ADVANCES IN NANOPARTICLE-BASED MEDICAL DIAGNOSTIC AND THERAPEUTIC TECHNIQUES

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9.1 INTRODUCTION

Advances in modern medicine have eliminated several major causes of human mortality and considerably extended life expectancies around the world; however, this increase in the global age average has also boosted the incidences of age-associated disorders. These conditions, such as cancer, neurodegenerative disorders, and cardiovascular disease, severely decrease the quality of life for the affected but are highly polymorphic and often difficult to treat. Their variable nature often necessitates the customization of treatment methods for each individual patient, which in turn requires close monitoring of the disease in question. In addition, a good prognosis is typically possible only when an early diagnosis has been made, which further emphasizes the need for rapid and accurate diagnostic methods. As medical diagnosis involves a combination of biological (e.g., throat cultures, blood and urine tests), chemical (e.g., immunosorbent and other colorimetric

assays), and physical (e.g., imaging techniques) components, disease detection methods can be advanced through any of these channels, and the multidisciplinary nature and obvious importance of disease diagnostics attracted much attention to this topic over the last few decades.

Imaging techniques are of paramount importance for both *in vivo* and *ex vivo* diagnostic efforts and, alongside biosensors and biochemical assays, form the backbone of nearly all medical analysis. Histopathological analyses are usually performed by light microscopy and chemical stains that visualize different cell types and metabolic states in an excised tissue section; however, such an approach is by necessity invasive and therefore is not amenable to the diagnosis of every disease. In addition, histological analysis often requires a preliminary diagnosis, as biopsy samples are typically taken from regions suspected to be diseased (e.g., based on the patient's symptoms or in areas with gross appearances that suggest disease). As such, diseases such as early stage cancers, which are largely asymptomatic and indistinguishable from the surrounding tissue, are difficult to detect by biopsy or visual investigation (Betz et al., 2002; Strong et al., 1968). Medical imaging techniques, in contrast, are capable of yielding information without any surgical intervention and may therefore detect early stages of a disease through regular screenings. These techniques vary in the electromagnetic region they use but generally work by scanning or rotating an electromagnetic field over the target area and measuring the response given either by the affected tissue or a specific tracer administered prior to imaging (Table 9.1).

Although medical imaging techniques offer invaluable benefits as noninvasive diagnostic methods, they also suffer from a set of limitations. They are limited in resolution compared to histological analysis and therefore cannot detect diseased tissues below a certain threshold volume (this volume changes depending on the technique and the wavelength or field strength used). By extension, they are also limited in their sensitivity and early detection capacity, as tumors may be below this detection threshold during their early development (Ekberg et al., 1988). They are further limited in their ability to identify the physiological characteristics of the disease they detect; while magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT) are capable of distinguishing between healthy and damaged tissues, immunoassays are nonetheless an easier means of evaluating the physiological state of the organ of interest. In addition, as medical imaging often requires the administration of contrast agents (CAs) or exposure to potentially harmful electromagnetic radiation, safety concerns have been raised against the prospect of their overuse (Dawson, 1985).

TABLE 9.1 Medical imaging techniques and the contrast agents used for their enhancement

Technique	Resolution	Tracers and Contrast Agents	References
MRI	<i>c.</i> 100 μm (ideally), millimeter scale (in practice)	Gadolinium chelates; manganese chelates; nanoparticles of gadolinium, manganese, and iron oxides (with or without cobalt, nickel, or manganese doping); metal alloys	Eastwood et al. (2014), Pan et al. (2011), Yablonskiy and Sukstanskii (2015)
CT	<i>c.</i> 1 μm (ideally), <i>c.</i> 130 μm (in practice)	Iodinated; nanoparticles of gold, silver, bismuth, tantalum, and other heavy transition metals	Burghardt et al. (2011)
PET and SPECT	Millimeter to centimeter scale	Radiotracers—water, salts, or biological molecules with radioactive atom substitutions. ^{11}C , ^{13}N , ^{15}O , and ^{18}F are common replacements	Bateman et al. (2006), Ciernik et al. (2003)
Optical imaging and fluoroscopy	Abbe limit (ideally), millimeter scale (in practice, NIR)	Fluorophores, quantum dots, gold and silver nanoparticles, upconverting nanoparticles	Chang et al. (2003)
Ultrasound	<i>c.</i> 20 μm for high-frequency ultrasound (but low penetration), <i>c.</i> 100–150 μm typical	Gas microbubbles	Calliada et al. (1998), Stanga and Bird (2001), Wright et al. (2006)

Much work has been performed on potential methods to alleviate these deficiencies. The resolution limit of the MRI can be enhanced by using a stronger field, which requires more powerful (and often expensive) magnets (Lee et al., 2009). Likewise, the resolution of positron emission tomography (PET) can be increased by the use of thinner detector elements, the crystal components of which become exponentially more expensive to produce (Thompson et al., 1994). Consequently, high-resolution medical imaging devices are mostly limited to academic research, and the modification of the tracers and CAs is a more convenient means of circumventing the disadvantages of these methods. These modifications can be performed to allow the imaging agent to track a specific biological moiety, to decrease its toxicity, to prevent an immune response from being raised against the agent, to increase or decrease its renal clearance, or to grant it therapeutic functions in addition to its diagnostic capacity. In addition, the size and morphology of the agent may be modified, and the material may be designed so as to serve as an imaging agent for multiple complementary techniques (Lee et al., 2008; Xie et al., 2010).

Due to their ease of functionalization, relative lack of toxicity, suitable magnetic properties, and applicability to multiple imaging techniques, nanoparticles (NPs) in particular have been popular targets of CA research. Typically, these NPs possess a metal core that grants them their contrast-enhancing properties and are coated by one or more “shell” layers that decrease the toxicity of the metal used, increase the circulation time of the complex, or allow specific targeting of a specific tumor or tissue type. However, despite the variety of NPs developed for imaging enhancement, a broad overview of their properties, advantages, disadvantages, and potential uses in medical diagnosis has so far been lacking. In this chapter, we describe the characteristics of NP CAs proposed for use in medical imaging, detail the surface modification methods used to designate specific targets for their attachment, compare their effectiveness and toxicity compared to conventional methods of contrast enhancement, and discuss the contribution that nanoscience has had, and will have, on medical imaging and disease diagnosis at large.

9.2 NPs USED IN MRI

MRI is one of the most valuable noninvasive imaging modalities and is frequently used in both clinical and research studies to diagnose various disorders. In addition to its high spatial resolution, MRI utilizes nonionizing radiation and is therefore capable of gathering three-dimensional

tomographic images with negligible damage to the imaged organ or tissue (Lodhia et al., 2010). Longitudinal (T_1) and transverse (T_2) proton relaxation times of water (or, less frequently, other molecules) are the two main parameters that affect the signal quality of MRI; consequently, contrast in MR images is a result of differences in proton relaxation times. Proton density differences between different tissues are another factor that affects the contrast in MRI: Since the altered physiological environment of a diseased region may yield a different intrinsic relaxation time compared to the rest of an organ or system, a specific contrast between healthy and aberrant tissues may be observed in MR images at more advanced stages of many disorders (Mulder et al., 2006). However, early diagnosis by MRI is feasible only for a select number of tissues and diseases; where in many other cases, the resolutions obtained are too low to conclusively determine the presence of disease. A variety of CAs have been designed to enhance the diagnostic value of MRI and lower the threshold at which the technique is able to differentiate between healthy and diseased tissue, which is accomplished by shortening T_1 or T_2 relaxation times or, in other words, increasing r_1 ($1/T_1$) or r_2 ($1/T_2$) relaxivities (Wang and Chen, 2009). Paramagnetic and superparamagnetic materials are typically used as T_1 and T_2 CAs, respectively, and applied in clinical imaging (Liu et al., 2012a).

