Luminescent and Magnetic Nanoparticulates as Biomarkers

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Abstract

Non invasive imaging modalities such as computed x-ray tomography, ultrasound, magnetic resonance imaging and positron emission tomography are used clinically for diagnostic medical applications. Contrast agents are frequently used for providing better spatial resolution with higher sensitivities. In recent years, advances in the field of nanotechnology have further fueled the research and development of contrast agents and introduced nanoplatforms to obtain sensitive imagery and detect changes at cellular and molecular level. Nanoparticles such as fluorescent silica, quantum dots, iron oxides, magnetically and optically labeled liposomes, dendrimer's are routinely employed in research investigations. A rising trend in this area is the development and use of multimodal contrast agents which enable multiple imaging modalities using a single entity and offer the possibility of improved diagnostics, preclinical research and therapeutic monitoring. This review paper focuses on the synthesis and application of optical and magnetic nanoparticulate probes and their integration into single multimodal nanoparticulate entities.

Keywords: Nanoparticles, multimodal, multifunctional, MRI, magnetofluorescent, magnetic, fluorescent

1. Introduction

Since early developments in x-ray imaging, medicine has become more and more reliant on bioimaging for the diagnosis of disease, the identification of physiological abnormalities, and the selection of therapeutic interventions. Modern medicine has been transformed by robust imaging modalities such as optical and magnetic resonance imaging (MRI) and continues to evolve with the ever changing technology landscape. These imaging methodologies and their corresponding contrast agents have made profound contributions to human health; a feature that is being further augmented with the advent of engineered nanotechnology based contrast agents.

The potential of X-rays for medical imaging was re-

alized soon after their discovery by William Rontgen in 1895. The advancement of X-rays based imaging techniques was continued for next several decades and resulted in the development of computed tomography (CT), an indispensible tool for medical imaging. During this period other imaging techniques eg MRI and Nuclear imaging (PET, SPECT) based on different physical principles have been developed. These imaging modalities have been employed clinically and in research to visualize the anatomical structures and to obtained information about the physiological function of various tissues and organs. Over the past decade, the research in bioimaging has become focused on the visualization of cellular and molecular activities in real time for an intact organism. However, no single imaging modality has the sensitivity and resolution to allow the direct observation of these processes at that level. Each imaging modality has its own unique strengths and weaknesses (Table 1). For instance CT provides high resolution but uses potentially harmful ionizing radiation and offers poor soft tissue contrast. MRI provides excellent soft tissue contrast but suffers form low sensitivity. Nuclear imaging has high sensi-
tivity but provides limited anatomical information. In the recent past attempts have been made to integrate imaging modalities synergistically to enable better diagnosis and help plan for effective therapeutic strategies. For example, by combining CT with MRI detailed information about both soft and dense tissue can be obtained. Combination of MRI and optical imaging provides the high resolution (spatial and temporal) and deep tissue penetration of MRI and the sensitivity of optical imaging probes.

Contrast agents are frequently used in various imaging modalities to improve the signal to noise ratio and provide better spatial resolution and/or higher sensitivities. In recent years, advances in the field of nanotechnology have introduced novel nanoparticle systems to obtain sensitive imagery and detect changes at cellular and molecular level. Nanoparticles such as fluorescent dye doped silica, quantum dots, iron oxides, magnetically and optically labeled liposomes, dendrimers are routinely employed in research investigations. Incorporation of contrast agent in nanoparticles can lead to improved stability and sensitivity of the encapsulated imaging agents. Further, multimodal contrast agents, or the incorporation of multiple types of contrast agents into a single nanomaterial, may offer improvements in patient care and at the same time can reduce costs and enhance safety by limiting the number of contrast agent administrations required for imaging purposes.

This review focuses on the synthesis and application of optical and magnetic nanoparticle probes and various challenges faced by researchers for their integration into single multimodal nanoparticle entities. In general, nanoparticles based probes should possess following properties

1) High sensitivity for their administration at ultra low concentrations
2) Chemical and physical stability in the biological environment
3) Amenable to surface functionalization for easy conjugation with targeting moieties
4) High dispersibility in biological environment
5) Stealth properties to avoid uptake by reticuloendothelial system (size, shape and surface modification)
6) Elimination from the body after achieving de-

