Molecular Imaging of the Cardiac Extracellular Matrix

Hans J. de Haas, Eloisa Arbustini, Valentin Fuster, Christopher M. Kramer, Jagat Narula

Abstract: In almost all cardiac diseases, an increase in extracellular matrix (ECM) deposition or fibrosis occurs, mostly consisting of collagen I. Whereas replacement fibrosis follows cardiomyocyte loss in myocardial infarction, reactive fibrosis is triggered by myocardial stress or inflammatory mediators and often results in ventricular stiffening, functional deterioration, and development of heart failure. Given the importance of ECM deposition in cardiac disease, ECM imaging could be a valuable clinical tool. Molecular imaging of ECM may help understand pathology, evaluate impact of novel therapy, and may eventually find a role in predicting the extent of ECM expansion and development of personalized treatment. In the current review, we provide an overview of ECM imaging including the assessment of ECM volume and molecular targeting of key players involved in ECM deposition and degradation. The targets comprise myofibroblasts, intracardiac renin-angiotensin axis, matrix metalloproteinases, and matricellular proteins. (Circ Res. 2014;114:903-915.)

Key Words: fibrosis ■ heart failure ■ molecular imaging ■ myofibroblasts

The structure and composition of extracellular matrix (ECM; Figure 1A) guarantee the harmonic structure (microscopic and macroscopic anatomy) and function of the heart and mediates cell to cell as well as cell to ECM molecular signaling and interactions. In cardiac disease, increased ECM deposition is a key compensatory and repair mechanism. Replacement fibrosis, which occurs after cardiomyocyte loss typically post–myocardial infarction (MI), contributes to maintaining the macroanatomy of the heart (Figure 1B). Reactive fibrosis follows cardiac stress and occurs in most cardiac diseases with pressure1,2 and volume overload,3 cardiomyopathies4–6 (Figure 1C), and post-MI remodeling in the noninfarcted area.7 Reactive fibrosis may result in ventricular wall stiffening,8 contribute to arrhythmogenesis,9 hamper oxygen and nutrient diffusion,9,10 and, in a downward spiral, induce functional decline and eventually heart failure. Myofibroblasts contribute to increased ECM production; they share the expression of contractile α-smooth muscle actin with smooth muscle cells. Beside resident myofibroblasts, circulating macrophages, smooth muscle cells, endothelial, and fibrocytes may differentiate into myofibroblasts and contribute to fibrosis.11–13 The differentiation is triggered by molecules upregulated during cardiac damage, such as the renin–angiotensin system (RAS), transforming growth factor-β, and tumor necrosis factor-α. The activated myofibroblasts synthesize procollagens, mostly type 1 and also of type 3, in the ECM. Procollagen proteinases convert precursor molecules into mature collagen fibers.14 Subsequently, collagen fibrils mature through lysyl-oxide–mediated crosslinking of collagen fibrils. Various...
molecules, including tissue transglutaminase and matricellular proteins such as tenascin C and secreted protein acidic and rich in cysteine (SPARC), regulate collagen assembly. Matrix metalloproteinases (MMPs) are also upregulated in cardiac diseases, which result in ECM degradation that facilitates adaptive changes (such as removal of cellular debris by inflammatory cells and migration of myofibroblasts) and also contributes to weakening the structure, promoting dilatation and even aneurysm formation. Because of its excellent spatial and temporal resolution, MRI has evolved as the gold standard for structural cardiac imaging. Using late-gadolinium enhancement (LGE), MRI can accurately detect fibrosis and quantify ECM volume. Molecular imaging probes labeled with magnetic contrast molecules, such as iron oxide nanoparticles or gadolinium, have been developed to allow molecular MRI; however, the sensitivity of this approach remains limited. Whereas, molecular imaging using single photon emission computed tomography (SPECT) or positron emission tomography (PET) offers sensitivity in the nanomolar range, but offers limited spatial resolution; PET has a higher resolution compared with SPECT. Several optical imaging approaches, including fluorescence imaging and near-infrared fluorescence (NIRF) imaging, are also becoming available for molecular imaging. These modalities constitute excellent research tools, because they are highly sensitive, relatively fast, simple, and inexpensive. However, the low tissue penetration does not allow clinical imaging, and their use is limited to small animal models. This review provides an update on structural and molecular imaging of ECM and potential future applications; Table 1 lists the studies regarding molecular imaging of the cardiac ECM.

