Advances in Cardiovascular Imaging

Multimodality Cardiovascular Molecular Imaging, Part I

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There has been a shift in emphasis from treatment of disease to the primary or secondary prevention of disease, primarily to advance human health through preservation of quality of life and improvement in survival, but also in part to control the escalating costs of health care. The prevention of disease necessitates early detection and risk stratification before the manifestation of disease, which may be facilitated by genotyping, assessment of circulating biomarkers, or noninvasive imaging. Moreover, it is becoming increasingly apparent that the combination of an individual’s genotype, level of gene expression, and clinical information can be used for individualized disease prevention, risk stratification, and therapy, thus leading to more successful and efficient health care. Taken together, the new directions in health care present new challenges to both the basic research and clinical practice communities and involve technological adaptations and integration into novel diagnostic paradigms. Traditionally, the detection, evaluation, and prognostication of cardiovascular disease or therapeutic interventions were assessed by studying physiological consequences expressed in changes in flow, metabolism, and function or on the detection of late anatomic changes. The development of biologically targeted markers to genetic and cellular processes of disease and disease amelioration has become possible with advances in genomics and proteomics. The application of imaging using these biologically targeted markers (eg, molecular imaging) in preclinical models and the translation of these approaches to patients has become possible with advent of a number of technological advances. The application of molecular imaging may provide additional unique molecular and pathophysiological insight that will allow a more personalized approach to evaluation and management of cardiovascular disease.

In this 2-part consensus article, the current state-of-the-art of cardiovascular molecular imaging will be reviewed. In Part I, the focus will be on the imaging methodology, evolving imaging technology, and the development of novel targeted molecular probes. The role of metabolic and neuroreceptor imaging will be reviewed, because these approaches represent the roots of molecular imaging within the cardiovascular system. Newer reporter gene and reporter probe imaging approaches for tracking of cardiac transgene expression will also be reviewed. Part II of this consensus article will summarize the available targeted imaging probes for identification and evaluation of critical pathophysiological processes of the cardiovascular system. These include novel imaging strategies for evaluation of processes like inflammation, thrombosis, apoptosis, necrosis, vascular remodeling, and angiogenesis. The second part will also review the role of targeted imaging of a host of interrelated diseases, including atherosclerosis, ischemic injury, postinfarction remodeling, and heart failure, as well as regenerative, genetic, or cell-based therapies. The second article will finally review the opportunities and challenges associated with the implementation and advancement of targeted molecular imaging in clinical practice for the realization of truly personalized medicine.

Molecular Imaging

The concept and practice of molecular imaging is defined as the visualization, characterization, and noninvasive measurement of biological processes at the molecular and cellular levels in humans and other living systems. Molecular imaging typically includes 2- or 3-dimensional imaging as well as quantification over time. Molecular imaging has been around for decades and originated with targeted nuclear imaging. However, other molecular imaging techniques include magnetic resonance (MR) imaging, MR spectroscopy (MRS), optical fluorescence and bioluminescence imaging, and ultrasound imaging, along with other developing imaging technologies. The true quantification of the absolute uptake or retention of targeted agents often requires dynamic imaging and the fusion of functional images with structural or anatomic images.

Targeted imaging can be defined in terms of the detection of an interaction between a probe and target. Localization and quantification of the probe is directly related to the interaction of the probe with the target epitope or peptide. Molecular imaging agents can include both endogenous molecules and...
exogenous probes. The strengths and weaknesses of all of the imaging technologies available for targeted molecular imaging will be outlined, followed by a review of the design and development of the targeted probes.

**Imaging Technology**

There have been tremendous advances in instrumentation and imaging technology that have been critical for the advancement and growth of cardiovascular molecular imaging. These advances include an array of high-resolution and high-sensitivity imaging systems dedicated for small animal imaging. Each imaging methodology has unique advantages as well as practical limitations. The relative strengths and weaknesses of each of the imaging modalities relative to their potential for molecular imaging are summarized in Table. The relative value of each modality for imaging anatomic structure, physiology, metabolism, and molecular events has been outlined in previous reviews.\(^1\)

**Nuclear Imaging**

Radiotracer-based imaging either using single-photon emission computed tomography (SPECT) or positron-emission tomography (PET) is particularly suited for targeted in vivo molecular imaging because of the relatively high sensitivity of the nuclear imaging approaches. The nuclear approaches include the use of monoclonal antibody targeting of a particular cell membrane epitope, targeted imaging of a receptor with radiolabeled peptides or peptidomimetics, imaging the activity of a particular enzyme, or even a transporter-specific probe.

