Seeing Within
Molecular Imaging of the Cardiovascular System
Farouc A. Jaffer, Ralph Weissleder

Abstract—Molecular imaging is a rapidly evolving discipline with the goal of developing tools to display and quantify molecular and cellular targets in vivo. The heart of this field is based on the rational design and screening of targeted and activatable imaging reporter agents to sense fundamental processes of biology. Parallel advances in small animal imaging systems and in agent synthesis have allowed molecular imaging applications to extend into the in vivo arena. These advances have permitted, for example, in vivo sensing of inflammation, apoptosis, cell trafficking, and gene expression. In this review, we first review core principles of molecular imaging with an emphasis on smart, activatable agent technology. We then discuss applications of state-of-the-art molecular probes to interrogate important aspects of cardiovascular biology, with a focus on atherosclerosis, thrombosis, and heart failure. In the ensuing years, we anticipate that fundamental aspects of cardiovascular biology will be detectable in vivo, and that promising molecular imaging agents will be translated into the clinical arena to guide diagnosis and therapy of human cardiovascular illness. (Circ Res. 2004;94:433-445.)

Key Words: molecular imaging ■ atherosclerosis ■ thrombosis ■ heart failure ■ contrast agents

Conventional imaging technologies typically rely on anatomic, physiological, or metabolic heterogeneity to provide image contrast. In comparison, the emerging field of molecular imaging uses targeted and activatable imaging agents to exploit specific molecular targets, pathways, or cellular processes to generate image contrast. The underpinning hypothesis of this newer approach to imaging is that most disease processes have a molecular basis that can be exploited to (1) detect disease earlier, (2) stratify disease subsets (eg, active versus inactive), (3) objectively monitor novel therapies by imaging molecular biomarkers, and to (4) prognosticate disease. The goal of this review is to briefly highlight advances and capabilities in molecular imaging hardware and to provide an overview of novel imaging agents. We then focus on three important aspects of cardiovascular disease, namely atherosclerosis, thrombosis, and heart failure. We will review salient molecular imaging applications in these fields and discuss how new applications could be used to further our understanding of these diseases.

Imaging Technologies
Imaging technologies allow for visualization of the body based on different forms of energy-tissue interactions. They can be used to image 3-D cardiovascular structures, assess biophysical parameters such as ventricular function, stress, and strain, and monitor physiological events such as changes in vascular blood flow and myocardial perfusion. Although some imaging methods [magnetic resonance imaging (MRI),
ultrasound (US) rely mainly on energy/tissue interactions, others [fluorescence reflectance imaging (FRI), fluorescence mediated tomography (FMT), bioluminescence imaging (BLI), single photon emission computed tomography (SPECT), positron emission tomography (PET)] require the administration of imaging agents to generate a physical signal. Common to all of these methods is the ability to transform a detected signal into an image. For a more detailed discussion of high-resolution imaging systems useful for mouse imaging, the interested reader is referred to the online data supplement available at http://circres.ahajournals.org and several recent review articles.

Proteins as Transgenic Imaging Reporters

Transgenic animal models are essential tools in understanding biological processes, and many experimental and clinical applications of molecular medicine such as gene therapy, stem cell transfer, and adoptive immunotherapy dictate the use of transgenes. Furthermore, drug discovery and testing frequently utilize transgenic marine models to better understand the biology governing drug-target interaction. Several recent articles have reviewed reporter transgenes and fusion proteins for PET and SPECT,10–12 multicolor fluorescence,13 bioluminescence,14,15 and MRI.16–20 Some transgenes are directly detectable (eg, photoproteins by optical methods, tyrosinase by MRI16), whereas others require additional injectable imaging substrates discussed later. For noninvasive detection of myocardial gene expression, PET,21 BLI,22 and injectable imaging substrates discussed later. For noninvasive detection of myocardial gene expression, PET,21 BLI,22 and noninvasive imaging of green fluorescent protein are good options, although at relatively lower spatial resolutions.

Injectable Imaging Agents With Molecular Specificity

Imaging agents with molecular specificity have been designed for MR, nuclear, optical, and ultrasound imaging. Agents are generally classified as targeted or activatable (Figure 1; Table).

Targeted Imaging Agents

Targeted imaging agents are generally created by chemically attaching an affinity ligand such as an antibody, peptide, or small molecule to an isotope, fluorochrome, magnetic compound, or acoustically reflective microbubble. The oldest form of targeted agents represent isotope-labeled antibodies such as antimyosin,23 Although many antibody-based conjugates can be easily constructed and provide molecular specificity in vitro, some limitations diminish their ultimate utility in vivo. One drawback of targeted (as opposed to activatable) agents is that both the unbound and nonspecifically bound fractions of an agent produce an active signal, commonly resulting in high levels of background noise. Targeted agents thus require time for washout, a particularly important point to consider when using rapidly decaying isotopes. Long washout times are also impractical in certain acute disease scenarios (eg, myocardial infarction or vascular thrombosis).

A second drawback of labeled antibodies has been their limited bioavailability beyond endothelial targets.24 Because of these limitations, most newer targeted imaging agents are based on modified antibodies or fragments,25 peptides and peptidomimetics,26 carbohydrate or lipid modified peptides,27 labeled small molecule agonists, binders and antagonists,28 multivalent constructs,29 or polyvalent nanoparticles30 among others. In addition, biological amplification strategies such as cellular trapping of imaging agents through targeting internalizing receptors,31 enzymatic conversion,32,33 and/or local binders34,35 are often used to further boost target/background ratios. Additional efforts to improve the imaging behavior of a given agent include optimization of pharmacological properties, size,36 and charge.

