Molecular Imaging of Coronary Atherosclerosis and Myocardial Infarction
Considerations for the Bench and Perspectives for the Clinic

Florian Leuschner, Matthias Nahrendorf

Abstract: Motivated by the promise to transform preclinical research and clinical care, cardiovascular molecular imaging has made advances toward targeting coronary atherosclerosis and heart failure. Here, we discuss recent progress in the field, highlight how molecular imaging may facilitate preventive patient care, and review specific challenges associated with coronary and heart failure imaging. Practical considerations stress the potential of fluorescence imaging for basic research and discuss hybrid protocols such as FMT-CT and PET-MRI. (Circ Res. 2011;108:593-606.)

Key Words: molecular imaging | hybrid imaging | atherosclerosis | myocardial infarction | heart failure

Ground-breaking advances in atherosclerosis and heart failure research have improved our understanding of disease etiology and identified effective therapeutic strategies. Many such discoveries have been translated into the clinical setting and have saved millions of lives. One tangible example is the substantially reduced 30-day mortality of myocardial infarction (MI). Despite this progress; however, today cardiovascular medicine faces greater challenges than ever. Heart failure causes 300,000 deaths per year and has an annual cost of $39 billion. Driven by an obesity epidemic and by improved survival rates of patients with acute MI, the number of heart failure patients in the US has risen to a staggering 5.8 million.

New approaches that reverse this trend are thus needed to reduce the prevalence of coronary events and improve therapy for patients with coronary heart disease and MI. We increasingly recognize preventive measures as an effective avenue toward this goal. As succinctly put by Dr Braunwald, “...treating such events is analogous to locking the barn door after the horse has been stolen.” Novel diagnostic solutions that identify individuals at risk could enable early initiation of personalized therapy before irreversible damage occurs. For instance, molecular imaging could identify culprit lesions in the coronary artery tree and initiate treatment to prevent the rupture of the plaque and infarction of the myocardium. Furthermore, imaging has the potential to identify patients with acute MI that are at risk for enhanced remodeling, and to steer yet-to-be-defined therapies that stop this process early before heart failure occurs. Our review focuses on how molecular imaging of the cardiovascular system might contribute to (1) preventing MI by identifying coronary culprit lesions before the ischemic event, and (2) preventing remodeling and left ventricular (LV) dilation in infarct patients. Ultimately, these advances could strengthen preventive care with the goal of reducing the prevalence of heart failure.

Targets in Inflamed Coronary Plaque

X-ray coronary angiography, which has enabled percutaneous coronary intervention (PCI) in patients with acute MI, is an impressive success story. Timely reperfusion therapy results in substantial and sustained survival benefits. With that said, the situation is less clear when it comes to PCI that does not target a culprit lesion in acute MI. Several clinical studies failed to show a survival benefit if significant coronary artery stenoses found on x-ray coronary angiography were treated with the aim to restore perfusion deficits. Taken together with autopsy results, which suggest that the majority of infarcts are triggered by a culprit lesion of less than 50% lumen loss, these studies imply that we possibly treat the “wrong” coronary lesions to prevent ischemic events. Coronary anatomy as assessed by fluoroscopic lumenography is an unreliable criterion for identification of atherosclerotic plaque that is at risk of rupture.

Molecular imaging, unlike anatomic imaging, focusses on the immunobiology hidden behind the endothelium and may therefore be able to identify prospective culprit lesions in coronary arteries. Once we can locate an inflamed plaque at risk of rupture, we may be able to use decisive measures, systemically or locally, to prevent MI. Imaging biology could also better triage treatment: which lesion should be treated, and which should be left alone? The decision to not implant a stent may avoid unnecessary complications and reinterventions. Thus, there is significant need for next-

Original received September 25, 2010; revision received November 24, 2010; accepted November 29, 2010. In October 2010, the average time from submission to first decision for all original research papers submitted to Circulation Research was 13.9 days.
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© 2011 American Heart Association, Inc.
Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/CIRCRESAHA.110.232678
generation imaging strategies that build on the increased knowledge in vascular biology. Molecular imaging of coronary arteries should be able to assess the regional risk that is specific to a lesion, which can then be used in concert with global risk factors to personalize the therapeutic strategy.\textsuperscript{10}

The identification of imaging targets has benefited from increased understanding of the inflammatory nature of atherosclerosis. In recent decades inflammation has been shown to have a key role in initiation, maturation and complication of atherosclerotic plaque.\textsuperscript{11,12} In the following, we discuss selected strategies that either show promise for clinical coronary imaging or are well positioned to enhance preclinical drug discovery.

