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Nanobiomaterials for Molecular Imaging

17.1 Introduction to Molecular Imaging

Molecular imaging is an emerging field that can be broadly defined as in vivo visualization, characterization, and quantification of biological processes at the cellular and molecular levels (Weissleder and Mahmood 2001; Massoud and Gambhir 2003). Conventional diagnostic imaging mostly measures non-specific anatomic, physiological, or metabolic changes that mark pathological differentiation of diseased tissues or conditions from healthy tissues. Molecular imaging, on the other hand, aims to probe the cellular and molecular pathways and changes that are the fundamentals of diseases.

This field offers a noninvasive tool to investigate the cellular and molecular phenomena inside a living animal, where cellular and molecular studies can be performed in physiological conditions representative of a clinical scenario. Serial studies can be carried out in the same animal without the need to sacrifice animals for in vitro analysis by histopathological means, providing continuous and more meaningful data. The development of a noninvasive imaging method to detect and predict the state and progress of diseases has the potential to shift the use of medical imaging from clinical diagnosis to prognosis. Detection of diseases can be achieved at a much earlier time point where it is possible to treat and potentially even cure diseases. In contrast to some current procedures, molecular imaging techniques are noninvasive, therefore significantly reducing patients’ discomfort. Likewise, these techniques can be used to evaluate the therapeutic effects, efficacy and/or toxicity of a pharmaceutical agent or a treatment regime.

Molecular imaging typically requires an agent to create signals detectable by the imaging systems. These agents are called contrast agents, molecular probes, tracers, or reporters. Molecular probes can broadly be categorized as either passive or responsive (“smart”) probes. Passive tracers facilitate in vivo visualization of tissues, cells, or molecules of interest. For smart probes, changes in biochemical or physiological parameters, or the presence of a particular biological species, affect the signals produced.
by them (Sherry and Woods 2008). Therefore, a “smart” probe reports on the biological activities of the
target tissues, cells, and molecules. This chapter aims to summarize the properties, applications, and
limitations of different molecular probes used with each imaging modality.

17.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) offers an excellent soft tissue contrast and a spatial resolution close
to the size of a single cell. Biological as well as anatomical information can be simultaneously obtained
by MRI. MR manipulation is mostly performed on water protons (1H), taking advantage of the water
abundance in human and animal bodies. The most common 1H MRI protocols are T1-weighted and T2
or T2*-weighted. MR probes can broadly be categorized as T2-weighted or negative contrast agents and
T1-weighted or positive contrast agents. Negative agents attenuate MR signals and appear as dark sig-
nals or hypointensities. In contrast, positive probes cause enhancement of signals or hyperintensities.
Examples of negative and positive contrast MRI are shown in Figure 17.1.

17.2.1 Negative MR Contrast Agents

Superparamagnetic iron oxide-based nanoparticles (SPIOs) are the most common and widely used
negative MR contrast agents. SPIOs are composed of nanocrystalline magnetite (Fe3O4) or maghemite
(γFe2O3) cores and typically have a median diameter around 50 nm. Those with a median diameter
smaller than 50 nm are called ultrasmall superparamagnetic iron oxide-based nanoparticles (USPIOs).
To improve their biocompatibility and colloidal stability, iron oxide nanoparticles are coated with a
biocompatible material: dextran (these nanoparticles are called ferumoxides), carboxydextran (fer-
rixans or ferucarbotrans), or silicone (ferumoxsils). Feridex® (AMAG Pharmaceutical Inc., this prod-
uct is now discontinued) is an FDA-approved ferumoxide and is being sold in Europe as Endorem™.
GastroMARK® (AMAG Pharmaceutical Inc.) and Lumirem® (European brand name) are commercial
ferumoxsils, while Resovist® (Bayer Schering Pharma AG) is a ferucarbotran approved for the European
market.

Inside the body, iron oxide nanoparticles are taken up by the reticuloendothelial system of the liver
(Kupffer cells) and the spleen (macrophages). These nanoparticles biodegrade into their coating and
their iron core, with the latter subsequently entering the normal iron metabolic pathway. SPIOs were
first formulated for noninvasive MR detection and visualization of hepatic tumors. Hepatic tumor cells
do not take up injected SPIOs, whereas Kupffer cells in the normal liver do, resulting in MR-signal
differences between healthy and cancerous cells.