Nuclear imaging has become a standard approach for physiological imaging in patients with the application of newer targeted approaches growing at a steady pace. The development of these targeted imaging approaches has been facilitated by the availability of commercial microSPECT and microPET imaging systems, which are now under widespread use in preclinical models of cardiovascular disease. MicroSPECT imaging offers several advantages over microPET imaging in small animal models of cardiovascular disease, and these include availability of a host of targeted radiotracers with a longer half-life, ability to image multiple tracers simultaneously, improved spatial resolution (<1 mm), and greater availability and affordability of the SPECT technology. The inherent resolution of PET radiotracers is fundamentally limited by physical behavior of positron decay (1 to 3 mm), associated with the significant movement of positron before annihilation, and deviation from exact 180º angular separation. SPECT imaging approaches also allow for simultaneous multiple-isotope imaging capability. On the other hand, microPET technology offers several unique advantages over microSPECT imaging. The clear advantages of PET imaging include higher sensitivity enabling dynamic imaging, creation of radiolabeled probes with natural radioisotopes such as \(^{11}\)C that do not alter chemical behavior, well-established approach for attenuation correction, and a greater potential for absolute image quantification.

**Optical Imaging**

The optical imaging approaches like the nuclear imaging approaches offer relatively high sensitivity, an important issue for detection and imaging of localized molecular or cellular processes. The optical approaches included both fluorescence and bioluminescence imaging. Microscopic bioluminescent and fluorescent optical imaging has been used for decades by molecular biologists, but only recently have these technologies been applied for in vivo molecular imaging of living animals.\(^2,3\) Fluorescence imaging systems provide excellent ex vivo spatial and temporal resolution, good molecular sensitivity, and favorable chemistry for the development of molecular imaging agents.\(^4\) These optical approaches provide the best means to evaluate molecular or cellular processes ex vivo. However, because of their relatively poor spatial resolution for in vivo imaging, the application of optical approaches for imaging anatomic structure is suboptimal, which is is increasingly addressed with hybrid fluorescence imaging approaches that also incorporate x-ray or MRI modalities for anatomic reference.\(^5\) Recently, investigators have developed approaches that allow for 3D optical imaging. Fluorescence molecular tomography (FMT) collects photons that have propagated through tissue at multiple projections and combines these measurements topographically to obtain the distribution of fluorochromes in deep tissues. As a single-point source illuminates into tissue, the photon field will propagate and excite a given distribution of fluorochromes. The fluorochromes act as secondary sources of fluorescence and bioluminescence imaging. Microscopic bioluminescent and fluorescent optical imaging has been used for decades by molecular biologists, but only recently have these technologies been applied for in vivo molecular imaging of living animals.\(^2,3\) Fluorescence imaging systems provide excellent ex vivo spatial and temporal resolution, good molecular sensitivity, and favorable chemistry for the development of molecular imaging agents.\(^4\) These optical approaches provide the best means to evaluate molecular or cellular processes ex vivo. However, because of their relatively poor spatial resolution for in vivo imaging, the application of optical approaches for imaging anatomic structure is suboptimal, which is is increasingly addressed with hybrid fluorescence imaging approaches that also incorporate x-ray or MRI modalities for anatomic reference.\(^5\) Recently, investigators have developed approaches that allow for 3D optical imaging. Fluorescence molecular tomography (FMT) collects photons that have propagated through tissue at multiple projections and combines these measurements topographically to obtain the distribution of fluorochromes in deep tissues. As a single-point source illuminates into tissue, the photon field will propagate and excite a given distribution of fluorochromes. The fluorochromes act as secondary sources at a higher wavelength, with an intensity that depends on the position of the light source. Fluorescence data are collected with a charge-coupled device camera and normalized for

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Spatial Resolution</th>
<th>Depth of Penetration</th>
<th>Temporal Resolution</th>
<th>Sensitivity (mol/L)</th>
<th>Molecular Probe</th>
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<tbody>
<tr>
<td>PET</td>
<td>1–2 mm</td>
<td>No limit</td>
<td>sec–min</td>
<td>10(^{-11})–10(^{-12})</td>
<td>ng</td>
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<tr>
<td>SPECT</td>
<td>0.3–1 mm</td>
<td>No limit</td>
<td>min</td>
<td>10(^{-10})–10(^{-11})</td>
<td>ng</td>
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<tr>
<td>Optical</td>
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<td>Bioluminescence</td>
<td>2–3 mm</td>
<td>1–2 mm</td>
<td>sec–min</td>
<td>10(^{-10})–10(^{-11})</td>
<td>ng–μg</td>
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<tr>
<td>Fluorescence</td>
<td>5–10 mm</td>
<td>&lt;1 mm</td>
<td>sec–min</td>
<td>10(^{-10})–10(^{-11})</td>
<td>ng–μg</td>
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<tr>
<td>FMT</td>
<td>1–3 mm</td>
<td>&lt;5 cm</td>
<td>min</td>
<td>10(^{-10})–10(^{-11})</td>
<td>μg</td>
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<tr>
<td>MRI</td>
<td>50–250 μm</td>
<td>No limit</td>
<td>min–hrs</td>
<td>10(^{-3})–10(^{-5})</td>
<td>μg–mg</td>
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<tr>
<td>X-ray CT</td>
<td>25–150 μm</td>
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<td>min</td>
<td>?</td>
<td>mg</td>
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<tr>
<td>Ultrasound</td>
<td>30–500 μm</td>
<td>mm–cm</td>
<td>sec–min</td>
<td>10(^{-6})–10(^{-8})</td>
<td>μg–mg</td>
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PET indicates positron emission tomography; SPECT, single photon emission computed tomography; FMT, Fluorescence molecular tomography.
Ultrasound Imaging