All imaging modalities, including PET, SPECT, MRI, CT, ultrasound and optical imaging, have been used in animal models of atherosclerosis, primarily in apoE\textsuperscript{−/−} and LDLr\textsuperscript{−/−} mice and in hyperlipidemic rabbits. Typically, animals are kept on a high fat diet to accelerate lesion development. The lack of a widely accepted animal model for vulnerable, rupture-prone plaque has been a hurdle,\textsuperscript{13,14} but inflammatory lesions, which share important hallmarks with human culprit lesions, serve as reasonable surrogates. Criteria include endothelial denudation, high macrophage content, presence of foam cells, a thin fibrous cap, and a lipid or necrotic core, among others (Figure 1).\textsuperscript{15} Because the rodent coronary artery tree is extremely small, the aorta has been used as an alternate vascular target region to model coronary imaging in humans. In mice, the aorta’s diameter is \(1\) mm, which is comparable to small coronaries in humans. For serial imaging studies, it is important to locate the target region previously imaged. Anatomic landmarks, such as the aortic root, can serve as guides. In apoE\textsuperscript{−/−} and LDLr\textsuperscript{−/−} mice the aortic root is particularly suitable because it features high plaque loads and is distant from the liver and the kidneys, organs that excrete imaging agents and therefore often have high background signal.

Atherosclerotic plaque begins with recruitment of circulating leukocytes, predominantly monocytes, to the vessel wall. The recruitment process is driven by chemokines such as MCP-1 and fractalkine, and by adhesion molecules that are expressed by endothelial cells.\textsuperscript{12} Adhesion molecules on endothelial cells bind ligands on the surface of rolling leukocytes. Adhesion molecule expression is limited to activated endothelium that either lines an inflamed plaque, or is located at a site of lesion initiation. Understanding of the leukocyte recruitment mechanism has resulted in particular

\begin{table}[h]
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\begin{tabular}{|l|}
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\textbf{Non-standard Abbreviations and Acronyms} \\
\hline
CCD & charge coupled device \\
CT & x-ray computed tomography \\
Gd & gadolinium \\
FACS & fluorescence-activated cell sorting \\
FDG & fluorodeoxyglucose \\
FMT-CT & fluorescence molecular tomography in conjunction with x-ray computed tomography \\
FXIII & factor XIII (plasma transglutaminase) \\
IVM & intravital microscopy \\
LAD & left anterior descending coronary artery \\
LV & left ventricular \\
MI & myocardial infarction \\
MPO & myeloperoxidase \\
PCI & percutaneous coronary intervention \\
PET & positron emission tomography \\
PS & phosphatidylserine \\
SPECT & single-photon emission computed tomography \\
Sv & Sievert \\
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interest in imaging selectins, integrins, and Vascular Cell Adhesion Molecule-1 (VCAM-1), all of which are in direct contact with the imaging agent circulating in the blood pool. Antibodies and peptides have served as affinity ligands and nuclear (Figure 1), ultrasound and MRI detected the respective reporter moieties.

Inflammatory cells residing in the plaque, in particular monocytes, macrophages, and foam cells, are excellent imaging targets because they play an important role in the evolution and complication of atherosclerosis. After recruitment, these cells scavenge lipids, secrete cytokines that further amplify inflammation, and produce proteases such as metalloproteinases and cathepsins. In concert with the inflammatory cells’ phagocytic function, proteases destabilize the plaque by damaging the extracellular matrix and thinning of the fibrous cap. Targeted imaging of macrophages is based on their phagocytic function. Differently sized and composed nanoparticles are readily taken up by monocytes and macrophages. This mechanism has been exploited using a variety of nanomaterials. Typical labels for MRI detection of these nanoparticles include liposome-encapsulated Gd-DTPA that increases T1 contrast (bright signal) and ultrasmall superparamagnetic iron oxide nanoparticles that enhance T2* contrast (dark signal). Nanoparticles have also been labeled with isotope reporters, including fluorine-18 and copper-64.

Proteases are attractive imaging biomarkers because of their role in plaque destabilization. They have been targeted with two major strategies. Nuclear imaging probes, which could be used in noninvasive coronary imaging in patients, are based on small-molecule protease inhibitors. These bind to the active site of the enzyme and enrich in tissue that has a high protease content. An alternative approach uses an optical prodrug that is activated when it comes into contact with proteases. The enzymes cleave defined peptide sequences, liberate the attached fluorochromes and thereby render them fluorescent. Only these liberated fluorochromes can be excited with a laser, and the resulting emission is recorded as a measure of enzyme activity. The ability of one enzyme to activate several reporter moieties acts as an efficient amplification mechanism. Fluorescence molecular tomography (FMT) has resolved this process noninvasively in mice (Figure 1). Physical hurdles likely preclude using FMT to assess coronary arteries in patients. Instead, clinical fluorescence imaging may be pursued with intravascular catheters, which are inserted into the vascular lumen and sense protease activity in the adjacent vessel wall. Current catheter prototypes are small enough to be used in coronaries and combine white light angiography with two-dimensional fluorescence imaging in the near infrared. Although invasive, this approach may be useful to supplement the anatomic information obtained by x-ray coronary angiography with a biological readout.

Additional molecular targets with promise for imaging atherosclerotic plaques in the coronary artery tree are integrins upregulated in angiogenesis, myeloperoxidase, clotting-related biomarkers such as fibrin and platelets, and lipid components of the plaque.