| Techniques | Labels | Signal Measured | Strengths | Weaknesses | Cost | Through-put | Sensitivity | Resolution |
|------------|--------|----------------|----------|------------|------|------------|-------------|------------|
| PET        | Radiolabeled molecules | Positron from radionuclides | Highly sensitive | Can detect only one radionuclide, requires radioactivity | High | Low | $10^{-12}$ | 1-2 mm |
| SPECT      | Radiolabeled molecules | $\gamma$-rays | Can distinguish between radionuclides, so more processes can be imaged at once | Requires radioactivity | High | Low | $10^{-14}$ | 1-2 mm |
| CT         | None | X-rays | Fast cross-sectional images | Poor resolution of soft tissues | High | Low | $10^{-6}$ | 50 µm |
| MRI        | Can use isotope labeled molecular tracers | Alterations in magnetic fields | Harmless, high resolution of soft tissues | Can not follow many labels | High | Low | $10^{-8}$-$10^{-6}$ | 50 µm |
| Optical    | Genetically engineered proteins and bioluminescent and fluorescently labeled probes | Light, particularly in the infrared | Easy, non-damaging technique readily adapted to study specific molecular events | Poor depth penetration | Low | High | $10^{-22}$ | 1-2 mm |
| Photonoacoustic | Probes that absorb light and create sound signals | Sound | Better depth resolution than light | Information processing and machines still being optimized | Low | High | $10^{-12}$ | 50 µm |
| Ultrasound | Microbubbles, which can be combined with targeted contrast agents | Sound | Quick, harmless | Poor image contrast, works poorly in air-containing organs | Low | High | $10^{-4}$ | 50 µm |

Table 1: Comparison of commonly used imaging modalities for small animal imaging (Reprinted, with permission, from Reference [98])
2.1. Endogenous fluorophores

Some of the most commonly used endogenous fluorophores are Flavins, Lipofuscin, Elastin and collagen. Detailed information about their properties including excitation and emission wavelengths is given elsewhere. Green fluorescent protein (GFP) is another example of endogenous fluorophore. Due to its unique properties, such as high stability, negligible toxicity, high quantum yield, and intrinsic fluorescence, GFP has become a versatile non-invasive fluorescent marker for a wide range of biological applications. At low concentrations of GFP its fluorescence signal is significantly affected by autofluorescence of other endogenous fluorophores present in the system and consequentially imaging contrast diminishes. Various methods for eliminating autofluorescence are systematically and critically reviewed by Billinton et al.

2.2. Exogenous fluorophores

Biological structures which do not possess autofluorescence e.g. DNA and lipids, are generally labeled with exogenous fluorophores. These exogenous fluorophores based on their specificity and sensitivity can be used to image a specific site, a particular cell, or an organelle inside a cell. Some examples of exogenous fluorophores are Alexa Fluor dyes, Cyanine dyes, Fluoroscien, Rhodamine and Texas red dyes. For bioimaging applications organic fluorophores are used more frequently due to their high quantum efficiency, high absorptivity, nontoxic behavior and availability in wide range of excitation and emission wavelengths.

The resolution and penetration depth of optical imaging, particularly in tissues, is limited by the high light scattering, autofluorescence, and high absorption by most relevant tissue chromophores, deoxy and oxyhemoglobin (HbO2), hemoglobin, myoglobin and other heme proteins in the visible region of electromagnetic spectrum. Depending on the wavelength of emission light, different penetration depths are achieved. For example UV-VIS spectral range photons are strongly absorbed within the first few micrometers to a millimeter of tissue thickness while near-infrared (NIR) light of 650 to 900 nm achieves the highest tissue penetration (up to centimeter depth) due to minimal absorbency of the surface tissue in this spectral region. In addition autofluorescence effects are minimized when the excitation wavelength is in NIR range. Thus in order to eliminate autofluorescence effects and reduce scattering, NIR dyes are currently being used as optical probes for bioimaging (table 2). Common problems associated with NIR dyes are related to their biological, environmental and photochemical stability. These dyes tend to form aggregates in water, often causing self-quenching of fluorescence. Also, these dyes are more prone to photobleaching compared to dyes that have their emission spectrum in visible region.

2.3. Dye doped silica nanoparticles

Amorphous silica nanoparticles have been extensively used to encapsulate dyes in order to enhance their stability and brightness for in vitro and in vivo applications. Silica is an optically transparent material and allows emission and excitation light to pass through it without any alteration. Silica is water dispersible, resistant to microbial attack and practically nontoxic at the dose levels required for diagnostic and therapeutic purposes. Silica particles are also resistant to swelling which allows their synthesis in a...
wide selection of solvents without altering the porosity and minimizing the leaching of dye. These chemically stable and highly fluorescent optical probes improve detection sensitivity and provide enhanced contrast when used for bioimaging.

In addition, for targeted delivery of the nanoparticles several biomolecules such as proteins, peptides, antibodies, oligonucleotides can be easily conjugated to the surface of silica using silane based chemistry. Both inorganic as well as organic dyes can be incorporated in the silica matrix either by covalent linkage to the silica (silane precursor), which prevents leakage from the matrix or simply by encapsulation (physical entrapment).