Activatable Imaging Agents

Activatable imaging agents (also known as “smart” agents, sensors, or beacons) are chemically engineered substrates that undergo a physicochemical change after interacting with their intended target. The resultant product is either easier to detect (eg, increase in fluorescence) or can be detected in a different channel (eg, shift reagents), thus resulting in increased target-to-background ratios through background suppression. Activatable imaging agents have been developed for magnetic compounds (with 2- to 10-fold signal increases18,30,37,38) and for fluorescent compounds (2- to 1000-fold signal increases39–43). Different chemical amplification strategies are summarized in Figure 1. These strategies can be further combined with biological amplification schemes to further increase target-to-background ratios.

Agents for Different Imaging Modalities

Please see the Table for a comprehensive list of cardiovascular molecular imaging agents.

Magnetic Resonance Agents

Targeted MRI agents have largely been based on either superparamagnetic iron oxide nanoparticles44 or gadolinium chelates. Superparamagnetic iron oxide nanoparticles exert...
### Examples of Molecular Imaging Targets and Agents Relevant to Cardiovascular Imaging

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<td><strong>FXIIIa</strong> indicates activated factor XIII; <strong>MMP</strong>, matrix metalloproteinase; <strong>HIV</strong>, human immunodeficiency virus; <strong>HSV</strong>, herpes simplex virus; <strong>NF</strong>, nuclear factor; <strong>EGF</strong>, epidermal growth factor; <strong>VEGF</strong>, vascular endothelial growth factor; <strong>TGF</strong>, transforming growth factor; <strong>HSV-Tk</strong>, herpes simplex virus thymidine kinase; <strong>VCAM-1</strong>, human vascular cell adhesion molecule-1; <strong>ICAM-1</strong>, human intercellular adhesion molecule-1; <strong>TRAIL</strong>, tumor necrosis factor-related apoptosis-inducing ligand; <strong>luc</strong>, luciferase; <strong>CLIO</strong>, cross-linked iron oxide; <strong>MB</strong>, microbubbles; and <strong>GFP</strong>, green fluorescent protein.</td>
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strong and reversible relaxation effects on their surrounding environment. Several forms of such iron oxides are in use, with some preparations under clinical investigation. Although these agents vary in size (10 to 300 nm), dispersion (monocrystalline, polycrystalline), surface coating (eg, dextran, carboxy dextran, carboxymethyl dextran, starch), and magnetic properties (R1, R2, susceptibility), only a few leading preparations will have utility as clinical imaging agents, targeting agents, or sensors. Efficient targeting often requires strategies such as caging of the dextran coat, which otherwise exists in an equilibrium of free and bound states surrounding the iron oxide core. Dextran-caging has been achieved by cross-linking the coating and resultant particles (cross-linked iron oxide, CLIO) that have already been used as a platform to target receptors, enzymes, integrins, and specific cells. Using phage display technology and high-throughput screening approaches, it is likely that a myriad of CLIO-based cardiovascular imaging agents will be developed over the next few years, including VCAM-1, E-selectin, and macrophage-targeted preparations.

Gadolinium has also been used in the development of targeted MRI contrast agents, but its lower intrinsic relaxivity often necessitates larger-sized nanoparticles constructs such as polymerized liposomes, dendrimers, or perfluorocarbons nanoparticles to achieve high-magnetic payloads and longer intravascular lifetimes. Nonetheless, these agents have been successfully used to specifically image angiogenesis, 31,52 progenitor cells, and thrombosis in vivo. Activatable smar agents have recently been developed for MRI and are generally based on one of two chemical principles: (1) enzymatic conversion of paramagnetic compounds or (2) assembly-disassembly of paramagnetic substrates or nanoparticles. In the first approach, investigators developed a contrast agent that is nearly magnetically silent at baseline. Suppression of the baseline MR signal occurs when cleavable high-affinity chelators are attached and block the access of water molecules to gadolinium, inhibiting its relaxation effects. The agent can be cleaved by β-galactosidase (β-gal), an enzyme encoded by the lacZ gene. After β-gal cleavage, water access to the gadolinium molecule is irreversibly restored, and the measured T1 relaxivity triples, permitting high-resolution 3-D images of β-gal mRNA inheritance patterns and lacZ gene expression in vivo.18

Enzyme-mediated polymerization of paramagnetic substrates into oligomers of higher magnetic relaxivity has been used to image nanomolar amounts of peroxidases and different affinity targets (eg, E-selectin) when used in conjunction with an anti–target-peroxidase conjugate. Another assembly/disassembly strategy for chemical amplification uses magnetic relaxation switches (MRSW). MRSW are iron oxide compounds that have been modified to include multiple copies of an affinity ligand on its dextran coat. When the target for the affinity ligand is encountered, the iron oxide particles begin form nanoassemblies. Of recent interest is the application of MRSW to sense the activity of myeloperoxidase, which has been recently shown to be predictive of myocardial infarction in patients with chest pain.