Techniques like ultrasound, x-ray CT, or MRI offer much better spatial resolution, although provide much lower sensitivity for evaluation of molecular processes. However, advances have been made in ultrasound imaging technology, which have helped to advance targeted molecular imaging. The major advance regarding molecular imaging with ultrasound has been associated with the development of targeted ultrasound contrast agents. The ultrasound contrast agents are typically gas-filled microspheres (microbubbles) of varying chemical composition that can be induced to expand and contract (resonate) in the presence of ultrasound delivered at the resonance frequency of the microbubbles. The natural resonance frequency of the microbubbles is determined predominantly by their size (typically <4 μm) and shell characteristics, and ranges from 1 to 5 MHz, which is fortuitously the frequency range of transducers for clinical diagnostic echocardiography. At sufficiently high transmitted acoustic pressures, the bubbles expand and contract in nonlinear fashion, emitting signals (harmonics) that are acoustically distinct from tissue backscatter.

Ultrasound imaging systems have been developed to receive these microbubble-specific signals in the harmonic frequency range, whereas suppressing tissue backscatter in the fundamental frequency range, allowing for enhanced detection of the microbubbles above and beyond tissue noise. Such systems use phase and amplitude modulation of the transmitted ultrasound waveform, coupled with subtraction methods to cancel out linear backscatter from tissue, to specifically detect the nonlinear microbubble signal.

Microbubbles 1 to 4 μm in size are used both clinically and in experimental models as red cell tracers during ultrasound imaging. For molecular imaging, the microbubble surface is modified to display ligands that cause microbubble binding to specific endothelial epitopes associated with disease processes. This binding results in a persistent contrast effect modified to display ligands that cause microbubble binding to specific endothelial epitopes associated with disease processes. This binding results in a persistent contrast effect.

Ultrasound Imaging systems are available with small-profile high-frequency transducers that facilitate imaging in small animals, including transgenic mice and even transcutaneous imaging of intrauterine fetuses. A challenge for ultrasound imaging systems is the simultaneous anatomic definition provided by the 2D ultrasound image, which obviates the need for hybrid imaging to spatially localize “hot spots” of activity.
sound molecular imaging with high-frequency systems (20 to 30 MHz) is their relative insensitivity for the detection of distinct microbubble signals. Because most microbubbles of the size used for molecular imaging do not resonate at high frequencies, a nonlinear imaging approach to suppress tissue noise while highlighting distinct microbubble signals is inherently difficult to implement on high-frequency platforms. Similarly, catheter-based ultrasound strategies (intravascular ultrasound), which require high frequencies to achieve adequate near-field resolution of the vessel wall, operate outside the resonance frequency range of micron-sized microbubbles. Thus, intravascular ultrasound is relatively insensitive for the detection of signals from microbubbles adhered to the wall. Recently, the design of a nonlinear intravascular ultrasound transducer in conjunction with smaller microbubbles (higher resonance frequency) has raised promise for microbubble detection on high-frequency imaging systems. This would be a key advance, as application of catheter-based intravascular ultrasound imaging of adhesion molecules might permit identification of early atherosclerosis or unstable atherosclerotic plaque facilitating early detection and treatment of disease.

The optimization of systems for ultrasound molecular imaging will require customization to the specific acoustic characteristics of microbubbles to maximize sensitivity for detecting the contrast agents. Both acoustic and optical characterization of microbubble behavior in an ultrasound field using ultrahigh speed imaging of microbubble resonance have yielded information on unique acoustic emissions from microbubbles that are adhered to a surface compared with those of a freely circulating microbubble. Such analyses should provide a rational basis for the prospective design of imaging systems optimized for the detection of microbubbles that have adhered to their specific target.

**X-Ray CT Imaging**

Many investigators and commercial vendors have now integrated high-resolution x-ray CT imaging devices with other higher sensitivity imaging systems to create hybrid imaging systems that are optimized for targeted imaging in small animals as well as patients. The introduction of these hybrid systems for imaging of small animals (microSPECT/CT and microPET/CT, and in the future FMT/CT) has greatly enhanced the performance and accuracy of nuclear imaging. The CT component is generally used for anatomic localization, as well as attenuation correction, and correction of partial volume errors, which may limit the nuclear approaches. The same types of hybrid imaging devices have also been developed for imaging patients and are quickly becoming the standard for these imaging systems. These hybrid imaging systems will facilitate the translation of molecular-based imaging to patient care.

Recent studies suggest that CT imaging alone may also have a unique role for targeted imaging of the vasculature. Macrophages in atherosclerotic plaques of rabbits can be detected with a clinical x-ray CT scanner after the intravenous injection of a contrast agent formed of iodinated nanoparticles. The use of these novel CT contrast agents may become an important adjunct to the clinical evaluation of coronary arteries with CT.