While the technologies discussed above are currently negotiating the discovery pipeline, 18F-fluorodeoxyglucose (18F-FDG) has been used to image coronary arteries in patients with PET-CT (Figure 1). 18F-FDG is a glucose analog in which an oxygen atom is replaced with 18F, a PET isotope with a half-life of 110 minutes. Because of its uptake in analogy to glucose, 18F-FDG enriches in cells with high metabolic rates. Once inside the cell, 18F-FDG is phosphorylated and becomes trapped. Originally, this imaging technique has been designed to follow metabolically active tumor cells. Although it has been very successful, an emerging limitation for oncological imaging is that 18F-FDG is not specific to cancer cells. Recruitment of metabolically active host leukocytes to other sites of local infection and/or inflammation may also increase 18F-FDG signal. Likewise, inflammatory atherosclerotic plaques are metabolically active tissue, and therefore show high signal on PET images. Several studies have correlated the 18F-FDG signal to the number of macrophages in a lesion; however, 18F-FDG is not specific to these cells either. The clinical availability of 18F-FDG PET creates an opportunity to overcome the considerable technical difficulties of imaging a small, fast moving target in coronary arteries with this modality.

### Noninvasive Coronary Molecular Imaging: What Modality, When, and For Whom?

There are specific hurdles associated with each modality for imaging atherosclerosis in coronary arteries (Table). The plaques in these vessels are small, move rapidly with the heart and respiration, and are close to tissue interfaces with lung, blood and myocardium, all of which can lead to artifacts or background signal. In addition to its high spatial and temporal resolution for anatomic imaging, MRI shows promise for molecular imaging in the coronary arteries, despite its somewhat lower sensitivity in comparison to PET. High sensitivity translates into low agent doses, which in turn reduces toxicity concerns. Although PET is the most sensitive and quantitative modality, it has a limited spatial resolution (~4 mm clinically, ~1 mm in small animal imaging). Unfortunately,

### Table. Advantages and Disadvantages of Modalities for Molecular Imaging of the Heart

<table>
<thead>
<tr>
<th>Modalities</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Optical</td>
<td>High resolution, may aid intervention, multispectral</td>
<td>Invasive (catheter-based)</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>High spatial resolution, real-time imaging, inexpensive, widely available</td>
<td>Interobserver variability, limitation with adjacent bone or lung structures, not quantitative</td>
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<tr>
<td>CT</td>
<td>High spatial resolution</td>
<td>Lower sensitivity, radiation exposure</td>
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<tr>
<td>Nuclear</td>
<td>Highest sensitivity, quantitative</td>
<td>Low spatial resolution, exposure to radioactivity, expensive</td>
</tr>
<tr>
<td>MRI</td>
<td>High sensitivity, high spatial and temporal resolution, functional imaging possible</td>
<td>Expensive, metallic implants (currently most pace makers and defibrillators are contraindication)</td>
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physical constraints make it unlikely that PET’s spatial resolution will improve further. A positron travels a considerable distance from emission by the isotope—the location of the probe—until annihilation during collision with an electron creates the gamma ray detected by the PET ring—the location of imaging signal. Partial compensation for limited spatial information can be achieved through combination with an anatomic modality. There have also been reports of CT agents that target vascular inflammation.46 The excellent spatial resolution and the recently discussed potential of multispectral CT imaging49 make this modality attractive, even though it is less sensitive to molecular markers than PET or MRI. It should be noted that coronary CT can cause considerable exposure to radiation (3 to 16 mSv).50 Newer devices can reduce the overall exposure from PET-CT to <10 mSv51; however, this is still considerable exposure that precludes uncritical use for screening in lower-risk individuals. For comparison, the exposure caused by a chest x-ray is 0.1 mSv, a sestamibi stress test is 9 mSv, and a coronary catheterization is 7 mSv.50

Once molecular imaging tools that assess coronary atherosclerosis become available for use in patients, we will need to analyze their clinical value, including considerations on radiation exposure mentioned above. Clinical trials will investigate how a specific imaging tool improves decision making and outcome, and assess cost-benefit ratios. Routine imaging should only be adopted after these data have been collected and appropriate patient selection procedure has been determined. A recent editorial proposes a possible scenario for integrating coronary imaging into clinical care to improve risk assessment and guide therapy.4 Global risk assessment using tools such as the Framingham risk score should precede imaging, resulting in low, intermediate and high risk cohorts. The latter comprises one-sixth of the adult population, who have a >2% chance of an event within the next year. Within this population, it could be beneficial to identify individuals at very high risk and regionalize risk by identifying specific vulnerable coronary plaques. These patients may potentially benefit from intense global risk factor reduction, medical therapy aiming at cooling down of inflamed coronary lesions,15 and local therapy such as implantation of drug eluting stents52 or preventive coronary bypass surgery.4 Because imaging technology targeting vulnerable plaque is a fairly recent development, our knowledge on the fate of vulnerable plaques is still limited. This renders considerations on therapy, although very important, also extremely speculative. For instance, there is a paucity of prospective clinical studies that investigate the fate of individual vulnerable plaques over time. An important aspect is the number of vulnerable plaques in an individual patient. If vulnerable plaques are predominantly short lived, often resolve spontaneously, are numerous in any one patient, or only infrequently result in infarction, then local therapy may not be the most practical approach. Autopsies53 and intravascular ultrasound studies54 suggest occurrence of more than one and up to 5 vulnerable lesions at a given time.