Nuclear Imaging Agents (SPECT and PET)

Nuclear imaging agents have been developed for a larger number of cardiovascular processes and targets including apoptosis and angiogenesis, viability, atherosclerosis, and thrombosis (see reviews). Targeted nuclear agents can also report on gene expression, for example by targeting a gene-transcribed extracellular protein or by detecting expression of a reporter gene such as herpes simplex virus thymidine kinase (HSV-Tk). In mammalian cells, exogenous HSV-Tk phosphorylates acycloguanosine residues, generating biological signal amplification when the phosphorylated radioisotopes are intracellularly trapped. Further improvements in PET imaging of gene expression are expected with modified reporter genes encoding highly active mutant thymidine kinases and substrates with improved biological behavior. Some of these paradigms have already been tested in clinical trials.

Ultrasound Imaging Agents

Targeted ultrasound contrast agents have been developed by conjugating affinity ligands to acoustically active particulates, such as encapsulated microbubbles, liposomes, or perfluorocarbon nanoparticles. Biologically, targeted agents have been developed for imaging thrombosis, endothelial cell adhesion molecules, tissue factor, activated leukocytes, and angiogenesis. Possible limitations of the current generation of ultrasound-targeted contrast agents are their relatively larger size (>250 nm), impeding efficient tissue penetration and limiting many applications to endothelial targets.

Fluorescent Imaging Agents

Several groups have exploited the near infrared (NIR) bandwidth to develop more efficient fluorescent imaging agents for in vivo applications. A number of targeted NIR fluorescent agents have been reported (see review), including those with specificity for tumor molecular signatures, apoptosis, and osteoblastic cellular activity. More recently, activatable NIRF agents have been developed for protease imaging. Molecular specificity is obtained by interposing a protease-specific peptide substrate between the fluorochrome and carrier vehicle. In the presence of the targeted proteolytic enzyme, the peptide substrate is cleaved, resulting in separation of the fluorochrome from the delivery vehicle. As a result, the NIRF signal increases by up to several hundred-fold in vivo. Protease-activatable agents have been designed to interrogate a number of enzymes including cathepsin S, matrix metalloproteinases, thrombin, and viral proteases. Limitations of NIR activatable agents include their higher synthetic complexity.

Bioluminescent Agents

Bioluminescence imaging (BLI) is performed on genetically modified cells and animals and refers to photocounting of a light-producing chemical reaction inside an organism without the need for an excitation light. Light is produced when a substrate, generically known as a luciferin, encounters its target enzyme, generically known as a luciferase. Although the vast majority of BLI studies rely on firefly and sea pansy
luciferases (see review78), alternative red-shifted luciferases and luciferins are being developed.14,85 Collectively these different luciferase/luciferin combinations potentially allow for “multichannel imaging.”13,86 Additional amplification strategies include incorporation of highly active transcriptional promoters to increase luciferase expression,87 as well as the development of activatable luciferases.88 In addition, luciferases can be modified so that they are only recognized by luciferase when they are chemically converted by an enzyme of interest. For example in our laboratory we have made synthetic luciferins with specificity for caspase-3, eg, Z-DEVD-luciferin.

In Vivo Molecular Imaging of Cardiovascular Disease

Atherosclerosis

Atherosclerosis is the leading cause of morbidity and mortality in developed countries. Biologically, atherosclerosis is an inflammatory disease, with inflammation implicated in atheroma progression and plaque disruption.89,90 Consequently, a considerable research effort is aimed at detecting highly inflamed (high risk or “vulnerable”) atherosclerotic lesions. By imaging high-risk lesions, investigators hope to better understand the link between inflammation and atherosclerosis, and to provide a new measure of clinical cardiovascular risk. A number of targeted nuclear imaging agents have already been developed to report on various high-risk features of atherosclerosis.84 In this section, we focus on recent advances in imaging agent development that permit high-resolution imaging of atherosclerosis.

Imaging of Protease Activity

Recently, our laboratory has demonstrated the ability to noninvasively image protease activity using a near-infrared fluorescent activatable imaging beacon.39 At baseline, the agent is self-quenched, but after cleavage of lysine-lysine bonds, its NIR fluorescence increases significantly. In particular, this agent can be cleaved by cathepsin B, an enzyme present in biologically active macrophages91 and implicated in the pathogenesis of atherosclerosis.92 We hypothesized that cathepsin B activity in atherosclerotic macrophages could activate this agent and serve as a new biomarker of plaque inflammation and vulnerability.84 To test this hypothesis, we injected the agent into atherosclerotic-prone apolipoprotein E–deficient (apoE−/−) mice and performed in vivo fluorescence mediated tomography (FMT). After 24 hours, submicromolar concentrations of the imaging agent were detectable (Figure 2), reflecting internal conversion by the enzyme. Animals were then euthanized, and the resected aortas underwent fluorescence reflectance imaging (FRI) followed by immunohistochemistry, Western blotting, and RT-PCR. FRI demonstrated substantial NIR signal from the aorta of apoE−/− but not from control mice, which correlated well with Sudan-stained lipid-rich areas (Figure 3). This study was the first to show that a protease can serve as an imaging biomarker for inflamed atherosclerotic lesions.