![Figure 17.1](image-url)
Currently, transplantation of therapeutic cells is performed "blindly." Cells may be injected into the wrong site and/or never reach the intended target site. To allay these potential problems, cells can be labeled with SPIOs before engraftment. Hence, the in vivo location, migration, and cell–tissue interaction of transplanted cells can be followed and studied in real time by MRI (Figure 17.1B). It is estimated that only 1.4–3 pg of iron per cell is required for the visualization of a single cell using a clinical MRI setup (Walter et al. 2007). Cells can be labeled by prolonged incubation with SPIOs, i.e., via phagocytosis (Bulte and Kraitchman 2004; Kraitchman and Bulte 2009), using an electroporation technique (Walczak et al. 2005) or using polycationic transfection agents, such as poly-L-lysine (Frank et al. 2003) or protamine sulfate (Arbab et al. 2006). USPIOs are taken up by macrophages following intravenous injections, and can thus be used as an endogenous macrophage labeling probe (Bulte and Kraitchman 2004).

A relatively new approach is to encapsulate cells and SPIOs inside immunoprotective alginate microcapsules (Figure 17.2A) (Barnett et al. 2007). An advantage here is that a high concentration of SPIOs can be confined inside the capsules, providing a stronger hypointense MR signal. Ruptured capsules exhibit weaker signals due to the release of nanoparticles (Figure 17.2B and C). However, this strategy only reports on the integrity of the capsules but not on the viability and functionality of the encapsulated cells.

New kinds of negative contrast agents are continuously being discovered and developed. A major improvement on iron oxide nanoparticles is the so-called magnetism-engineered iron oxide (MEIO) nanoprobes or metal-doped Fe$_3$O$_4$ nanoparticles (Lee et al. 2007) with enhanced MR-sensitivity (lower concentration offers sufficient MR-contrast) compared to regular SPIOs. The use of ferritin, a natural globular protein complex and the main intracellular iron storage vehicle, has also been explored. Free Fe$^{3+}$ ions are toxic, but inside the ferritin shell, Fe$^{3+}$ ions form crystallites with phosphate and hydroxides. Wild-type ferritin is a poor negative contrast agent. To improve MR-sensitivity, ferritin aggregates were fabricated by the conjugation of ferritin to actin, followed by the polymerization of actin (Bennett et al. 2008). Bouchard et al. (2009) created cobalt nanoparticles coated with bioinert gold that are seven times more sensitive than SPIOs. Cobalt nanoparticles were an unattractive choice due to their oxidation-induced instability and toxicity, but these issues could be circumvented with gold coating.

### 17.2.2 Positive MR Contrast Agents

Hyperintense MR signals are easier to be detected in vivo than hypointense signals due to the endogenous hypointense background in T$_1$ or T$_2^*$-weighted images. The interpretation of these hypointensities may be ambiguous and can have a physiological origin, such as iron-containing hemoglobin in blood, or pathological origin, such as blood clots due to trauma. Paramagnetic gadolinium (Gd) is the most effective positive contrast MR agent due to its seven unpaired electrons, but free Gd$^{3+}$ ions are toxic and
must be chelated for clinical and biomedical applications. Diethylene triamine pentaacetic acid (DTPA),
tetraazacyclododecane tetraacetic acid (DOTA), and their derivatives are examples of clinically used
Gd-chelates. A Gd-DTPA enhanced clinical MRI exam is shown in Figure 17.1A.

Gd-chelates can easily be inserted into cells through pinocytosis but their MR sensitivity is much
lower than iron oxide nanoparticles. A myriad of strategies has been explored to increase the sensitiv-
ity of this contrast agent. Multiple Gd-chelates were bound inside a fullerene cage (Shu et al. 2006;
MacFarland et al. 2008) or into a dendrimer (Bryant et al. 1999) or inside a nanotube (Sitharaman and
Wilson 2007). Gd-chelates were encapsulated inside vesicles that can be further functionalized (Mulder
et al. 2006; Sofou and Sgouros 2008), such as liposomes (Mulder et al. 2006) or porous polymeric shells
(Cheng and Tsourkas 2008). Gd-based nanoparticles have also been fabricated, for example, nanopar-
ticles coated or embedded with gadolinium (Gerion et al. 2006; Zhu et al. 2008) and gadolinium oxide
(Gd₂O₃) nanocrystals capped with a biopolymer (Engstrom et al. 2006). Multiple Gd³⁺ ions were che-
lated on the surface of gold nanoparticles to create MR and X-ray compatible agents (Alric et al. 2008).
Gd-agents can also be encapsulated inside immunoprotective capsules.

