Cardiovascular Molecular Imaging
Focus on Clinical Translation

Ian Y. Chen, MD, PhD; Joseph C. Wu, MD, PhD

The past few decades have seen an explosion in the knowledge of the molecular basis of cardiovascular diseases owing to rapid advances in molecular biology research. An improved understanding of disease pathogenesis at the genomic, transcripational, and proteomic levels has led to the discovery of promising experimental strategies for the prevention, diagnosis, and treatment of cardiovascular disease. Unfortunately, only a minute number of these strategies have survived the rigors of preclinical and clinical trials to become therapeutically useful. Furthermore, even these successful strategies must endure a prolonged process of translation from bench to bedside, partially owing to the lack of tools to directly interrogate the molecular events in patients. The strong impetus to develop noninvasive imaging techniques to visualize molecular changes in patients has given rise to the field of molecular imaging.

Molecular imaging has its roots in nuclear medicine, in which radiolabeled imaging probes are injected into living subjects to assess the functionality of different organ systems. Unlike conventional diagnostic imaging techniques (eg, radiography and computed tomography [CT]) that delineate the anatomy of the cardiovascular system (eg, coronary luminal diameter), molecular imaging techniques have been designed and validated to study much smaller-scale molecular events (eg, gene expression) that may underlie disease processes. The complexity of molecular imaging lies in the requirement for molecular targeting, the design of which requires a solid understanding of the pharmacokinetics of the imaging probe and how it interacts with the molecular target. When the target is proven to be a biomarker, molecular imaging becomes a valuable tool for detecting disease before its clinical manifestation, stratifying disease severity, predicting disease progression, monitoring treatment efficacy, and prognosticating disease. These challenges must be met before the true potential of personalized medicine can be fully realized.

Significant advances have been made in molecular imaging to make it a clinical reality in many areas. Instrumental in its development has been the advancement of small-animal imaging technologies over the past 2 decades, including fluorescence imaging (FI), bioluminescence imaging (BLI), ultrasound, micro-posion emission tomography (micro-PET), high-field small animal magnetic resonance imaging (MRI), and micro–computed tomography (microCT; Figure 1A). A parallel development has been the construction of sophisticated imaging probes with high specificity for various molecular targets. The use of these imaging systems and probes has facilitated the validation of molecular imaging techniques in animal models. Ongoing translation of these techniques into the clinical arena is being achieved by use of either clinical versions of small-animal scanners or special imaging platforms specifically developed for clinical translation. A wide range of exciting cardiovascular molecular imaging applications have now been developed and reviewed previously.1,2 After a brief overview of the fundamentals of molecular imaging approaches, the present review will focus on the latest advances in the areas of atherosclerosis, heart failure, and stem cell therapy. The article will conclude with discussions on the future prospects of molecular imaging in the clinical arena, as well as future directions that will shape molecular imaging in the postgenomics era.

Fundamentals of Molecular Imaging Approaches

Imaging of molecular events requires the selection of a molecular target, an imaging probe, and an imaging system. The molecular target can be DNA, RNA, or protein in the form of an intracellular enzyme, a cell surface receptor, a membrane transporter, or an extracellular enzyme. Proteins are of most practical interest because of their abundance within a cell (0.01 to 1 million compared with 1 to 2 DNA and 10 to 1000 RNA), which contributes to the imaging sensitivity. The cellular compartment in which the target resides needs special consideration, because a transport mechanism (eg, transporter) may be required to bring the probe across the cell or organelle membrane for intracellular target-probe interaction. In contrast, imaging of cell surface or extracellular targets can circumvent cellular transport barriers and can be performed with a generalized platform, as shown in Figure 1B. The commonly used molecular imaging modalities are discussed below to highlight their relative strengths and weaknesses (see also Table 1).
**Fluorescence Imaging**

Fluorescence imaging (FI) is typically performed by exogenous delivery of a fluorescent probe (e.g., an organic dye) that interacts with the target or by direct imaging of an endogenously expressed fluorescent protein. Signal generation is achieved by exciting the fluorescent probe or protein at a given light wavelength ($\lambda$) and detecting light emission at another $\lambda$ with a charge-coupled device camera built into either a planar (fluorescence reflectance imaging) or a tomographic (fluorescence molecular tomography) imaging system.\(^6\) Because light attenuation by tissue is wavelength dependent, fluorescent probes or proteins with more red-shifted emission $\lambda$ (near-infrared probes or fluorescent proteins with $\lambda = 700$ to $900$ nm) have been developed to maximize imaging sensitivity and specificity. More recently, fluorescent semiconductor nanoparticles (2 to 8 nm), also known as quantum dots, have been customized to allow simultaneous imaging of multiple targets with a single excitation wavelength and quantum dots of varying sizes.\(^7\) The advent of quantum dot technology alleviates the problems associated with organic fluorophores (e.g., photobleaching, low quantum yield, low absorbance, and broad emission band). The cytotoxicity of quantum dots, however, needs to be addressed before they can be used clinically.\(^8\) Magnetofluorescent nanoparticles for both MRI and FI represent yet another class of fluorescent probes, which have found wide preclinical applications in the study of inflammatory atherosclerosis,\(^9\) postinfarction healing,\(^10\) transplant rejection,\(^11\) and early aortic valve disease.\(^12\) These agents enable the attainment of both high imaging sensitivity from FI and high spatial resolution from MRI, which also helps to compensate for the limited imaging depths of FI. More recently, an intravascular near-infrared fluorescence-sensing catheter has been developed to allow imaging close to the vascular target.\(^13\) This novel imaging technique should help open the door for many promising human applications, including imaging of microthrombi associated with vulnerable coronary plaques.

**Bioluminescence Imaging**

Bioluminescence imaging (BLI) uses light generated from an enzyme-substrate pair as an imaging signal and an ultrasensitive cooled charge-coupled device camera for signal detection. Exogenous expression of a luciferase enzyme, followed by systemic delivery of its substrate, forms the basis of in vivo BLI. To date, luciferases...
Table 1. Characteristics and Performance of Common Molecular Imaging Modalities

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Spatial Resolution, mm</th>
<th>Imaging Sensitivity, mol/L Probe</th>
<th>Imaging Time</th>
<th>Clinical Translation</th>
<th>Imaging Cost*</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>&lt;1 (FRI); 1 (FMT)</td>
<td>Not well characterized, likely $10^{-12}$</td>
<td>Seconds to minutes (FRI), minutes (FMT)</td>
<td>Yes (FRI; IO/EV); in development (FMT)</td>
<td>$ (FRI) $$$ (FMT)</td>
<td>High sensitivity, multiplexed imaging, immense catalogue of probes</td>
<td>Surface weighted (FRI), relatively low spatial resolution, planar (FRI), mass quantity ($\mu$g to mg) of probe needed</td>
</tr>
<tr>
<td>BLI</td>
<td>3–5</td>
<td>Not well characterized, likely $10^{-15}$</td>
<td>Minutes</td>
<td>No</td>
<td>$</td>
<td>High sensitivity, high-throughput, easy, low cost</td>
<td>Surface weighted, planar, low spatial resolution, not translational, mass quantity ($\mu$g to mg) of probe needed</td>
</tr>
<tr>
<td>US</td>
<td>0.04–0.1 (Small-animal US); 0.15–1 (clinical US)</td>
<td>Single MB can be visualized</td>
<td>Seconds to minutes</td>
<td>Yes</td>
<td>$$$$</td>
<td>Real-time, portable, low cost, high sensitivity</td>
<td>High operator dependency, limited target choices, poor probe adhesion efficiency, limited bone imaging</td>
</tr>
<tr>
<td>PET</td>
<td>1–2 (microPET); 6–10 (clinical PET)</td>
<td>$10^{-11}$–$10^{-12}$</td>
<td>Minutes</td>
<td>Yes</td>
<td>$$</td>
<td>High sensitivity, translational, quantitative, trace amount (ng) of probe needed</td>
<td>Radiation, relatively low spatial resolution, cyclotron or generator needed</td>
</tr>
<tr>
<td>SPECT</td>
<td>0.5–2 (microSPECT); 7–15 (clinical SPECT)</td>
<td>$10^{-10}$–$10^{-11}$</td>
<td>Minutes</td>
<td>Yes</td>
<td>$$</td>
<td>High sensitivity, translational, multiplexed imaging, trace amount (ng) of probe needed</td>
<td>Radiation, relatively low spatial resolution</td>
</tr>
<tr>
<td>MRI</td>
<td>0.01–0.1 (Small-animal MRI); 0.5–1.5 (clinical MRI)</td>
<td>$10^{-3}$–$10^{-5}$</td>
<td>Minutes to hours</td>
<td>Yes</td>
<td>$$$</td>
<td>High spatial resolution; superb soft-tissue discrimination; combined anatomic, functional, and molecular imaging</td>
<td>Relatively low sensitivity, long scan or postprocessing time, mass quantity ($\mu$g to mg) of probe needed</td>
</tr>
<tr>
<td>CT</td>
<td>0.02–0.3 (microCT); 0.5–2 (clinical CT)</td>
<td>$10^{-2}$–$10^{-3}$, potentially $10^{-2}$–$10^{-10}$ with NP probes</td>
<td>Minutes</td>
<td>Yes</td>
<td>$$</td>
<td>High spatial resolution, superb bone imaging</td>
<td>Radiation, limited soft-tissue discrimination, mass quantity ($\mu$g to mg) of probe needed</td>
</tr>
</tbody>
</table>

*Cost is represented in relative terms by dollar signs, with $ representing the least expensive modality and $$$ the most expensive.