**Imaging of Matrix Metalloproteinase Activity**

Matrix metalloproteinases (MMPs) are a diverse group of zinc-dependent proteolytic enzymes involved in degradation of the extracellular matrix.93 MMPs are classified based on their resident location (secreted or membrane-bound) and proteolytic target (eg, collagen, gelatin, stromelysin, or matrilysin). MMPs have been broadly implicated in a number of cardiovascular diseases, including atherosclerosis,90,94 aortic aneurysms,95 and heart failure,96 and therefore represent an

![Figure 2](image-url)

**Figure 2.** Imaging of an apoE−/− mouse by fluorescence-mediated tomography (FMT). a, Sagittal magnetic resonance (MR) image showing highlighted axial sections b and c for anatomic reference. d and e, FMT images corresponding to the MR sections shown in b and c. Note that there is a signal emanating from the descending aorta in a distribution similar to that shown in Figure 2 (color map, 0 to 6×10−7 mol/L concentration of Cy 5.5; numbers on x- and y-axes represent millimeter bars).


![Figure 3](image-url)

**Figure 3.** Aortas from 2 apoE−/− mice fed a Western-type diet. a and b, Intact vessel; c and d, Vessel opened longitudinally. a, Photograph of unstained intact vessel, with normal areas filled with blood (red) and atherosclerotic lesions appearing white. b, Corresponding NIRF image showing cathepsin B–activated fluorescent areas in the arch and abdominal aorta. c, Sudan IV staining of the longitudinally opened aorta, where red areas represent lipid-rich areas stained with Sudan IV. d, Corresponding NIRF image showing prominent cathepsin B signal from atherosclerotic lesions that matches Sudan staining. Native atherosclerotic lesions had near-infrared autofluorescence similar to that in normal aorta. Reproduced from Chen J, Tung CH, Mahmood U, Ntziachristos V, Gyurko R, Fishman MC, Huang PL, Weissleder R. In vivo imaging of proteolytic activity in atherosclerosis. *Circulation.* 2002;105:2766–2771, by permission of the American Heart Association ©2002.
important target for cardiovascular molecular imaging. In atherosclerosis, MMPs are expressed in macrophages and vascular cells, and are involved in atherosclerotic plaque disruption via enzymatic degradation of the fibrous cap or endothelial basement membrane.\textsuperscript{90,94} MMPs have also been associated with atherogenic vascular remodeling via several mechanisms, including smooth muscle cell migration and inflammatory cell recruitment to the atherosclerotic intima.\textsuperscript{94}

Recently, we have demonstrated the ability to noninvasively image gelatinase MMP activity and therapeutic inhibition in vivo.\textsuperscript{41} Building on the NIRF activatable framework described earlier,\textsuperscript{39} the gelatinase MMP-2 peptide substrate PLGVR was conjugated to the carrier backbone. Using an MMP-2–positive human fibrosarcoma model, we demonstrated a 3-fold NIR tumor signal increase after injection of the agent. Furthermore, the NIRF signal was suppressible by a potent MMP-2–specific inhibitor.\textsuperscript{41} As MMP-2 has been suggested to be a specific mediator of fibrous cap destabilization,\textsuperscript{97} we are currently imaging gelatinase activity in human carotid endarterectomy specimens to better understand their role in the pathogenesis of stroke (Figure 4).

Future efforts are aimed at developing more specific activatable MMP imaging agents.

**Imaging of Activated Macrophages**

Macrophages are an essential component of the inflammatory response governing atherosclerosis and are implicated in lesion formation, progression, and disruption.\textsuperscript{90,98} Given their broad role in atherosclerosis, macrophages, particularly activated macrophages, are being increasingly recognized as an important treatment target for atherosclerosis.\textsuperscript{98}

As described earlier, NIRF imaging of macrophage protease activity is one strategy for detecting activated macrophages.\textsuperscript{84} Another approach uses superparamagnetic iron oxide nanoparticles for high-resolution MRI. Atherosclerotic macrophages are known to phagocytose ferritin,\textsuperscript{99} and several groups have successfully imaged iron oxide–laden macrophages in atherosclerosis\textsuperscript{96,100–103} and cardiac transplant rejection.\textsuperscript{104} Mechanistically, macrophages appear to internalize dextranated nanoparticles, and the resultant iron oxide accumulation generates strong T2 relaxation and MRI contrast (Figure 4). It is anticipated that some iron oxide agents will become commercially available, allowing for clinical
detection of atherosclerotic macrophages in situ, as well as for potentially detecting monocyte recruitment to atherosclerotic lesions.105

**Imaging of Activated Endothelium**

Via their interaction with leukocytes and platelets, activated endothelial cells are instrumental in atherogenesis.90,106 Although under normal conditions endothelial cells resist cell adhesion, atherogenic stimuli induce the expression of adhesion molecules on the endothelial cell surface, facilitating firm adhesion and transmigration of leukocytes into the arterial intima.90

Several ultrasound-targeted agents have been described for interrogating endothelial cell receptor presence. Specifically, acoustically reflective microbubbles or liposomes have been conjugated to monoclonal antibodies to target ICAM-170,71 and P-Selectin.72 One particular study using an ICAM-1–targeted liposome demonstrated significant transvascular and intravascular ultrasound enhancement of early atheroma in a swine model,70 although a limitation of this approach may be the limited biological role of ICAM-1 in atherogenesis.107

**Thrombosis**

Thrombosis is the pathological hallmark of a number of cardiovascular diseases, including myocardial infarction, stroke, and pulmonary embolism. The ability to specifically image molecules important in thrombogenesis could provide insight into their biological function and also serve as a highly specific diagnostic thrombosis imaging method. Several thrombosis-targeted nuclear imaging agents have been developed,65 including a clinically approved agent.108 In this section, we highlight new thrombosis agents for use with high-resolution MR, US, and NIF imaging systems.