**MRI and Spectroscopy**

MRI is also emerging as a technique for molecular imaging. MRI provides high spatial resolution and the unique capability to elicit both anatomic and physiological information simultaneously. The MR signal is modulated by the interaction of the tissue water magnetic moment (proton spin) and the magnetic properties of the surrounding environment. This scenario provides an opportunity for the development of contrast agents that magnetically modify this environment, leading to either positive (hot spot, T1-targeted) and negative (cold spot, T2-targeted) enhancement. The most commonly used nonspecific contrast agents are based on gadolinium. These agents shorten the spin-lattice relaxation time (T1) of nearby water protons, providing an increase in signal on T1-weighted images. Additionally, superparamagnetic iron oxide particles have been developed that shorten the spin–spin relaxation times (T2 and T2*) of water protons and create signal voids on T2- and T2*-weighted MR images. Some other less popular MRI methods also demonstrate high potential for molecular imaging. For example, one approach is to use nanoparticles that carry a fluorine (19F) payload, and then detect the 19F MR signal instead of the 1H signal. This method has the advantage that there is no background signal, but the disadvantage of lower target signal. Another novel method makes use of the chemical exchange of 1H protons between tissue water and the contrast agent. Specifically, chemical exchange saturation transfer (CEST) contrast agents are designed to contain a narrow band of off-resonance 1H protons that exchange with tissue water. The contrast agent takes effect only when the imaging pulse sequence applies radiofrequency saturation pulses at the shifted CEST resonance. When the saturated CEST 1H protons exchange with tissue water 1H protons, the on-resonance water signal decreases, causing decreased signal intensity in the region of the image containing the contrast agent. The CEST contrast agent can effectively be turned on or off, depending on the specific pulse sequence that is applied. Also, it may be possible to generate families of CEST agents with different off-resonance frequencies to separately and simultaneously label different targets. In general, CEST agents have the disadvantage of low sensitivity; however, recent advances in their design suggest that this limitation may be overcome. Although MRI generally offers much lower sensitivity than optical or radiotracer-based approaches for targeted molecular imaging, novel approaches are now being applied to increase the contrast payload of MR targeted contrast agents and to better amplify the signal change, making molecular imaging with MRI systems feasible.

Alterations in myocardial substrate metabolism are critical in the pathogenesis of many cardiovascular diseases. MRS can provide highly sensitive and quantitative measures of metabolic processes in the heart, which may complement MRI. MRS can be accomplished with evaluation of hydrogen (1H), carbon (13C), fluorine (19F), sodium (23Na), and phosphorous (31P). Although MRS provides limited sensitivity...
and spatial resolution for in vivo imaging, important insight has been gained about in vivo myocardial energetics associated with myocardial ischemia and infarction. The availability of higher field (>3 T) large bore magnets may enhance the future clinical utilization of MRS in evaluation of cardiovascular disease.

**Image Quantification**
Quantification of molecular imaging refers to the determination of regional concentrations of molecular imaging agents and biological parameters. This quantification is a key element of molecular imaging data and image analysis, especially for inter- and intrasubject comparisons. The accuracy of microSPECT imaging is fundamentally limited by the attenuation of the low-energy photons by body tissues. This introduces an error in relating the density of detected photons to the concentration of the radiopharmaceutical in an organ. Moreover, the presence of photon scatter limits spatial resolution. The microPET imaging systems generally provide better attenuation correction. However, most state-of-the-art microSPECT and microPET imaging systems are fused with microCT, which can facilitate accurate attenuation correction as well correction for partial volume errors that result in underestimation of true regional radiotracer activity without these corrections. Fluorescence reflectance imaging faces limitations when deep structures such as vessels or the heart are targeted. The attenuation of photons depends on the length of their travel through tissue, and absorption diminishes the signal of deeper sources. This limitation is overcome by FMT, which is not surface weighted but is 3D and normalizes the signal for absorption and scatter.

**Molecular Probe Development and Design**

**Radiolabeled Probes**
SPECT and PET imaging represent the prototypic in vivo methods for imaging and quantification of biochemical and molecular processes. Organic synthesis and combinatorial chemistry, solid-phase peptide syntheses, and phage display approaches to be easily translated from the laboratory to clinical trials, whereas nanotechnology is used for targeting and signal amplification.

**Optical Probes**
Near-infrared fluorochromes have been coupled to antibodies, peptides, or nonpeptide small molecules and nanoparticles to target specific biological processes, including apoptosis, angiogenesis, expression of adhesion molecules by activated endothelium, and phagocytic uptake by macrophages. Several activatable optical reporters have been developed to image proteases, and harbor a powerful signal amplification strategy by targeting protease activity rather than imaging enzyme presence. Cathespin- and metalloproteinase-targeted optical reporters have a polymeric scaffold, which consists of near infrared fluorochromes, specific protease substrate peptides and partially methoxypolyethylene glycol graft copolymers. The sensor is injected in an inactive state, when the closely approximated fluorochromes are not excitable because of auto-quenching. Proteolytic cleavage of the scaffold releases the fluorochromes and results in extensive fluorescence generation (dequenching). Amplification is achieved because one active moiety of enzyme can activate multiple reporters. These probes have been used to image enzyme activity in intact mice by FMT and with cellular resolution by fluorescence microscopy and flow cytometry in a variety of inflammatory conditions including myocardial infarction (Figure 2) and atherosclerosis.