Another strong positive contrast MR agent is manganese ions. Manganese salt (MnCl₂) crystals (Aoki
et al. 2006), manganese carbonate (MnCO₃) (Shapiro and Koretsky 2008), and manganese oxide (MnO,
MnO₂, and Mn₃O₄) nanoparticles (Gilad et al. 2008; Shapiro and Koretsky 2008) were formulated for cell
labeling applications. Intact particles are detected by T₂*-weighted MRI. However, they dissociate into
manganese ions upon internalization by cells, producing hyperintense signals (Figure 17.1B). Similar to
Gd-based agents, the biocompatibility of these compounds is a major issue and currently under investi-
gation. When combined with negative contrast agents, positive contrast agents offer a method of MR
“double labeling” of two different cell populations.

17.2.3 Perfluorocarbons for ¹⁹F MR Imaging

Perfluorocarbons (PFC) are derived from hydrocarbons by replacing hydrogen atoms with fluorine
atoms. These compounds are bioinert, and chemically and thermally stable. Their fluorine nuclei can be
detected by ¹⁹F MRI. ¹⁹F MRI cannot provide anatomic information since an abundance of fluoride
is not naturally present in a living body and therefore its use is always combined with ¹H MRI. The use
of PFCs offers the possibility of “hot spot” MR imaging, analogous to nuclear imaging (exemplified in
Figure 17.3A). PFCs also function as X-ray and ultrasound agents, an advantageous feature, which will
be discussed in the later sections. PFCs currently being used for molecular imaging applications are
perfluorooctyl bromide (PFOB) and perfluoro-15-crown-5-ether (PFCE). These compounds are liquid

![FIGURE 17.3](See color insert.) (A) 9.4T MR image of a mouse brain 3 days post-engraftment of PFC-labeled
neural stem cells, with the ¹⁹F signal superimposed on the ¹H MR image. The cells labeled with fluorinated emul-
sions appear as “hot spots.” (From Ruiz-Cabello, J. et al., Magn. Resonance Med., 60 (6), 1506, 2008.) (B,C) PET
(B) and bioluminescent (C) images of a mouse injected with stem cells carrying thymidine kinase (PET) and lucif-
erase gene 4 weeks post-injection. (Courtesy of Dr. Joseph C. Wu, Stanford University, Stanford, CA.) Cells were
visible as “hot spots” on the PET and BL images.
at room temperature and have to be emulsified in order to be used as a contrast agent. Nanoscale emulsions of PFCs are prepared with surfactants, the most common being phospholipids, to maintain their colloidal stability.

Neural stem cells were directly labeled with cationic PFCE-phospholipid emulsions without the use of transfection agents (Ruiz-Cabello et al. 2008). The authors reported an MR-sensitivity of approximately 140 pmol of PFCE per cell at 9.4 T (Figure 17.3A), and no observable detrimental effects on the viability and proliferation of stem cells. Emulsions of PFOB were co-encapsulated with mesenchymal stem cells inside immunoprotective alginate microcapsules to create 19F MR, X-ray, and ultrasound-trackable capsules (Fu et al. 2009). Oxygen dissolves in PFOB in a linear relationship, hence co-encapsulated PFOB emulsions also function as an oxygen carrier for the encapsulated cells. The surface of PFC emulsions can be conjugated with paramagnetic Gd-chelates (Winter et al. 2007) or with targeting-ligands (Schmieder et al. 2005) to improve MR imaging. To date, PFCs have not been used in clinics but have shown promising results in animal models.

17.2.4 Chemical Exchange Saturation Transfer Imaging

Chemical exchange saturation transfer (CEST) imaging is an emerging MRI technique that detects signals arising from the proton exchange between the imaging target and the surrounding water molecules. Some endogenous species can directly be detected by CEST imaging without the need of contrast agents, for example, the hydroxyl protons of glycogen and the amide protons in brain tissues. CEST imaging has been used for real-time, noninvasive studies of glycogen metabolism in a mouse liver (van Zijl et al. 2007) and ischemic regions in a rat brain (Zhou et al. 2003).