9.2.1 T_1 CAs

Paramagnetic metals possess a large number of unpaired electrons, which create magnetic dipole moments when exposed to a magnetic field. Among all transition and lanthanide metals, gadolinium (Gd^{3+}), manganese (Mn^{2+}), and iron (Fe^{3+}) ions are known to have the strongest paramagnetic properties and show the most effective T_1 relaxation times, which increases both the signal intensity and the brightness of the image. This relaxation is enhanced as a result of dipolar interactions between the protons of water molecule inside the tissue and unpaired electrons of the metal when the paramagnetic compound is in close proximity to the tissue. The brightness of this signal makes anatomic details discernible in either pathogenic or ordinary conditions, and the paramagnetic nature of these compounds precludes them from altering the magnetic homogeneity of the body, effectively preventing them from disrupting the background of MR images. The r_2/r_1 ratio is the factor of interest for T_1 CAs (also called positive agents) and must ideally be as low as possible (i.e., a high r_1 value and a low r_2 value). T_1 agents have been used in either NP (Gd_2O_3 , GdF_3 , MnO , Mn_3O_4 , Fe_2O_3 , etc.) or complex form (Gd-DTPA, Mn-DOTA, etc.) (Hu and Zhao, 2012). In addition, there are several examples regarding the incorporation of Gd^{3+}

into nanostructured frameworks such as dendrimers, liposomes, polymers, and metal-organic frameworks (MOFs). Although they exhibit enhanced relaxivity and sensitivity compared to a single ionic chelate, the production of these nanostructures is laborious and costly, and their large sizes impose further limitations on their potential utility. Since NP-based T_1 CAs are superior to complex-based agents in terms of toxicity, sensitivity, relaxivity, contrast, retention time, and tailoring and targeting abilities, studies related to the development of the former have garnered great interest in recent years (Liang et al., 2013). As such, the present section will focus on inorganic NP-based systems designed for T_1 contrast enhancement.

9.2.1.1 Gd-Based CAs Recently, significant effort has been directed toward the utilization of gadolinium NPs, including gadolinium oxide (Gd_2O_3), gadolinium fluoride (GdF_3), and gadolinium phosphate (GdPO_4), in MRI. Synthetic approaches to produce these NPs show great variation: Reduction–coprecipitation, polyol, and hydrothermal processes are all utilized for Gd-based nanoparticle (GdNP) synthesis. The first technique results in the formation of polydisperse NPs and aggregates and is consequently not an efficient way to produce NPs for MRI. In polyol processes, crystal growth is controlled by a stabilizing agent in polyol, while extraction and exchange steps are performed with another, stronger stabilizing agent. In the last technique, hydrophobic NPs are prepared in high-boiling organic solvents, and the organic solvent is replaced by water in the presence of a hydrophilic ligand that solubilizes the NP in aqueous environment (Johnson et al., 2011). Following synthesis, the NPs must be functionalized in order to control their solubility, biocompatibility, stability, and retention times in the tissue of interest. Dextran, polyethylene glycol (PEG), polysiloxane, citrate, aminoethyl phosphate (AEP), and PEG-silica are some of the materials that impart these properties to metallic NPs and are commonly used in NP functionalization. Surface modification also enables tethering bioactive moieties onto the NP surface for targeting purposes (Na and Hyeon, 2009). Gd NPs can demonstrate different relaxivities depending on their size, shape, composition, and method of assembly (Hu et al., 2011). Park et al. prepared D-glucuronic acid-coated ultrasmall Gd_2O_3 NPs with an average diameter of 1 nm (Park et al., 2009). While the NP core provided a large longitudinal relaxivity of $9.9 \text{ mM}^{-1} \cdot \text{s}^{-1}$ (1.5 T) with a low r_1/r_2 ratio (1.1), the coating allowed *in vivo* T_1 -weighted MRI of rat brain tumors. However, despite the effective visualization of subanatomic details of organs with Gd-based CAs, the toxicity of the metal remains as an obstacle to the use of these materials in clinical practice.

9.2.1.2 Mn-Based CAs Another paramagnetic ion used for T_1 -weighted MRI is Mn^{2+} . Among manganese oxides, MnO and Mn_3O_4 are the two most commonly synthesized NPs and can be produced with great control over particle size. Since paramagnetic Mn^{2+} ions present on the NP surface is the main factor that shortens the longitudinal relaxation time T_1 , the total surface area of the NP is a key factor to obtain high r_1 relaxivity values in Mn-based NPs. The pronounced effect of the NP surface-to-volume ratios on the r_1 value is exemplified by a study by Shin et al., in which solid MnO NPs were prepared with a hydrothermal method and stirred in an acidic buffer solution to create hollow structures. According to the results, r_1 values were found as 0.21 and $1.42 \text{ mM}^{-1} \cdot \text{s}^{-1}$ at 3 T for solid and hollow MnO nanoparticles (HMON), respectively (Shin et al., 2009). Since the inner surfaces of hollow NPs contain a higher concentration of Mn^{2+} ions compared to their solid counterparts, the enhancement probably stems from the fact that the former interacts more with water molecules. Other than HMON, mesoporous silica-coated MnO NPs were also synthesized to increase the accessibility of water to manganese ions with the help of the porous coating (Zhu et al., 2013). Different preparations of Mn-based NPs were used to visualize various organs and tissues, such as brain, liver, and kidney, using MRI. Although these agents effectively depict detailed structures in the brain and can detect neuronal activity in T_1 -weighted MR, they are not applicable in humans because of the hepatic and cardiovascular toxicity effect of free manganese ions (Na and Hyeon, 2009).

9.2.1.3 Fe-Based CAs Both Gd-based and Mn-based T_1 -weighted CAs show detrimental effects due to the dissociation and accumulation of these ions in the body. Consequently, much research has been performed on nontoxic formulations of these metals, as well as on nontoxic alternatives to traditional T_1 CAs. Since iron atoms are naturally found in the human blood and their excess can be stored as ferritin in the body, iron oxide is a promising candidate for use as a more biocompatible CA. Although Fe-based NPs are considered to be superparamagnetic T_2 CAs because of their high magnetic moments, the small-sized iron oxide NPs allow them to exhibit low r_2/r_1 ratios, which makes them appealing for T_1 -weighted MRI. As the size of an NP decreases, so does the effects of magnetic anisotropy and spin disorders at the particle surface (Jun et al., 2008). However, uniformity and stability are two requirements for NPs to maintain a high T_1 contrast effect, and the synthesis of uniform NPs at very small sizes is problematic. Several approaches have been attempted to achieve the required sizes and uniformities in iron oxide NPs; these are principally classified under (i) hydrophobic and (ii) hydrophilic phase syntheses.

The former is performed at high temperatures ($>200^{\circ}\text{C}$) to allow the decomposition of the iron precursor and the formation of homogeneous, extremely small iron oxide nanoparticles (ESPIONs), which then are made water soluble through ligand exchange (Tromsdorf et al., 2009). The latter is a simpler, one-step approach in which triethylene glycol (or diethylene glycol) acts as both a reductant and a surfactant to react with iron precursors at high temperatures, resulting in hydrophilic ESPIONs (Park et al., 2008). Hyeon's group synthesized PEG-derivatized phosphine oxide-capped ESPIONs with sizes down to 1.5 nm by a heat-up process and used them as T_1 MRI CAs for high-resolution angiography. They suggested that these NPs have the potential to be used in the diagnosis of several diseases, such as myocardial infarction, renal failure, atherosclerotic plaque, and thrombosis, because of their low toxicity, high r_1 relaxivity, long circulation times, and cost-effective synthesis (Kim et al., 2011). Very recently, Liu et al. also prepared glutathione-coated ESPIONs with 3.7 nm diameters using a single-step reaction in mild conditions, under atmospheric pressure and room temperature. The authors anticipate that these biocompatible high T_1 CAs ($3.63\text{ mM}^{-1}\cdot\text{s}^{-1}$ at 4.7 T and 40°C) can be employed to great effect in vascular diagnosis, especially in stroke, venous thrombosis, renal disease, and urinary tract tumor diagnosis (Liu et al., 2014).