### 2.3.1. Synthesis

#### 2.3.1.1. Sol-gel synthesis of silica nanoparticles

Sol-gel based method for synthesizing monodisperse silica nanoparticles was first developed by Stöber. This method has been widely used for synthesizing spherical, monodisperse and electrostatically stabilized silica particles in the range from tens of nanometers to submicron regime. In this method hydrolysis and condensation reactions of alkoxysilanes [tetraethylortosilicate (TEOS) and tetramethylortosilicate (TMOS)] are performed in ethanol solution in the presence of water and ammonia which is used as a catalyst. Commonly used organic fluorophores are hydrophobic in nature and hence there incorporation in hydrophilic silica matrix is achieved by modification of either dye or silica itself. For example, fluorescein isothycynate (FITC) dye can be chemically modified with an amine containing silane agent (e.g. APTS, aminopropyltriethoxy silane) and then this conjugated dye is allowed to hydrolyze and condense to form FITC conjugated silica particles. It is shown that using modified Stöber’s method organic fluorophores can be covalently incorporated in to silica matrix by coupling them with reactive organosilicates.

#### 2.3.1.2. Microemulsion synthesis of silica nanoparticles

Another approach for synthesizing dye-doped silica particles is by using oil-in-water (o/w) or water-in-oil (w/o) microemulsions. Particles down to a size of 15 nm can be synthesized by using this method. W/o microemulsions consist of an oil (continuous phase) and water (as surfactant coated nanosize droplets). These water droplets serve as nano-reactors and their collision, coalescence and de-coalescence causes nucleation and growth of silica particles inside the confined volume of nanoreactor. This elegant synthesis approach facilitates the size-tuning of silica particles by varying the water to surfactant molar ratio and the dynamic properties of the microemulsion system. W/o emulsion mediated sol-gel synthesis is currently used for the synthesis of organic as well as inorganic dye-doped silica nanoparticles.

Dye doped silica nanoparticles are currently being used for a range of biological diagnostics and studies involving DNA and cells. Zhao et al. have developed a bioassay for precise and accurate determination of a single bacteria (Escherichia coli) cell using antibody-conjugated fluorescent (RuBpy dye doped) silica nanoparticles. Highly fluorescent dye doped silica particles were also used for the ultrasensitive detection of gene products down to subfemtomolar concentrations.

### 2.4. Quantum Dots

Owing to their small size (in most cases less than 10nm) and unique tunable optical features, QDs are widely being used in place of organic dyes for imaging applications in biological systems. Some of the unique features of QDs include- bandgap energy
dependence on size, broad excitation spectra, narrow emission spectra, high quantum yield, large separation between excitation spectra and emission spectra and high photostability. The broad excitation spectra permit use of a single excitation source to excite QDs of different colors while narrow emission spectra reduce overlap of emission and excitation spectra. The large Stoke’s shift enables the collection of whole spectra by detector and therefore improves sensitivity of detection.

2.4.1. Synthesis

Initially top down lithographic techniques were employed for the synthesis of QDs e.g. first QDs were fabricated from GaAs/AlGaAs quantum well structures by using deep etching technique. However, even currently available lithographic techniques require significant improvement in producing feature sizes for routinely fabrication of QDs. With the feature size limitation on lithographic approaches another technique based on colloidal methods came in to existence in early 1980s when Henglin and Rossetti synthesized CdS QDs by mixing cadmium and sulfide salts in an aqueous buffer.

For the synthesis of highly crystalline and monodisperse organometallic QDs a simple method was introduced by Murray et al. This method is based on producing temporally discrete homogenous nucleation by rapid injection of organometallic reagents into a hot coordinating solvent. Organometallic CdSe QDs of size ranging from 1.1-11.5 nm were produced by this method. After achieving nucleation final size of QDs was controlled by changing the growth temperature. Photo-oxidation of QDs is generally prevented by capping them with a protective coating which minimizes the interaction with environmental oxygen and limits photobleaching. In general, the shell should be a wide band gap material and structurally similar to the core (QD) material. For example encapsulation of CdSe (QD) core by ZnS shell (ZnS has larger bandgap energy compared to CdSe) reduces the photochemical bleaching and drastically increases its quantum yield.

The well studied and most commonly used CdS QDs were synthesized using a novel one step solid state reaction between CdCl₂.2.5H₂O and Na₂S.9H₂O
by Wang et al. The average size of the particles formed was 5 nm. It was hypothesized that nonionic surfactant, C18EO10, used in this synthesis forms a shell surrounding the CdS particles which helped in preventing aggregation by steric hindrance and by absorbing heat of the reaction during grinding process.

In another approach using reverse micelle technique CdS QDs were synthesized by mixing two w/o emulsions viz. Cd(ClO4)2 in water/Heptane/AOT(surfactant) and Na2S in water/Heptane/AOT (surfactant). In this work water to surfactant ratio was kept constant ([H2O]/[AOT]=6) and CdS particles of mean size 6 nm were synthesized. However, the size of QDs can easily be controlled by changing the amount of water in the emulsion e.g. particles of the size 1 nm were obtained when water to surfactant ratio of 1 was used and the particle size was increased to 5 nm when water to surfactant ratio was changed to 10.