**Imaging Targets in Heart Failure**

Even when the considerations above may turn into clinical reality, it is unlikely that they achieve complete prevention of MI. Thus, for patients with acute MI, we need additional tools to identify individuals that will undergo accelerated remodeling and have a high risk of developing heart failure. Anatomic imaging is already an integral element of the care provided after MI.55 Patients undergo serial echocardiography, and MR and nuclear imaging are used to measure function, perfusion and infarct size. The prognostic value of infarct size is widely recognized. Although mortality has been the cornerstone of drug efficacy trials, imaging is increasingly used to follow surrogate end points related to LV volumes and function. However, many of these parameters change late in the course of disease. To enable preventive measures we will need upstream biomarkers that allow us to intervene before disease has progressed beyond the point of no return. In the following, we will discuss selected molecular imaging techniques in the sequence of disease progression, starting with the initial injury.

Within hours of ischemia onset, myocytes begin to die from necrosis and apoptosis (Figure 2). Although delayed enhancement MRI after injection of Gd-DTPA yields an excellent high-resolution image of myocyte loss and infarct size, it cannot differentiate between necrosis and apoptosis. Apoptosis is a reversible process, and thus a therapeutic target, whereas necrosis is not. A number of apoptosis imaging approaches have been proposed, most of which involve the use of annexin V as an affinity ligand.56–59 Annexin V binds to phosphatidylserine (PS), a molecule that flips to the outside of the cell membrane. An elegant approach proposed by Sosnovik et al combined MRI of apoptosis with an annexin V–decorated nanoparticle and delayed enhancement MRI in a mouse model of cardiac ischemia (Figure 3).60 Here, identifying necrotic versus apoptotic myocardium was possible because T1-weighted delayed enhancement after injection of Gd-DTPA enhanced the entire infarct, whereas T2* signal changes attributable to nanoparticle binding reported on apoptotic myocardium only. MR agents that bind to exposed DNA are also being developed.61 Apoptotic myocytes have been imaged in patients with chronic heart failure by SPECT after injection of technetium-99m-labeled annexin V.52

Injury to the myocardium triggers an inflammatory response which initiates the wound healing process.63 An initial neutrophil surge is followed by monocytes, which are recruited in large numbers from their splenic reservoir64 and the bone marrow. These cells are essential for proper repair (Figure 2), and their either insufficient or excessive presence in the infarct may impair wound healing, increase infarct expansion, and consequently enhance LV dilation.65 Two monocyte subsets are recruited to the heart sequentially.66 In the mouse, inflammatory Ly6-C<sup>high</sup> monocytes dominate on days 1 to 3 and promote removal of debris. Thereafter, Ly6-C<sup>low</sup> monocytes promote tissue repair and resolution of inflammation.66 A similar biphasic pattern, with an initial peak of inflammatory CD16<sup>+</sup> monocytes, was also reported for blood monocyte levels in patients with acute MI.67 Monocytes' central role in infarct healing65 make these cells interesting imaging targets. Imaging may detect excessive or prolonged inflammation after MI, and so identify individuals at risk for progressive remodeling. This hypothesis has been tested in preclinical studies, in which monocyte/macrophage
imaging was combined with serial MR volumetry. Mice with increased monocyte/macrophage signal on day 5 after coronary ligation had worse ejection fraction on day 21, despite similar initial infarct size. In patients, monocyte/macrophage content in infarcts has not yet been directly imaged, however, blood levels of the inflammatory CD14+CD16− subset were inversely correlated with MRI-derived ejection fraction 6 months after MI. Imaging tools that noninvasively report on these cells’ presence could serially follow the myeloid cell flux from the bone marrow and spleen toward the injured heart and thus enhance understanding of system kinetics. This, in turn, will help to identify how monocyte/macrophage responses can be modulated therapeutically.

T2*-weighted MRI can follow uptake of iron oxide nanoparticles by macrophages, an attractive approach because MRI also reports on wall motion and global heart function (Figures 2 and 4). Fluorescent labeling of nanoparticles has precisely determined nanoparticles’ cellular uptake profile by microscopy and fluorescence-activated cell sorting (FACS). After digestion of infarct tissue, the cell suspension retrieved...
from infarcts was stained with a cocktail of fluorescently labeled antibodies to identify cells of interest by their specific surface marker expression. In a separate channel of the flow cytometer, the fluorescence intensity of the nanoparticle fluorochrome label in each cell type (ie, the cellular uptake of the probe) was then quantified. With certain nanoparticle preparations, more than 80% of the signal is derived from monocytes/macrophages. Timing of the imaging (ie, day after coronary ligation, injection-imaging sequence) may influence the uptake profile.