9.2.1.4 Hybrid Systems T_1 -weighted MR contrast can be further enhanced using a variety of derivative methods, such as by embedding paramagnetic species into NPs (Zhou et al., 2013), developing surface-doped metal oxides (Sook Choi et al., 2010), or doping of Gd^{3+} to NaYF_4 NPs (Hou et al., 2013). Zhou et al. synthesized gadolinium-embedded iron oxide (GdIO) NPs ($\sim 5\text{ nm}$ diameter) and coated them with zwitterionic dopamine sulfonate molecules to create a hydrophilic surface, decrease nonspecific protein adsorption, prevent agglomeration, and provide a fast renal clearance. The embedding of Gd species in iron oxide NPs enhances the spin-canting effect, resulting in increased r_1 relaxivity ($7.85\text{ mM}^{-1}\cdot\text{s}^{-1}$ at 7 T) and a lower r_2/r_1 ratio (5.24) for 4.8 nm diameter GdIO NPs compared to bare iron oxide NPs with similar sizes. Furthermore, the passive targeting ability of the NPs was investigated using a subcutaneous SKOV3 ovarian cancer model, and the NPs were found to create a marked improvement in T_1 contrast enhancement in the tumor site (Zhou et al., 2013). A similar system was reported by Choi et al., who doped Gd_2O_3 NPs with MnO and further functionalized the hybrid complex with lactobionic acid to increase biocompatibility and surface hydrophilicity. The complex was tested on the mouse model, and the r_1 relaxivity was found to increase from 9.9 to $12.8\text{ mM}^{-1}\cdot\text{s}^{-1}$ after MnO doping; in addition, the r_2/r_1 ratio rose to 2.1 due

to the dopant effect of MnO on r_2 relaxivity (Sook Choi et al., 2010). Among upconverting nanoparticles (UCNPs), Gd species-doped UCNPs demonstrate good MR contrast potential. Hou et al. synthesized NaGdF₄ NPs (~20 nm) exhibiting an r_1 relaxivity of 8.78 mM⁻¹·s⁻¹, which is higher than that of Gd chelate (Gd-DTPA). In addition, a T_1 contrast enhancement was observed in tumor-bearing mice when these NPs were conjugated with an anti-EGFR monoclonal antibody (Hou et al., 2013).

9.2.2 T_2 CAs

Superparamagnetic materials exhibit a high susceptibility similar to ferromagnetic materials in the presence of an external magnetic field and a rapid demagnetization similar to paramagnetic molecules when the magnetic field is removed (Schaeffer, 1997). These materials, called T_2 or negative CAs, are typically associated with low T_2 relaxation times and the acquisition of darker images with low signal intensities, which stem from microscopic field inhomogeneities and the activation of proton dephasing. The spin-spin relaxivity r_2 , which depends firmly on the magnetic moment and the relaxation processes of the magnetic spin, is a key factor for determining the degree of T_2 contrast effect on the NPs, with higher values resulting in greater contrast. The presence of T_2 agents creates darker MR images, and their signals may closely resemble the signatures associated with bleeding, calcification, or metal deposits; in addition, susceptibility artifacts that alter the background image are hard to distinguish from genuine signals with these CAs. Nonetheless, T_2 agents are still promising for use in clinical applications on account of their high r_2 and biocompatibility, as well as their prolonged circulation times in the body (Lee et al., 2012). T_2 CAs generally incorporate pure iron and cobalt metals; alloys such as CoPt₃, FePt, and FeZn; and iron oxides such as magnetite (Fe₃O₄) and maghemite (γ -Fe₂O₃). Unfortunately, problems associated with toxicity and susceptibility to oxidation limit the use of cobalt- and nickel-containing NPs in biomedical applications. Nonetheless, divalent cations such as Mn, Fe, Co, or Ni can be used as dopants for iron oxides to improve the magnetic properties of MFe₂O₄ structures (Veisheh et al., 2010).

9.2.2.1 Iron Oxide NPs Maghemite (γ -Fe₂O₃), magnetite (Fe₃O₄), and hematite (α -Fe₂O₃) are the main forms of iron oxide; and among these, magnetites are the most effective CAs and have received much attention as “next-generation” imaging agents due to their biocompatibility, biodegradability, and low levels of toxicity (Yu et al., 2008). In order to display superparamagnetic properties, a particle should have a suitable

crystal structure, size, and shape (Jun et al., 2008). Several techniques allow the control of these features in iron oxide NPs, in addition to other factors such as solubility and size distribution. These synthetic approaches can be listed as (i) coprecipitation of iron salt solutions, (ii) thermal decomposition and/or reduction, (iii) hydrothermal synthesis, and (iv) polyol synthesis (Laurent et al., 2008). Among these techniques, thermal decomposition is the most widely used method for the production of NPs with tunable sizes (4–50 nm) and narrow size distributions, despite the limitations faced in large-scale production. In addition to scaling problems, a phase transfer into an aqueous solution is also required for these hydrophobic NPs to be used in biomedical applications (Barreto et al., 2011).

Since MRI CAs are administrated intravenously, the solubility, stability, and dispersity of NPs in water are also essential for NP CAs to be used effectively for any clinical application (Caravan, 2009). Carboxylates, phosphates, sulfonates, silicon compounds, gold, and polymers such as dextran, PEG, and polyvinyl alcohol (PVA) are frequently used as stabilizing agents to improve the stability of NPs in water (Barreto et al., 2011). Water-stabilized NPs can be further functionalized by incorporating targeting moieties (proteins, antibodies, peptides) and drug molecules to detect a specific disease or track cellular processes (Mahmoudi et al., 2011). In terms of MR contrast enhancement, NP size is the predominant parameter and should be taken into consideration in the design of both T_1 and T_2 CAs. Jun et al. investigated the effect of size on the spin–spin relaxivity (r_2) value and demonstrated that r_2 gradually increases with the size of Fe_3O_4 NPs, due to the enhanced magnetic moment produced by larger particles (Jun et al., 2005). Recently, Zhao et al. developed a new strategy to increase r_2 relaxivity by altering the morphology of iron oxide NPs. This method allows the production of size-controllable octapod iron oxide NPs with ultrahigh transverse relaxivity values ($679.3 \pm 30 \text{ mM}^{-1} \cdot \text{s}^{-1}$), which are of potential interest in applications involving *in vivo* imaging and small tumor detection (Zhao et al., 2013). Additionally, surface hydrophilicity and coating thickness are known to contribute greatly to MR contrast by affecting the proton relaxivities of iron oxide NPs (Huang et al., 2012).

9.2.2.2 Metal-Doped Iron Oxides Transition metal dopants (M^{2+} , where $\text{M} = \text{Co}$, Ni , or Mn) may be used instead of Fe^{2+} ions in MFe_2O_4 NPs to create significant MR contrast enhancement effects. Among all ferrites, MnFe_2O_4 NPs exhibit the highest mass magnetization value of 110 (emu/mass of magnetic atoms). In accordance with magnetization results, Mn-doped MnFe_2O_4 shows the strongest MR contrast effect, with an r_2 relaxivity value of $358 \text{ mM}^{-1} \cdot \text{s}^{-1}$ at 1.5 T. Thus, the material composition of NP complexes

plays an important role in controlling the spin–spin relaxation processes of protons in the water molecules. Furthermore, herceptin-conjugated MnFe_2O_4 was shown to have a superior T_2 contrast effect compared to an undoped control in both *in vitro* cancer detection experiments and *in vivo* small tumor MRI (Lee et al., 2006).