FI indicates fluorescence imaging; FRI, fluorescence reflectance imaging; FMT, fluorescence molecular tomography; IO, intraoperative; EV, endovascular; BLI, bioluminescence imaging; US, ultrasound; MB, microbubble; PET, positron emission tomography; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; CT, computed tomography; and NP, nanoparticle.

cloned from beetles (firefly luciferase [FLuc] and click beetle red [CBR]) and sea pansy (Renilla luciferase [RLuc]) have been used together with their respective substrates (D-luciferin and coelenterazine) for in vivo BLI. The beetle luciferases are generally preferred over the sea pansey luciferase, because their greater red-shifted emission spectrum produces better tissue penetration. Their light generation, however, requires O₂, magnesium, and ATP, whereas the sea pansy luciferase can function well in extracellular environments that lack ATP. For optimal in vivo application, a mutant sea pansy luciferase with red-shifted emission wavelength ($\lambda=535$ nm) has been developed for improved imaging sensitivity and extracellular targeting. BLI has excellent imaging sensitivity ($10^{-15}$ to $10^{-17}$ mol/L) owing to its negligible background signal in living animals. Its low cost and high-throughput capability make BLI the preferred modality for tracking stem cell fate and gene therapy in small-animal models. However, because of the limited light penetration, the requirement for exogenous reporter genes, and the need to inject mass amounts (micrograms to milligrams) of potentially immunogenic substrates, it is unlikely that BLI will be clinically useful.

Molecular Ultrasound Imaging

Molecular ultrasound is performed with microbubbles ($\approx 1$ to 10 $\mu$m) that are composed of gas (eg, perfluorocarbon) enclosed in spherical shells of lipid, protein, or biocompatible polymer. Targeting of microbubbles to diseased vasculature can be achieved either by direct conjugation of ligands to the microbubble shell or indirectly via an interjacent molecule (eg, streptavidin) with biotinylated ligands. The size of the microbubbles, however, precludes their vascular extravasation, thereby limiting the selection of molecular targets to the endothelium. Signal enhancement from the microbubbles depends on their size and compressibility, as well as the frequency and power of the incident ultrasound wave. Imaging of microbubbles at their resonance frequency with medium power can stimulate nonlinear oscillation around their equilibrium dimension, producing harmonic signals that can be separated from the surrounding tissue signal. Microbubble-specific signal can otherwise be produced by destroying the microbubbles with high acoustic powers, thereby releasing highly echogenic free gas. Molecular ultrasound retains the advantages of conventional ultrasound (eg, real-time imaging, portability, and low cost) in addition
to being highly sensitive. The disadvantages include its high degree of operator dependency, limited access to extravascular targets, low microbubble adhesion efficiency, and poor ability to image past bony structures.

Radionuclide Imaging

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) are radionuclide-based techniques commonly used for molecular imaging that differ mainly in the radionuclide used and the mechanism for signal generation and detection. SPECT imaging probes (tracers) are labeled with γ-emitting radionuclides (eg, technetium Tc-99m [\(^{99m}\text{Tc}\]), indium-111 [\(^{111}\text{In}\]), iodine-123 [\(^{123}\text{I}\]), and iodine-131 [\(^{131}\text{I}\])], some of which can be milked from a generator, whereas PET tracers are labeled with positron-emitting radionuclides (eg, oxygen-15 [\(^{15}\text{O}\]), nitrogen-13 [\(^{13}\text{N}\]), carbon-11 [\(^{11}\text{C}\]), and fluorine-18 [\(^{18}\text{F}\)]) that are mainly produced in an on-site cyclotron. The former set of radionuclides produce high-energy gamma rays of different energy levels, which can be detected with a SPECT camera equipped with lead collimators. The latter set of radionuclides produce signal via annihilation events, in which each positron emitted collides with a nearby electron to produce two 511-keV γ-rays 180° apart, which can then be coincidentally detected and localized by a PET scanner. Because both SPECT and PET use high-energy γ-rays as signal, these techniques can be performed in large animals and humans, as well as in small animals. Coregistration of SPECT/PET and CT images acquired sequentially allows anatomic localization of probe activity. The combination of PET imaging with MRI can further enhance anatomic localization by taking advantage of the superb ability of MRI to differentiate soft-tissue boundaries. Currently, PET and SPECT are the most translatable noninvasive molecular imaging platforms because of the availability of existing clinical scanners, the availability of versatile radiochemistry options, and the ongoing development of new imaging agents that only need to be administered in a small, nonpharmacological dose (nanograms) for highly sensitive imaging (SPECT \(10^{-10}\) to \(10^{-11}\) mol/L; PET \(10^{-11}\) to \(10^{-12}\) mol/L). The last merit should greatly facilitate the translation of new radionuclide-based imaging probes into clinical trials, in which toxicity from the trace amount of probe injected is least expected. The disadvantages of radionuclide imaging include low spatial resolution (clinical SPECT 7 to 15 mm; clinical PET 6 to 10 mm; microSPECT 0.5 to 2 mm; microPET 1 to 2 mm), radiation exposure, lack of anatomic information (without CT), and the need for a generator/cyclotron for radionuclide production.

Magnetic Resonance Imaging

MRI detects the net magnetic moment of a collection of nuclei in a strong magnetic field (\(B_0\)) after a radiofrequency pulse. The rates at which the net magnetic moment recovers or decays in the axes parallel and perpendicular to the direction of \(B_0\) are characterized by the longitudinal and transverse relaxation times, \(T_1\) and \(T_2/T_2^*\). Hydrogen-1 (\(\text{H}\)) is the most commonly studied nucleus, but carbon-13, fluorine-19 (\(\text{F}\)), sodium-23 (\(\text{Na}\)), phosphorus-31 (\(\text{P}\)), and others have also been used for imaging. Molecular MRI is performed with targeted contrast agents that can alter either the \(T_1\) or \(T_2/T_2^*\) relaxation of water protons near the target, thereby creating signal contrast. Gadolinium (Gd) chelates and superparamagnetic iron oxide nanoparticles (SPIO; 60 to 150 nm), including the ultrasmall (USPIO; 10 to 40 nm) and micron-sized (MPIO; 0.9 to 8 μm) versions, are the 2 most popular classes of imaging agents that can generate contrast by shortening \(T_1\) and \(T_2/T_2^*\), respectively. The lower intrinsic moment of gadolinium, however, generally requires multiple gadolinium chelates to be linked together via a carrier (eg, nanoparticle or peptide) to improve its sensitivity. Conjugation of gadolinium chelates (or nanoparticles) to antibodies, peptides, or peptidomimetics is needed for molecular targeting. Note that more signal amplification can be accomplished by incorporating multiple gadolinium chelates into antibody-coated nanoparticles or more sophisticated protein/liposome assemblies (eg, recombinant high-density lipoprotein–like nanoparticles or immunomicelles). Alternatively, the MRI signal can be further enhanced and made more specific by the use of novel gadolinium-based contrast agents that oligomerize in response to target enzyme activation. Other exciting contrast agents that are under active development include exchange saturation transfer agents and \(\text{F}\) perfluorocarbon nanoparticles. The latter agents, which confer both positive contrast and high specificity, have been used successfully to label circulating monocytes or macrophages in vivo. The main advantages of MRI include its superb spatial resolution (1.5T, 0.5 to 1.5 mm; ≥3T, 10 to 100 μm), great soft-tissue discrimination, lack of radiation exposure, good clinical translatability, and the ability to combine functional, anatomic, and molecular information. Its disadvantages include its low imaging sensitivity (\(10^{-3}\) to \(10^{-5}\) mol/L), long scan or postprocessing time, and high maintenance cost.

Computed Tomography

CT measures the relative ability of different tissues to attenuate incident x-ray by rotating an x-ray source and a detector around the 3-dimensional volume of a subject. Traditionally, the role of CT in molecular imaging has been limited to providing anatomic roadmaps for functional SPECT or PET scans (eg, SPECT/CT or PET/CT applications), largely because of its low imaging sensitivity (\(10^{-2}\) to \(10^{-3}\) mol/L). However, recent development of an iodinated nanoparticulate contrast agent, N1177, has made it feasible to image cellular activity in vivo (ie, macrophage accumulation in atherosclerotic plaques), beyond just outlining the adjacent anatomic structures. The ability to manufacture polymeric nanoparticulate contrast agents that contain high concentrations of organometallics or radiopaque organically soluble elements has further pushed the limit of imaging sensitivity for CT (\(10^{-9}\) to \(10^{-10}\) mol/L). The main advantages of CT include its high spatial resolution (microCT, 20 to 300 μm; CT, 0.5 to 2 mm), excellent hard-tissue imaging, and short scan time. Its disadvantages include its low imaging sensitivity, limited soft-tissue discrimination, and radiation exposure.