**Imaging of Platelets**

Platelets are an integral cellular component of arterial thrombi, where they become activated in the presence of abnormal endothelium or high shear.109 Historically, platelets have been a well-recognized target for nuclear imaging.65 Several radiolabeled platelet-targeted agents have been targeted to the activated αIIbβ3 integrin (also known as the GP IIb-IIIa receptor), known to mediate platelet stable adhesion and platelet aggregation.109 The activated αIIbβ3 integrin is a favorable molecular imaging target due to its dynamically high concentration (50 000 to 100 000 receptors per platelet), and has been targeted using linear peptides, cyclic peptides (typically incorporating an RGD sequence), and natural polypeptides.65 Encouragingly, a recent report of an FDA-approved platelet GP IIb-IIIa receptor–targeted nuclear agent (99mTc-apcitide) has shown good sensitivity and specificity for detecting recurrent DVT.108 Similarly, activated αIIbβ3 integrin–targeted agents have been created for in vivo US and MR imaging of thrombi in vivo.

**Imaging of Enzymes in Thrombosis**

**Imaging of Thrombin Activity**

Thrombin, a serine protease, is an important enzyme in a wide array of normal and pathological processes including thrombogenesis, embryogenesis, and angiogenesis.111 During thrombogenesis, thrombin cleaves fibrinogen to form fibrin monomers, which subsequently polymerizes to form fibrin, the scaffolding of thrombus. Thrombin also directly activates platelets and induces vasoconstriction to further promote thrombus propagation. The ability to locally image thrombin activity in vivo could provide new insight into the effects of thrombin in a range of homeostatic and pathological conditions and allow more precise assessment of anti-thrombin pharmacological therapies.

To image thrombin activity, we developed a new NIR activatable agent93–41 to report on thrombin enzyme activity.112 In human blood, thrombin strongly activated this NIR agent.42 Activation of the agent was suppressed by hirudin, a direct thrombin inhibitor. Using in vivo murine thrombosis models, the thrombin agent enabled high-resolution imaging of thrombin activity in experimental thrombi with intravital fluorescence microscopy (Figure 5).42 Strong NIF fluorescence was evident in acute thrombi and at the leading edges of thrombi, as expected biologically. In conjunction with a NIRF imaging catheter, the thrombin agent could permit detection of acute coronary thrombi. The NIR activatable agent approach is also well suited to study other important proteases involved in thrombosis and fibrinolysis, such as activated factor X and plasmin, respectively.

**Imaging of Activated Factor XIII Activity**

Activated coagulation factor XIII (FXIIIa) is a tissue transglutaminase that cross-links fibrin chains and plasmin inhibitors such as α2-antiplasmin (α2-AP) to form mechanically and proteolytically stable thrombi.113 FXIIIa is important in fibrinolytic resistance114 and is a hallmark of biologically acute thrombi.115 Imaging of FXIIIa activity could provide important insight into thrombus formation and aging, anti-FXIIIa therapies, and factor XIII genetic polymorphisms.

Recently, a new FXIIIa-sensitive iron oxide agent has been described for MRI.35 This agent (F13-CLIO) consists of a dextran-coated caged iron oxide particle (CLIO) conjugated to an α2-AP peptide fragment that can be cross-linked by factor XIIIa. In vitro F13-CLIO experiments with human plasma thrombi demonstrated marked thrombus contrast enhancement over control agents (Figure 5). Furthermore, gel electrophoresis and scanning electron microscopy revealed that F13-CLIO was covalently cross-linked into the thrombus. Additional factor XIIIa agents have been recently characterized for MRI and NIF imaging,116 and are also under evaluation for imaging of FXIIIa activity in vivo.

**Imaging of Fibrin**

Through a series of proteolytic reactions of activated blood coagulation factors, fibrinogen is ultimately cleaved to produce fibrin, the scaffolding of thrombi.111 Fibrin is a favorable molecular imaging target because it is usually present in all types of thrombi (arterial and venous, acute and chronic) and is unlikely to be modified by standard antithrombotic pharmacological therapies such as aspirin, thrombin inhibitors (eg, heparin), or GP IIb-IIIa antagonists. Furthermore, fibrin is present in low plasma concentrations, minimizing background signal.

Fibrin-targeted molecular imaging agents have been developed for nuclear imaging,65 ultrasound imaging,68,70 and most...
Thrombus contrast is significantly enhanced on both T1w and T2w images with the F13-CLIO agents where as otherwise rectangular thrombi are barely detectable in solution. Right, Scanning electron microscopy of saline and F13-CLIO-incubated clots reveal F13-CLIO cross-linking to fibrin fibrils (white arrows). Data obtained in collaboration with Dr Ching Tung, Center for Molecular Imaging Research, Massachusetts General Hospital.