9.2.2.3 Metal Alloy NPs Another class of MR probes, metal alloys, is exemplified by iron alloys such as FeCo and FePt. In these NPs, the parallel alignment of magnetic spins to the external magnetic field results in a higher magnetic moment compared to ferrimagnetic NPs. For instance, FeCo/graphitic shell nanocrystals, which were further coated with phospholipid–poly(ethylene glycol) (PL-PEG) to provide colloidal stability, were found to have a ultrahigh magnetization value of 215 emu/g metal and an r_2 relaxivity value of 644 $\text{mM}^{-1}\cdot\text{s}^{-1}$. Moreover, enhanced MR contrast was observed in FeCo NP-labeled mesenchymal stem cells, suggesting that these probes are able to provide excellent cellular MR signals. This study also shows that the toxic effect of highly reactive Co ions can be eliminated by using a suitable coating material, such as a graphite shell, to increase the safety of these heavy metal NPs in clinical practice (Seo et al., 2006).

9.2.3 Dual Modal Contrast Agents

In recent years, there has been a growing interest on the development of T_1 – T_2 dual model strategies for MRI, which offers more precise diagnostic information by simultaneously utilizing the T_1 and T_2 imaging modes. While T_1 imaging provides high tissue resolution, T_2 imaging allows the detection of tumor sites with high confidence; as such, their combination allows both high-resolution imaging and the acquisition of biologically meaningful information. Since each imaging technique has a distinct penetration depth and spatiotemporal resolution, dual imaging within a single device may increase the diagnostic potential of MRI beyond what is expected from the individual imaging modes. However, the creation of an effective T_1 – T_2 dual CA requires the prevention of direct contact between the two CAs, as the T_1 signal would otherwise be quenched due to the fact that the magnetic field generated by the T_2 CA perturbs the relaxation process of the T_1 CA. Cheon's group designed an inorganic core–shell model in which T_2 (MnFe_2O_4) and T_1 ($\text{Gd}_2\text{O}(\text{CO}_3)_2$) contrast materials were located in the core and the shell, respectively, and were separated by a SiO_2 layer with different thicknesses to modulate the degree of T_1 and T_2 coupling (Choi et al., 2010). Zhou and coauthors proposed a different core–shell model, in which superparamagnetic iron oxide nanoparticles

(SPIONs) and Gd species (e.g., Gd_2O_3) were integrated, and this system was used for the accurate detection of hepatic tumors in mice. This “core–shell” arrangement is also notable for facilitating a synergistic effect on r_1 and r_2 relaxivities by enhancing local magnetic field strengths under an external magnetic field (Zhou et al., 2012b).

9.3 NPs USED IN COMPUTED TOMOGRAPHY

Computed tomography (CT) imaging is another means by which high-resolution three-dimensional images of tissue structures can be acquired: While MRI scans change their focus by altering the applied magnetic field, a CT scanner physically moves around the patient to obtain a full “view” of the desired tissue. X-rays are used for imaging, and adverse effects caused by overexposure to radiation have long been a point of concern for CT scans (Chodick et al., 2007; Smith-Bindman et al., 2009). The images are interpreted on a similar basis as conventional X-ray radiography, in that the relative permittivity of the imaged area to the passage of X-rays is used to differentiate between tissues. This permittivity, called radiodensity, is evaluated using the Hounsfield scale, an arbitrary scale that sets the radiodensity of distilled water under standard conditions at zero Hounsfield units and the radiodensity of air under standard conditions at 1000 Hounsfield units (Forbes et al., 1978). X-ray attenuation, which is the combination of X-ray absorption and scattering, is also defined on the Hounsfield scale, and any given element has its own energy-dependent X-ray attenuation profile (Cormode et al., 2014). Damaged or diseased tissues typically possess different radiodensities, allowing their identification under CT, while natural differences between different tissue types allow the imaging of the local tissue structure. The latter property has led to the combination of the technique with PET scans, which visualize tissue activity rather than structure (von Schulthess et al., 2006; Wu et al., 2013).

The use of X-rays by this technique has resulted in frequent criticisms about its potential risks, especially on patients who may also benefit from less damaging imaging methods. Radiation exposure may be minimized by decreasing the exposure time, energy, or intensity of the X-ray beam; however, all three methods also decrease the resolution of the technique. Radiocontrast agents are typically administered both to increase the accuracy of the scan and to minimize the exposure to radiation. Presently, aromatic iodinated molecules are the clinical standard for *in vivo* contrast enhancement (Pasternak and Williamson, 2012). However, these CAs possess several

limitations that restrict their use in clinical settings, mainly due to issues based on their (i) toxicity, (ii) high osmolality and viscosity, (iii) low payload capacity for targeted imaging, and (iv) low molecular weight. Although new approaches have been developed to eliminate these limitations, no marked improvement has so far been reported, especially with regard to the low attenuation coefficients and nontargeting natures of iodinated molecules (Jakhmola et al., 2012). Therefore, new radiocontrast agents with improved functionalities are required to increase the diagnostic capacity of X-ray based imaging methods and eliminate the toxicity issues associated with current contrast enhancers. These “next-generation” CAs should ideally fulfill several requirements: (i) High solubility, (ii) chemical stability and inertness in the physiological environment, (iii) opacity to X-rays, (iv) cost effectiveness, (v) biocompatibility, (vi) ease of functionalization, and (vii) long *in vivo* circulation times are all essential properties for a potential radiocontrast agent (Liu et al., 2012a). NP-based CAs fulfill many of these criteria and have therefore been developed to overcome the potential shortcomings of iodinated CT CAs.

9.3.1 Noble Metal-Based NPs

There has been a recent surge of interest in the use of noble metal NPs, especially gold and silver, in the field of CT imaging. These NPs are easy to synthesize and can be functionalized through well-established surface chemistry methods; as such, gold and silver are ideal materials for the production of surface-functionalized NPs in specific morphologies with little batch-to-batch variance.

9.3.1.1 Gold Nanoparticles Gold nanoparticles (AuNPs) are useful for X-ray CT imaging due to the high signal-to-noise ratios they exhibit, which allows the reduction of exposure times and the associated radiation damage to the patient. AuNP radiocontrast agents can also replace their iodine-based counterparts for *in vivo* imaging, as gold is a relatively inert element and its higher atomic number and electron density increase the resolution of *in vivo* X-ray imaging results. The X-ray absorption coefficient of gold is higher than that of iodine (5.16 and 1.94 cm²/g, respectively, at 100 keV); as such, gold particles yield around 2.7 times higher contrast per unit weight than iodine (Hubbell and Seltzer, 1995).

AuNPs can be synthesized by the reduction of gold salts by a variety of reducing agents and can be further decorated with active molecules. Citrate reduction, also known as the Turkevich method, is the most popular technique for AuNP synthesis. Citrate is used both for the reduction of gold and as a

stabilizing agent, and the size and shape of the resulting AuNPs can be altered by changing the reduction conditions (Kimling et al., 2006). The seeding growth method is another popular technique, producing size-adjustable particles within a size range of about 5–40 nm (Jana et al., 2001). Xu et al. examined the effect of size on the X-ray attenuation of NPs and compared the results with clinically administered Omnipaque. In this study, NPs synthesized by different synthetic approaches had a size range of 4–60 nm, and X-ray attenuation was found to be greater in smaller NPs compared to either larger NPs or Omnipaque, which is probably due to the increased surface area (Xu et al., 2008).

AuNPs can also be modified to exhibit additional functionalities, such as targeting capacity (Eck et al., 2010; Sun et al., 2009) or antifouling (Kim et al., 2007) properties, through facile surface chemistry. Sun et al. conjugated heparin–3,4-dihydroxy-L-phenylalanine (DOPA) functional group to AuNPs by a facile coating technique and found that intravenously injected AuNPs enhanced the resolution of liver-specific CT images, while no marked contrast signal was observed in the liver tissue of mice treated with the commercialized iodine-based CA eXIA160 (Sun et al., 2009). Eck et al. likewise demonstrated that anti-CD4 antibody-conjugated AuNPs facilitated the enhancement of CT contrast in the peripheral lymph nodes of mice (Eck et al., 2010). Kim et al. covalently anchored PEG to citrate-reduced AuNPs in an aqueous environment via thiol chemistry, thus preventing the nonspecific binding of plasma proteins and salts to AuNPs and alleviating the rapid clearance issues faced by NPs in the bloodstream. These PEG-coated AuNPs were still present in the bloodstream after 4 h without significant loss of contrast, exhibiting longer half-lives compared to the clinically used iodine-based CA Ultravist (<10 min) (Kim et al., 2007).