Macrophage presence in the infarct has also been imaged by MRI after injection of 19F-labeled liposomes (Figure 4). 19F MRI does not rely on protons, which are abundant in the body as part of water, as a signal source. Unlike Gd- or iron oxide–based MR agents, which indirectly modify signal by accelerating proton relaxation, 19F is imaged directly. Because it does not naturally occur in the body, the background signal in this imaging technique is very low, as only 19F incorporated in the imaging agent is detected. Typically, 19F is combined with 1H MRI for anatomic coregistration (Figure 4). On the downside, 19F MRI lacks the typical amplification mechanism of Gd or iron oxide, in which one moiety of agent interacts with many surrounding protons. Thus, the sensitivity of 19F MRI could be lower than that of 1H MRI.

Comparable to the strategy of 18F-FDG PET imaging of inflammatory atherosclerosis, French et al have followed inflammation in a mouse after myocardial ischemia with 18F-FDG (Figure 4). The high background uptake of the myocardium is a hindrance in cases where the ischemic area...
is not well defined, especially because ischemia shifts myocardial metabolism toward increased glucose utilization.\textsuperscript{73} Nanoparticles labeled with PET isotopes such as \textsuperscript{64}Cu are not taken up by myocytes and may thus be a suitable tool to quantify post-MI inflammation.\textsuperscript{26} Current efforts aim at developing clinically viable nanoparticle PET reporters.\textsuperscript{25,74}

Ultrasound imaging is a less quantitative but very cost-efficient modality and has been used for macrophage imaging with lipid microbubbles (Figure 4). These microbubbles are retained in phagocytes in the myocardium\textsuperscript{75} and may thus facilitate bedside estimation of inflammatory macrophage content in an infarct of a patient.

Monocytes/macrophages synthesize and secrete a wealth of cytokines and enzymes, including proteases. In acute MI, matrix metalloproteinases and cathepsins participate actively in the wound healing process.\textsuperscript{76} They digest the preexisting extracellular matrix and so enable the generation of granulation tissue and formation of a stable scar. Their function is delicately regulated between protease generation, activation and inhibition by TIMPs (Tissue Inhibitor of Metalloproteinases).\textsuperscript{77} If the balance of matrix degradation and synthesis is disturbed, infarct healing is impaired, and this may result in ventricular rupture or infarct expansion. In later stages of LV remodeling, these enzymes also attack the extracellular matrix in the remote, noninfarcted myocardium and promote ventricular dilation.\textsuperscript{78} The same molecular agents used for imaging in inflamed atherosclerotic lesions, as described above, have also been used to characterize proteases in MI by \textit{SPECT}\textsuperscript{79} and fluorescence imaging (Figure 2).\textsuperscript{60,70,80} These studies reported increased activity after MI, which, if too high, may counteract matrix generation, favor infarct expansion and thus contribute to ventricular dilation. A typical example of how increased protease activity worsens outcome can be seen in a study that investigated infarct healing in apoE\textsuperscript{-/-} mice.\textsuperscript{68} Because of their atherosclerotic “comorbidity,” which implies higher monocyte blood levels, apoE\textsuperscript{-/-} mice show increased and prolonged monocyte recruitment to the heart, increased protease activity, prolonged infarct inflammation, and accelerated LV dilation. Myeloperoxidase (MPO), which also serves as a biomarker for inflammatory monocytes and neutrophils in ex vivo clinical pathology, is an inflammatory enzyme that has been imaged after MI with the activatable T1-targeted MRI agent MPO-Gd (Figure 2).\textsuperscript{81} In addition, MPO-Gd enhances rejecting heart transplants.\textsuperscript{82} The probe is based on a Gd-encaging chelator derivatized with serotonin moieties. If in contact with the target enzyme, the serotonin residues are radicalized and react with each other and the surrounding matrix, which leads to delayed wash out kinetics and higher signal intensity on T1-weighted MRI.\textsuperscript{83} Although MPO content is even higher in neutrophils,\textsuperscript{82} this approach can, with the right timing, be used to follow inflammatory monocyte levels in the infarct. A fluorescent nanoparticle-based sensor for peroxynitrite (ONOO\textsuperscript{-}) and myeloperoxidase mediated hypochlorous acid (HOCl/OCl\textsuperscript{-}) production has been used to sense inflammation in infarct tissue,\textsuperscript{84} however this approach has not yet been evaluated for in vivo imaging.