Figure 5. In vivo optical imaging of thrombin activity in thrombosis. A through C, Molecular imaging of thrombi using a thrombin-activatable NIR agent. A, In an acute thrombus model (thrombin agent injected 1 hour after thrombus formation), the light image demonstrates darker clotted segments within the femoral vein after application of FeCl3 (arrows). B, NIRF image demonstrates focal signal in areas of thrombosis, particularly within side branches (arrows and dashed box). C, Fusion NIR image shows focal areas of high-fluorescence signal within microthrombi. Images were acquired 60 to 90 minutes after agent injection and have been windowed individually. V indicates vein; A, artery. Reproduced from Jaffer FA, Tung CH, Gerszten RE, Weissleder R. In vivo imaging of thrombin activity in experimental thrombi with thrombin-sensitive near-infrared molecular probe. Arterioscler Thromb Vasc Biol. 2002;22:1929–1935, by permission of the American Heart Association ©2002.

D, Molecular MR imaging of thrombi using an activated factor XIII-targeted imaging agent. Left, T1- and T2-weighted (T1w and T2w) images with the F13-CLIO agents where as otherwise rectangular thrombi are barely detectable in solution.35 Right, Scanning electron microscopy of saline and F13-CLIO-incubated clots reveal F13-CLIO cross-linking to fibrin fibrils (white arrows). Data obtained in collaboration with Dr Ching Tung, Center for Molecular Imaging Research, Massachusetts General Hospital.

recently, for high-resolution MRI.54,117 In one MRI example, investigators coated perfluorocarbon microemulsions with high densities of gadolinium using an avidin-biotin conjugation scheme, and then attached a modified antifibrin monoclonal antibody.54 In a canine model, surgically formed carotid thrombi were incubated with the fibrin-targeted nanoparticles during cessation of blood flow. After restoration of flow, nanoparticle-incubated thrombi demonstrated significant MR signal enhancement in vivo over native thrombi. In human carotid endarterectomy specimens, the nanoparticles also enhanced microthrombi overlying the atherosclerotic intima, demonstrating the potential for vulnerable plaque detection.54 Finally, another gadolinium-based fibrin-targeted probe has been recently reported in abstract form117 and shows promise for rapidly diagnosing vascular thrombi.

Heart Failure
Heart failure is a disabling illness that encompasses a broad array of pathological and clinical entities, and may be classified based on structural, functional, or molecular frameworks. Strategies to image-specific molecular and cellular targets are currently being developed with the ultimate goal of improving diagnosis, predicting prognosis, and assessing response to therapy.

Imaging of Myocardial Apoptosis
Cardiomyocyte apoptosis is a pathological feature of heart failure, ischemia/reperfusion injury, and transplant rejection.118 In explanted failing human hearts, cardiomyocyte apoptosis occurs much more frequently than in normal hearts.119 Recently, even a low-level of apoptosis has been shown to be sufficient to cause a lethal dilated cardiomyopathy in genetically engineered mice.120 Accordingly, several apoptosis-targeted imaging agents have been developed and are discussed herein.

Imaging of Phosphatidylserine
Phosphatidylserine (PS) is a phospholipid normally located on and restricted to the internal cell membrane by an energy-dependent aminophospholipid translocase.121 During apoptosis, this translocase is inactivated whereas other nearby enzymes such as phospholipid scramblase are activated, resulting in PS externalization to the outer cell membrane. Externalized PS avidly binds several proteins, including annexin V. Using intravital fluorescence microscopy, fluorescently labeled annexin V can image real-time apoptotic cell-membrane changes in the beating heart.122 Using radio-labeled annexin V, noninvasive imaging of apoptosis has been performed clinically in patients with acute myocardial infarction123 or transplant rejection.124 Because differentiation between bound and nonspecifically accumulated (ie, unbound) annexin V is difficult, we have recently developed dual-wavelength annexin reporters with active and inactive PS binding sites.125 Furthermore, to improve fluorescence imaging at greater depths, near infrared fluorochrome tagged annexins have been described.82,125

To overcome limitations of lower spatial resolution involved in nuclear imaging, alternative PS-targeted agents have been developed for high-resolution MRI. In one example, SPIO was conjugated to synaptotagmin I, another PS-binding protein, and used to image apoptotic tumor cells in vivo.126 Our laboratory has also developed an annexin V conjugated to MION capable of detecting apoptotic cells using 100-fold lower concentration than the synaptotagmin MR agent.127

Imaging of Caspase Activity
Caspases are specialized cysteine-dependent proteases involved in apoptosis via cytoplasmic and nuclear protein cleavage.128 In particular, caspase-3 activation has important
functional myocardial consequences, including impaired contraction and infarct expansion. Recently, a novel caspase-3 reporter has been developed for in vivo BLI by using a luciferase-based fusion protein construct. Because BLI has already been used to detect cardiac gene expression, this agent could be readily applied for bioluminescence imaging of myocardial apoptosis, although at relatively low spatial resolution because of scattering of photons emanating from the heart.

Another approach to caspase imaging uses injectable reporters, ie, constructs that contain the caspase-3 cleavage region, a reporter label (eg, 99mTc or a quenched NIR fluorochrome), and a membrane translocation signal such as tat so that the molecule can be efficiently internalized into cells. Whereas the nuclear agents are trapped inside cells, the NIRF compounds could also potentially benefit from fluorescence activation.

**Imaging of Matrix Metalloproteinase Activity in Heart Failure**

MMPs have been implicated in heart failure and ventricular remodeling. In human explanted hearts, both ischemic and dilated cardiomyopathic tissue demonstrate altered patterns of collagenase, gelatinase, stromelysin, and TIMP expression compared with normal myocardium. In concert, transgenic murine experiments have confirmed a pathophysiological role for various MMPs in heart failure. Accordingly, in vivo detection of MMP activity will be important in both understanding the biology and treatment of heart failure.