AuNPs can also be delivered as part of a therapeutic complex. Dendrimers have been used in the fabrication of organic/inorganic hybrid NPs either by entrapping or stabilizing AuNPs: While single or multiple NPs are entrapped within each dendrimer molecule in the formation of dendrimer-entrapped nanoparticles (DENPs), multiple dendrimer molecules surround the surface of a single NP in dendrimer-stabilized nanoparticles (DSNP). Dendrimer-entrapped gold nanoparticles (Au DENPs) are not only stable in different environments such as water, phosphate-buffered saline, and cell culture media but also retain their stability at various temperatures and pH (Guo et al., 2010). Fast reduction and nucleation chemistry have been used in the synthesis of Au DENPs, resulting in NPs smaller than 5 nm (Shen and Shi, 2010). Although the highly positive charges in the terminal amines of PAMAM dendrimers are known to result in high cytotoxicity and nonspecific binding to the cell membrane, these dendrimers are nonetheless used as templates due to their

commercial availability. The surface of Au DENPs is commonly acetylated to minimize the risk of toxicity and nonspecific binding (Shi et al., 2008). Hybrid gold–dendrimer systems can also be modified with additional functionalities through the use of targeting molecules, such as folic acid (Wang et al., 2013) or the RGD peptide motif (Shukla et al., 2008). Au DSNPs can be synthesized in a similar manner as Au DENPs, as long as low-generation dendrimers are used. However, the production of Au DSNPs using higher-generation dendrimers requires complexation of gold salts with PAMAM dendrimers followed by physical or chemical reduction (Shen and Shi, 2010).

9.3.1.2 Silver NPs Silver NPs intended for use in biomedical applications generally suffer from toxicity and stability issues; however, these problems can be remedied using surface modifications. Liu et al., for example, formed size-tunable (8.8–23.2 nm) acetylated Ag DSNPs, which exhibited prolonged contrast enhancement in CT imaging performed on mice and did not show any toxic effects. Even though elemental silver has a lower X-ray absorption coefficient than iodine (due to the former's lower atomic weight), silver NPs with diameters of 16.1 nm were reported to show similar X-ray attenuation profiles with a clinically used iodine-based CA (Omnipaque), which may stem from the longer circulation times of silver NPs in the blood (Liu et al., 2010). The same group recently prepared spherical Au–Ag alloy DSNPs by using a G5 PAMAM dendrimer and folic acid as the stabilizer and targeting agent, respectively. Although the NPs have not yet been tried for targeted tumor imaging *in vivo*, the folic acid-modified particles were observed to be uptaken to a greater extent by cancer cells overexpressing folic acid receptor (Liu et al., 2013a).

9.3.2 Heavy Metal-Based NPs

Heavy metals possess good X-ray absorption properties because of their high density and atomic weights. More importantly, their X-ray attenuation is not significantly affected by changes in their X-ray energy, which is related to the K-edge values of heavy metals (Jakhmola et al., 2012). Unfortunately, the accumulation of certain heavy metals in the body may result in serious health problems, up to and including fatalities. Nevertheless, bismuth- and tantalum-based NPs have been produced and used for CT imaging without showing considerable toxic effects.

9.3.2.1 Bismuth-Based NPs The use of bismuth-based NPs in CT imaging has attracted great interest in recent years, due to high atomic number ($Z=83$) and large X-ray attenuation coefficient ($5.74\text{ cm}^2/\text{kg}$ at 100 keV) of bismuth.

In addition, bismuth has low toxicity for a heavy metal and is readily cleared from the body without leaving residues. Since the synthesis of elemental bismuth NPs suffers from hydrolytic instability issues and the need for stringent purification steps, bismuth-based NPs often use compounds of bismuth, such as bismuth(III) sulfide (Bi_2S_3) (Brown and Goforth, 2012; Cormode et al., 2014). Rabin et al. reported the synthesis of polyvinylpyrrolidone (PVP)-coated Bi_2S_3 NPs with low cytotoxicity and good aqueous stability and demonstrated that the NPs display enhanced *in vivo* contrast efficacy and circulation times compared to commercialized iodine-based agents (Rabin et al., 2006). Ai et al. also synthesized PVP- Bi_2S_3 nanodots, which became water soluble after facile ligand exchange in dichloromethane. Their contrast efficacy was 1.9 times higher than that of the clinically used CA iobitridol, and X-ray CT imaging results on rats suggested that these particles had the potential to be used in vascular imaging and the detection of hepatic metastases (Ai et al., 2011). Sailor and his group reported the synthesis of Bi_2S_3 NPs conjugated to a cyclic peptide containing nine amino acid residues (CGNKRTRGC, LyP-1) and demonstrated that the NPs had a pronounced accumulation in tumor sites when administered to $4T_1$ breast tumors in mice (Kinsella et al., 2011).

9.3.2.2 Tantalum Oxide NPs Bioinertness, cost effectiveness, and a high X-ray attenuation coefficient value ($4.3\text{ cm}^2/\text{kg}$ at 100 keV) all make elemental tantalum a good candidate for CT imaging. Bonitatibus et al. reported a two-step synthesis of Ta_2O_5 core-shell NPs, in which the core is prepared through the controlled hydrolysis of tantalum ethoxide, $\text{Ta}(\text{OEt})_5$, and the surrounding shell is produced by coating with (2-diethylphosphatoethyl) triethoxysilane. The resulting NPs were water soluble and stable, had diameters of 6 nm, and showed enhanced CT contrast compared to iodine. Although no toxic effects were observed in rats at the concentrations administered, further analyses are required to establish whether the NPs are safe for use as CT CAs (Bonitatibus, 2010). Oh et al. reported large-scale synthesis of TaO_x NPs by a microemulsion method and further modified these core NPs with silane derivatives using an *in situ* sol-gel reaction. Following silane modification, the surface was modified with a secondary layer of PEG and/or Rhodamine B to allow prolonged circulation times and fluorescence imaging capacity, respectively. The addition of the fluorescent dye increased the *in vivo* circulation time, which is an important issue for CT agents intended for image-guided lymph node mapping and CT angiography (Oh et al., 2011).

Other elements, such as platinum, ytterbium, yttrium, gadolinium, palladium, holmium, and tungsten, have been used for CT imaging, albeit less frequently

(Cormode et al., 2014; Jakhmola et al., 2012). These will not be discussed in this chapter, as their methods of functionalization are similar to the ones that were outlined. From a biological standpoint, the major requirements for a radiocontrast agent are biocompatibility and solubility in biological environments, which are principally achieved by coating the core with a lipid, polymer, protein, silica, or any other suitable moiety and can be performed on both “conventional” and more uncommon metal NPs.

9.4 NPs USED IN OPTICAL AND FLUORESCENCE IMAGING

Although organs such as the eye and the outer ear are amenable to unaided visual inspection and endoscopic techniques such as colonoscopy and angioscopy can be used to visualize a broader range of tissues, the majority of the body is opaque to visible light. Nonetheless, it is possible to use light in the near-infrared (NIR) region, which displays a greater depth of penetration compared to the visible spectrum, to inspect the structure and function of a broader range of tissues. NIR light penetrates to a depth of up to 10 cm in the human body and, depending on the technique, can be used for the detection of tissue abnormalities at depths ranging from the superficial (such as burns and skin lesions) to deep tissue (such as brain and other solid organ tumors) (Huang et al., 2006; Still et al., 2001; Wang et al., 2004). However, tissues typically offer little NIR contrast by themselves, limiting the effectiveness of unenhanced NIR imaging and necessitating the use of CAs to increase imaging resolution (one exception is the detection of hemoglobin, which naturally absorbs in the NIR and shows different absorption patterns in the presence and absence of oxygen) (Pogue et al., 2001; Villringer and Chance, 1997). These CAs are typically fluorescent dyes that absorb in the NIR region and can be functionalized with one or more targeting molecules to ensure their delivery into a specific region (usually a tumor site).