Atherosclerosis

Atherosclerosis, a leading cause of mortality in the developed world, is a systemic inflammatory process characterized by...
PET Imaging of Plaque Inflammation

The predominant form of vulnerable plaque, termed thin-cap fibroatheroma, has been shown by ex vivo histology of human cadavers to have a thin fibrous cap (<65 μm), a large necrotic core (>10% plaque area), a paucity of smooth muscle cells, a lack of thrombus, and heavy infiltration of the fibrous cap by inflammatory cells (ie, macrophages). The initiation of plaque rupture has been linked to macrophages that contribute to the digestion of the fibrous cap by upregulating metalloproteinases. PET imaging of plaque macrophages with 18F-fluorodeoxyglucose (18F-FDG; radiolabeled glucose analog) represents an attractive strategy to study plaque progression because (1) macrophage density is greater in ruptured plaques than stable plaques, (2) macrophages in the antherosclerotic plaque interior use glucose as substrate, and (3) activated macrophages in cell culture express the high levels of glucose transporters and hexokinase needed for 18F-FDG uptake and trapping.

The potential to use 18F-FDG PET to image atherosclerosis was first suggested more than a decade ago on the basis of the observation that deoxyglucose can be significantly trapped by macrophages that reside within a tumor. Subsequently, the feasibility of 18F-FDG plaque imaging was established in animal models of aortic atherosclerosis (eg, rabbits with heritable hyperlipidemia or exposed to balloon denudation plus atherogenic diet), with results that showed up to 3- to 5-fold greater 18F-FDG uptake in atherosclerotic aortas than in normal aortas. The clinical feasibility of 18F-FDG plaque imaging has since been demonstrated in patients with carotid, aortic, vertebral, femoral, and iliac atherosclerosis. Although it has not been fully validated that 18F-FDG reports on plaque macrophage activity, a positive correlation has been observed between 18F-FDG PET activity and macrophage content in human plaque samples. The short-term (1 to 2 weeks) reproducibility of 18F-FDG PET for imaging systemic atherosclerosis is excellent, reportedly better than that of MRI, intravascular ultrasound, and CT angiography for aortic and carotid lesions, with negligible intraobserver and interobserver variabilities (<5%). Plaque inflammation assessed by 18F-FDG imaging after statin therapy (Figure 2) has been noted to precede changes in plaque morphology by ~9 months, which demonstrates the importance of imaging the molecular or cellular changes that often precede morphological change.

The 18F-FDG PET/CT imaging of coronary plaque inflammation has been attempted with preliminary success despite suboptimal image contrast, which results from elevated myocardial 18F-FDG uptake, motion artifact, and partial volume effect due to small vessel size (typically 2 to 6 mm). The first challenge has been addressed by use of an overnight low-carbohydrate, high-fat diet regimen, which can suppress myocardial 18F-FDG uptake by 3.5-fold more than fasting. Ongoing efforts are aimed at reducing motion artifacts by instituting cardiac gating and breath-hold techniques and minimizing partial volume errors by performing postreconstruction corrections with anatomic information obtained from prior MRI scans. Efforts to standardize imaging protocols and data analysis are under way to help improve image quality and data accuracy. Ultimately, large prospective trials to test the power of 18F-FDG at predicting cardiovascular event risks are needed.

The translatability of 18F-FDG PET as a technique to study atherosclerosis in humans is excellent owing to increased use of PET scanners worldwide for routine oncological investigations. Furthermore, 18F-FDG can now be made available to sites without cyclotrons at reasonable costs. With pharmaceutical companies eager to develop treatment strategies to either prevent or stabilize vulnerable plaques, 18F-FDG PET is poised to take on an essential role in the area of clinical atherosclerosis imaging.

MRI of Plaque Inflammation

Imaging of human plaque inflammation has also been achieved with USPIO as a magnetic resonance contrast agent. USPIO is preferred over SPIO because of its longer circulation time in humans (>24 hours), which maximizes probe-
improve the diagnostic accuracy of the USPIO-enhanced contrast signal are under development and should help to erroneous interpretation of plaque USPIO uptake. Newer from motion, tissue voids, or heavy calcification, which leads hypointensity is sometimes masked by signal loss that arises can lead to signal loss in areas of USPIO uptake. This

target interaction. After intravenous delivery, USPIO is predominantly engulfed by macrophages and, to a limited degree, by endothelial or smooth muscle cells within the inflamed plaques. The propensity of USPIO to accumulate in ruptured or rupture-prone plaques (75%) compared with stable plaques (7%) makes it a promising probe for the imaging of plaque vulnerability.52

Early studies involving symptomatic patients undergoing carotid endarterectomy found that up to a 24% change in MRI signal could be observed for plaques 24 hours after USPIO delivery with histological proof of USPIO uptake.52 A subsequent optimization study found an imaging time between 24 and 36 hours after USPIO injection yielded the greatest detectability.53 No target image contrast could be observed after 72 hours because of either USPIO “washout” from the plaques or physiological recycling of endocytosed iron. The fast iron turnover renders repetitive imaging with this technique feasible and potentially useful to monitor treatment efficacy. In fact, a recent prospective human study, ATHEROMA (Atorvastatin Therapy: Effects on Reduction of Macrophage Activity), showed the feasibility of using USPIO-MRI to serially monitor the effect of atorvastatin on plaque inflammation at 6-week intervals (Figure 3).54 Reduction of USPIO uptake was detected as early as 6 weeks after high-dose (80 mg) atorvastatin therapy, months to years earlier than changes in plaque morphology. These findings reaffirmed the advantage of molecular imaging over morphometric changes in plaque morphology. These findings reaffirmed

MRI of Plaque Thrombosis

Plaque erosion represents another process (besides plaque rupture) by which luminal thrombosis can occur. More

commonly seen in premenopausal women and in the coronary circulation, it accounts for ~40% of coronary thrombi in patients who die suddenly of coronary artery atherosclerosis.56 Histologically, plaque erosion is characterized by a luminal thrombus in an area of endothelial denudation, with the underlying intima enriched with smooth muscle cells and proteoglycan matrix but almost devoid of inflammation or calcification.57 Recent studies have shown that the luminal thrombus typically undergoes days to weeks of organization and healing before causing an acute event.57 Therefore, a time window exists during which early detection of thrombosis with imaging can help guide prompt intervention and prevent coronary accidents.

MRI of fibrin-rich thrombus has been demonstrated in swine models of coronary thrombosis by use of EP-2104R (a novel gadolinium-based fibrin-binding peptide manufactured by EPIX Pharmaceuticals), a clinical 1.5T magnetic resonance scanner, and a T1-weighted cardiac-triggered inversion-recovery black-blood gradient echo sequence.58,59 Early validation of this agent was performed by imaging an ex vivo engineered human clot that had been delivered previously into the swine left main coronary artery.59 The fibrin-rich clot used was larger than the size of a typical subclinical microthrombus overlying a vulnerable plaque. Nevertheless, as a proof of principle, high contrast-to-noise ratios (>17) comparing clot to blood pool were demonstrated after systemic delivery of EP-2104R. In a subsequent study, the MRI signal was further proven to correlate with ex vivo thrombus size.26 Afterward, EP-2104R was validated in a phase II clinical trial that involved a subset of patients with thrombi in different vessel territories potentially responsible for stroke (left ventricle, left atrium, thoracic aorta, and carotid artery), with results that showed comparable postcontrast contrast-to-noise ratios (>20).60 More recent data from the phase II trial showed a sensitivity of 84% for detection of known thrombi in the aforementioned vascular territories with this agent.61 As the application of this versatile imaging agent continues to expand (eg, imaging of deep vein thrombosis62), ongoing efforts are aimed at further increasing its imaging sensitivity by optimizing the contrast dose and various imaging parameters. For now, this agent is more suitable for the imaging of advanced large plaque thrombosis...
rather than subclinical microthrombi that overlie eroded plaques.

MRI of thrombus has been otherwise achieved at high fields (9.4T) in mouse models of carotid thrombosis by use of single-chain antibody-conjugated MPIOs (1 μm) targeted to the glycoprotein IIb/IIIa integrin of activated platelets.63 Compared with EP-2104R, this agent reportedly has the ability to detect smaller thrombi because of the high imaging sensitivity associated with MPIOs. The MRI signal achieved with this targeted agent has been found to correlate well with ex vivo thrombus size and response to thrombolytic treatment. The lack of toxicity observed with the use of these MPIOs in animal models is reassuring and should help propel their further testing in humans.

More recently, a novel imaging agent (P975) composed of a single gadolinium moiety attached to a cyclic arginine-glycine-aspartic acid (RGD) peptide has been developed to target activated platelets within thrombi via binding to their α<sub>IIb</sub>β<sub>3</sub> integrin. In mouse models of arachidonic-acid–induced carotid thrombosis, this agent was capable of leading to a 4-fold greater change in contrast-to-noise ratio after contrast injection than sham surgery controls.64 Importantly, P975 was able to bind specifically to α<sub>IIb</sub>β<sub>3</sub> integrin, even though other integrin subtypes (eg, α<sub>v</sub>β<sub>3</sub> on smooth muscle cells) could potentially compete with its binding. Thus far, only nearly occlusive thrombi have been imaged with this agent. It remains to be seen whether this single-gadolinium–containing agent would allow imaging of even smaller thrombi.