Recent work on exogeneous CEST agents includes diamagnetic peptide-based (DIACEST) agents and paramagnetic CEST (PARACEST) agents. A lysine-rich protein (LRP) reporter (Gilad et al. 2007), a prototype CEST reporter gene, was developed for transfection into cells. Lysine is a peptide rich in exchangeable amide protons and a strong CEST agent. This reporter presents a biocompatible and biodegradable agent that provides a constant endogenous CEST signal even after cell proliferation. Since LRP is strongly pH-dependent, the contrast is lowered by an order of magnitude during ischemia or cell death, offering a noninvasive method to monitor cell viability in vivo (Gilad et al. 2007). The CEST potential of a library of prototype polypeptides with exchangeable amine, amide, and hydroxyl protons was further investigated (McMahon et al. 2008). These three exchangeable groups have different chemical shifts and therefore different “colors” can be assigned to each of them, presenting a possibility of multiple labeling and simultaneous imaging of multiple targets.

Paramagnetic CEST (PARACEST) agents are complexes of paramagnetic lanthanides, such as Eu³⁺, Ho³⁺, Dy³⁺, Tb³⁺, Pr³⁺, and Yb³⁺. The exchange rate of these agents increases with increasing temperature, therefore PARACEST agents have the potential for detecting temperature changes (Zhang et al. 2002, 2005) in addition to passive CEST imaging. Moreover Pr- and YbDOTA-tetraamide complexes are sensitive to pH changes (Aime et al. 2002; Terreno et al. 2004). However, lanthanide ions are toxic, therefore their chelated forms must have a high in vivo stability and a safe route of clearance from the body. Zhang et al. (2003) predicted that only a few lanthanides may function as PARACEST agents in a low magnetic field. Since clinical MRI is performed at 1.5–3 T magnetic field strength, this will narrow the use of most PARACEST agents to research applications. DIACEST and PARACEST agents can be functionalized for targeting a tissue, cell, or molecule of interest.

A current drawback of CEST agents is its relatively low sensitivity, requiring a concentration in the range of 1–10 mM (Sherry and Woods 2008). Sensitivity can be enhanced by increasing the number of exchangeable groups on a probe or by assembling multiple probes in/on a substrate. Multiple Eu³⁺ complexes were conjugated on the surface of PFC-filled nanoparticles, resulting in a PARACEST and ¹⁹F MR agent (Schmieder et al. 2005). Complexes were easily encapsulated in the aqueous cores of liposomes (LIPOCEST) (Aime et al. 2005). Liposomes are an attractive option due to their biocompatibility and potential for surface-functionalization: with targeting ligands, with poly(ethylene glycol) to prolong in vivo circulation.
life, and/or with an extra contrast-generating agent such as Gd chelates (Torchilin 2005; Zheng et al. 2006). Moreover, the exchange of water molecules across the phospholipid membranes can be manipulated by varying the membrane composition (Sherry and Woods 2008). Terreno et al. (2009) improved the sensitivity by creating osmotically shrunken LIPOCESTs. Other inventions include polypeptides rich in exchangeable protons (McMahon et al. 2008) and multiple agents bound to a dendrimer (Snoussi et al. 2003).

17.3 Nuclear Imaging

Positron emission tomography (PET) is a nuclear imaging system that detects pairs of gamma rays emitted by positron-emitting radionuclides. Common positron-emitting radionuclides are $^{18}$F, $^{15}$O, $^{13}$N, and $^{11}$C. Less common ones are $^{64}$Cu, $^{62}$Cu, $^{14}$O, $^{124}$I, $^{78}$Br, $^{68}$Ga, and $^{82}$Rb. Single photon emission computed tomography (SPECT) utilizes a gamma camera that rotates around the subject and directly measured the gamma rays emitted by the radionuclides. Some SPECT radionuclides are $^{111}$In, $^{123}$I, $^{131}$I and $^{99m}$Tc. SPECT agents typically have longer half-lives than PET radionuclides. Most radionuclides are produced in a cyclotron and a few of them by a generator. Radionuclide production sites should be in close proximity to the hospitals since the half-lives of some radionuclides are short, for example, the half-life of $^{18}$F is 110 min. $^{99m}$Tc is the most clinically used agent since it is a gamma-emitter of low energy, has a moderate half-life (6 h), and a $^{99}$Mo/$^{99m}$Tc generator that is relatively inexpensive.