**ECM Volume Assessment Using CMR**

LGE MRI is used clinically to visualize macroscopic fibrosis due to MI or any other cause that is visible to the naked eye of the imager. Gadolinium leaks into the interstitial space as it extravasates, but is not taken up by cells. In areas of fibrosis, in addition to increased volume of distribution of gadolinium, there is delayed washout. Gadolinium affects the magnetic properties of water molecules in the tissue, thereby shortening the T1 time of water, and thus appears bright on T1-weighted images. For scar due to MI, areas of LGE typically must demonstrate signal intensity >5 SD higher than remote normal myocardium to be characterized as infarct-related scar. Fibrosis as visualized by LGE in the setting of other cardiomyopathies may be more patchy or diffuse. No matter the clinical setting, the finding of macroscopic fibrosis, as identified by LGE, is a marker of adverse cardiac prognosis. LGE cannot be visualized in cardiovascular diseases where fibrosis is diffuse and microscopic. In addition, there may not be normal reference regions in these conditions even if there is diffuse extravasation of gadolinium. An MRI technique of T1 mapping has been proposed, which is able to measure changes in extracellular volume (ECV) as a surrogate marker of diffuse fibrosis. Of these, an extensively studied technique is modified Look-Locker inversion recovery (MOLLI) in which diastolic single-shot images are obtained with multiple different T1 sensitivities >17 heartbeats in a single breathhold. The image data are then fit to a T1 recovery function to create a map of T1. A newer technique that allows a shorter breathhold, such as shortened MOLLI, has been developed, which reduced the heart rate dependence of the technique. Nonrigid registration measures have further improved the reliability and clinical applicability of these MRI methods, and they are now being applied widely in several myocardial pathologies (Figure 2). A consensus statement has recently been developed that makes recommendations for use of these techniques. The measurement of postcontrast T1 as a marker of interstitial fibrosis was validated in endomyocardial biopsy specimens from transplant recipients that showed an inverse correlation between postcontrast T1 time and histologically verified collagen content. In another study of patients with heart failure, postgadolinium T1 time was inversely correlated with histological fibrosis identified by endomyocardial biopsy (r=0.57; P<0.0001). Prognostic data are now emerging as in a recent study of 100 patients with heart failure and preserved ejection fraction; T1 times below the median were associated with an increased risk of cardiac events compared with those above the median. T1 maps of the myocardium can be made without contrast, measuring the so-called native T1. For example, native T1 is increased in amyloidosis as compared with both normal controls and patients with aortic stenosis because of the markedly expanded extracellular space. Native T1 is also elevated in both dilated and hypertrophic cardiomyopathies as compared with controls. Thus, native T1 mapping identifies myocardial diseases associated with expanded extracellular space. This approach may prove particularly useful in patients with stage 4 or 5 kidney disease who are not candidates for receiving gadolinium contrast due to concerns regarding nephrogenic systemic fibrosis. Native T1 mapping gives investigators a general idea of the state of extracellular space, but is not equally as quantitative as the methods to measure ECV as described below. Measuring ECV is performed by mapping T1 before and after either a bolus injection or equilibrium infusion of gadolinium. The latter 2 methods have been shown to be essentially equivalent. ECV is determined by calculating the volume of

### Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
</tr>
<tr>
<td>AT1R</td>
<td>angiotensin II receptor type 1</td>
</tr>
<tr>
<td>CRIP</td>
<td>Cy5.5 RGD imaging peptide</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>ECV</td>
<td>extracellular volume</td>
</tr>
<tr>
<td>FMT</td>
<td>fluorescent molecular tomography</td>
</tr>
<tr>
<td>FXIII</td>
<td>blood coagulation factor XIII</td>
</tr>
<tr>
<td>LGE</td>
<td>late-gadolinium enhancement</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>MOLLI</td>
<td>modified Look-Locker inversion</td>
</tr>
<tr>
<td>NIRF</td>
<td>near-infrared fluorescence</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>RAS</td>
<td>renin-angiotensin system</td>
</tr>
<tr>
<td>SPARC</td>
<td>secreted protein acidic and rich in cysteine</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
</tbody>
</table>

---

**Nonstandard Abbreviations and Acronyms**

- **ACE**: angiotensin-converting enzyme
- **AT1R**: angiotensin II receptor type 1
- **CRIP**: Cy5.5 RGD imaging peptide
- **ECM**: extracellular matrix
- **ECV**: extracellular volume
- **FMT**: fluorescent molecular tomography
- **FXIII**: blood coagulation factor XIII
- **LGE**: late-gadolinium enhancement
- **MI**: myocardial infarction
- **MMP**: matrix metalloproteinase
- **MOLLI**: modified Look-Locker inversion
- **NIRF**: near-infrared fluorescence
- **PET**: positron emission tomography
- **RAS**: renin-angiotensin system
- **SPARC**: secreted protein acidic and rich in cysteine
- **SPECT**: single photon emission computed tomography

**ECM Volume Assessment Using CMR**

LGE MRI is used clinically to visualize macroscopic fibrosis due to MI or any other cause that is visible to the naked eye of the imager. Gadolinium leaks into the interstitial space as it extravasates, but is not taken up by cells. In areas of fibrosis, in addition to increased volume of distribution of gadolinium, there is delayed washout. Gadolinium affects the magnetic properties of water molecules in the tissue, thereby shortening the T1 time of water, and thus appears bright on T1-weighted images. For scar due to MI, areas of LGE typically must demonstrate signal intensity >5 SD higher than remote normal myocardium to be characterized as infarct-related scar. Fibrosis as visualized by LGE in the setting of other cardiomyopathies may be more patchy or diffuse. No matter the clinical setting, the finding of macroscopic fibrosis, as identified by LGE, is a marker of adverse cardiac prognosis. LGE cannot be visualized in cardiovascular diseases where fibrosis is diffuse and microscopic. In addition, there may not be normal reference regions in these conditions even if there is diffuse extravasation of gadolinium. An MRI technique of T1 mapping has been proposed, which is able to measure changes in extracellular volume (ECV) as a surrogate marker of diffuse fibrosis. Of these, an extensively studied technique is modified Look-Locker inversion recovery (MOLLI) in which diastolic single-shot images are obtained with multiple different T1 sensitivities >17 heartbeats in a single breathhold. The image data are then fit to a T1 recovery function to create a map of T1. A newer technique that allows a shorter breathhold, such as shortened MOLLI, has been developed, which reduced the heart rate dependence of the technique. Nonrigid registration measures have further improved the reliability and clinical applicability of these MRI methods, and they are now being applied widely in several myocardial pathologies (Figure 2). A consensus statement has recently been developed that makes recommendations for use of these techniques. The measurement of postcontrast T1 as a marker of interstitial fibrosis was validated in endomyocardial biopsy specimens from transplant recipients that showed an inverse correlation between postcontrast T1 time and histologically verified collagen content. In another study of patients with heart failure, postgadolinium T1 time was inversely correlated with histological fibrosis identified by endomyocardial biopsy (r=0.57; P<0.0001). Prognostic data are now emerging as in a recent study of 100 patients with heart failure and preserved ejection fraction; T1 times below the median were associated with an increased risk of cardiac events compared with those above the median. T1 maps of the myocardium can be made without contrast, measuring the so-called native T1. For example, native T1 is increased in amyloidosis as compared with both normal controls and patients with aortic stenosis because of the markedly expanded extracellular space. Native T1 is also elevated in both dilated and hypertrophic cardiomyopathies as compared with controls. Thus, native T1 mapping identifies myocardial diseases associated with expanded extracellular space. This approach may prove particularly useful in patients with stage 4 or 5 kidney disease who are not candidates for receiving gadolinium contrast due to concerns regarding nephrogenic systemic fibrosis. Native T1 mapping gives investigators a general idea of the state of extracellular space, but is not equally as quantitative as the methods to measure ECV as described below. Measuring ECV is performed by mapping T1 before and after either a bolus injection or equilibrium infusion of gadolinium. The latter 2 methods have been shown to be essentially equivalent. ECV is determined by calculating the volume of...
distribution ($V_d$) of gadolinium assuming an equilibrium between its concentration in the myocardium and blood pool at multiple time points postinfusion and must be corrected for the hematocrit.\textsuperscript{20} Normal ECV is generally in the range of 24% to 28%.\textsuperscript{40} The measurement of ECV and $V_d$ has been performed in several myocardial conditions to assess fibrosis, including dilated cardiomyopathy,\textsuperscript{41} aortic stenosis, and hypertrophic cardiomyopathy.\textsuperscript{42} $V_d$ measured by T1 mapping in aortic stenosis and hypertrophic cardiomyopathy correlated strongly with histological measures in surgical biopsy with $r^2$ values of 0.86 and 0.62, respectively. Collagen content averaged between 17% and 21% in the 2 conditions. Thus, T1 mapping with measurement of ECV is a promising method to assess the state of interstitium in health and myocardial disease.