Similarly as described in the atherosclerosis section, the gelatinase probe has been used to study MMP activity in cardiac remodeling after myocardial infarction (J. Chen, C.H. Tung, Q. Zhen, R. Weissleder, P.L. Huang, unpublished data, 2004). After imaging agent injection and subsequent euthanasia, NIRF imaging of myocardium correlated well with immunohistochemical staining for gelatinases. Increased MMP activity was confirmed by gelatinase zymography and Western blot analysis. Dual-label
in vivo confocal microscopy showed colocalization of gelatinase activity with neutrophils on day 1, and colocalization with macrophages at later time points. These preliminary studies indicate that gelatinases may serve as a biomarker of tissue remodeling after myocardial infarction, and that specific agents can be used for biological imaging of MMP activity (J. Chen, C.H. Tung, Q. Zhen, R. Weissleder, P.L. Huang, unpublished data, 2004).

**Imaging of Stem Cells for Myocardial Regeneration**

The concept that stem cells could repair failing myocardium has engaged the scientific community.137 Several recent experimental investigations have demonstrated that transplanted adult bone marrow stem cells (BMSCs) can engraft in myocardium and differentiate into cardiomyocytes, endothelial cells, and vascular smooth muscle cells.138,139 In addition, stem cell therapy has been shown to improve cardiac function and survival in animal models of heart failure.140–142 Of note, two recent clinical studies of intracoronary stem cell therapy after acute myocardial infarction have demonstrated safety, feasibility, and improved ventricular function.143,144

Given the potential to serially track the presence, distribution, migration, durability, and molecular function of transplanted stem cells, cardiovascular imaging will likely play an important role in myocardial regeneration studies. Several imaging possibilities exist for detecting and tracking of stem cells in vivo. One approach is to transfect stem cells containing optical or nuclear reporter genes that can be noninvasively imaged in the heart.21,22,145 The other genetically intact approach, more likely to be used in human stem cell trials, is to label stem cells with magnetic nanoparticles. Encouragingly, MRI of magnetically labeled mesenchymal stem cells (MSCs) injected into porcine myocardium has recently been performed in vivo.146,147 In a myocardial infarction model, allogeneic, fluorescently labeled MSCs were incubated with commercially available magnetic particles for 24 to 48 hours. After ischemia and reperfusion, 400 million magnetically labeled MSCS were intramyocardially injected under x-ray fluoroscopy. Serial MRI demonstrated progressively less intensely hypoenhancing lesions (Figure 6). Prussian blue staining demonstrated colocalization of the iron signal with the MSC-labeled fluorescent dyes, confirming that the magnetic particles remained within the intracellular space. In addition, another study demonstrated that relatively large magnetic and fluorescent particles (~1-μm diameter) could also be used to image magnetically labeled MSCS injected into infarcted and normal porcine myocardium.147 These particles may be advantageous for stem cell labeling due to their high relaxivity and longer intracellular lifetime (at least 3 weeks), although labeling times are relatively long. Of note, other schemes for cell labeling have also been pursued. Using the transferrin receptor to shuttle iron oxide into the cell interior,19 oligodendrocyte progenitor cells have been tracked in spinal cords in vivo.148 Magnetodendrimers represent another class of derivatized magnetic particles useful for cell labeling. In addition, gadolinium labels have also been used for ex vivo confirmation of stem cell tracking.149

Newer magnetic nanoparticle preparations such as CLIO-tat offer significant advantages including higher labeling efficiency, faster labeling (minutes instead of hours to days), chemical inertness, lack of immunogenicity and toxicity, dual labeling (magnetic and fluorescence capability), and higher signal (relaxivity) effects.150 These agents consist of cross-linked iron oxide (CLIO) conjugated to membrane translocation signals of the human immunodeficiency virus (HIV) Tat protein.49 Given the availability of the various magnetic cell labeling agents, and the ability to assess myocardial function and anatomy, MRI should play a leading role in assessing myocardial stem cell therapy.

**Conclusions**

With the parallel advances in small animal imaging and reporter agent technology, investigators in the cardiovascular arena are well poised to apply and extend current capabilities to assay cardiovascular gene expression, stem cell biology, inflammation, apoptosis, and protease activity. It is further expected that clinical translation of molecular imaging technology will ultimately aid in the diagnosis and treatment of human cardiovascular disease.

**Acknowledgments**

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Molecular Imaging of the Cardiovascular System

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Seeing Within: Molecular Imaging of the Cardiovascular System
Farouc A. Jaffer and Ralph Weissleder

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Molecular Imaging System Technology