The most attractive feature of nuclear imaging is the small size of radiotracers compared to the probes of other imaging techniques. Atoms in molecules, proteins, or drugs can be easily substituted with radionuclides without perturbing their native properties. For example, hydrogen atoms can readily be substituted with $^{18}$F. Nuclear imaging allows the quantification of injected radiotracers, a technique that can be translated to the quantification of therapeutic agents. This modality has an excellent sensitivity, and agents in a mere nanomolar concentration can be detected. Similar to $^{19}$F MRI, nuclear imaging has to be combined with $^1$H MRI or X-ray imaging to obtain both metabolic and anatomical information. “Hot spot” PET imaging is shown in Figure 17.3B. However, it is unsuitable for repeated and extensive imaging due to the short half-lives of radionuclides. $^{111}$In for SPECT and $^{64}$Cu for PET have relatively longer half-lives (2.8 days and 12.7 h, respectively) compared to other radioligands. Cells were labeled with these agents via direct incubation. In the case of $^{111}$In-labeled cells, serial tracking by SPECT was attainable for only 5–7 days (Kraitchman and Bulte 2009). Radionuclides are potentially radiotoxic, therefore these agents are clinically used in a conservative manner or avoided if possible.

Cells can be transduced with a reporter gene for nuclear imaging applications. Transduced cells produce a particular substance that accumulates and can be detected by the administration of a reporter probe. The main advantage is that only viable cells are detectable, providing a way to track viable versus nonviable cells. Herpes simplex virus 1 thymidine kinase (HSV-Tk) is the best known reporter gene for nuclear imaging (Cao et al. 2006). Prodrugs (ganciclovir, penciclovir, or fialuridine) labeled with radioactive $^{18}$F or $^{124}$I easily penetrate cell membranes. Inside transduced cells, prodrugs are phosphorylated by the viral thymidine kinase and are trapped. This accumulation of radioactivity allows the monitoring of viable, engrafted cells inside the host. Another example is sodium-iodide sympporter (NIS) labeled with radioactive iodine and $^{99m}$Tc-pertechnetate (Kang et al. 2005).

To improve the payload of radiotracers, radiocolloids and liposome-based radiotracers have been fabricated. Radiocolloids are colloidal aggregates consisting of or containing radionuclides. An example of these is $^{99m}$Tc-sulphur colloids that have been utilized to image lymph nodes in oncology patients and bone marrows in patients with hematological and muscoskeletal diseases (Moffat and Gulec 2007).

Liposomes are biocompatible, can be surface-functionalized, and shield radionuclides from potentially destabilizing external environment. Radiolabeled liposomes were formulated with various methods but the following two yield the best labeling efficiency and $in vivo$ stability: (1) the incorporation of radiolabeled amphiphilic chelators into the bilayers of preformed liposomes (Laverman et al. 1999); and (2) the after-loading method: radionuclides chelated with lipophilic molecules are shuttled across the bilayers of liposomes, which encapsulate chelators with higher affinity for the radionuclides, thereby
trapping the radionuclides inside the liposomes (Bao et al. 2003). A variation of the first method is the use of polychelating polymers containing a hydrophobic, phospholipid fragment to amplify radionuclide loading (Torchilin 2000; Erdogan et al. 2006). Phospholipids with saturated long acyl chains and cholesterol are chosen as the membrane composition to improve the stability and rigidity of bilayers, therefore reducing the leakage or dissociation of radionuclides. Liposomes are removed by the reticuloendothelial system in the liver and spleen. Hence, radiolabeled liposomes can be used to identify and visualize hepatic and splenic tumors in a fashion similar to iron oxide nanoparticles for MRI. A few clinical studies on radiolabeled liposomes have been completed with promising results.

17.4 Optical Imaging

Optical imaging typically requires an external light source to excite the fluorophores or optical agents. An ideal fluorescence-based probe should have the following properties: high photo- and chemical stability, sufficient biocompatibility, emission in the near-infrared range (650–900 nm where tissue absorption is low), high quantum yield, and a chemical matrix for multifunctionalization (Bremer et al. 2003; Sharma et al. 2006).