The extracellular matrix is a tissue component that preserves the structure of the heart. A delicate network of collagen fibers, linked to each other and to cells via integrins, supports the LV geometry. Because it weakens this network, increased protease activity favors ventricular dilation.\textsuperscript{79} On the other hand, overabundant collagen is also harmful because it hinders diastolic function. In addition, the extracellular matrix lends stability to the infarct scar, which prevents rupture and/or infarct expansion. Several imaging approaches directly target components of the extracellular matrix (Figure 2). A technetium-99m-labeled RGD peptide was used to image \(\alpha_1\beta_1\) integrin expression in mice after MI.\textsuperscript{85} The highest uptake was observed 2 weeks after coronary ligation in the infarct, whereas the signal was higher in the remote myocardium at 12 weeks. The signal was colocalized with myofibroblasts, which are important producers of collagen. This study detected the effects of pharmacological inhibition of angiotensin-2, a peptide with known profibrotic activity. These data suggest that it is possible to image active construction of the extracellular matrix, as integrin expression declines in a mature scar. The same agent was then used in 10 patients with MI in conjunction with delayed enhancement \textit{MRI}.\textsuperscript{86} In this first-in-human study, integrin SPECT signal at 3 and 8 weeks colocalized with the infarct scar on follow-up \textit{MRI} 1 year after the ischemic event. The findings on SPECT-positive area as well as signal intensity were heterogeneous. Future clinical studies will explore the predictive value of these data. A similar peptide, labeled with the PET isotope \(^{18}\text{F}\) (\(^{18}\text{F}-\text{Galakto-RGD}\)), has been used in a patient 2 weeks after MI.\textsuperscript{87} The authors describe that the likely target is integrin expression attributable to angiogenesis in the healing infarct, based on a study in rats after coronary ligation.\textsuperscript{88} A wealth of publications describe the use of the RGD peptide to target angiogenesis in the setting of atherosclerosis\textsuperscript{89} and cancer.\textsuperscript{90} In addition, integrins are also expressed by leukocytes, which are abundant in the wound shortly after MI. Thus, although RGD-based probes have high affinity to integrin, imaging timing is essential because the manifold cellular source of integrin expression varies as disease progresses.

Once collagen fibers are synthesized, they are cross-linked into an interconnected matrix. Tissue and plasma transglutaminases (FXIII) are involved in this process. FXIII\textsuperscript{-/-} mice showed an increased ventricular rupture rate after coronary ligation.\textsuperscript{91} The role of FXIII in infarct healing was also suggested by 2 clinical observations: patients who died because of infarct rupture had lower FXIII protein content in the infarct when compared to a control cohort.\textsuperscript{92} Furthermore, a study that analyzed the FXIII genotype and followed acute MI patients over time suggested a protective effect of a FXIII-A-34L mutation associated with increased FXIII activity.\textsuperscript{93} A FXIII targeted imaging probe, which is based on a transglutaminase substrate peptide labeled with Indium-111, was used to study infarct healing in mice after MI (Figure 2). Decreased FXIII SPECT signal in the infarct associated with accelerated LV dilation, suggesting that this probe could be used to predict prognosis.\textsuperscript{94} Collagen was also imaged directly in rats 6 weeks after coronary ligation.\textsuperscript{94} A Gd-based T1 shortening probe that relies on a cyclic peptide as an affinity ligand to collagen\textsuperscript{95} caused strong enhancement and delayed wash out of the agent from the infarct scar (Figure 2).
Other imaging strategies used to investigate heart failure include imaging of therapeutic targets (angiotensin-2 receptors, β-receptors), sympathetic innervation, and imbalances in myocardial metabolism (we refer to a recent comprehensive review by Peterson and Gropler).

“How-To” Considerations for Bench Research
Anatomic and molecular imaging are attractive research tools because their noninvasive character enables serial studies. In the laboratory, a molecular imaging biomarker often represents a molecule or cell that is central to system regulation and is thus a possible therapeutic target. A noninvasive tool that can quantitate a regulatory molecule or cell would allow its serial investigation, for instance before and after a therapeutic intervention. Imaging may quantify such a target, and because the animal survives, one can later obtain ejection fraction in the same individual to study impact on outcome and prognostic value.

The investigation of biology in the undisturbed in vivo environment prevents many artifacts introduced by in vitro techniques. Some dynamic phenomena, such as cell-to-cell interactions, are only accessible via in vivo imaging; cell-to-cell interactions, to continue with the specific example, require in vivo microscopy. The advantages of in vivo methodologies have resulted in an increased interest in imaging to supplement invasive and ex vivo techniques in basic science.

Some imaging techniques, such as echocardiography in mice, have already been embraced by a large research community. The example of echocardiography shows what is required to broadly disseminate imaging: the tool must be straightforward, capable of high throughput, and cost effective. Unfortunately, more technology-intensive modalities like MRI and PET do not meet these criteria. These scanners are expensive to purchase, and their operation and maintenance requires expertise and sustained financial commitment. The higher costs of equipment and required on-site expertise will likely limit widespread installation of these devices. One possible solution is the creation of centralized facilities that make their services available to outside collaborators. Supported by specific grant mechanisms from the National Cancer Institute, this strategy has been successfully implemented for cancer imaging. Dedicated cardiovascular imaging centers would likely advance the use of molecular imaging tools in basic cardiovascular research, share the financial burden, and make these technologies more accessible to the community.