**Ultrasound Probes**
Ultrasound probes or contrast agents for molecular imaging have been synthesized using varying compositions, but share in common the property of “acoustic activity” in an ultrasound field. In the presence of the appropriate energy and frequency of ultrasound, the probes, which are particulate in nature, scatter the ultrasound or become acoustic emitters themselves, resulting in a received acoustic signal, which can be displayed on a simultaneous 2D ultrasound image. Unlike freely circulating ultrasound contrast agents used for ventricular opacification and perfusion imaging, targeted ultrasound probes are designed to adhere to endothelium via specific ligand-receptor interactions that are prespecified in the probe design. To achieve this, a targeting ligand is attached to the surface of the probe, causing it to adhere to a specific endothelial marker. The first generation of targeted contrast agents for ultrasound-based molecular imaging were...
liquid perfluorocarbon nanoparticle emulsions\textsuperscript{49} and liposomes,\textsuperscript{50} which are relatively weakly echogenic until aggregated in large numbers to the target. These agents have been used to target thrombus and leukocyte adhesion molecules in plaques, respectively. Subsequently, a number of investigators developed targeted microbubbles for ultrasound imaging, which are larger in size.\textsuperscript{51–53} and comprised of perfluorocarbon or nitrogen gas encapsulated by shells of various composition, such as phospholipids, albumin, or biodegradable polymers. Targeting ligands initially used for proof of concept were monoclonal antibodies directed against endothelial targets.\textsuperscript{52–54} Other targeting moieties that would be more suitable for clinical translation have been reported, including peptides sequences\textsuperscript{55,56} and naturally occurring ligands for the given receptor, including carbohydrates or proteins.\textsuperscript{21,57–58}

Adhesion of microbubbles to their target is influenced by fluid shear stress levels at the vessel wall and is proportional to receptor density on the endothelial cell.\textsuperscript{59} Ligand density on the microbubbles, ligand-receptor bond affinity, and the conformational presentation of the ligand to the receptor are properties that affect net microbubble adhesion and that pose opportunities for optimization of microbubble design.\textsuperscript{59–61} By virtue of their size (1 to 4 \( \mu \text{m} \)), microbubbles remain within the intravascular space, such that the targets for imaging using this modality are most often endoluminal in location. Submicron agents that are acoustically active, though not gas-encapsulated microspheres, have been shown to home to extravascular sites.\textsuperscript{62}

**MR Probes**

As outlined above, there are 2 major classes of molecular probes for MRI. The first includes gadolinium (Gd)-based paramagnetic probes that enhance the T1-weighted images, and the second includes superparamagnetic iron oxide nanoparticles that provide negative contrast on T2-weighted images. Manganese is another important paramagnetic agent that enhances the signal by shortening T1. Investigators have also synthesized protein-based macromolecular molecular probes (albumin-Gd conjugates), monoclonal antibody-based molecular probes, polyamidoamine dendrimers of Gd-based probes, and nanoparticle-based molecular probes for targeted MRI.\textsuperscript{63} Micelles, liposomes, and emulsions have been used to carry large payloads of gadolinium,\textsuperscript{64} \( ^{19} \text{F} \),\textsuperscript{31,32} and CEST agents\textsuperscript{34} and can be targeted by incorporating ligands into their lipid bilayer. Activatable agents have also been developed for MRI. These include Gd complexes that when cleaved by an enzyme, expose the shielded Gd to water resulting in alterations of T1 relaxivity.\textsuperscript{65} A different approach involves the derivatization of Gd chelators with 5-hydroxytryptamide \( \text{[bis-5HT-DTPA(Gd)]} \). Myeloperoxidase activates the small-molecule substrate, which then polymerizes and exhibits increased T1 relaxivity, protein binding, and “trapping” in areas of high myeloperoxidase (MPO) activity, all leading to increased enhancement on T1-weighted MRI (Figure 3).\textsuperscript{66} Others have engineered magnetic

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**Figure 2.** Protease sensors are activated in the healing infarct. A, FMT of mouse with myocardial infarction after injection of Prosense-680. In this maximum-intensity projection, focal signal can be observed in the heart region. B, FMT of mouse without myocardial infarction (sham surgery involves thoracotomy only) 24 hours after injection of Prosense-680. Very little fluorescent signal is detected in the heart region, because no cathepsin activity occurs in the uninjured heart to activate Prosense-680. C, Fluorescence reflectance image of excised heart 4 days after myocardial infarction. The fusion of fluorescence image with the white light image shows Prosense activation mainly in the thin infarct scar (top). Almost no signal is observed in the remote myocardium in the septum and right ventricle (bottom). The infarct can be readily identified as the unstained, pale area in the triphenyl tetrazolium chloride (TTC) stain (D). E, Time course of protease activity after coronary ligation. Adapted from reference 8.