The most commonly used fluorophores for passive imaging are organic fluorescent dyes, such as fluorescein isothiocyanate (FITC) and derivatives of rhodamine. Targeting-ligands can be conjugated to these dyes. Some dyes are currently used in clinical screening (Pierce et al. 2008). However, they undergo rapid photo-bleaching, are detectable only at a superficial tissue depth, and are not well suited for simultaneous multicolor imaging of different target populations. Some dyes have even been reported to adversely affect cell viability and proliferation (Modo 2008). New generations of dyes such as Alexa Fluors® (Molecular Probes) and DyLight Fluors™ (Thermo Fisher Scientific) are more photo-stable, brighter, and less pH-sensitive.

Fluorescent nanoparticles are an attractive alternative since their signal intensity can be stronger than organic dyes. Silica nanoparticles doped with fluorescent dyes suffer from the drawbacks mentioned above (Veiseh et al. 2005). Porous silicon nanoparticles are naturally luminescent but they biodegrade in less than a day and hence are not suitable for extended in vivo imaging. On the other hand, these nanoparticles are appealing for the image guided-delivery of therapeutic agents (Canham 1990; Park et al. 2009). The potential of gold nanoparticles for fluorescence imaging is currently being explored (Medley et al. 2008).

Quantum dots (QDs) are comprised of a semiconductor core, capped by a coating of a second semiconductor material, the most common being CdSe/ZnS core/shell. The core/shell structures are coated with silica, biopolymers, amphiphilic, or hydrophilic ligands, for biocompatibility and solubility, followed by conjugation with functional molecules. QDs are brighter and more resistant to photo-bleaching than standard dyes, and have tuneable optical properties for multiplexed imaging and/or in vivo imaging of deep tissues (Zimmer et al. 2006; Pierce et al. 2008; Walling et al. 2009). The size of QDs is typically 2–10 nm. The core of QDs is toxic and a careful design is pertinent to ensure their in vivo biocompatibility and stability. Moreover, the fluorescence signals of QDs display intermittent on/off behavior, which can complicate measurements (Pierce et al. 2008; Walling et al. 2009).

The following two agents produce signals only when the cells are viable, providing means to monitor dead versus live cells in vivo. When the genes encoding for these proteins are inserted into the DNA, proteins will be continuously produced and not diluted by cell proliferation and therefore can be used to study cell lineages in vivo. The first one is green fluorescent protein (GFP), extracted from bioluminescent jellyfish Aequorea Victoria. A library of mutagenized fluorescent proteins with excitation wavelengths in the 350–630 nm range and emission wavelengths in the 450–650 nm range was developed for multicolor labeling (Hadjantonakis et al. 2003). However, these proteins have a shallow penetration depth since their emission is in the visible light range, which is highly adsorbed by the tissues (Bremer et al. 2003).

Bioluminescence imaging does not require an external source of light. Molecules or cells of interest are engineered to carry the enzyme luciferase. Luciferase genes have been cloned from a variety of
sources: fireflies, coral, jellyfish, and bacteria. When the substrate luciferin is introduced, luciferase converts luciferin to oxyluciferin with the simultaneous emission of light. An example of in vivo bioluminescence imaging is shown in Figure 17.3C. However, bioluminescent signals are attenuated by tissue thickness due to nonhomogeneous scattering (Modo 2008). The use of this technique is limited to research applications since luciferin is unlikely to be administered to patients (Bremer et al. 2003).

17.5 X-Ray and Ultrasound Imaging

X-ray or computed tomography (CT) and ultrasound imaging systems are traditionally not considered sensitive enough for molecular imaging applications. However, recent development in X-ray and ultrasound probes has opened the possibility of using these modalities for molecular imaging in the near future.

Radiopaque iodine-based agents such as iohexol, iopromide, iomeprol, or iodinated oil droplets were encapsulated inside liposomes (Zheng et al. 2006) or polymeric micelles (Trubetskoy et al. 1997) to increase their sensitivity. Silica nanoparticles carrying electron-dense metal ions such as ruthenium and gadolinium (Santra et al. 2005) are a potential CT-probe. Gold nanoparticles are radiopaque with better sensitivity than iodine-based agents, bioinert, and their fabrication method is simple (Figure 17.4A and B) (Hainfeld et al. 2006). PFCs, particularly PFOB, are radiopaque, bioinert, and can multifunction as an oxygen carrier (Sanchez et al. 1995). Arifin et al. (unpublished results) fabricated a new type of immunoprotective Ba\(^{2+}\)-gelled alginate microcapsules with inherent radiopacity. Hence, these capsules can potentially be imaged by CT without co-encapsulation of a contrast agent.

