days of synthesis. The quantum yield is four times higher than that of CdTe alone, and in the presence of *E. coli* cells, it provides enhanced fluorescence emission properties, in which the shell of ZnS controls the stability of the core CdTe nanoparticle. Ganjin et al. synthesized CdTe-PVA, CdTe-PSS, and CdTe-PDDA towards bioimaging. The MTT, PVA, and PSS analysis shows the material has less toxicity and suggested for bioimaging in Ca127 and HeLa cells with the quantum yield about 65%. Zdenka Bujňáková et al. (2017) synthesized InAs/ZnS nanocrystal mixture by high-energy milling in chitosan to get the stable suspension. The bioimaging was done on cancer cell lines such as CaCo-2, HCT116, MCF7, and HeLa. From this study, the materials entered into the cytoplasm and surrounded the nuclei.

In general, zinc sulfide-based quantum dots are nontoxic materials, but the major drawback is the excitation in the UV region bandgap, which limits its beneficial for bioimaging. Whereas when it is combined with cadmium-based quantum dots, the bandgap shifts towards visible region, but it is not desirable for bioimaging due to its cytotoxicity. In the earlier work, the glutathione (GSH)-capped Mn-ZnS was studied for RTP sensing of Pb^{2+}. It showed GSH capping had good sensitivity and selectivity. Hence it was extended to bioimaging by the reason of fluorescence by Manju Singhal et al. (2019). In that work GSH was acted as a functionalization agent for ZnS:Mn^{2+} quantum dots. From the multiphoton-excited time-resolved photoluminescence study, it was very stable, and lifetime is in millisecond; thus it

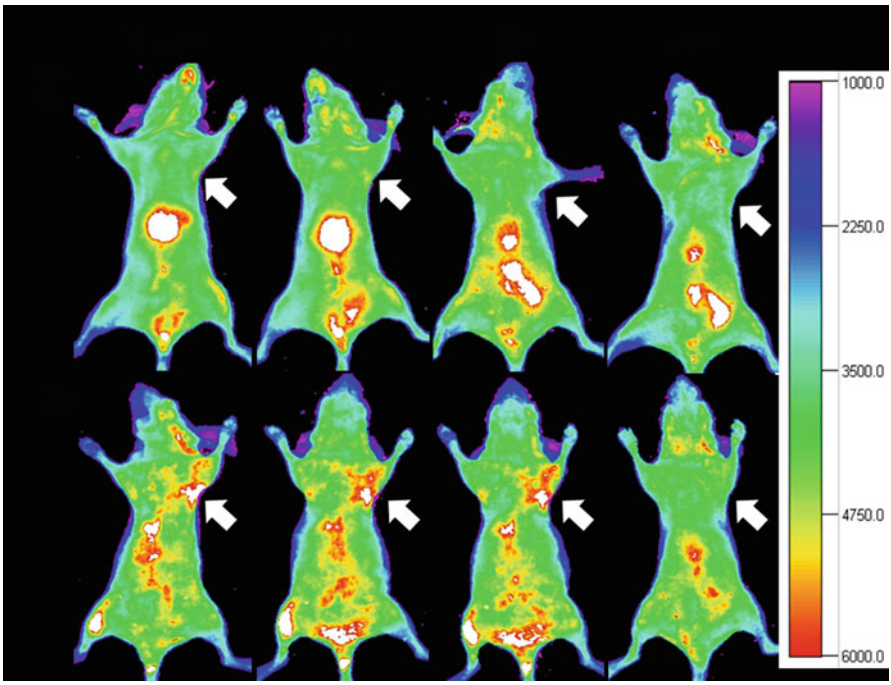


Fig. 8.5 In vivo NIR fluorescence imaging of U87MG tumor-bearing mice (arrows) injected with (**a**) ZCIS/ZnS QDs and (**b**) ZCIS/ZnS-RGD QDs (Guo 2013)

avoided autofluorescence. Hence, this is a promising material for bioimaging. The core@shell of Zn-Cu-In-S (ZCIS)@ ZnS conjugated with cRGD peptide were also examined for in vivo NIR fluorescence imaging. The cytotoxicity and biocompatibility test with 3T3 cells shows the results of lesser toxicity, and hence it was injected into U87MG tumor of nude mice through the tail vein. The MR images (Fig. 8.5) showed signal from the tumor region injected with ZCIS@ZnS.