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**Figure 3.** MR-based MPO imaging. A, The infarct of a wild-type mouse 2 hours after injection of MPO-Gd is brightly enhanced. Imaging was performed on day 2 after myocardial infarction in all mice. B, Intermediate-level enhancement is observed in heterozygous MPO-deficient mice, corresponding to 50% enzyme activity levels. C, In homozygous MPO-deficient mice, enhancement after injection of MPO-Gd is significantly reduced, establishing the specificity of MPO-Gd. D, Contrast-to-noise ratio in respective genotypes show significantly diminished enhancement in MPO-deficient mice. E, Polymerase chain reaction PCR and Western blotting of representative genotypes shown in A–C. Adapted with permission from reference 66.
nanoparticles that assemble and disassemble, which can be used for detection of enzymatic activity.67

**Hybrid Nanoparticles**

Effort has been recently directed to developing multimodality imaging instruments, resulting in an increased interest in the synthesis of hybrid targeted molecular probes for these new hybrid instruments. Multimodality imaging with these hybrid probes can potentially provide both anatomic information and as well as functional, metabolic, or molecular information simultaneously.63 Several groups have synthesized multifunction probes for preclinical imaging. These hybrid probes allow initial in vivo 3D imaging with radiotracer or MR probes and subsequent high-resolution ex vivo postmortem cellular localization with optical probes. Alternatively, hybrid optical-nuclear/MR probes might facilitate rapid screening of animals with an optical probe and selective more time intensive imaging of nuclear or MR probe. A trimodal nanoparticle with avidity for macrophages has been used to image inflamed atherosclerotic lesions with PET, MR, and fluorescence imaging (Figure 4).68

Engineered nanoparticles provide an excellent platform for creating such hybrid probes.69 Investigators have developed polymer coated superparamagnetic ion oxide nanoparticles for molecular imaging.70 These can be engineered to optimize the intravascular residence time and can be targeted44,71 or made activatable.69 Phage display biopanning is often used to identify novel ligands for specific molecular targets of interest.

Nanoparticles offer the ability to incorporate drugs or genes into a detectable nanosystem, facilitating targeted therapeutics and leading to image-based drug delivery.72 Payloads of therapeutic agents, including drugs, genes, or radionuclides, can be linked to or dissolved within carrier lipid coatings, deposited in subsurface oil layers, or trapped within the carrier systems themselves. The use of these targeted agents can concentrate therapy within selected tissues by nonspecific or specific uptake mechanisms, minimizing toxicity, and harmful side effects. However, investigators developing nanoparticles for targeted drug delivery face important challenges. These nanoparticles must be able to avoid elimination by the reticuloendothelial system and have sufficient circulation time to reach and accumulate at the target site.

**Reporter Gene and Reporter Probe Approach**

The reporter gene/probe approach has been used in the evaluation of gene- and cell-based therapies of the cardiovascular system. Reporter gene imaging was initially developed for analysis of postmortem tissues; however, the feasibility for in vivo noninvasive imaging of reporter genes is now well established. A suitable reporter gene product is generally not present in the target tissue and produces minimal effects on tissue function. An appropriate reporter gene can produce, an enzyme that catalyzes accumulation of an imaging reporter probe, a receptor that accumulates specific receptor ligand probe, or a transport protein that results in intracellular accumulation of specific probe molecules.73 This principle is illustrated in Figure 5.
The herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene is the most widely used reporter gene. Although the (HSV1-tk) reporter gene technology was developed for application in oncology, the first application in cardiac cells demonstrated the specific accumulation of a radioiodinated pyrimidine derivative 2V-fluoro-2Vdeoxy-5-iodo-1-“-D-arabinofuranosyluracil (FIAU) in the area of in vivo myocardial gene transfer using autoradiographic analyses. Additional studies by these investigators showed that adenoviral titers as low as 1×10⁷ produced enough signal for noninvasive identification. Serial studies demonstrated that the reporter gene signal peaked at 3 to 5 days after gene transfer but was no longer detectable after 10 to 17 days. Subsequently, the feasibility of this reporter gene approach for tracking gene expression was demonstrated in a more clinically relevant pig model using 124I-labeled FIAU and a clinical PET imaging system, as well as 18F-labeled FHBG and a clinical PET imaging system.