Microbubbles are biocompatible shells containing acoustically trackable gases with PFC gases as the most ideal agent. The typical diameter is 1–3μm. The shells maintain the colloidal stability of the cores and should accommodate acoustic contraction and expansion of the cores, therefore flexible phospholipids (liposomes) are the best choice. The surface can be further functionalized (Klibanov 2007). Microbubbles can be destroyed in vivo with high ultrasound energies, opening the potential for targeted-drug and gene delivery (Mayer et al. 2008). A few microbubbles have been approved for clinical use (Figure 17.4C) (Blomley et al. 2001; Feinstein 2004). Alginate microcapsules were found to have an acoustic property similar to microbubbles and empty microcapsules appeared in an ultrasound image (Arifin et al. 2011). The signals were significantly enhanced with co-encapsulation of contrast agents (Arifin et al. 2011; Fu et al. 2009; Figure 17.5D and E).

![Figure 17.4](https://example.com/image1.png) (A,B) Clinical X-ray images of a mouse before (A) and after (B) tail-vein injection of gold nanoparticles. (Courtesy of Dr. James F. Hainfeld, Nanoprobes, Inc., Yaphank, NY, and Dr. Henry M. Smilowitz, University of Connecticut Health Center, Farmington, CT.) Gold nanoparticles flowing inside the blood vessels appear bright (arrow). (C) Ultrasound image of the carotid artery of a patient with an atherosclerotic plaque. The artery lumen appears white and the intraluminal plaque appears black. Within the plaque, microbubbles appear as white dots (arrows). ( Courtesy of Dr. Steven B. Feinstein, Rush University Medical Center, Chicago, IL.)
17.6 Multimodal Imaging Probes

A multimodal imaging probe combines the strengths and applications of multiple imaging techniques. Moreover, more complete information on a target can be gathered from each imaging modality. PFCs are a natural multimodal imaging probe and are detectable by $^{19}$F MR and X-ray imaging. Nanoparticles or nanoshells are a convenient platform for creating a multimodal contrast agent. The core can contain an agent visible to one system, while the surface can be decorated with a second agent visible with the second imaging system. Encapsulation of more than one agent is a possibility as long as a quenching effect does not exist. Liposomes, silica nanoparticles, or a combination of both are popular choices for the shells/cores. Examples of these are silica nanoparticles doped with fluorescent dyes (Veiseh et al. 2005) or quantum dots (Koole et al. 2008) with Gd-chelates on the surface for MRI and optical imaging; gold nanoparticles (GadoGold, Figure 17.5) (Alric et al. 2008) or iodinated liposomes (Zheng et al. 2006) with Gd$^{3+}$ ions incorporated on the surface for X-ray and MR imaging; PFC or iodinated cores

![Figure 17.5](image-url)
surface-decorated with Gd-chelates for $^{19}$F and $^1$H MRI and X-ray imaging (Winter et al. 2007); liposomes encapsulating radionuclides and Gd-chelates for nuclear and MR imaging (Zielhuis et al. 2006).

Santra et al. (2005) fabricated a trimodal imaging probe: silica nanoparticles doped with organometallic Ru(bpy) and surface-conjugated with Gd-chelates, for MR, X-ray and optical imaging. An ingenious use of bimodal imaging was demonstrated by Langereis et al. (2009), who created liposomes containing Tm$^{3+}$-complexes (CEST) and $\text{NH}_4\text{PF}_6$ ($^{19}$F) for image guided-drug delivery. Intact liposomes were trackable only by CEST imaging due to the quenching of the $^{19}$F effect. When the liposomes were ruptured, resulting in the release of drugs, the CEST effect vanished and $^{19}$F agents were detected.

Cells can be designed to carry more than one probe for the multimodal imaging of engrafted cells. For example, stem cells were engineered to carry thymidine kinase and luciferase genes (Cao et al. 2006) for PET and bioluminescence imaging, respectively (Figure 17.3B and C); cells were “housed” inside immunoprotective microcapsules with a multimodal probe (Arifin et al. 2011; Fu et al. 2009). Encapsulating these probes inside acoustically reflective alginate microcapsules may impart an ultrasound-visibility. The use of Ba$^{2+}$/alginate microcapsules with an intrinsic radiopacity (Arifin et al. 2011) may amplify or add CT-visibility. GadoGold microcapsules were readily imaged by MR, X-ray, and ultrasound imaging (Figure 17.5) (Arifin et al. 2011), while PFOB-loaded capsules were visible in X-ray, $^{19}$FMRI, and ultrasound images (Fu et al. 2009).