QDs synthesized using organometallic reagents described above are hydrophobic due to surface capping with coordinating surfactants. In order to use QDs for biological applications it is necessary to make them polar or water dispersible. Several coating methods have been developed for efficiently dispersing these particles in water such as by encapsulation in phospholipid micelles, derivatization of surface using mercaptoacetic acid, ligand exchange, adsorption of amphiphilic molecules/polymers or coating them with silica. The hydrophilic coating process can be selected to introduce organic functional groups such as COOH and NH2 for conjugation to biorecognition molecules such as proteins, oligonucleotides, antibodies, peptides etc. Due to the success of hydrophilic surface treatment and conjugation to biorecognition molecules, QDs were readily adopted for bioimaging applications. Some of the main application areas include nucleic acid detection, immunoassays, cell tracking, tumor targeting and fluorescence resonance energy transfer (FRET) sensing. However, toxicity of QDs remains a concern for invivo applications.

3. Magnetic Resonance Imaging (MRI)

MRI, primarily used for clinical imaging, is a non-invasive imaging method which provides information about physiological and pathological alteration of living tissues inside the body. MRI uses nuclear magnetic resonance (NMR) signals, generated by hydrogen nuclei located in different physiological environments, to generate contrast in tissue imaging. When a specimen consisting of hydrogen nuclei is placed in an external homogeneous magnetic field, their magnetic moment vectors align themselves either parallel or antiparallel to the direction of magnetic field. Upon application of radio frequency (RF) pulse at the appropriate resonant frequency the net magnetization of hydrogen nuclei changes. When the RF pulse stops and the nuclei spins relax back to their equilibrium state, electromagnetic signals are produced. These signals depend on time required for a paramagnetic substance to become magnetic (T1, longitudinal relaxation time) and on how long these nuclei remain precess (rotating) in phase following the RF pulse (T2, transverse relaxation time). In other words T1 represents dissipation of energy from excited state to surrounding lattice and T2 represents the loss of phase coherence of the precessing nuclei spins. T2* is also an important parameter, which is same as T2, but also contains local heterogeneities in the magnetic field. These signals are used to construct three dimension images of the body. Areas with faster T1 relaxation appear bright in T1 weighted MRI, while area with faster T2 relaxation looks darker in T2 weighted MRI. The inverse of relaxation time is referred to as relaxation rate.

Contrast agents (CA) are frequently employed in clinical MRI to improve the sensitivity and/or specificity of the process and provide much more accurate diagnosis in many applications such as cancer detection, inflammation and angiography. The ability of a CA to generate contrast is characterized by its “relaxivity” value, which is defined as the ratio of change in the relaxation rate (of water protons) to the concentration of CA. The CAs accumulate in targeted region (by conjugating to antibodies or peptides), and manipulate relaxation times (T1 and T2) so that these subregions appear darker or brighter. On the basis of their magnetic properties contrast agents can broadly be classified as paramagnetic CA and super-paramagnetic CA.

3.1. Paramagnetic CA

Paramagnetic materials possess magnetic dipole moment even in the absence of external magnetic field but due to their random orientation the net magnetic moment remains zero. In presence of external magnetic field these materials have positive magnetic susceptibility and can be used as MRI contrast agents. Gadolinium (Gd) chelates are the most commonly used paramagnetic CA in MRI. Gd CA shorten T1 more compared to T2, improve signal
in T1 weighted images and generate bright contrast. For this reason Gd CA are sometime referred to as positive CA. Since Gd is highly toxic in its ionic form and causes disruption of Ca^{2+}-required signaling, it is used in a complexed form with different kinds of ligands. These ligands not only form thermodynamically and kinetically stable chelates but also increase the relaxation rates of the protons. Commonly used chelating ligands for contrast enhancement include derivatives of polyaminocarboxylic acids such as DTPA (diethylenetriaminepentaacetic acid), macrocylic Gd chelates such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid).

Paramagnetic CA increase the relaxation rate through interactions between the electron spins of the paramagnetic center and the proton nuclei. These CAs influence both outer coordination sphere (long range interactions) and inner coordination sphere protons relaxivity (short range interactions). For the CA of same size and composition the outer sphere interaction does not vary significantly. However, the effect on protons relaxivity related to inner coordination sphere is substantially high. Hence the relaxivity of paramagnetic agents is generally enhanced by tuning the various parameters, such as rotational correlation time Tr (tumbling time of CA in water), water proton residence time TM (exchange rate of single inner coordinated water molecule with the bulk water) and electron spin relaxation time Te, related to inner sphere interaction.