Organic dyes and inorganic NPs are both used for contrast enhancement in *in vivo* NIR imaging applications. Organic fluorophores, as exemplified by the FDA-approved diagnostic agent indocyanine green, are limited in their effectiveness by their low quantum yields, tendency for rapid photobleaching, hard-to-control excitation and emission wavelengths, and inability to secondarily enhance targeting efficiency by functionalizing the CA with a large number of targeting molecules (Frangioni, 2003). The limitations of organic fluorophores can be overcome with the use of NP CAs, which display higher quantum yields, are resistant to photobleaching, and can be synthesized with great control over excitation and emission

wavelengths. In addition, these NPs can be modified for *in vivo* applications by surface modification of the metal NP, which may be performed to introduce additional functionalities such as biocompatibility, enhanced clearance profiles, or targeting capacity.

It must be noted here that, in addition to NIR fluorescence imaging, a number of spectroscopic techniques have been repurposed for *in vivo* applications. The laser and detector elements of Raman and FTIR spectrometers, for example, can be mounted on the tip of a fiber-optic catheter and used in endoscopy in order to assist in diagnosis (Li et al., 2005; Qian et al., 2008). Although these techniques offer highly accurate diagnostic capacities and are sometimes used in tandem with NPs for contrast enhancement, they are not currently in clinical use and will not be covered by the present chapter (Qian et al., 2008).

9.4.1 Quantum Dots

Element pairs from the groups III–V, II–IV, or IV–VI of the periodic table can be used to produce fluorescent semiconductor nanocrystals called quantum dots (QDs), typically in sizes between 1 and 10 nm. These NPs are highly resistant to photobleaching and chemical degradation and display very high quantum yields, making them prime candidates for fluorescence imaging. They have been synthesized using different methodologies, resulting in either hydrophobic or hydrophilic QDs. The former necessitates further modification to increase their water solubility and biocompatibility and have been synthesized via organometallic and chalcogen precursor pyrolysis. Either ligand exchange with thiolated molecules or encapsulation by a variety of different moieties such as polymers, silica, or phospholipid micelles can be employed to allow the use of hydrophobic QDs in aqueous systems, such as in *in vivo* imaging. Hydrophilic QDs are synthesized using thiol-containing molecules as stabilizers, and the synthesis reactions are performed in aqueous conditions (Li and Zhu, 2013). In addition, higher band gap semiconductors, such as ZnS and ZnSe, are commonly used as a secondary layer around the core, not only to impede chemical degradation and oxidation but also to improve QD stability and photoluminescence (PL) (Barreto et al., 2011).

On account of the quantum confinement effect, absorption and PL spectra of QDs, as well as the color of emitted light, show size-dependent variance (Algar and Krull, 2009). In addition to size, the chemical compositions and core structures of QDs are the other two critical parameters to be considered for the control of the photophysical properties of QDs (Cooper and Nadeau, 2009). As opposed to organic fluorophores, QDs have a broad absorption

spectra and a narrow emission peak, allowing multicolor fluorescence imaging by concurrently exciting multiple QDs with distinct emission wavelengths. Nie and coworkers devised a multifunctional NP system, in which encapsulation of QDs into ABC triblock copolymers was followed by the anchorage of tumor targeting ligands to an amphiphilic polymer, ultimately creating an NP complex for multicolor fluorescence imaging in live animals. The delivery and accumulation of QDs in tumor sites were investigated under passive and active targeting modes, the former with the help of the enhanced permeability and retention effect of the NPs and the latter through the specific interaction between antibody-conjugated QDs and cancer-specific cell surface biomarkers (Gao et al., 2004). Furthermore, Kobayashi et al. synthesized five different polymer-coated carboxyl-functionalized QDs (CdSe or CdTe) with similar sizes but different emission wavelengths (varying from 565 to 800 nm) for multiplexed lymphatic imaging. Intracutaneous injection of all QDs was performed into five different drainage sites of the lymphatic system in the upper body of mice, and the results revealed that no pronounced decrease would occur in fluorescence intensity for up to 3 h postinjection, which is longer than other NIR dye-labeled molecular systems (Kobayashi et al., 2007). Previously, Kim et al. showed that polydentate phosphine-coated CdTe/CdSe core-shell QDs could be used to detect sentinel lymph nodes in mice and pigs. The NPs had 15–20 nm diameters and high aqueous quantum yields, were stable in aqueous environments, and exhibited good retention in lymph nodes (Kim et al., 2003). Shuhendler and coauthors developed a hybrid system for deeper tissue penetration in optical imaging using NIR PbSe QDs embedded into solid fatty ester nanoparticles (QD-FENs). Following the intravenous administration, noticeable accumulation of QD-FENs was observed in the tumor sites of breast tumor-bearing mice after 30 min. Although the precise depth profile of the QD-FENs was difficult to measure under *in vivo* conditions, the system nonetheless displayed capacity for macroscopic deep tissue fluorescence imaging (Shuhendler et al., 2011).

In addition to QDs, considerable interest has been focused on the fabrication of anisotropic quantum wires (QWs) and quantum rods (QRs) with tailorable optical properties. More controlled synthetic approaches and surface modification techniques may allow the use of these one-dimensional semiconductors as effective probes for bioimaging applications. Prasad's group demonstrated successful *in vivo* tumor imaging of pancreatic cancer-bearing mice by using cyclic RGD (cRGD) peptide-conjugated CdSe/CdS/ZnS QRs. These QRs were first encapsulated in PEG-grafted phospholipid micelles, followed by surface conjugation of cRGD peptide for specific tumor targeting and imaging (Yong et al., 2009).

9.4.2 AuNPs

AuNPs are commonly used in optical cancer imaging due to their unique optical properties. As noble metal NPs, AuNPs exhibit strong light scattering at their localized surface plasmon resonance (SPR) frequency, which is related to the interaction between light and the conduction electrons of AuNPs. NP sizes and shapes as well as the dielectric constant of the medium change the SPR properties of AuNPs (Sharma et al., 2006). Spherical NPs are associated with a number of drawbacks, such as photochemical degradation and lack of deep tissue penetration because of strong absorption in the optical visible range; as such, AuNPs with alternative morphologies have been synthesized for tissue imaging purposes. AuNP rods, urchins, prisms, wires, disks, shells, and stars with more desirable SPR properties have been reported for use in imaging in the infrared region (Hutter and Maysinger, 2011). The surfaces of these NPs can also be modified to increase their aqueous dispersibility and to provide active sites for further biofunctionalization, which is crucial for targeting applications that are commonly employed in bioimaging.

Choi et al. used a seed-mediated growth approach to produce gold nanorods (GNRs) and functionalized the core NPs with cRGD peptide by EDC/NHS chemistry after heterobifunctional PEGylation. GNRs with aspect ratios of 4 were shown to exhibit maximum absorption at 780 nm and could be used to target and image glioblastoma under both *in vitro* and *in vivo* conditions. Gold accumulation in *in vivo* brain tumors was found to be higher for cRGD-tethered GNRs compared to control, while the opposite result was obtained in the case of liver uptake (Choi et al., 2011). In addition to GNRs, gold nanoclusters (GNCs) have also been used for the development of NP systems for optical imaging (Quek and Leong, 2012). Wu et al. produced ultrasmall GNCs with hydrodynamic sizes of 2.7 nm, which were tested on MDA-MB-45 and HeLa tumor xenograft models. Discernible differences in *ex vivo* fluorescence images were observed between tumor tissue and the muscle tissue surrounding the tumor, suggesting that the GNCs could be used as a CA in fluorescence tumor imaging (Wu et al., 2010).