Wider dissemination of a modality beyond imaging centers requires “push button” ease of use, limited costs, and seamless integration with traditional laboratory techniques. Optical imaging has matured to fulfill these requirements and is therefore attractive for scientists without extensive expertise in imaging. In comparison to other modalities, optical imaging equipment is less expensive to buy and to maintain, because it does not require radiation permits, shielding, or cryogen fills. Optical imaging agents are harmless and nontoxic, do not decay, and can be stored on the shelf. As shown in Figure 5, optical imaging is highly versatile and spans applications with high spatial resolution, such as intravital microscopy, to applications with high sensitivity of noninvasively probing molecular targets, as with bioluminescence and FMT.

A decisive advantage of fluorescence imaging is its capacity to integrate well with traditional laboratory techniques. For instance, microscopy and FACS can measure fluorescent molecular agents following in vivo imaging (Figure 5). The multichannel capabilities of optical imaging, which are already widely exploited in multicolor flow cytometry or immunofluorescence histology, are increasingly used in vivo to interrogate several targets simultaneously. This allows investigators to pursue systems approaches not limited to a single biomarker. Examples for multichannel imaging are shown for intravital microscopy and FMT (Figure 5).

Role of Multimodal Imaging: Preclinical
Imaging modalities are complementary. Nuclear imaging has a limited spatial resolution, CT has low sensitivity to molecular targets, MRI is often semiquantitative, and optical imaging cannot penetrate more than ~5 to 8 cm of tissue. Combining modalities can compensate for some of these limitations while building on their respective strengths (Table). This is particularly important in the heart, a challenging imaging target with fast moving, small structures. A modality with high resolution and good soft-tissue contrast can provide anatomic structures, whereas a second, more sensitive modality may sample molecular information.

An example of how advantages can be gained from fusing modalities is the combination of x-ray CT with FMT. Like PET imaging, FMT is quantitative and sensitive but provides limited anatomic information. Stand-alone FMT imaging is sufficient in large targets such as subcutaneously implanted tumors. Vascular and myocardial FMT, however, benefit from adding a modality that provides precise anatomic information. Two strategies have been proposed. An integrated FMT-CT device that combines laser, CCD cameras and X-ray source on one gantry facilitates the use of CT priors for improved image reconstruction of FMT data sets. An alternative approach uses separate devices. Here, the animal is transferred from the FMT imager to the CT or MRI scanner and image fusion is based on fiducials incorporated in an imaging cassette holding the anesthetized mouse. The advantage of the second approach lies in its versatility. It allows integration of any modality (FMT with PET, CT, MRI, or multiple combinations). Spectrally resolved FMT provides several channels for simultaneous quantification of molecular markers. In the commercially available system, the excitation/emission wavelengths are 635/655, 680/700, 750/780, and 785/815 nm. Theoretically, PET and MRI could be added to yield a total of 6 channels. The most complex study to date combined 3 FMT channels with PET and CT and indicates that hybrid imaging of multiple targets is feasible. For instance, an infarct imaging study could use both the commercially available FMT system and probes, to quantify myocyte apoptosis, monocyte/macrophage numbers, protease activity, and integrin expression, and MRI, to measure collagen content, in one single study. These parameters could then be followed over time, and correlated to LV function and infarct size. Such a multimodal protocol would...
provide insight into the generation of heart failure on a systems level by following a network of interdependent biomarkers. The use of quantum dots as fluorescence reporters may add even more channels, because these materials’ emission profiles are narrow. Another interesting development is the quantitation of near infrared reporter proteins by FMT, which could be used to investigate gene expression or stem cell survival in the cardiovascular system in concert with the biomarkers noted above.