Relaxivity of the protons associated with inner sphere can be increased by increasing the tumbling time of CA which can be achieved by increasing the effective size of the CA either by using polymer or dendrimer scaffold or by conjugating them with slowly tumbling macromolecules. Relaxivity can also be increased by decreasing the TM which depends on the coordination number, charge and geometry of the paramagnetic CA. For increasing the relaxation rate of Gd, complexes bearing hydrophobic moities were designed and with the aid of the hydrophobic groups, human serum albumin (HSA) proteins were conjugated to Gd. In presence of HSA higher relaxations rates were achieved. In another
particles have been synthesized using various methods. Monodisperse and homogeneous iron oxide nanoparticles (MION) are preferred due to its superior magnetic properties. For biological applications iron oxide nanoparticles should have homogeneous composition and narrow particle size distribution. The most common method for synthesizing these nanoparticles is by the coprecipitation of iron salts with a base in an oxygen free environment. Large amount of particles can be synthesized using this method however; control on particle size remains a concern with this method. Monodisperse and homogenous iron oxide nanoparticles have been synthesized using various methods and are reviewed in details elsewhere.\(^{56}\)

Particles synthesized by above methods are hydrophobic in nature and thus unsuitable for applications in aqueous biological environments. Several hydrophilic coatings encompassing organic and inorganic materials have been used to render these particles water soluble (Table 3). For biological applications polymer coatings are preferred as they not only provide the steric stabilization to the particles but also offer additional functional groups for further bioconjugations.

### 4. Multimodal Magnetofluorescent Particles

The desirable physical and chemical properties of contrast agents needed for bimodal optical and magnetic imaging can be combined in a single nanoparticle. In the following section three different types of commonly used magnetic and fluorescent (magnetofluorescent) particles and various synthesis approaches used for their integration in to a single nanoconstructs are described.

#### 4.1. Luminescent core -magnetic shell

Multimodal magnetic and fluorescent particles have been synthesized by first synthesizing a fluorescent core and then attaching a magnetic component on its surface. The fluorescent core consist of either dye doped silica or semiconducting QDs. The magnetic shell was constructed by attaching either Gd or iron oxide nanoparticles on the surface of core particles.

##### 4.1.1. Dye doped silica particles coated with magnetic shell

In this construct first a fluorescent dye doped silica particle is synthesized and then it is coated with either paramagnetic chelated Gd shell or iron oxide nanoparticles to make it MR active. Santra et al.\(^{56}\) have reported the synthesis of monodisperse (\(\sim 100nm\)) Rubpy dye doped silica nanoparticles coated with Gd-TSPETE silane. These particles were synthesized in AOT/heptane/water microemulsion. The MR and optical contrast generating ability of these nanoparticles was demonstrated in phantom and in \textit{in vitro} experiments. The high relaxivity values of the Gd chelated silica nanoparticles as compared to the Gd-chelates was explained on the basis of (a) decrease in the tumbling rate of silica nanoparticles and (b) high Gd loading per nanoparticle. Santra et al. have shown presence of nearly 16,000 Gd atoms per nanoparticle\(^{58}\). Additionally the presence of Gd on the surface of nanoparticles also permits a rapid water exchange of the inner coordination sphere water with the bulk. The Gd chelation to ligands on the surface of smaller (40nm) silica nanoparticles was improved by using higher coordinating ligands e.g. mono (Gd DTTA) and bis (Gd DTPA) silylated gadolinium chelates\(^{59}\).

Lee et al.\(^{60}\) have developed 30 nm “core-satellite” particles by attaching 9 nm water soluble iron oxide (WSIO) nanoparticles on the surface of rhodamine dye doped silica particles (Fig. 3). Maleimide groups, incorporated on the surface of silica, were reacted with thiol groups of WSIO particles using well known sulfo maleimide chemistry. The hybrid silica-WSIO particles were \(\sim 45nm\) in size with an average of 10 WSIO particles per silica particle. Synergistic magnetism of multiple WSIO satellites surrounding the silica particles caused 3.4 fold increase in T2 relaxivity. Furthermore, it was reported that the fluo-
Fe₃O₄ nanoparticles have been assembled on dye-doped mesoporous silica nanoparticles (MSNs) as multifunctional nanoparticles for bimodal imaging and drug delivery. MR contrast was significantly enhanced due to the synergistic magnetism of iron oxide nanoparticle assembly. For the synthesis of these particles, 3-aminopropyl triethoxy silane (APTS) modified dye (Rhodamine isothiocyanate or Fluorescein isothiocyanate) was co-condensed during the synthesis of mesoporous silica nanoparticles using Cetyl trimethylammonium bromide templates. The surface of MSN was functionalized with amine groups by refluxing with APTS in ethanol. 2-bromo-2-methylpropionic acid (BMPA) functionalized Fe₃O₄ nanoparticles were coated on amine-functionalized MSN by direct nucleophilic substitution between the terminal bromine groups of BMPA and the amine groups of MSN.