The appropriate imaging system for a particular experiment depends primarily on three main questions (Table 1): (1) what is the required spatial, temporal, and depth resolution; (2) what are the currently available imaging reporters; and (3) is the experiment aimed at experimental (e.g. mice, rats, zebrafish) or human imaging? For applications requiring very high spatial resolutions (< 20 µm), intravital microscopy (confocal or multiphoton) offers a most useful approach.\(^1\,^2\) If lower resolution suffices (20-200 µm voxel resolution) or if intravital microscopy is not feasible, preferred imaging modalities include largely MRI and US. MRI is a versatile all-around imaging modality with several advantages including high-resolution, noninvasive and true three-dimensional volumetric imaging capabilities, useful for imaging complex anatomical structures like the heart; concurrent imaging of myocardial flow, mechanical, and metabolic biophysical parameters; and excellent intrinsic soft-tissue contrast, permitting convenient registration of anatomic and molecular information. Furthermore, a number of targeted and activatable magnetic imaging agents are currently being developed which undoubtedly will further increase the utility of MRI. Limitations of MRI primarily include its lower inherent sensitivity towards agent detection (µM for paramagnetic agents and nM for superparamagnetic agents) compared to nuclear techniques, and the lack of radiofrequency penetration deep into the chest, as coil sensitivities fall off with distance. Ultrasound, the cornerstone of clinical cardiovascular imaging, offers portability, high temporal and spatial resolution, can reliably assess cardiovascular
anatomy, function, and physiology. With the advent of high-frequency transducers (40-50 MHz), high-resolution in vivo imaging of the murine heart is now feasible.

For applications with less stringent resolution requirements (~1-2mm), nuclear imaging (PET and SPECT) is frequently used. Nuclear imaging approaches have the advantages of high intrinsic sensitivity and a wide array of imaging agents. PET has the advantage of being fully quantitative, although PET imaging systems and cyclotrons are expensive. Limited target/blood ratios of imaging agents can also result in limited image quality unless imaging is performed at later time points when the blood pool activity of an imaging agent has cleared.

Macroscopic optical imaging approaches such as near infrared (NIR) fluorescence imaging (FRI and FMT) and bioluminescence imaging (BLI) represent the latest addition to the imaging armamentarium and possess a number of significant advantages. There exist excellent reporter systems, many of which can be induced or constitutively expressed in murine models (green fluorescent protein (GFP) and/or luciferase (luc) transgenic mice). In addition, there are now a series of activatable (i.e. amplifiable such as protease sensors) and targeted imaging agents. Limitations of FRI included the surface-weighted nature of the image and its semi-quantitative analysis. FMT is more quantitative and also provides 3D tomographic images. Current instrumentation efforts are underway to further increase penetration depth, spatial resolution and quantitative capabilities.
**Clinical Translation of Molecular Imaging**

An important goal of molecular imaging is to develop imaging systems and imaging agents that can aid in the diagnosis, risk stratification, and treatment of human clinical disease. From a clinical perspective, patient-based imaging systems are routinely available for magnetic resonance, ultrasound, and nuclear imaging, and therefore molecular probes with these imaging readouts are likely to have the broadest immediate clinical impact. From a cardiovascular standpoint, the leading molecular imaging platforms are likely to be magnetic resonance imaging and ultrasound imaging, as both have the ability to provide important clinical information (high-resolution images of cardiovascular anatomy, function, perfusion) as well as molecular information via activatable and targeted probes.

Optical technologies such as fluorescence reflectance imaging also appear to be promising in the cardiovascular arena. FRI systems have available for clinical use for some time in the endoscopy suite,\(^{16}\) and more recently in the operating room for cardiovascular surgery.\(^{17}\) In addition, clinical catheter-based NIR imaging systems are under development for human coronary arterial imaging (InfraReDx, Cambridge, MA). On the noninvasive front, fluorescence mediated tomography (FMT) is a noninvasive quantitative 3D imaging method that has been tested in small animals. Computer simulations suggest that NIR light may be detected from 7-14cm into the body,\(^ {13}\) potentially allowing fluorescence imaging of the anterior wall of the heart as well as the carotid, brachial, and femoral arteries. Development of clinical FMT systems is therefore underway in our laboratory as well as other centers. Lastly, bioluminescence
imaging, another optical imaging method useful for cellular and animal imaging, would require genetic modification of the subject’s DNA, and therefore is unlikely to translate into clinical practice.

As in oncology, clinical trials of cardiovascular molecular imaging agents have utilized nuclear imaging systems, with specific applications to the detection of myocarditis and myocardial infarction,\textsuperscript{18,19} deep venous thrombosis,\textsuperscript{20,21} and cardiac allograft rejection.\textsuperscript{22,23} More recently, superparamagnetic iron oxide particles have been used clinically to image macrophages in inflamed human carotid atherosclerotic lesions.\textsuperscript{24} Future clinical molecular imaging agents are under development for all platforms including magnetic resonance imaging, ultrasound imaging, near infrared fluorescence imaging, and nuclear imaging.
REFERENCES


Table 1: Overview of small-animal imaging systems for molecular/cell based applications

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Spatial Resolution</th>
<th>Temporal Resolution</th>
<th>Volumetric Imaging (True 3D)</th>
<th>Targeted Probes</th>
<th>Activatable Probes</th>
<th>Clinical Use?</th>
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<td>Magnetic resonance imaging (MRI)*</td>
<td>10 µm</td>
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<tr>
<td></td>
<td>100 µm</td>
<td>Minutes</td>
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<tr>
<td></td>
<td>1000 µm</td>
<td>Seconds</td>
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<td>YES</td>
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<td>Intravital fluorescence microscopy (IVFM)</td>
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<td>cm</td>
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<tr>
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<tr>
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<td>Bioluminescence imaging (BLI)*</td>
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<td></td>
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<td>cm</td>
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<td></td>
<td>50 µm</td>
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<td>cm</td>
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<td>Nuclear imaging</td>
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<td>Positron emission tomography (PET)</td>
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<td>YES</td>
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</table>

*currently or potentially catheter based  **via stimulated emission of microbubbles