8.4 The Advanced Technique of FRET in Present and Future

Forster resonance energy transfer (FRET) is a powerful technique used to study nano-bio combination for bioimaging applications. In 1922, the FRET concept was discovered by Cario and Franck using mercury to thallium atomic vapor. In 1927, it was theoretically explained by Jean Perrin as the molecular transfer of energy, and Perrin reported dipole interaction was responsible for energy transfer when the intermolecular distance was 1000 Å. After that, the influence of spectral overlap between donor emission and acceptor absorption on energy efficiency was reported by Perrin's son Francis using quantum mechanical theory; and he calculated that the

average distance between the intermolecular was 250 Å. However, it was larger than the experimental result. Then Forster with Jean and Francis developed the equation depending on the spectral overlap and intermolecular distance, and he calculated the distance to be 10–100 Å. Thus, FRET is a more sensitive distance-dependent fluorescence signal to monitor the molecular interaction in vitro and in vivo (Shi et al. 2015; Cario and Franck 1922; Förster 1948).

FRET based on organic dye has simple preparation and low cost. But short lifetime and low chemical stability are the challenges of FRET in organic dyes. FRET involves the energy transfer between donor and acceptor, i.e., one fluorophore to another, by the fluorescence emission shift. It also provides the spatial and kinetic information about the interaction between the fluorescent nanoparticle and cells or protein labeled by the fluorescent. It is a growing area in imaging application by using materials such as luminescent semiconductor quantum dots, upconversion material, inorganic material, and dye-doped nanoparticles. This technique provides more advantages compared with the conventional fluorescent labeling technique. The mechanism involves the non-radiative energy transfer from an excited donor to a ground-state acceptor. Excitation of the donor results in emission by the acceptor and at the same time quenching of donor fluorescence. The emission of the donor must be able to excite the acceptor. Thus, the donor fluorescence overlaps with the acceptor excitation spectrum. The FRET efficiency is higher, when donor and acceptor molecules are within 10 nm from each other and their dipoles are parallel (Fig. 8.6). To increase the FRET efficiency, the traditional organic dyes were replaced by nanoparticles such as quantum dots, graphene quantum dots, and upconversion nanoparticle. The table shows different biological targets with nanoparticle in FRET. In the future, the nanoparticle with FRET assay will govern a predominant role in bioimaging applications owing to low cost, high sensitivity, specificity, and stability for both in vivo and in vitro applications (Charron and Zheng 2018). (Table 8.4)

Normalized excitation and emission spectra of four different FRET pairs with spectral overlaps are shown in Fig. 8.7. The four FRET pairs are protein–protein,

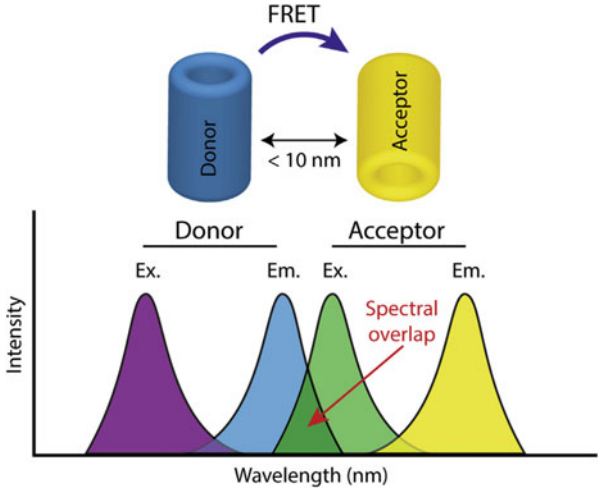


Fig. 8.6 Requirements for FRET (Broussard and Green 2017)