### 4.1.2. Quantum dots coated with magnetic shell

Mulder et al. have reported the first example of paramagnetic quantum dots (pQDs) that can be

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**Table 3**: Commonly used molecules for the surface functionalization of iron oxide nanoparticles for biological applications

| Type                        | Example                              | References          |
|-----------------------------|--------------------------------------|---------------------|
| Surfactant / Small Molecules| (Citric, Gluconic, carboxylic, phosphonic) Acids | 66,99,100          |
|                             | Polyethylene glycol nonylphenyl ether | 101                 |
| Polymers                    | silanes                              | 102-104             |
|                             | Dextran                              | 105-109             |
|                             | Starch                               | 110                 |
|                             | Gelatin                              | 111                 |
|                             | Chitosan                             | 112-113             |
|                             | Polyethyleneglycol                   | 104,114-121         |
|                             | Poly(vinyl alcohol)                  | 122-125             |
| Biological Molecules        | APTs                                  | 126,127             |
|                             | Peptides                             | 76-78,81,128,129    |
|                             | Proteins                             | 74,106,130-132      |
|                             | Antibodies                           | 133-136             |
| Inorganic Materials         | Titanium, Zirconium, Aluminum         | 137-139             |
| Metal Oxides                | Gold                                 | 140-144             |
| Precious Metals             | Silica                               | 93,145-148          |
utilized for MR and optical imaging. These high quality CdSe/ZnS core/shell pQDs were synthesized by hot solvent method and coated with PEGylated phospholipids and paramagnetic lipids to make them water dispersible. PEG chains forms a hydrophilic coating at the surface and enhances the half life time significantly for invivo applications. The Qdots were coating at the surface and enhances the half life time (Omniscan and Prohance).

4.2. Magnetic core luminescent shell

This class of particles consists of a magnetic core that is modified with a fluorescent moiety. Direct conjugation of a fluorophore with a magnetic core results in the quenching of its fluorescence. Based on the various strategies used by researchers to overcome this problem, this section is divided into following subsections:

4.2.1. Fluorophore labeling of magnetic particles using molecular spacers

Using a sufficiently long molecular linker between magnetic core and fluorophore minimizes the quenching effect. The simplest strategy adopted for this purpose was to use the functional groups of stabilizing agents already present on iron oxide nanoparticle surface. Sahoo et al.46 have used carboxylic acid groups of citrate stabilized magnetic nanoparticles for their conjugation with rhodamine 110 using EDC coupling. In another approach large excess of 2,3-dimercaptosuccinic acid (DMSA) was used to make Fe3O4 nanoparticles water dispersible during there phase transfer from toluene to DMSO66. Thiol groups available on DMSA were used to attach FITC labeled Herceptin as the targeting antibody for HER2/neu receptor overexpressed from breast cancer cells59. In an attempt to make magnetofluorescent particles that could potentially be used for photodynamic therapy, Gu et al have conjugated porphyrin derivative with Fe3O4 nanoparticles. Porphyrin is traditionally used for photodynamic therapy and has fluorescence in the visible range. Catechol chemistry was used for linkage of porphyrin with magnetic nanoparticles to avoid quenching of its fluorescence69. In another study a negatively charged carboxylic acid containing porphyrin was electrostatically attached to amine containing polyhedral silsesquioxane coated Fe3O4 nanoparticles70.

4.2.2. Fluorescently labeled polymer coated magnetic particles

Iron oxide nanoparticles are coated with various polymeric moieties such as dextran, PEG, polyelectrolytes to render them water dispersible and available for various bioconjugations. Dextran (a branched polysaccharide) has been used widely to coat iron oxide nanoparticles for biological applications. Dextran forms hydrogen bonds with iron oxide surface and is susceptible for detachment. To solve this problem Weissleder and coworkers cross-linked the dextran on the surface of magnetic nanoparticles.
by using epichlorohydrin to form cross-linked iron oxide (CLIO) nanoparticles. These particles were reacted with ammonia to yield amine groups on the surface for their further chemical modifications. CLIO nanoparticles were conjugated with various organic fluorescent dyes such as FITC, Cy3.5, Cy5.5, and Alexa-488 to yield contrast agent for both MR and optical imaging. Josephson et al. have reacted CLIO particles with N-succinimidyl 3-(2-pyridyldisulphide group on the particle surface. These groups were reacted with FITC labeled membrane translocating signal peptide (HIV-Tat) through a disulphide exchange reaction. These HIV-Tat modified particles were internalized into lymphocytes over 100-fold more efficiently than unmodified particles. Similar particles were radiolabeled with 111In by reacting the dextran coating with DTPA bisanhydride. CLIO-Tat particles showed high uptake efficiency into hematopoietic and neural progenitor cells as verified by flow cytometry and nuclear accumulation. Further it was shown that upon intravenous injection of CLIO-Tat labeled CD34 cells into immunodeficient mice, 4% of CD34 cells homed to bone marrow per gram of tissue. The author suggested that localization and retrieval of cell populations in vivo could enable detailed analysis of specific stem cell and organ interactions, critical for the advancement of stem cell-based therapies.

Bioconjugation of fluorescent CLIO nanoparticles has been carried out extensively to synthesize targeted probes towards phosphatidylserine, vascular adhesion molecule-1 (VCAM-1), mucin-1, αv β3 integrins, and lymphocytes. Some other applications of fluorescent CLIO nanoparticles include monitoring in changes in tumor volume caused by treatment with a chemotherapeutic agent and design of smart probes whose position can be located by MR imaging while their luminescence can be activated through cleavage of dye molecule by a specific enzyme.