Figure 5. Four different strategies of imaging reporter gene/reporter probe. A, Enzyme-based bioluminescence imaging. Expression of the firefly luciferase reporter gene leads to the firefly luciferase reporter enzyme, which catalyzes the reporter probe (D-Luciferin) that results in a photochemical reaction. This yields low levels of photons that can be detected, collected, and quantified by a charge-coupled device camera. B, Enzyme-based PET imaging. Expression of the herpes simplex virus type 1 thymidine kinase (HSV1-tk) reporter gene leads to the thymidine kinase reporter enzyme, which phosphorylates and traps the reporter probe (18F)-FHBG intracellularly. Radioactive decay of (18F) isotopes can be detected using PET. C, Receptor-based PET imaging. The (18F)-FESP is a reporter probe that interacts with the dopamine 2 receptor (D2R) to result in probe trapping on or in cells expressing the D2R gene. D, Receptor-based MRI imaging. Overexpression of engineered transferrin receptors results in increased cell uptake of the transferrin-monocrystalline iron oxide nanoparticles. These changes result in a detectable contrast change on MRI. Reprinted with permission from reference 96.
PET/CT scanner. These investigators were able to coregister the in vivo PET images of transgene expression with PET images of perfusion and metabolism or with CT images, highlighting the potential value of this imaging approach for evaluating novel gene or cell-based therapies.

**Metabolic Imaging**

To date, the primary emphasis of metabolic imaging has been in the study of myocardial intermediary metabolism. Approaches that have been used include MRS, PET, or SPECT imaging. Each approach offers specific advantages in evaluation of myocardial metabolism. MRS has provided critical information about high energy phosphate and carbon metabolism but has been primarily restricted to preclinical studies, because of limit sensitivity and spatial resolution.

PET and to a lesser degree SPECT are the most commonly used radionuclide methods to assess myocardial metabolism. In the case of PET, 2 different radiolabeling strategies have been used. One approach is to radiolabel naturally occurring substrates such as various fatty acids (1-11C-palmitate or 1-11C-acetate), glucose (1-11C-glucose), and lactate (L-3-11C-lactate) in specific carbon locations with carbon-11.79–82

Advantages of this approach are that the metabolism of the radiolabeled substrates are identical to the unlabeled substrate and when combined with appropriate mathematical modeling schemes permits in-depth assessments of myocardial uptake and downstream metabolism of these substrates (Figure 6). Disadvantages include the requirement for an on-site cyclotron and advanced radiochemistry capabilities both of which the widespread applicability of these methods. The second approach is to radiolabel a substrate-analog with fluorine-18. Typically, the myocardial kinetics of the substrate analog will reflect a specific but limited set of metabolic pathways and be trapped in tissue. As a consequence, image quality is high and the use fluorine-18 permits distribution of the radiotracer to PET sites without radiochemistry capability. Quantification of a limited number of metabolic processes is possible (eg, uptake and/or oxidation) but subject to the requirement of correcting for differences in the kinetics between the substrate analog and the naturally occurring substrate it mimics. The most prominent example in this category is 2-(18F)-fluoro-2-deoxy-D-glucose (FDG). The uptake and retention of FDG reflects the activity of the various glucose transporters and hexokinase mediated phosphorylation in a manner similar to the handling of unlabeled glucose. However, unlike glucose-6-phosphate, FDG-6-phosphate is not further metabolized and gets trapped in the cells enhancing image quality. The myocardial kinetics of FDG provides an integrated in vivo assay of increased glycolytic activity, increased hexokinase activity, and upregulation or sarcolemmal translocation of glucose transporters.63 Thus, this imaging approach is a non-invasive assay of increased glycolytic activity, increased hexokinase-mediated phosphorylation, and upregulation of glucose transporters.63

Figure 6. Correlation between PET and arterial-coronary sinus (ART/CS) measurements of fractional glycolysis (A) and glucose oxidation (B). The close correlations demonstrate the potential of compartmental modeling of 1-11C-glucose myocardial kinetics to estimate the fate of extracted glucose. Reprint with permission from *Journal of Nuclear Medicine.*

Over the past 25 years, numerous radiotracers have been developed to assess myocardial fatty acid metabolism with SPECT. All of these radiotracers were either a straight or branched chain fatty acid analog. One of the first successful examples of the former was 15-(p-iodophenyl)-pentadecanoic acid (IPPA), which contained an aromatic ring at the omega position radiolabeled with radioiodine.83 The aromatic nature of the iodine-carbon bond resulted in greater in vivo stability, making it less likely to be degraded by dehalogenases and defluorination. This radiotracer demonstrated rapid accumulation in the heart and exhibited clearance kinetics that followed a biexponential function characteristic for unlabeled palmitate. Moreover, the clearance rates correlated directly with β-oxidation in animal models of disease and in humans. Unfortunately, SPECT systems did not have the temporal resolution to take advantage of the rapid turnover of IPPA. As a consequence, quantification of myocardial fatty acid metabolism was not possible and image quality was reduced. These challenges led to the development of branched-chain analogs of IPPA, such as 125I-β-methyl-p-iodophenylpentadecanoic acid (BMIPP). Alkyl branching inhibited β-oxidation, thereby increasing radiotracer retention and improving SPECT image quality. Tissue retention seems to reflect initial activation of extracted fatty acids by acyl-coenzyme A (CoA)
carboxylase and their incorporation into triglycerides. Consequently, static images provide an index of the initial portions of fatty acid metabolism. However, quantification of myocardial substrate use is difficult because of the technical limitations of SPECT (relatively poor temporal and spatial resolution and inaccurate correction for photon attenuation) and incomplete metabolism of BMIPP relative to unlabeled fatty acid use.