Toxicity and the accumulation of the CA in the body are two major concerns that should be taken into consideration when dealing with NPs. Although factors such as size, shape, concentration, surface charge, surface coating, and aggregation status are known to alter the toxicity of AuNPs, special care must be taken while selecting targeted cell lines, exposure times, and determination methods for toxicity assessment. Toxicity is generally recognized to be unavoidable for AuNPs with very small sizes, negative charges,

and functionalization with intrinsically toxic chemicals or with excessive amounts of coating materials (Ng et al., 2013). Surface modifiers have been developed to circumvent these issues by lowering the toxicity or preventing the long-term accumulation of NPs. Zheng's group synthesized glutathione-coated luminescent AuNPs and compared their results with a renal clearable organic fluorophore, IRDye 800CW. AuNPs were found not only to exhibit rapid normal tissue clearance but also to display prolonged tumor accumulation, which is a highly desirable result for clinical trials (Liu et al., 2013b).

9.4.3 UCNPs

UCNPs are lanthanide-doped nanocrystals and are composed of three major components: activators, sensitizers, and the host matrix. Lanthanide-based sensitizers (Yb^{3+}) and activators (Er^{3+} , Tm^{3+} , Ho^{3+}) are used as dopants to alter the emission properties of the nanocrystal composite. Host materials provide a suitable environment for upconversion by isolating the dopant from its surrounding and promoting energy transfers between dopant ions and are generally fluoride-based materials such as NaYF_4 , LaF_3 , and NaYbF_4 or oxide-based materials such as Y_2O_3 , CeO_2 , and YVO_4 (Nam et al., 2013).

In contrast to other fluorescent agents, UCNPs are excited at longer wavelengths (generally in the NIR region) by absorbing low-energy photons and emit at shorter wavelengths by releasing a high-energy photon through anti-Stokes emission. This upconversion process can be explained under three main mechanisms, which can be applicable alone or in combination: excited state absorption (ESA), photon avalanche (PA), and energy transfer upconversion (ETU) (Chen and Zhao, 2012). The upconversion behavior makes UCNPs enticing candidates for applications in bioimaging and provides superior properties over other fluorescent agents, such as decreased autofluorescence background, photobleaching resistivity, deeper penetration in tissues, and larger anti-Stokes shifts (Li et al., 2014).

Advances in the synthesis of lanthanide NPs have spurred the development of small UCNPs in the last decade (Bao et al., 2013). Thermal decomposition and hydrothermal synthesis are the most widely applied methods for the production of UCNPs with well-defined shapes and low variation in particle sizes. Since these two methods result in the production of hydrophobic NPs, further steps are necessary to render them water dispersible. Methods such as ligand exchange, organic ligand-free synthesis, ligand oxidation reaction, silanization, layer-by-layer methods, hydrophobic-hydrophobic interactions, or host-guest interactions can be used for this purpose (Wang et al., 2010; Zhou et al., 2012a). UCNPs can be further modified with specific

functional groups by tethering bioactive moieties such as peptides, antibodies, DNA, and sugars (Zhou et al., 2012b). In terms of *in vivo* upconversion luminescence imaging, they can be used in whole-body imaging (Liu et al., 2011; Nyk et al., 2008), tumor imaging (Xiong et al., 2009), lymphatic imaging (Cao et al., 2011), and vascular imaging (Hilderbrand et al., 2009).

Since rapid progress has been made in the development of UCNPs, investigations on the short- and long-term cytotoxic effects of UCNPs are currently incomplete. Although these NPs have so far been reported to be biocompatible, further toxicological assessments are necessary to fully evaluate the risks associated with these novel CAs (Xiong et al., 2010).

9.5 THERANOSTIC APPROACHES AND MULTIMODAL SYSTEMS

Theranostics is a relatively new approach that proposes the integration of properties related to therapy and diagnostic imaging into a single system, and nanotechnology, as a developing area of science, is especially suitable for the development of such combined approaches. The coordination of therapeutic and imaging functionalities is an ideal means of increasing the efficacy of treatment, since doing so allows the medical practitioner to monitor and control each individual step of the therapy. However, the development of powerful imaging strategies is paramount to gain a better understanding of the nature of the disease at hand, which is of fundamental importance to theranostic approaches. Due to their ease of synthesis and functionalization, as well as their imaging potential, NPs are particularly suitable for the development of theranostic systems and have received much attention in this capacity (Brigger et al., 2002; Choi et al., 2012; Janib et al., 2010). A wide variety of NPs have been developed as powerful platforms to improve our understanding of the basic framework underlying NP-based diagnosis and therapy; however, clinical applications of these structures are limited. Compared to conventional methods of diagnosis and therapy, NPs present several advantages, such as (i) a high surface-to-volume ratio, (ii) adjustable size and surface properties, (iii) ease of functionalization, (iv) ability to form conjugates with specific targeting moieties, (v) prolonged circulation times in the blood, and (vi) enhanced uptake into the reticuloendothelial system. These properties allow multifunctional NPs to be produced as effective imaging and therapeutic systems with potentially promising applications in clinical settings (Brigger et al., 2002).

Theranostic NPs (Lee et al., 2012), such as iron oxide NPs, AuNPs, and GdNPs, can be specifically delivered to their target region by using passive

and active targeting approaches. Passive targeting utilizes the pathophysiological characteristics of the target region to facilitate the accumulation of NPs. In particular, the enhanced permeability and retention (EPR) effect inherent to tumor sites allows NPs to selectively accumulate in these regions, as the high metabolic rates, elevated nutrient and oxygen demands, and acidic character of tumor tissues all enhance NP deposition. Uptake into specific cell types, however, usually necessitates the use of an active targeting strategy, which involves functionalization of the NP with a ligand molecule. This ligand may be a peptide, antibody, oligonucleotide, or small organic molecule and typically binds to a target moiety on the surface of the cell of interest (Danhier et al., 2010). In order to be applicable in theranostic approaches, NPs must be detectable through noninvasive imaging modalities, which include MRI, CT, PET, single-photon emission computed tomography (SPECT), and optical imaging. Each of these techniques has its own advantages and drawbacks, especially with regard to sensitivity and spatial resolution. Limits in the detection capacity of one method can be overcome by another; as such, combined imaging techniques such as PET/MRI (Pichler et al., 2010), PET/CT (Zhou et al., 2010), and MRI/CT (Chou et al., 2010) are particularly effective for disease diagnosis.

MRI is one of the most effective noninvasive imaging techniques in clinical diagnosis. Many types of NPs can be used as CAs in theranostic MRI. Of these, SPIONs have unique physical properties that make them particularly suitable for use as CAs in MRI, as well as in other techniques, and these NPs have been popular platforms for the development of theranostic methods (Cohen and Shoushan, 2013). One such example has been reported by Fang et al., who have produced a theranostic platform that combines SPIONs, a biodegradable and pH-sensitive poly (beta-amino ester) (PBAE) copolymer, and a chemotherapeutic agent (doxorubicin (Dox)) for an effective diagnostic–therapeutic delivery system. Their system demonstrated a pH-responsive drug release profile over 72 h of incubation: While Dox release was rapid between pH 5.5 and 6.4, the rate of release was slower at pH 7.4. In addition, theranostic NPs displayed a strong r_2 relaxivity of $146 \text{ mM}^{-1} \cdot \text{s}^{-1}$, which makes them suitable for MRI. *In vitro* studies with drug-resistant C6/ADR cells demonstrated that, compared to free Dox, the drug complex undergoes cellular internalization at significantly higher rates (Fang et al., 2012).

Magnetic NPs also display substantial potential for combining MRI with magnetic hyperthermia treatment (MHT), as they typically generate heat under alternating current (AC) magnetic fields (Kumar and Mohammad, 2011). In one such study, Hayashi et al. designed FA- and PEG-modified SPION nanoclusters (FA-PEG-SPION NCs) for cancer

treatment. Twenty-four hours after the intravenous injection of NCs, the mice were exposed to an AC magnetic field with $H=8\text{ kA/m}$ and $f=230\text{ kHz}$ ($Hf=1.8\times 10^9\text{ A/m s}$) for 20 min, resulting in a temperature increase of 6°C above ambient at the tumor region. After 35 days of treatment, the tumor volume was found to be smaller compared to control mice (Hayashi et al., 2013).