For targeting most common and lethal brain tumor cells, gliomas, a magnetofluorescent probe was developed by Veiseh et al. This probe was synthesized by coating Fe3O4 nanoparticles with amine terminated PEG silane polymer. The amine groups on the surface of iron oxide particles were used to covalently bind a NIR dye (Cy 5.5) for optical imaging and sulphhydryl-modified chlorotoxin for specific targeting of 9 L gliomas cells. The preferential uptake of these multimodal probes was demonstrated invivo by both MR and optical imaging.

Magnetic and luminescent nanocomposites were also synthesized by multilayer deposition of CdTe QDs and polyelectrolytes on Fe3O4 nanoparticles. Two oppositely charged polyelectrolytes polyallylamine hydrochloride (positively charged) and poly sodium styrene sulfonate (negatively charged) were deposited alternatively on the surface of iron oxide nanoparticles until the desired thickness of polymer coating was obtained. The outermost positively charged polyelectrolyte layer was then coated with negatively charged CdTe QDs to incorporate the OI modality in the nanoconstruct. By using this construct it was demonstrated that photoluminescence intensity of the construct increases with the increase in number of polyelectrolytes layers and remained constant after 21 layers of polyelectrolytes.

Polymer coated γ-Fe2O3 superparamagnetic nanoparticles were coated with dimercapto-succinimid acid (DMSA) to stabilize and functionalize the ferrofluid with both SH and carboxyl acid groups in the ratio 3:20. Trioctylphosphine oxide (TOPO) capped CdSe/ZnS QDs were coupled to magnetic particles using thiol chemistry in 10:5:1 mixture of chloroform/methanol/water. The resulting particles had approximately 3 times lower quantum yield compared to the bare quantum dots in chloroform. This relatively lower quantum yield was attributed to the quenching effect from magnetic core and closely packed structure of quantum dots. These water dispersible nanocomposites were functionalized with anticyclone E using EDC coupling for the detection and separation of breast cancer specific marker cycline E expressing cells in serum.

4.2.3. Fluorescently labeled lipid coated magnetic particles

Biocompatible amphiphilic lipid molecules have been used for the stabilization and further conjugation of nanoparticles. Becker et al. have synthesized oleate lipid bilayer coated Fe3O4 nanoparticles. These particles were further functionalized with biotin in order to attach streptavidin-FITC conjugate. It was shown invivo that these nanoparticles localized in the lysosomal compartment of the HeLa cells through a receptor-mediated uptake mechanism. Van Tilborg et al. have used Annexin A5 functionalized lipid-coated Fe3O4 nanoparticles for the detection of apoptosis in Jurkat cells.

Nitin et al. have synthesized PEG-modified phospholipid micelle coated superparamagnetic iron oxide nanoparticles (mMIONs) for intracellular molecular probes for MRI based deep tissue imaging. Iron
oxide nanoparticles were synthesized with reverse micelle technique to achieve efficient control on the size of nanoparticles. These monodispersed particles were coated with PEG-phospholipid micelles to render them water dispersible and biocompatible. The amine groups available on the surface of micelles were used for attaching Texas Red dye molecules for OI and FTAT peptide for targeting.

4.2.4. Fluorescent inorganic shell coated magnetic particles

Fluorescent dyes exposed to harsh biological environment suffer from photobleaching. This problem was solved by the incorporation of these dye molecules into an optically transparent biocompatible matrix of silica. Silica provides a mechanically and chemically stable, biocompatible, and optically transparent coating that can be used for further functionalization of particles using well developed silica chemistry.

Lu et al. have described the synthesis of fluorescent silica coated superparamagnetic iron oxide nanoparticles. Commercially available ferrofluid (EMG 304, size 5-15 nm) and magnetic particles synthesized using wet chemical method were coated with silica utilizing base catalyzed hydrolysis and condensation of TEOS. The thickness of silica coating was controlled in the range from 2-100 nm by varying the concentration of TEOS used in the reaction. In addition fluorescent dyes such as 7-(dimethylamino)-4-methylcoumarin-3-isothiocyanate and tetramethylrhodamine-5-isothiocyanate were covalently incorporated in the silica shell. These nanocomposites form chain like structures in presence of an external magnetic field as demonstrated by fluorescence microscopy. In another approach Lin et al. have synthesized tumbling like magnetofluorescent particles for cell tracking and drug delivery applications. These multifunctional particles were synthesized by adding organic dye and silica coated Fe3O4 nanoparticles during the synthesis of mesoporous silica nanoparticles.

The application of magnetic core and fluorescent shell particles for targeted diagnostics and therapy was demonstrated by Levy et al. Fe3O4 particles were coated with a thin layer of silica using sodium silicate. A two photon dye (1-Methyl-4-(E)-2- ethenyl) pyridinium iodide or ASPI that absorbs two photons at 800 nm and emits a photon at 580 nm was encapsulated inside the silica shell. The surface of these nanocomposites was functionalized with a luteinizing-hormone-releasing-hormone (LH-RH) for specific targeting and selective lysing of KB cells in a dc magnetic field.