With the advent of microPET and microSPECT systems and appropriate image reconstruction and quantification schemes, it is now possible to perform many of the measurements described above in rodent heart (Figure 7). Thus, the potential for bidirectional cross-talk is enhanced during the investigation of myocardial metabolism in relation to cardiac disease.

Of note, cardiovascular metabolic imaging does not need to be limited to the study of myocardial processes. The most notable example here is the use of FDG imaging to evaluate macrophage activity within the atherosclerotic plaque as an index of plaque inflammation and instability. Other more specific PET tracers could potentially be available for the assessment of atherosclerotic plaques.

Neuroreceptor Imaging

Another classic targeted molecular imaging approach involves the imaging of cardiac neuroreceptors in the heart. Most of this work has focused on imaging of the sympathetic nervous system. Important alterations in presynaptic and postsynaptic cardiac sympathetic function occur in several cardiovascular diseases, including ischemic heart disease and heart failure. Presynaptic function can be measured using $^{11}$C-meta-hydroxyephedrine ($^{11}$C-HED), a PET radiotracer, or $^{123}$I-meta-iodobenzylguanidine ($^{123}$I-MIBG) a SPECT radiotracer. Postsynaptic function can be assessed with $^{11}$C-CGP12177, a radiolabeled $\beta$-blocker for PET imaging. This topic is the focus of several excellent reviews.

Many studies have demonstrated that $^{123}$I-MIBG imaging can provide powerful diagnostic and prognostic information in patients with heart failure. Several radiotracers for scintigraphic imaging of cardiac neurotransmission have been developed with radiolabeling of the neurotransmitters or their structural analogs (Figure 8). Of these radiotracers, $^{123}$I-MIBG shares many cellular uptake and storage properties with norepinephrine, and, thus, $^{123}$I-MIBG scans have been used to evaluate cardiac sympathetic nervous system distribution and function. In patients with heart failure, $^{123}$I-MIBG scans typically show a reduced heart-mediastinum uptake ratio, heterogeneous distribution within the myocardium, and increased $^{123}$I-MIBG washout from the heart. Arimoto et al showed that patients with abnormally rapid washout levels had a significantly higher cardiac event rate (57%) than did those with normal washout levels (12%; $P<0.0001$) during the follow-up period (6 to 30 months). Arora et al showed that patients with ICD discharge had a substantially lower $^{123}$I-MIBG heart-mediastinum tracer uptake ratio, higher $^{123}$I-MIBG defect scores, and more extensive sympathetic denervation. A large industry-sponsored trial is currently looking at $^{123}$I-MIBG imaging as a potential approach for AICD triage in patients at risk for sudden cardiac death (CAD, LV ejection fraction <35%). The National Institutes...
of Health–sponsored Prediction of A Rhythmic Events with PET trial at the University of Buffalo is assessing $^{13}$NH$_3$, $^{18}$F-FDG, and $^{11}$C-HED PET imaging in patients with heart failure. If the value of cardiac autonomic assessment using $^{123}$I-MIBG or $^{11}$C-HED imaging is confirmed, these neuroreceptor imaging approach may help in the selection of patients who would benefit the most from an ICD by means of identification of those at increased risk for potentially fatal arrhythmias, leading to more cost-effective implementation of this life-saving device.

A recent preclinical PET imaging study demonstrated that impairment of myocardial catecholamine uptake and storage exceeds the reduction of tissue perfusion after experimental myocardial infarction. These investigators performed both PET $^{13}$N-ammonia perfusion imaging along with $^{11}$C-epinephrine imaging and observed an association between sustained inducible ventricular tachycardia and the presence of more extensive dysinnervation of normally perfused viable myocardium in the infarct border zone. This mismatch of perfusion and neuronal function correlates regionally with reduced voltage and the earliest endocardial activation site of ventricular tachycardia. These results support the important role of impaired sympathetic innervation as a substrate of postinfarct ventricular tachycardia. This preclinical study provides a rationale for future clinical trials to evaluate the efficacy of molecular imaging in the work-up of postinfarct ventricular arrhythmia.

**Summary**

In Part I of this consensus article, the imaging methodology, evolving imaging technology, and development of novel targeted molecular probes relevant to the developing field of cardiovascular molecular imaging were reviewed. Novel reporter gene and reporter probe imaging approaches for tracking of cardiac transgene expression were also discussed and have important future implications for evaluation of gene- and cell-based therapies for the failing heart. The current role of metabolic and receptor imaging was also briefly reviewed, as these represent the beginning of our current role of metabolic and receptor imaging for identification and evaluation of contractile dysfunction in the failing myocardium. The recent preclinical study demonstrates the potential for future clinical trials to evaluate the efficacy of molecular imaging in the work-up of postinfarct ventricular tachycardia.

**Disclosures**

None.

**References**


Sinusas et al

Multimodality Cardiovascular Molecular Imaging


**Key Words:** cardiovascular diseases □ diagnosis □ imaging □ radionuclide imaging □ image three-dimensional □ molecular probes