To date, MRI of coronary thrombosis has been difficult owing to the small size of coronary vessels and the cardiac/respiratory motion that complicates high-resolution imaging. Nevertheless, recent successful imaging of human coronary plaques with gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA), a nontargeted magnetic resonance contrast agent, is encouraging and should pave the way for further testing of targeted coronary thrombosis imaging agents.65

**Intravascular Near-Infrared Fluorescence Imaging of Plaque Protease Activity**

Recent advances in intravascular ultrasound–virtual histology with radiofrequency analysis have made it possible to accurately (>92%) characterize plaque composition, with the potential to predict risk of plaque rupture.66 The detection of molecular events that precede changes in plaque composition should further accelerate the workup for vulnerable plaques. For example, near-infrared fluorescence imaging of aortic atheroma has been validated extensively in mouse models of atherosclerosis with activatable fluorescent probes.67 These probes have been custom designed to fluoresce in living subjects only on encountering cysteine proteases or metalloproteinases, the upregulation of which has been closely linked to plaque expansion and rupture.68 The clinical translation of these imaging approaches has been propelled by the latest development of a near-infrared fluorescence sensing catheter, which has allowed imaging of macrophage-associated cathepsin B protease activity in rabbit aortic atheroma with a protease-activatable probe.68 Images with target-to-background ratios >6 have been achieved despite a 30% light attenuation by blood. Current efforts are aimed at improving the specificity of the probe for cathepsin B and the sampling capability of the imaging device. Further refinement of this novel intravascular near-infrared fluorescence imaging platform may open the door for early intracoronary detection of vulnerable plaques.

**SPECT Imaging of Apoptosis Within Unstable Plaques**

The apoptosis of smooth muscle cells or macrophages within a plaque has been causally linked to plaque rupture and represents yet another promising imaging target for the detection of vulnerable plaques. Among many cellular or molecular changes that accompany apoptosis, the translocation of phosphatidylserine to the external surface of the cell membrane represents an integral event that precedes many morphological or nuclear changes. For this reason, 99m<sup>Tc</sup>-labeled annexin A5, a plasma protein with high affinity for phosphatidylserine, has been tested in a small pilot study of 4 patients for its ability to detect cellular apoptosis associated with unstable human carotid plaques.69 It is encouraging that after probe delivery, significant 99m<sup>Tc</sup>-annexin A5 uptake was found in the culprit lesions of only patients with a recent, but not remote, history of transient ischemic attack. Ex vivo histological analysis of the culprit lesions after endarterectomy further demonstrated annexin A5 binding to the macrophage membranes only in plaques with unstable characteristics. Therefore, although tested in a limited number of patients, this probe appears to be capable of distinguishing vulnerable from stable plaques. Future prospective outcome studies in a larger group of patients will help determine its true discriminatory potential.

**Other Molecular Imaging Techniques for Evaluation of Vulnerable Plaques**

Other promising plaque imaging techniques include but are not limited to (1) 99m<sup>Tc</sup>-interleukin-2 for imaging activated T cells in human carotid plaques;70 (2) α<sub>IIb</sub>β<sub>3</sub>–integrin–targeting nanoparticles for MRI of plaque-associated angiogenesis;71 (3) micelles that contain gadolinium and oxidation-specific antibodies for MRI of oxidation-rich plaques;72 and (4) 18F-labeled vascular cell adhesion molecule-1–targeting peptide or vascular cell adhesion molecule-1–targeted magnetic nanoparticles for PET/CT or MRI of activated endothelium in inflammatory atherosclerosis. The readers are referred to other excellent reviews for techniques not covered in this review.75–77

**Heart Failure**

Congestive heart failure (CHF) is a devastating condition responsible for 1 in 8.6 deaths in the United States as of 2006 and is expected to incur a cost of $39.2 billion in 2010.78 The astounding cost reflects the difficulty of managing CHF, a disease with complicated pathogenesis. Our current knowledge of CHF points to inflammation, oxidative stress, extracellular matrix remodeling, neurohormonal activation, and myocyte injury/stress as the main contributors. Serum-based biomarkers associated with these processes are currently being investigated for their ability to stratify disease severity, predict mortality, guide therapy, and assess treatment efficacy.79 How-
ever, not all valuable biomarkers are secretory, nor can they be specifically attributed to myocardial processes. The development of molecular imaging techniques to detect and localize myocardial biomarkers with high sensitivity should complement the use of serum-based biomarkers for better management of CHF.

123I-MIBG Imaging of Cardiac Sympathetic Innervation

CHF has been linked to a hyperadrenergic state in which an increased norepinephrine release from the cardiac sympathetic postganglionic nerve terminals causes desensitization of the postsynaptic β-adrenergic receptors, which leads to worsening of left ventricular systolic function.80 A decreased efficiency of norepinephrine reuptake through the presynaptic norepinephrine transporter-1 further contributes to norepinephrine spillover into the bloodstream in CHF patients.81 The clinical significance of such neuropathic derangement is evidenced by the known benefit of β-blockade for reducing mortality. Noninvasive investigation of the myocardial adrenergic postganglionic nerve terminals causes desensitization and sympathetic hyperactivity in CHF.82 If proven to be prognostic, 123I-MIBG planar imaging has been studied extensively. Previous studies have suggested that 123I-MIBG uptake (cutoff late H/M = 1.2) is a better predictor of 2-year event-free survival than plasma norepinephrine, echocardiography, or radionuclide ventriculography for CHF patients (New York Heart Association class II to IV; left ventricular ejection fraction [LVEF] < 45%) with either nonischemic or ischemic cardiomyopathy.86 A recent meta-analysis of 18 123I-MIBG studies (n = 52 to 205 patients each; n = 1755 total patients) has found that either a low late H/M or a high washout rate in patients with CHF predicts high mortality.87 However, the largest study to date (ADMIREF-HF [AdreView Myocardial Imaging for Risk Evaluation in Heart Failure]; phase 3; n = 961; New York Heart Association class II to III; LVEF < 35%) showed that only late H/M is consistently associated with risks for cardiac events in a multivariate analysis.88

A growing area of interest in 123I-MIBG imaging research is to investigate whether such a tool can be used to predict sudden death due to severe arrhythmia, which has been linked to sympathetic hyperactivity in CHF.88 If proven to be prognostic, 123I-MIBG can be used to identify patients who would benefit from placement of an implantable cardioverter defibrillator. Thus far, multiple single-center studies (around 100 or less patients) have shown that 123I-MIBG planar imaging with either an abnormal washout rate (≥ 27%)89 or abnormal late H/M (< 1.95) in combination with plasma brain natriuretic peptide > 187 pg/mL can independently predict sudden cardiac death, with positive and negative predictive values reaching 82% and 94%, respectively.90 Although larger studies are needed to confirm these results, these studies highlight the feasibility of combining 123I-MIBG with other serum markers to augment predictability.
18F-FDG PET Imaging of Myocardial Viability and Glucose Metabolism

A failing heart has been likened to “an engine out of fuel,” such that increasing its efficiency rather than its workload helps to maintain its lifetime.91 In CHF, the heart has an innate ability to increase its metabolic efficiency by switching from predominantly fatty acid metabolism at rest to glucose metabolism, which results in 11% more ATP produced per molecule of oxygen. Such metabolic transformation is also seen in myocardial ischemia, in which the myocardium exposed to limited oxygenation increases anaerobic glycolysis to preserve viability.92 In fact, the clinical use of 18F-FDG PET PET together with myocardial perfusion imaging to identify hibernating myocardium in patients undergoing consideration for coronary revascularization is based on this concept.89 The utility of 18F-FDG PET recently has been expanded to include studying therapies that affect myocardial metabolism, including cardiac resynchronization therapy (CRT). CRT has been shown in numerous trials to improve long-term LVEF and ventricular remodeling by minimizing contractile dyssynchrony, and Glucose Metabolism

18F-FDG uptake–to–blood flow ratio) commonly seen in these patients.93 Because approximately one third of CHF patients do not respond to CRT, this pattern has been used to predict response to CRT. With the receiver operating characteristic area under the curve reaching 0.93, this technique has been shown to be more predictive of positive CRT response than either LVEF (0.66) or QRS duration (0.75).96 In light of the recent multicenter PROSPECT trial, which established by use of a magnetofluorescent nanoparticle (CLIO-Cy5.5),101 The temporal kinetics of macrophage/neutrophil infiltration and their secretion of proteases was later studied noninvasively in a transgenic mouse model of impaired wound healing (FXIII−−) with CLIO-VT750 (a mag- netofluorescent nanoparticle for phagocyte imaging), Prosense-680 (a fluorescence reporter on cathepsin B activity), and a combination of MRI and multispectral fluorescence molecular tomography.102 The lower CLIO-VT750 and Prosense-680 heart signals in these animals compared with wild-type mice after coronary ligation suggested both impaired phagocyte recruitment and protease secretion, both of which could account for the previously observed inability of these mice to repair infarction. This dual-modality imaging technique thus offered new insights into the potential causes of pathological postinfarction remodeling. By enabling close monitoring of myocardial repair and healing, such a powerful imaging tool may one day allow physicians or scientists to devise strategies to ensure more optimized scar formation so as to prevent heart failure.