**Role of Multimodal Imaging: Clinical**

Multimodal scanners combine 2 or more modalities and fuse the resulting images, as, for instance, in PET-CT imaging of coronary atherosclerotic plaque (Figure 6A). Here, the limited spatial information derived from PET is overcome by the addition of anatomic CT, which helps to localize PET signal (but does not necessarily improve PET spatial resolution or solve the issue of partial volume effects). Although the integration of PET with MRI is particularly promising for imaging the heart, the engineering challenges are higher than in PET-CT because of the required nonmagnetic MRI environment. Experimental systems that feature a PET ring inside the magnet allow simultaneous acquisition in both modalities. This integration represents a break-through for functional brain imaging, in which simultaneous MRI and...
PET (e.g., fMRI for blood oxygenation in combination with disease-targeted PET probes) can provide novel insight. Unlike in multimodal functional brain imaging, the time component may be less important in cardiovascular imaging. Here, MRI and PET could be done consecutively as in PET-CT, somewhat lowering the technological hurdles because the PET ring would not have to reside inside the magnet. There are a number of arguments for the particular synergy of cardiovascular PET-MRI. First, using MRI instead of CT reduces radiation exposure, which is of paramount importance for the preventive approaches discussed in this review. Second, cardiovascular MRI is highly versatile and very powerful. It can identify precise anatomic structures and physiological parameters (including coronary anatomy, cardiac volumes, regional wall motion, global heart function, infarct size, perfusion, and myocardial strain) with excellent temporal resolution. A possible clinical scenario for coronary PET-MRI is the detection of plaque inflammation by PET (with 18F-FDG now, and in the near future with agents such as 18F-RGD, the VCAM-1 targeted probe 18F-4V17 or macrophage targeted nanomaterials 26). The molecular information can then be fused with MR coronary angiography, which reports the degree of stenosis and composition of the plaque. In addition, MRI can quantify downstream physiological effects such as perfusion (using first pass or spin labeling techniques) and wall motion (using cine or phase contrast), and identify an infarct scar (using delayed enhancement). Similar scenarios can be envisioned for patients after MI: coronary status, infarct size, and LV function are determined by MRI, and a wound-healing biomarker that predicts remodeling is tracked by PET. Figure 6B shows an example of multimodal imaging in heart failure. Wound healing was imaged with an integrin-targeting nuclear tracer, whereas anatomy was followed by MRI. This study did not combine both modalities in one session, but nevertheless illustrates the clinical potential of multimodal imaging.

Clinical Promise
The need for spatially resolved, patient-individualized risk assessment is driving the molecular imaging field toward clinical translation. The near future will likely see several molecular agents entering the clinical phase of development. The track record of nuclear tracers that are in clinical use, many of them in cancer imaging, positions PET as a modality that, in conjunction with CT and MRI, will assess inflamed coronary plaque and molecular events in remodeling and heart failure. A number of nuclear imaging agents have already been used in patients with heart disease, including agents targeting integrin expression, apoptosis and metabolic activity (18F-FDG). The promise of nuclear imaging lies in its high sensitivity and the design aspects of PET tracers. These often consist of a peptide and an isotope, whereas MR agents are more complex because of the need for signal amplification. However, the appeal of imaging techniques that do not cause radiation exposure and feature good spatial resolution favors MR, ultrasound and optical imaging. Cardiovascular MRI, in particular, is undergoing rapid technical advances that will increase acquisition speed, signal to noise ratio and sensitivity.

Will we be able to afford clinical molecular imaging? The increasing number of imaging procedures contribute to exploding costs in health care. Nevertheless, integrating molecular imaging into clinical decision-making with the goal of disease prevention should be cost effective. A procedure detecting a vulnerable plaque could result in intervention that prevents deleterious, and costly, events. Care for a patient with acute MI or heart failure includes admission to the hospital, coronary catheterization, possibly an implantable defibrillator, and perhaps even heart transplantation, all of which outweigh the cost of imaging by several orders of magnitude.

Conclusion
Motivated by the potential to transform preclinical research and clinical care, cardiovascular molecular imaging has made advances toward targeting coronary atherosclerosis and heart failure. Technology-driven proof of principle studies are abundant, have reached key technology milestones (Figure 7), and illustrate the promise of molecular imaging. In the next decade, our rapidly growing knowledge in cardiovascular biology promises identification of advanced targets, which will likely represent central check points of pathophysiological systems (Figure 7).
Basic research is increasingly integrating imaging data, although optical techniques hold particular promise for dissemination into nonimaging laboratories. The synergy created by multimodal imaging, in particular PET-MRI, will unite molecular and physiological information. $^{18}$F-FDG PET-CT is a clinically available tool currently being explored for coronary imaging. Imaging will become an integral part of clinical trials, in which imaging biomarkers may serve as surrogates for clinical translation still exist. To overcome these, the cardiovascular community can learn from the cancer field, which is quickly adopting imaging for both bench work and clinical translation. Dedicated grants have funded large imaging centers that combine expertise across medical physics, cancer biology, and probe chemistry. Basic scientists without extensive imaging expertise profit from this infrastructure, using high-end imaging tools on a collaborative base. Similar efforts in cardiovascular science could replicate this success.

Acknowledgments
We gratefully acknowledge Drs Juhani Knuutti, John Chen, and Claudio Vinegoni for providing previously unpublished image material. We acknowledge Drs Filip K. Swirski, Mikael J. Pittet, David E. Sonnokov, and Ralph Weissleder for fruitful discussions.

Sources of Funding
This work was supported, in part, by NIH grants R01HL095629, R01HL096576, R01EB006432, HHSN26820100004C; American Heart Association Grant SDG0835623D; and the Deutsche Herzstiftung e. V.

Disclosures
None.

References

Leuschner and Nahrendorf Molecular Imaging of Coronary Atherosclerosis and MI 603


Molecular Imaging of Coronary Atherosclerosis and Myocardial Infarction: Considerations for the Bench and Perspectives for the Clinic
Florian Leuschner and Matthias Nahrendorf

Circ Res. 2011;108:593-606
doi: 10.1161/CIRCRESAHA.110.232678

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/108/5/593

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