**Targeted Collagen Imaging**

Because collagen I is the main component of fibrosis, its targeting has been of wide interest for molecular imaging in cardiac disease. Most experience has been obtained with a collagen-avid peptide using nanoparticle-enhanced MRI probe GKWHCTTKFPHHYCLY (named EP3533) coupled with 3 gadolinium molecules. This nanoprobe demonstrates micromolar affinity ($K_d=1.8 \times 10^6$ mol/L) for collagen I\textsuperscript{43}, and its low molecular weight (5 kDa) allows easy diffusion into the interstitial space and efficient renal clearance. This targeted MRI has been successfully used to visualize collagen I in murine models of pulmonary\textsuperscript{44} and liver\textsuperscript{45} fibrosis. Successful collagen imaging using this particle has been demonstrated in a mouse model of MI (Figure 3A).\textsuperscript{21} Dynamic MRI at 6 weeks post-MI revealed probe uptake in infarcted areas as shown by gadolinium MR and histology. Collagen-avid peptide showed longer myocardial retention compared with gadolinium alone, mostly in the infarct zone (194.8±116.8 versus 25.5±4.2 minutes) as compared with normal myocardium (45.4±16.7 versus 25.1±9.7 minutes). CNA35 is another collagen-avid peptide with high affinity to collagen I. CNA35-based probes have been used for targeting vascular collagen in animal models of abdominal aneurysm, atherosclerosis, and colon cancer using MRI, 2-photon laser scanning microscopy, and $\gamma$-imaging, respectively.\textsuperscript{47–49} CNA35 has not been evaluated for cardiac