Kim et al. have synthesized magnetofluorescent Co/CdSe core shell nanoparticles by controlled deposition of CdSe shell onto preformed cobalt core. Cobalt nanoparticles were synthesized by thermal decomposition of octacarbonyldicobalt in solution in the presence of coordinating ligand trioctylphosphane oxide (TOPO). The low quantum yield (2-3%) of similar type of core shell (Fe3O4-CdSe) nanoparticles was improved to 10-15% by the deposition of ZnS shell on CdSe. The increase in quantum yield was attributed to decrease in the number of defects in the CdSe shell.

Gu et al. have described a one pot synthesis of sub 10 nm CdS-FePt heterodimer nanoparticle that has both fluorescent and superparamagnetic properties. Similar construct (CdSe-Fe3O4) was synthesized by Gao et al. for intracellular manipulation of magnetofluorescent Fe3O4– CdSe nanoparticles using magnetic force generated by a small magnet. Several other groups have synthesized similar heterodimer nanoparticles that could potentially be used for bioimaging applications.

4.3. Magnetic probes embedded inside organic/inorganic matrix

4.3.1. Iron oxide inside silica matrix

Yi et al. have encapsulated both Fe3O4 nanoparticles and QDs inside a silica matrix using reverse micelles of Igepal CO-520/cyclohexane/water microemulsion. It was observed that co-encapsulation of these particles in silica matrix resulted in decrease of quantum yield from 11.4% to 1.1% for CdSe QDs and from 14.5% to 4.8% for ZnS-capped CdSe QDs. Further, SiO2 coated Fe3O4 particles showed higher magnetization compared to the particles in which Fe3O4 and QDs were co-encapsulated.

Magnetic mesoporous silica nanoparticles covalently bonded with near-infrared (NIR) luminescent lanthanide complexes were synthesized by a two step method. Firstly, Fe3O4 nanoparticles were embedded in the mesoporous silica nanoparticles during their synthesis by surfactant templated method. Secondly, lanthanide complexes were introduced into the nanocomposites via a ligand exchange reaction.
hancing contrast agents due to their highly porous structure. Reiter and coworkers have incorporated a large payload of Gd chelates in mesoporous silica nanoparticles for the synthesis of highly efficient MR contrast agent (MSN-Gd particles). Mesoporous silica nanoparticles were coated with a mixture of rhodamine B-aminopropyl-triethoxysilane and Gd-Si-DTTA complex by refluxing the particles in toluene. The relaxivity values obtained for MSN-Gd are much larger than that of solid silica nanoparticles that are coated with multilayers of the Gd-DTPA derivative. The enhanced MR relaxivity of MSN-Gd was attributed to the ready access of water molecules through the nanochannels of the MSN?Gd particles. Using similar approach Gd incorporated mesoporous silica nanorods were developed for use as both T1 and T2 contrast agents.

Hsiao et al. have synthesized FITC and Gd incorporated fluorescent (Gd-Dye@MSN) T1 contrast agent for human stem cell tracking applications. Gd based contrast agents produce bright positive contrast that is desirable for distinguishing cells in tissues that have low intrinsic MR signal. These particles were synthesized by co-condensing a chelating ligand N-1-(3-trimethoxysilylpropyl)-N-2-(DTPA) phenyl thiourea (DTPA-ph-NCS-APTMS) and N-1-(3-trimethoxysilylpropyl)-N-fluoresceyl thiourea (FITC-APTS) with TEOS during the synthesis of mesoporous silica nanoparticles using CTAB as structure guiding templates. These particles were stirred in a methanolic solution of gadolinium chloride hexahydrate in order to load Gd3+ at the chelating sites. In vitro cellular uptake studies were performed with Gd-Dye@MSN particles using flow cytometry and MRI. Further, Gd-Dye@MSN labeled hMSCs cells implanted into the basal ganglions of nude mice were visualized using MR imaging.

5. Concluding Remarks

Multimodal nanoparticles can provide far more comprehensive and complementary information ranging from tissue structure to biological processes at molecular levels. Although multimodal nanoparticles for simultaneous MR and OI are still at an early stage of development, they offer multiple advantages for clinical diagnostics. In vivo optical imaging using NIR probes is a latest trend in small animal imaging for monitoring the progression of disease and therapy. The recent development of new NIR optical probes with high quantum yield and high photostability are expected to significantly enhance imaging sensitivity. Incorporation of therapeutic moieties within multimodal nanoparticles can be employed for image guided therapies. The ultimate goal is to develop nanoparticles for early stage diagnostics and treatment of diseases. Ability to monitor changes in organ/tissue in response to therapy will eventually enable selection of optimum strategy with minimal side effects, reduced patient discomfort and better prognosis. However, our limited knowledge on the targeted delivery and long term biocompatibility of these particles still remain a challenge that needs to be addressed.

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