Table 8.4 Some of the FRET molecules discovered

Biological targets	Donor/acceptor
DNA hybridization	QD/Cy5
DNA hybridization	QD/Cy3 on optical fiber
Estrogen receptor b (ER-b) antigen	QD/Alexa Fluor
Thrombin	QD/aptamer-dye
Cancer marker type IV collagenase	QD/AuNP
Cancer marker MUC1	QD/mCherry
Caspase-3 activity	QD/AuNP
Bacteria DNA	GQD/AuNP
DNA hybridization	GQD/CNT
Glucose	UCNP/GO
Virus gene	UCNP/AuNP

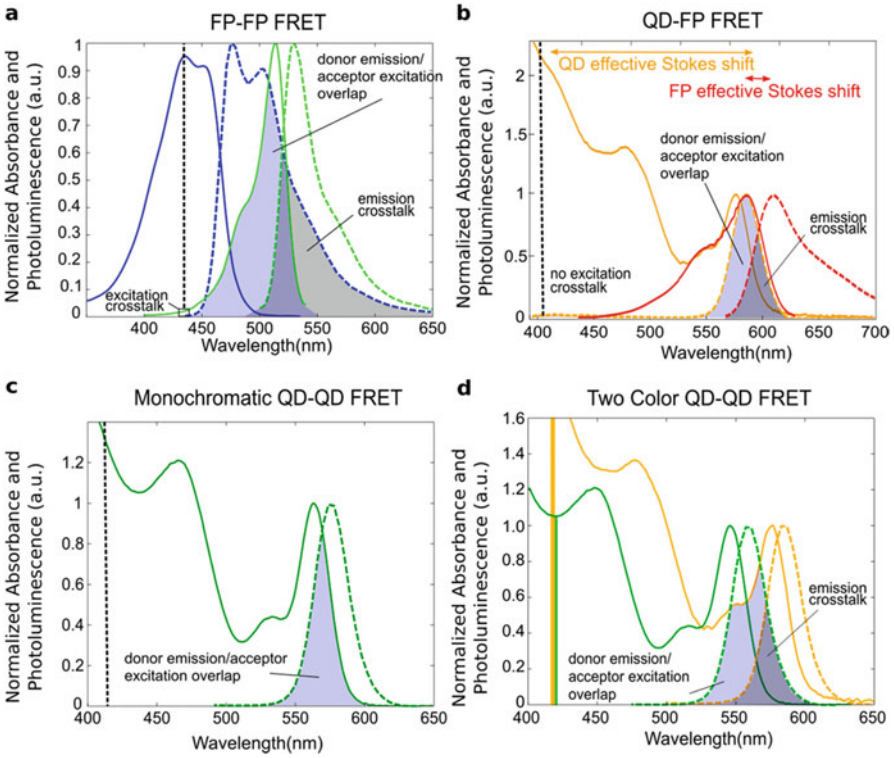


Fig. 8.7 (**a**) Enhanced cyan fluorescent protein (ECFP, blue, donor) and enhanced yellow fluorescent protein (EYFP, green, acceptor); (**b**) A 3.7 nm CdSe/ZnS QD (orange, donor) and the fluorescent protein mCherry (red, acceptor); (**c**) A 3.3 nm diameter CdSe/ZnS QD acting as both donor and acceptor; (**d**) A 2.9 nm diameter CdSe/ZnS QD (green, donor) and 3.7 nm diameter CdSe/ZnS QD (yellow, acceptor)

quantum dot–protein, monochromatic quantum dot that acts as both donor and acceptor, and two color quantum dots.

8.5 Summary

Nanostructuring material is a well-established field of research, which spreads its applications in wider field. Especially, the biocompatible inorganic nanoparticle with different functionalities marks an important role for biomedical applications and requires lot of innovation. In this field, shift is moving towards inorganic nanoparticle as it is more stable than organic nanoparticle. Inorganic nanoparticles not only involved in therapy but also involved in cell tracking, signaling, and interaction at the molecular and cellular levels.

Fluorescence is one of the techniques used in bioimaging. There are numerous fluorophores of organic dyes and also inorganic nanoparticles used for imaging. Inorganic nanoparticles portraying the enhanced light property than organic are highlighted in this chapter. In future, inorganic nanoparticle will play a prominent role as a diagnostic tool of bioimaging due to its advance technology of Forster resonance energy transfer (FRET).

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