**MRI of Ischemia-Reperfusion Injury and Postinfarction Repair**

The nature of tissue repair after myocardial ischemia or infarction can significantly influence the chance that further ventricular remodeling will lead to CHF. Understanding the kinetics of molecular or cellular events that accompany infarct repair (eg, cardiomyocyte apoptosis, leukocyte trafficking, and protease activation) through the use of molecular imaging can help facilitate the design of therapeutic strategies to both minimize ventricular remodeling and prevent CHF progression.

The apoptotic pathway plays a major role in the way cardiomyocytes die after myocardial ischemia reperfusion and represents a promising target for therapy. An early attempt to noninvasively study cardiomyocyte apoptosis in patients presenting with acute coronary syndromes used SPECT and 99mTc-labeled annexin.98 The dynamic range of this technique, however, was found to be limited by the high background signal within 12 hours of probe administration, which made early detection of apoptosis after ischemia difficult. Furthermore, this probe does not effectively differentiate apoptosis from necrosis, both of which expose the imaging target (phosphatidylserine) to annexin. Recent development of annexin-labeled magnetofluorescent nanoparticles (AnxCLIO-cy5.5) for high-resolution MRI made it more feasible to image cardiomyocyte apoptosis within 4 to 6 hours of ischemia reperfusion, when apoptosis is most prevalent.99 When used in conjunction with Gd-DTPA-NBD (a gadolinium-based probe for necrosis) in a cleverly designed mouse study, this agent allowed the apoptotic cells to be differentiated from necrotic cells, which further helped to identify a group of apoptotic yet viable cells in the midmyocardium (4 to 6 hours after ischemia reperfusion) that could benefit from antiapoptotic therapy. As demonstrated recently in a transgenic model of chronic heart failure, AnxCLIO-cy5.5 has an impressive ability to detect very low levels (2%) of cardiomyocyte apoptosis.100 The dual-contrast imaging technique with both AnxCLIO-cy5.5 and Gd-DTPA-NBD has great potential for clinical translation and will improve further with ongoing optimization of various imaging parameters (eg, echo time) and contrast agent dose.

Postinfarction myocardial remodeling represents another imaging target of significant interest owing to its central role in the development of ischemic cardiomyopathy. The initial phase of remodeling is triggered by the recruitment of phagocytes, followed by their release of proteases to degrade extracellular matrix in an attempt to repair the infarcted myocardium. The feasibility of imaging macrophage infiltration in the infarcted myocardium with a combination of fluorescence molecular tomography and MRI was initially established from predominantly fatty acid metabolism at rest to glucose metabolism, which results in 11% more ATP produced per molecule of oxygen. Such metabolic transformation is also seen in myocardial ischemia, in which the myocardium exposed to limited oxygenation increases anaerobic glycolysis to preserve viability.92 In fact, the clinical use of 18F-FDG PET recently has been expanded to include studying therapies that affect myocardial metabolism, including cardiac resynchronization therapy (CRT). CRT has been shown in numerous trials to improve long-term LVEF and ventricular remodeling by minimizing contractile dyssynchrony, and Glucose Metabolism

18F-FDG uptake–to–blood flow ratio) commonly seen in these patients.93 Because approximately one third of CHF patients do not respond to CRT, this pattern has been used to predict response to CRT. With the receiver operating characteristic area under the curve reaching 0.93, this technique has been shown to be more predictive of positive CRT response than either LVEF (0.66) or QRS duration (0.75).96 In light of the recent multicenter PROSPECT trial, which established by use of a magnetofluorescent nanoparticle (CLIO-Cy5.5),101 The temporal kinetics of macrophage/neutrophil infiltration and their secretion of proteases was later studied noninvasively in a transgenic mouse model of impaired wound healing (FXIII−−) with CLIO-VT750 (a magnetofluorescent nanoparticle for phagocyte imaging), Prosense-680 (a fluorescence reporter on cathepsin B activity), and a combination of MRI and multispectral fluorescence molecular tomography.102 The lower CLIO-VT750 and Prosense-680 heart signals in these animals compared with wild-type mice after coronary ligation suggested both impaired phagocyte recruitment and protease secretion, both of which could account for the previously observed inability of these mice to repair infarction. This dual-modality imaging technique thus offered new insights into the potential causes of pathological postinfarction remodeling. By enabling close monitoring of myocardial repair and healing, such a powerful imaging tool may one day allow physicians or scientists to devise strategies to ensure more optimized scar formation so as to prevent heart failure.

**Other Molecular Imaging Techniques for Evaluation of CHF Progression**

An impressive repertoire of molecular imaging techniques has been developed for studying heart failure. These novel techniques include (1) 99mTc-labeled Cy5.5-RGD imaging peptide (CRIP) for imaging fibrinogenesis by myofibroblasts during myocardial remodeling, (2) 99mTc-labeled losartan for imaging angiotensin receptor II upregulation after MI, and (3) 99mTc-labeled collagelin, a collagen-targeted pep-
tidoimmetic of the platelet collagen receptor glycoprotein VI, for imaging myocardial fibrosis105; (4) 111In-labeled affinity peptide (111In-DOTA-FXIII) for imaging transglutaminase factor XIII activity in the healing infarct106; and (5) 111In- or 99mTc-labeled radiotracers for imaging metalloproteinase activities in postinfarction remodeling.107 The readers are referred to other outstanding reviews on imaging techniques not covered here.108–110

**Stem Cell Therapy**

MI, an undesirable consequence of atherothrombosis, is associated with a considerable rate of progression to CHF.78 Currently, the only cure for end-stage CHF is heart transplantation, which is limited by organ shortage and high cost. Left ventricular assist devices can help prolong life by several months, but this is only a temporizing measure before heart transplantation. For these reasons, stem cell transplantation has been studied extensively for the past decade as a novel therapy to reverse or minimize myocardial injury, with the goals of improving cardiac function and halting progression from MI to CHF.111,112

The majority of preclinical studies thus far have shown that implantation of various cell types (eg, skeletal myoblasts, bone marrow–derived mononuclear stem cells, mesenchymal stem cells, circulating progenitor cells, embryonic stem cell–derived cardiac or endothelial cells, and cardiac resident stem cells) into ischemic or infarcted myocardium can lead to varying degrees of benefits, including reduced infarct size, reversed ventricular remodeling, and improved left ventricular systolic function.111,112 The exact mechanism by which cells exert their benefits is still being unraveled but generally has been linked to cells secreting growth factors locally (paracrine function) to either stimulate angiogenesis or recruit endogenous stem cells for enhanced cardiac repair, cells acting as mechanical scaffolds to strengthen the mechanically weak myocardium, or stem cells differentiating into cardiomyocytes capable of contracting in synchrony with the host myocardium.111,112

Despite encouraging findings from early animal and initial small-scale human studies, the more recent large-scale, randomized phase II trials have shown inconsistent results, mainly with the use of bone marrow–derived stem cells (BMCs). Two recent meta-analyses of 18 and 10 trials, respectively, on intracoronary infusion of BMCs for the treatment of acute MI have shown only marginal benefits (3% to 4% increase in LVEF).113,114 The reasons for these perplexing, if not disappointing, results are not entirely clear but are gradually being unraveled by the increasing use of imaging tools in preclinical studies and in clinical trials to monitor stem cell behavior.115

**Cell Labeling and Imaging Techniques**

Direct imaging of the behavior of cells after implantation into living subjects can offer great insights into their mechanisms of action, as well as their therapeutic efficacy. Cell imaging requires the use of a cell marker that ideally (1) generates signal only when the marker is associated with the cell while it is viable (imaging specificity), (2) emits adequate signal for detection (imaging sensitivity), (3) minimally perturbs cellular function (cytotoxicity), and (4) causes minimal toxicity to the subject when it (or its metabolite) is released into the circulation during excretion (systemic toxicity).