17.7 “Smart” Contrast Agents

The signals of “smart” probes are affected by changes in biochemical or physiological parameters (pH, temperature, ion concentration) or by the presence of a particular biological species (enzyme, glucose). Since a myriad of responsive agents is currently under development, this segment aims to present a few examples of “smart” agent designs.

A “smart” probe can differentiate between viable and nonviable cells by (1) direct measurement where only viable cells exhibit signals, for example, thymidine kinase transduced cells (PET or SPECT) (Cao et al. 2006) and GFP- (fluorescence) or luciferase-carrying cells (bioluminescence) (Bremer et al. 2003); (2) the indirect measurement of pH since dead cells release their acidic cellular contents, for example, cells transfected with LRP (CEST) (Gilad et al. 2007). Urano et al. (2009) developed a probe based on a boron-dipyrrmethene fluorophore, which is highly fluorescent only in the protonated form, i.e., in an intracellular acidic environment. This probe was conjugated to a cancer-targeting ligand for internalization by viable cells and hence for the detection of viable cancer cells.

DIACEST agents (Gilad et al. 2007; McMahon et al. 2008) and some PARACEST agents, such as Pr- and YbDOTA-tetraamide complexes (Aime et al. 2002; Terreno et al. 2004), can be used as a pH-sensor. Positive contrast MR-properties of gadofullerene derivatives depend on their aggregation state, which in turn depends on the local pH (Toth et al. 2005). PARACEST agents (Zhang et al. 2002, 2005) and quantum dots (Ngo et al. 2009) show potential for detecting temperature changes.

In the case of ion, protein, or enzyme-sensing probes, interaction with target ions/protein or enzymatic activity alters the pathways for signal production and therefore allows detection of the target. The simplest enzyme-indicator is multiple fluorescent dyes grafted on a biopolymer. The fluorescence is quenched when the dyes are in close vicinity. Enzymes cleave the polymer, releasing dyes which become fluorescent (Klohset al. 2008). An example of the mechanism of enzyme-detection is depicted in Figure 17.6A. In a Gd-based, Zn$^{2+}$-responsive agent, the binding of Zn$^{2+}$ ions opens the access of water protons to chelated Gd$^{3+}$ ions, eliciting an MR-positive contrast (Major et al. 2007). Louie et al. (2000) designed a Gd-based agent in which the access of water protons is blocked with a substrate that can be removed by marker enzyme galactosidase. An agent composed of two GFP-variants linked by a kinase-inducible domain (KID) is used to fluoroscently detect protein phosphorylation by cAMP-dependent protein kinase A (PKA). The phosphorylation of KID by PKA converts the fluorescence emission of the probe (Nagai et al. 2000). A MR-counterpart of this probe is ferritin-based nanoparticles, which aggregate in the presence of PKA, thereby altering their MRI contrast (Shapiro et al. 2009). Ren et al. (2008) fabricated a glucose-sensor: Eu$^{3+}$-chelated by a macrocyclic ligand containing a glucose recognition site.
The binding of glucose decreases the water exchange rate of Eu$^{3+}$ ions with bulk water, changing the CEST contrast (Figure 17.6B and C). Likewise, enzymatic cleavage by caspase-3 (Yoo and Pagel 2006) or the presence of Zn$^{2+}$ ions (Trokowski et al. 2005) causes the CEST effect to disappear.

### 17.8 Conclusion

Molecular imaging typically requires a contrast agent to facilitate visualization. The key points to consider in designing a contrast agent are biocompatibility, design feasibility, detection sensitivity, and specificity for the application. Although a variety of imaging modalities (MR, PET, SPECT, optical, X-ray, and ultrasound imaging) are available for molecular imaging applications, each system has its limitations and offers different information. Therefore, there is currently a high interest in developing a multimodal probe. Another novel design is a responsive or “smart” agent, which is able to provide information on the biological activities of a target in addition to passive imaging. To date, the majority of molecular probes are in their research and development stage, and their clinical suitability is yet to be determined.
References