Although the strong T_2 responses, versatile chemical functionalization capacity, and ability to track cells and tissues at low concentrations render SPIONs popular for a variety of biomedical applications, the practical use of these materials is limited by a number of drawbacks, especially with regard to their negative contrast characteristics and magnetic sensitivity. T_1 CAs, such as gadolinium (Gd) and manganese (Mn), are employed to bypass these limitations. Bae et al. have generated multifunctional HMON and functionalized them with a biocompatible adhesive ligand (DOPA) and a therapeutic monoclonal antibody specific to cancer cells (herceptin) for the targeted delivery of therapeutic siRNAs concurrent with MRI-based tumor monitoring. MRI and confocal microscopy results indicated that herceptin-conjugated HMON enabled the detection of HER2-overexpressing cancer cells in T_1 -weighted MRI and facilitated effective intracellular delivery of siRNA to inhibit VEGF expression (Bae et al., 2011).

Gadolinium chelates are commonly used as MRI CAs but suffer from drawbacks such as rapid clearance from the body, nonspecific biodistribution, and interference with Ca(II) ion-mediated processes (Cabella et al., 2006). GdNPs are capable of circumventing many of these problems and are further advantageous in that they can be functionalized with a variety of ligands and delivery platforms for theranostic purposes (Liu and Zhang, 2012). One example of a GdNP-based combined imaging/delivery system was provided by Liu et al., who reported production of PEGylated Eu(III)-doped mesoporous gadolinium oxide nanorods as CAs for MRI/X-ray imaging/PL imaging and vehicles for chemotherapeutic drug delivery. These NPs were shown to possess high biocompatibility, good photoluminescent and magnetic properties, well-ordered mesopores, and rodlike morphology that may serve to increase internalization rates during cellular uptake. The relaxivity of these nanorods was $23.5\text{ mM}^{-1}\cdot\text{s}^{-1}$, which is substantially higher than that of a commercially available CA Magnevist ($4.0\text{ mM}^{-1}\cdot\text{s}^{-1}$). The drug delivery potential of these nanorods was also evaluated by the use of the hydrophobic cancer drug camptothecin (CPT), the chemotherapeutic efficiency of which was greater when delivered with Gd nanorods compared to delivery by conventional CPT-loaded NPs (Liu et al., 2012b).

AuNPs are of considerable importance in many fields and are consequently one of the most well-studied nanomaterial types. Well-established synthesis

methods make AuNPs attractive candidates for the design of novel theranostic delivery vehicles, and their unique physical characteristics lend them well to multimodal imaging and photodynamic therapy (Huang et al., 2007). A particularly interesting example of an Au-based delivery system has been reported by Liu et al., who generated a quintuple-modality nanoprobe by functionalizing gold nanostars with a surface-enhanced Raman scattering (SERS) reporter and Gd(III) ions for detection by SERS, MRI, CT, and two-photon luminescence (TPL) imaging, as well as tumor ablation by photothermal therapy (PTT). These multifunctional NPs may allow the use of multiple preoperative (MRI and CT) and intraoperative (SERS and TPL) imaging systems on the same tumor site for enhanced diagnostic capacity, in addition to actively contributing to therapeutic efforts by serving as absorptive materials for PTT (Liu et al., 2013c).

In addition to nanomaterials composed of pure metals, NP alloys can also be used for diagnostic and therapeutic efforts. These materials may offer enhanced resolution, sensitivity, and biocompatibility compared to their single metal counterparts. Amendola et al. reported the generation of magneto-plasmonic Au–Fe alloy nanoparticles (AuFeNPs) as multimodal CAs. These AuFeNPs displayed many advantages over conventional metal NPs, including a synthesis method that does not include harmful chemicals, a simple conjugation mechanism through the use of thiolated molecules, longer retention times in bloodstream, and better accumulation in tumor sites, making them promising candidates for simultaneous MRI, CT, and Raman imaging (Amendola et al., 2014). Also worthy of note are luminescent NPs, such as QDs, which have drawn considerable attention due to their unique optical properties such as narrow, symmetric, and size-tunable emission spectra, broad and continuous excitation spectra, high stability against photobleaching, and high fluorescence quantum yields. These properties, in tandem with their high functionalization capacity, make QDs attractive agents for biomedical imaging and drug and gene delivery (Sreenivasan et al., 2013). Bagalkot et al., for example, developed a QD–aptamer (Apt)–doxorubicin (Dox) conjugate system for targeted synchronous cancer imaging and drug delivery. The NP surfaces were functionalized with A10 RNA Apt, which recognize prostate-specific membrane antigen (PSMA) expressed in LNCaP cells, to ensure specific delivery of QDs into these cells. Doxorubicin (Dox), a widely used anthracycline drug, was intercalated into Apt for targeted delivery. The system mainly relied on a Bi-FRET mechanism between the QD/Dox and RNA Apt/Dox pairs. With this design, the fluorescence of the QD was “turned off” by the quenching effect of Dox, and the fluorescence of Dox was quenched in turn by the Apt. When Dox is released from the QD–Apt(Dox) complex, the quenching

mechanism no longer functioned, resulting in fluorescence (Bagalkot et al., 2007). QDs can also be incorporated into other delivery vectors, such as liposomes, to increase their biocompatibility while also offering a strong fluorescent signal. Weng et al. reported that doxorubicin-loaded QD-immunoliposome complexes showed no cytotoxicity and displayed notably enhanced circulation times and anticancer activity (Weng et al., 2008).

Theranostic systems are as variable and versatile as the drug delivery and imaging components they incorporate, and this flexibility makes them attractive for the detection and treatment of many hard-to-diagnose, hard-to-treat diseases. There have been notable efforts to create multifunctional platforms for combined diagnostics and therapeutics, but challenges such as data reproducibility, NP biocompatibility and biodegradability, and targeting issues remain. If these limitations were to be overcome, theranostic systems can effectively combine several critical aspects of current diagnostic and therapeutic efforts to create a combined platform for the treatment of a wide range of disorders, from the common and mild to the rare and deadly.

9.6 OVERLOOK AND FUTURE DIRECTIONS

Despite their relatively recent addition to the roster of medical diagnostics tools, NPs are versatile and powerful imaging agents. The ability to modify a single NP with multiple targeting molecules enhances the targeting efficiency of the CA, and multiple steps of functionalization can be performed to produce an NP that can simultaneously perform several distinct functions (e.g., antibodies for targeting, PEG for avoiding complications with the immune system, and other molecules for lower toxicity and better clearance profiles). The contrast enhancements of NPs are typically greater than their conventional counterparts (e.g., iodinated contrast for CT, bulk metal chelates for MRI, and organic fluorophores for NIR imaging). In addition to acting as stand-alone contrast enhancers, the larger-sized NPs also can deliver a therapeutic molecule alongside the CA, making use of the targeting capacity that these NPs are often modified to display. In short, NPs are not so much an individual class of CAs, but more an all-purpose platform that can be modified to a great extent to serve the specific needs of the tissue and disease to be imaged. This diversity of function makes them especially suitable for the era of next-generation medical diagnostics, where personalized treatment for each individual patient is necessary to tackle complex diseases such as cancer, diabetes, obesity, and neurodegenerative disorders.

Nevertheless, it must be kept in mind that NP diagnostics is still largely an area of research, rather than a standard of clinical use. The approval of

any medical agent for human use requires very stringent conditions of testing, as well as a clear improvement over the previous standard of care. Despite their effectiveness in animal models and small-scale medical applications, issues such as long-term toxicity, environmental effects, and potential side effects on a large and genetically diverse human population should still be resolved for the widespread use of NPs for diagnostic purposes. The success of currently approved NP CAs, such as the SPIONs Feridex and Resovist (Wadajkar et al., 2013), stands as testament to the diagnostic potential of NPs, but much work remains to be done for NPs to truly realize this potential across all imaging methods and systems.

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