Cell labeling has been performed by use of 2 general approaches, namely, direct cell labeling and reporter gene/probe labeling (Figure 5).116 The former approach is accomplished by incubating cells with contrast agents that either bind to the cell surface or are imported into the cells via diffusion, endocytosis, or active transport (eg, 18F-FDG, SPIO). The agents may be trapped intracellularly or may leak out of the cell over time. The latter approach is performed by transfecting/transducing cells with a reporter gene, whose protein can be (1) a membrane transporter that actively imports exogenously delivered probes (eg, sodium iodide symporter [NIS]),117 (2) an intracellular enzyme that actively accumulates/interacts with exogenously delivered probes (eg, herpes simplex virus type 1 thymidine kinase [HSV1-TK] or its mutant [HSV1-sr39TK]),118 (3) an intracellular storage protein that actively concentrates endogenous contrast elements (eg, ferritin for concentrating iron),119 or (4) a cell surface receptor that binds to an exogenously delivered probe (eg, dopamine type 2 receptor).120

The main advantage of the reporter gene/probe labeling approach is that the imaging signal becomes specific to only viable implanted cells capable of mediating reporter protein–probe interaction and becomes reflective of cell number if the reporter gene is integrated into the genome and replicates with cell division. In contrast, with direct cell labeling, the contrast agent is diluted over time with cell division and may be engulfed by other cell types (eg, macrophages) on cell death, which leads to the signal either not being reflective of cell number or not being specific to the implanted cells.121,122 The main concerns regarding genetic-based stem cell labeling for human use are potential immunogenicity of some of the reporter genes (eg, HSV1-sr39tk) and random reporter gene integration into cellular chromosomes (eg, lentiviral transfection), which can lead to tumorigenesis. The former issue can either be circumvented by use of endogenous reporters (eg, human ferritin heavy chain) or addressed by use of a human version of the exogenous reporter gene (eg, human mitochondrial thymidine kinase type 2 for HSV1-sr39tk).123 The latter issue necessitates consideration of safe, site-specific transgene integration strategies, which are under development.124 Other barriers to translation of reporter gene technology are related to the need to achieve robust and persistent reporter gene expression for utmost imaging sensitivity without significantly perturbing the functionality (differentiation capacity) of the implanted stem cells. In this regard, the use of a strong endogenous promoter (eg, human ubiquitin promoter) to regulate reporter gene expression can minimize gene silencing due to promoter methylation.125 Enhanced reporter gene expression can be otherwise achieved by use of strategies to enhance promoter activity (eg, 2-step transcriptional amplification126), reporter protein stability (site-directed mutagenesis127), or potentially transcript stability, which needs further exploration. Because of the finite imaging sensitivity of any imaging device, it may be the case that even a lower number of cells than can be imaged must be assayed for clinical management. The development and concomitant use
Figure 5. Cell-labeling strategies for imaging of stem cells. Direct cell labeling: A, FI. Cell incubation with RGD peptide-conjugated quantum dots (QDs) leads to their endocytosis via integrin (α, β) receptors, followed by sequestration in early endosomes (EE). Excitation of endocytosed QDs with a light source (yellow λ) leads to fluorescence emission (pink λ) as cell signal. B, SPECT. Cell incubation with 111In-oxine molecules leads to their passive diffusion into the cytosol, where each molecule dissociates into 111In-In3⁻ and an oxine ion (Ox⁻), both of which can efflux from the cell, although only the former can bind reversibly to intracellular proteins. The γ-ray (γ) emission from intracellular 111In radioisotope is detected as cell signal by SPECT. C, PET. Cell exposure to 18F-FDG molecules leads to their uptake through the glucose transporter type 1 (GLUT1) into the cytosol, where each molecule undergoes phosphorylation by hexokinase and becomes trapped intracellularly. Positron (+) emitted from 18F radioisotope annihilates with a nearby electron (−) to produce oppositely directed γ-rays, which can be detected as cell signal by PET. 18F-FDG uptake mimics that of glucose (Glc), except that the phosphorylated product of glucose, glucose-6-phosphate (Glc-6-P), is further converted to pyruvate (Pyr), which enters the tricarboxylic acid (TCA) cycle. D, MRI. Cell incubation with SPIOs previously complexed with transfection agents (TAs) leads to SPIO uptake via nonspecific endocytosis and subsequent storage in endosomes. The water protons (1H) that surround each SPIO can emit a radiofrequency (RF) wave (gray) as magnetic resonance cell signal if excited with an RF wave (black) in the presence of a static magnetic field (B₀; not shown). Reporter gene/probe labeling: E, FI and BLI. Transcription of the firefly luciferase reporter gene (fluc) under the regulation of a promoter (P), followed by translation of its mRNA, leads to accumulation of firefly luciferase enzyme (FLuc), which catalyzes the oxidation of its substrate, D-luciferin, into oxyluciferin, accompanied by emission of bioluminescent light (yellow-orange λ) as cell signal. Expression of the enhanced green fluorescent protein reporter gene (eGFP) leads to cytosolic retention of enhanced green fluorescent protein (EGFP), which emits fluorescent light (green λ) as signal when excited with a light source (blue λ). F, PET. Transgene expression of a mutant herpes simplex virus type 1 thymidine kinase (HSV-sr39tk) reporter gene leads to the thymidine kinase enzyme (HSV1-sr39TK), which phosphorylates the PET reporter probe 9-(4-18F-fluoro-3-hydroxymethylbutyl)guanine (FHBG) and traps it intracellularly. Radioactive decay of 18F leads to positron (+) emission and subsequent annihilation with a nearby electron (−) to produce 2 oppositely directed γ-rays as cell signal. G, SPECT. Expression of sodium iodide symporter (NIS) reporter gene leads to insertion of sodium iodide symporters into the cell membrane, where they import either 123I⁻ or technetium pertechnetate (99mTcO₄⁻) as reporter probe, along with sodium ion (Na⁺), into the cytosol. Radioactive probes within the cytosol emit γ-rays as cell signal and...
of secretory reporters that can be sensitively assayed by ex vivo nanotechnology-powered diagnostic devices should help ensure long-term monitoring of stem cells after implantation. Lastly, the potential toxicity from high levels of reporter gene expression (or repeated accumulation of the reporter probe) must be assessed carefully by more sophisticated proteomic analyses, as shown previously.128 Adherence to the regulations implemented by the US Food and Drug Administration regarding reporter gene transfection of stem cells, in addition to the safe use of cell stem products and contrast agents, will also be key to the quick translation of reporter gene technology to the clinical setting. As demonstrated in a recent clinical study involving the use of PET to track the homing of cytolytic T cells stably expressing HSV1-TK in a glioblastoma patient undergoing adoptive cellular immunotherapy,129 reporter gene imaging can already be accomplished in the clinical setting; it should only be made easier and safer after all of the above issues are addressed.

**Clinical Imaging of Stem Cell Engraftment**

A variety of cell imaging techniques have been validated in preclinical models, with each having its unique strengths and weaknesses, as reviewed elsewhere.116 However, only the radionuclide-based imaging techniques (PET, SPECT, and γ-camera imaging) with direct cell labeling have been used in human studies (Table 2).130–137 These approaches can be quickly translated into the clinical setting because (1) radionuclide imaging techniques are routinely performed in nuclear medicine; (2) similar cell-labeling/imaging techniques have been used clinically for years to monitor the trafficking of other cell populations (eg,111In-oxine–labeled leukocytes for imaging infection),138 such that both the toxicity profile and the technical limitations are already known; and (3) their high imaging sensitivity allows the tracking of low numbers of cells.

To date, all cardiac-related human stem cell imaging studies have been performed with either BMCs130,131,133,135 or circulating progenitor cells.132,134,136,137 Direct labeling of stem cells has been achieved with 18F-FDG for PET imaging130,132,134,137 and 99mTc-HMPAO131,133,135 or 111In-oxine134,136 for SPECT/planar γ-camera imaging. The cytotoxicity associated with these techniques is generally low (cell viability >90% after labeling).130,131,133,135–137 The labeling efficiency, however, can vary greatly from study to study, even with the same cell marker (<10% to >70% for 18F-FDG).132,137 After intracoronary infusion, all stem cells have been found to engraft poorly (<10% and <5% at 2 and 24 hours, respectively), regardless of the cell type and the number of cells implanted (15 to 4000 million; Figure 6A).130–137 Cell retention has also been shown to correlate inversely with infarct age, presumably because more chromatic cytokine is released during acute MI.136 However, the limited number of subjects in all of these imaging studies has precluded an adequate analysis of therapeutic efficacy. Nevertheless, the systemic toxicity profile of these techniques appears to be excellent, with no reported morbidity/mortality or breach of current radiation safety standards.132 In all cases, intravenous injection of stem cells, regardless of cell type, leads to undetectable myocardial homing.130,132 This does not mean BMCs do not home to the myocardium after intravenous injection, but rather the number of BMCs homing to the heart is below the detection threshold of PET, because the more sensitive BLI approach has been able to illustrate the spatiotemporal kinetics of BMC homing after MI in small-animal models.139

A major limitation of the aforementioned studies concerns the inability to monitor cell viability beyond 4 to 5 days. This duration is technically limited by the half-lives of the common isotopes used (18F, ~110 minutes; 111In, ~2.8 days; and 99mTc, ~6 hours) and biologically limited by the poor cell engraftment and survival. The technical limitation can be circumvented with the use of PET reporter gene/probe cell labeling, which has allowed longitudinal PET imaging of stem cells for at least 10 days in porcine animal models140 and greater than 2 weeks in murine models (Figure 6B).118,125 The biological limitation has been addressed in preclinical studies using molecular imaging tools to study factors that influence stem cell survival (eg, stem cell type,16,141,142 timing of cell delivery after MI,143 route of cell delivery,139 and stem cell immunogenicity144), in addition to stem cell function (eg, differentiation145 and biology (eg, tumorigenicity146)). Effective genetic or pharmacological strategies to promote transplant engraftment and survival have also been developed and validated in small animals.147 With further refinement of these strategies, long-term monitoring of stem cell therapy in humans should be feasible and could help optimize its therapeutic efficacy.

**Imaging of Stem Cell–Mediated Therapeutic Angiogenesis**

The clinical assessment of stem cell therapy has relied on the use of echocardiography, MRI, and nuclear perfusion imaging to assess the physiological consequences (eg, changes in LVEF or myocardial blood flow) of stem cell implantation. However, none of these measurements reflects the impact of stem cells at the molecular level, which may occur long before any noticeable physiological changes. Because a major benefit of stem cell implantation arises from stimulated angiogenesis, a direct assessment of this should yield great insights into the effectiveness of stem cell therapy. Molecular imaging of angiogenesis has been performed with a wide variety of techniques (eg, PET, SPECT, MRI, and ultrasound) and has been well described in other reviews.148,149 Integrin (α,β3) is by far the best-validated target, and its overexpres-

---

Figure 5 (Continued). can slowly efflux from the cell over time. Sodium ions are pumped out of the cell by sodium potassium exchange (Na/K ATPase). H, MRI. Transgene expression of either ferritin heavy chain (FTH) or ferritin light chain (FTL) magnetic resonance reporter gene leads to the assembly of ferritin (FT) proteins, which can individually sequester intracellular iron (Fe), triggering an upregulation of transferrin receptor (TfR) to internalize Fe-bound transferrin (Tf) into endosomes, where Fe is further transferred to ferritin for storage, with TfR recycled to the cell surface. Water protons (H) within the local magnetic field of ferritin-sequestered iron molecules emit radiofrequency (RF) wave (gray) as cell signal when excited by an RF wave (black) in the presence of a static magnetic field (B0; not shown).
sion on activated endothelial cells plays an important role in angiogenesis. The clinical feasibility of imaging myocardial angiogenesis with PET has been demonstrated recently in a patient with subacute MI by use of 18F-Galacto-RGD as an integrin (αv,β3)-targeting probe.\(^\text{150}\) Focal probe uptake reflecting angiogenesis as a natural component of myocardial healing could be visualized clearly in the infarcted myocardium. Angiogenesis has also been assessed noninvasively in porcine models of chronic myocardial ischemia undergoing plasmid-mediated vascular endothelial growth factor (phVEGF\(_{165}\)) therapy with 123I-Gluco-RGD, another αvβ3-targeting PET imaging probe.\(^\text{151}\) Up to 1.7-fold greater probe accumulation could been seen in areas of gene delivery than in saline-injected controls, and this may be further enhanced with stem cell–mediated gene transfer in the future. Besides PET, ultrasound has been successfully used to image endogenous reactive angiogenesis and the effect produced by fibroblast growth factor supplementation in a rat model of chronic hindlimb ischemia. The ability of this technique to detect peak integrin expression 7 to 10 days before maximal blood flow recovery after fibroblast growth factor administration further lends support to the greater sensitivity of molecular imaging over conventional physiological imaging for assessing therapeutic interventions.\(^\text{152}\) With further validation in both animal models and humans, these aforementioned imaging technologies could greatly improve clinical treatment planning by helping to predict the long-term benefits of stem cell therapy on the basis of early cell engraftment or local stimulated angiogenesis.

**Future Outlook and Directions**

An immense repertoire of molecular imaging techniques have been developed for various cardiovascular targets. The translatability of these techniques hinges mainly on whether they have been validated previously for other human applications, whether they require additional platforms for clinical trans-

---

**Table 2. Selected Human Imaging-Based Studies of Stem Cell Engraftment**

<table>
<thead>
<tr>
<th>First Author/Year</th>
<th>Number of Patients</th>
<th>Cell Type</th>
<th>Delivery Method</th>
<th>Timing of Delivery</th>
<th>Total Cells Delivered (10^6)</th>
<th>Cell Imaging Modality</th>
<th>Cell-Labeling Technique</th>
<th>Labeling Efficiency/Viability (%)</th>
<th>Myocardial Cell Retention, %</th>
<th>Notable Study Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hofmann et al(^\text{130}) (2005)</td>
<td>9</td>
<td>BMNC/ BM CD34+</td>
<td>IC, IV</td>
<td>5–10 d post-PCI</td>
<td>2486 ± 654 (US; 5% LB); 1754 ± 835 (100% CD34+ plus 40% CD34*)</td>
<td>PET (^{18}) F-FDG</td>
<td>99/92–96</td>
<td>3 ± 2 (US, IC); UD (US, IV), 26 ± 13 (CD34+, IC) (50–75 min)</td>
<td>CD34+ enrichment ↑ homing, more to infarct border zone</td>
<td></td>
</tr>
<tr>
<td>Karpov et al(^\text{131}) (2005)</td>
<td>44</td>
<td>BMNC</td>
<td>IC</td>
<td>7–21 d post-MI</td>
<td>89 ± 49</td>
<td>SPECT (^{99m}) Tc-HMPAO</td>
<td>NQ/96 ± 4</td>
<td>8 (30 min), 7 (2.5 h), 3 (24 h)</td>
<td>No ↑ LVEF at 6 mo; ↓ TNF-α (d2), ↓ IL-1β (d5), ↑ IGF (d12)</td>
<td></td>
</tr>
<tr>
<td>Kang et al(^\text{132}) (2006)</td>
<td>20</td>
<td>CPC (+ G-CSF)</td>
<td>IC, IV</td>
<td>&lt;13 d/&gt;90 d post-MI</td>
<td>450</td>
<td>PET (^{18}) F-FDG</td>
<td>73 ± 17</td>
<td>2 ± 1 (IC), 1 ± (IV) (2 h)</td>
<td>% Cell retention not different between acute and old infarcts</td>
<td></td>
</tr>
<tr>
<td>Goussetis et al(^\text{133}) (2006)</td>
<td>8</td>
<td>BMNC CD133+ and BMNC CD34+</td>
<td>IC</td>
<td>45 ± 36 mo post-MI</td>
<td>16 ± 5</td>
<td>GCI (^{99m}) Tc-HMPAO</td>
<td>29 ± 5/&gt;95</td>
<td>9 ± 4 (1 h), 7 ± 2 (24 h)</td>
<td>3% ↑ LVEF at 10 mo</td>
<td></td>
</tr>
<tr>
<td>Blocklet et al(^\text{134}) (2006)</td>
<td>6</td>
<td>CPC CD34+</td>
<td>IC</td>
<td>7–21 d post-MI</td>
<td>15 ± 9 (27–54% LB)</td>
<td>PET WB GCI (^{111}) In-Oxine and (^{18}) F-FDG</td>
<td>65 ± 8 and 6 ± 1/NQ</td>
<td>6 ± 1 (1 h), UD/faint (19 h and 43 h)</td>
<td>No cells recirculated to heart by 43 h; limited homing with CD34+ enrichment</td>
<td></td>
</tr>
<tr>
<td>Penicka et al(^\text{135}) (2007)</td>
<td>10</td>
<td>BMNC</td>
<td>IC</td>
<td>3–10 d post-PCI / 2–5 y post-MI</td>
<td>3934 ± 954 (20% LB)</td>
<td>SPECT WB GCI (^{99m}) Tc-HMPAO</td>
<td>90/94–99</td>
<td>3 ± 2 (2 h), 1 ± (20 h) / 2 ± 1 (2 h), UD (20 h)</td>
<td>5% ↑ LVEF at 4 mo for AMI; no cell engraftment at 20 h or ↑ LVEF at 4 mo for CMI</td>
<td></td>
</tr>
<tr>
<td>Schacherer et al(^\text{136}) (2008)</td>
<td>19</td>
<td>CPC</td>
<td>IC</td>
<td>≤14 d/&gt;14 d and &lt;1 y/&gt;1 y post-MI</td>
<td>15 ± 6 (10% LB)</td>
<td>WB GCI (^{111}) In-Oxine</td>
<td>29 ± 12/</td>
<td>7 ± 5 (1 h) 2 ± 1 (3–4 d)</td>
<td>↑ Cell homing to areas of low viability or reduced CFR; cell retention at 24 h inversely correlates with infarct age</td>
<td></td>
</tr>
<tr>
<td>Dedobbeneur et al(^\text{137}) (2009)</td>
<td>12</td>
<td>CPC CD34+</td>
<td>IC</td>
<td>20±2 mo post-MI</td>
<td>23 ± 7 (0% LB)</td>
<td>PET (^{18}) F-FDG</td>
<td>5 ± 1/96 ± 1</td>
<td>3 ± 1 (1 h)</td>
<td>No ↑ LVEF, endothelial function, or neointimal thickening at 3 mo</td>
<td></td>
</tr>
</tbody>
</table>

US indicates unselected; BMNC, bone marrow–derived mononuclear cells; BM, bone marrow; IC, intracoronary; IV, intravenous; PCI, percutaneous coronary intervention; LB, labeled; UD, undetectable; \(^{99m}\) Tc-HMPAO, \(^{99m}\) Tc-hexamethylenepropylene-oxime oxide; NQ, not quantified; TNF, tumor necrosis factor; d2, day 2; d5, day 5; IL, interleukin; IGF, insulin-like growth factor; d12, day 12; CPC, circulating progenitor cells; G-CSF, granulocyte colony-stimulating factor; GCI, γ-camera imaging; WB GCI, whole-body γ-camera imaging; AMI, acute myocardial infarction; CMI, chronic myocardial infarction; CFR, coronary flow reserve; ↑, increased or improved; and ↓, decreased or worsened. All original values have been rounded to full digits.

---
Cao et al. 125 implanted cells (arrowheads) in the anterolateral wall of the LV, FDG fusion (bottom right) images show the location of and myocardial glucose metabolism. Representative transverse middle), and transverse PET/CT (bottom middle) images of 18F-FHBG and 18F-FDG scans 2 weeks later to assess cell viability and proliferation (grayscale for the coronal image; color scale for the transverse images) is clearly visualized in the anteroseptal wall of a patient with 92-day-old anteroseptal wall infarction. Cell accumulation (grayscale for the coronal image; color scale for the transverse images) is clearly visualized in the anteroseptal wall of heart (H; 2% at 4 hours), liver (L), spleen (S), and bone marrow within the skeleton. Images courtesy of Won Jun Kang at Yonsei University, South Korea. 132 B, PET imaging of reporter gene/probe-labeled embryonic stem cells. A mouse underwent intramyocardial injection of mouse embryonic stem cells expressing HSV1-sr39tk PET reporter gene, followed by 18F-FHBG and 18F-FDG scans 2 weeks later to assess cell viability and myocardial glucose metabolism. Representative transverse 18F-FHBG (top right), 18F-FDG (middle right), and 18F-FHBG/18F-FDG fusion (bottom right) images show the location of implanted cells (arrowheads) in the anterolateral wall of the LV, where cells were implanted. Reproduced with permission from Cao et al. 133

Figure 6. Clinical and preclinical PET imaging of stem cell transplantation. A, Human PET imaging of the engraftment of 18F-FDG–labeled circulating progenitor cells after intracoronary infusion. Representative coronal PET (left), transverse PET (top middle), and transverse PET/CT (bottom middle) images of 18F-FDG–labeled circulating progenitor cells 4 hours after intracoronary infusion via the left anterior descending coronary artery in a patient with 92-day-old anteroseptal wall infarction. Cell accumulation (grayscale for the coronal image; color scale for the transverse images) is clearly visualized in the anteroseptal wall of heart (H; 2% at 4 hours), liver (L), spleen (S), and bone marrow within the skeleton. Images courtesy of Won Jun Kang at Yonsei University, South Korea. 132 B, PET imaging of reporter gene/probe-labeled embryonic stem cells. A mouse underwent intramyocardial injection of mouse embryonic stem cells expressing HSV1-sr39tk PET reporter gene, followed by 18F-FHBG and 18F-FDG scans 2 weeks later to assess cell viability and myocardial glucose metabolism. Representative transverse 18F-FHBG (top right), 18F-FDG (middle right), and 18F-FHBG/18F-FDG fusion (bottom right) images show the location of implanted cells (arrowheads) in the anterolateral wall of the LV, where cells were implanted. Reproduced with permission from Cao et al. 133

lation, and whether there are additional safety concerns regarding the use of new imaging probes or other biologics (eg, stem cells and reporter genes). With regard to these issues, radionuclide-based techniques are most ideal for clinical use in the short-term (next ~1 to 3 years), because many of the imaging probes (eg, 18F-FDG) have already been used routinely in clinics and are now finding newer applications in cardiovascular medicine. The requirement for only a trace, nonpharmacological dose of imaging probe, coupled with high imaging sensitivity, further accelerates Food and Drug Administration approval and the translation of newer radionuclide-based imaging techniques into clinical trials. Although PET/CT is now routinely performed in humans, newer applications that focus on detailed anatomies within the heart (eg, coronary plaques) may suffer significantly from partial volume errors due to the relatively poor spatial resolution of PET. A dedicated PET/CT scanner optimized for heart imaging may be the key to ensuring successful imaging of small and sparse molecular imaging targets within the heart. The MRI-based molecular imaging techniques are likely to be successful in the midterm (~3 to 5 years), pending more demonstrations of clinical safety for both gadolinium-based and iron oxide–based compounds, especially after repetitive imaging. As the imaging sensitivity of MRI continues to improve with the optimization of nanoparticle agents, MRI may become the most clinically versatile modality owing to its high spatial resolution and its ability to provide anatomic, physiological, and molecular information, all in one imaging session. Fluorescence imaging will likely take longer for clinical translation (~6 to 8 years) pending full maturation of a clinically feasible technology (eg, intravascular near-infrared fluorescence-sensing catheter for coronary imaging). Although fluorescence molecular tomography cannot currently be applied to humans owing to current detector technology, noninvasive imaging of superficial vessels may still be possible. The large number of fluorescent probes already available for cell imaging will likely make in vivo fluorescence imaging very versatile in the long run. Molecular ultrasound and molecular CT are currently the 2 least-explored molecular imaging modalities and are expected to take the longest for clinical translation. Imaging applications that involve manipulation of biologics (reporter genes) will also take longer to flourish because of the need to ensure clinical safety from these biologics and the challenges in imaging them. Ultimately, each of the above imaging modalities may find a unique role in clinical molecular imaging that best utilizes its strengths.

As the field of molecular imaging moves forward in parallel with the advancement in molecular biology and human genetics, an increasing number of molecular targets will be discovered, many of which will become worthwhile to image noninvasively. It is likely that a high-throughput approach will need to be used to quickly develop and screen for imaging probes with the ideal pharmacodynamic and pharmacokinetic properties, which primarily determine the performance of a molecular imaging technique. In this regard, high-throughput screening of peptides or small scaffold proteins from either 1-bead–1-compound combinatorial libraries or phage/yeast display libraries, in conjunction with a high-stringency screening method, represents a promising strategy to quickly identify a few peptide- or protein-based probes (out of billions) that can interact favorably with the imaging target. 153 The in vivo pharmacokinetics of these selected peptide or protein probes can then be further fine-tuned by modification of the amino acid sequences after repeated testing in preclinical animal models. Such a systematic, highly efficient approach has been demonstrated for the development of peptide ligands targeted to relevant physiological targets, including integrin. 154, 155 Other high-throughput screening techniques have been used to identify nanoparticles with improved uptake by different cell types. 156 Perhaps with a high-throughput manufacturing scheme like this, it will be possible to satisfy the preclinical and clinical needs for imaging a large pool of new molecular targets.

Summary
Significant technological developments in the field of molecular imaging over the past 2 decades have made molecular imaging a clinically feasible tool for the interrogation of important disease-related molecular events in a wide spectrum of clinical conditions ranging from atherosclerosis to advanced heart failure. As demonstrated in this review, molecular imaging has the potential to detect vulnerable plaques before the clinical manifestation of coronary artery...
disease to guide early intervention; assess the severity and prognosis of CHF via sympathetic innervation and glucose metabolism to help identify patient subpopulations who are more suitable for specific treatments (eg, implantable cardioverter defibrillator and CRT); study postischemia cardiomyocyte apoptosis and postinfarction repair so that therapeutic strategies can be designed to prevent ventricular remodeling and heart failure; and monitor novel stem cell therapies to both predict treatment outcomes and guide therapy. As molecular imaging techniques continue to be translated into the clinical setting, future efforts will focus on standardizing existing imaging protocols for particular applications (eg, $^{125}$I-MIBG imaging) to facilitate comparison across studies, developing generalizable platforms by which molecular imaging probes can be either screened or tailored for optimal pharmacodynamics or pharmacokinetics, exploring the combined use of different imaging modalities to utilize the strength of each technique (eg, PET/MRI), and combining molecular imaging assays with highly sensitive serum-based assays to maximize the sampling of relevant disease-specific biomarkers (secretory and nonsecretory) and to improve their sensitivity and predictability. The proper implementation of these measures will help propel molecular imaging to the forefront of clinical cardiology, where diagnostic imaging continues to be crucial for effective day-to-day patient management.

Acknowledgments
We thank Blake Wu, Jim Strommer, and Dr Patricia Nguyen for assistance with manuscript preparation. We also thank Drs Craig Levin and Frezghi Habte for helpful discussions. Due to space limit, we are unable to include all of the important papers relevant to cardiovascular molecular imaging; we apologize to the investigators who have made significant contributions to this field.

Sources of Funding
This work was in part supported by HL093172, HL099117, and EB009689 (Dr Wu).

Disclosures
None.

References


**Key Words:** molecular imaging | atherosclerosis | heart failure | sympathetic innervation | apoptosis | angiogenesis
Cardiovascular Molecular Imaging: Focus on Clinical Translation
Ian Y. Chen and Joseph C. Wu

Circulation. 2011;123:425-443
doi: 10.1161/CIRCULATIONAHA.109.916338

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/123/4/425

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/