---

**Figure 1.** Electron microscopic and histopathologic characterization of extracellular matrix. **A.** Electron microscopic image of extracellular matrix, showing large bundles of dense collagen, a capillary vessel with visible nucleus of the endothelial cell (EC), and an extravasated red cell (RC). Microphotographs of collagen deposition in replacement fibrosis in healed myocardial infarction (HMI; B1–B4) and reactive fibrosis in dilated cardiomyopathy (DCM; C1–C4), stained with Movat pentachrome (MSC; B1, B2, C1, and C2) and antibodies against collagen I (Col I; B3, B4, C3, and C4). MSC clearly distinguishes between collagen (green) and muscle (red) tissue. For overview, graphs at low magnification (B1, B3, C1, and C3) and graphs at higher magnification (B2, B3, C2, and C4) are shown. In HMI, MSC overview graph (B1) shows subendocardial replacement fibrosis that has followed necrotic cardiomyocyte loss. High-magnification graph (B2) demonstrates severe subendocardial and interstitial fibrosis and bundles and isles of cardiomyocytes that, although still viable, show myofibrillolysis. Col I staining (B3 and B4) highlights the areas of replacement fibrosis. In DCM, MSC overview graph (C1) shows prominent endocardial fibrous thickening and fine, focal interstitial fibrosis. High-magnification graph (C2) shows high-magnification graph (C4) shows patchy areas of interstitial fibrosis in more detail. All overview microphotographs were acquired with Aperio ScanScope CS (Aperio Technologies, Inc, Vista, CA), and detailed graphs were obtained from these microphotographs.
Imaging Myofibroblasts and RAS

The cardiomyocyte stretch (resulting from overload states or ECM destruction) triggers the production of angiotensin-converting enzyme (ACE) and angiotensin I, and hence angiotensin II. Angiotensin confers most of its cardiac effects through angiotensin II receptor type 1 (AT1R), which in the myocardium is mainly present on myofibroblasts and also fibroblasts. This induces transforming growth factor-β, which is responsible for fibroblast differentiation into collagen-secreting myofibroblast subtype and cardiomyocyte hypertrophy. Because RAS and myofibroblasts are key determinants of fibrosis, they constitute attractive targets for molecular imaging. Given their close relationship, imaging studies are discussed together here. Dilsizian et al provided the proof of concept for imaging the local RAS system. They incubated sections of explanted hearts from 3 patients with ischemic cardiomyopathy with 18F-benzoyl-lisinopril and showed uptake adjacent to regions with collagen deposition. Also, lisinopril was labeled with 99mTc and evaluated for myocardial ACE-1 targeting in ACE-1–overexpressing transgenic rats (Figure 4A). Using in vivo SPECT imaging, they confirmed higher radiotracer uptake in transgenic than in control animals. Gamma well counting confirmed 5-fold higher uptake. AT1R has been evaluated as a target for myofibroblast imaging. In a study by Verjans et al, 2 probes targeted at angiotensin receptors were evaluated in a mouse model of MI (Figure 4B and 4C). First, a fluorescent-labeled angiotensin peptide analog was used. Intravital microscopy revealed low uptake in unmanipulated animals or early after MI. Accumulation substantially increased from week 1 to week 12 after MI, peaking at 3 weeks. Immunofluorescent colocalization study showed that angiotensin peptide analog targeted α-smooth muscle actin–positive collagen-producing myofibroblasts. In a second set of animals, they performed in vivo and ex vivo SPECT imaging with 99mTc-losartan, which specifically targets AT1R. Uptake was compared in animals at 3 weeks post-MI compared with unmanipulated controls. Gamma well counting revealed a 2.5-fold higher uptake in the MI group. Although this is an interesting proof of principle study, this target-to-background ratio may be too low for imaging of myocardial scarring in clinical situation. Moreover, although tracer uptake was also higher in border zone and remote zone in infarct animals, the increase was smaller and did not reach statistical significance. Therefore, imaging of collagen deposition in remodeling myocardium will likely be difficult with aforementioned tracers. Similar results were recently obtained using a nonpeptide AT1R-selective antagonist (SK-1080) labeled with C-11 (and referred as KR31173). In a pig model of MI, there was increased myocardial retention of the tracer in infarct and remote areas. Pilot clinical imaging experiments were also conducted as part of the same study, which showed that imaging was feasible and safe. Although tracer uptake was lower than in porcine experiments, radiotracer retention was high enough to allow PET imaging; tracer specificity might be limited because the administration of cold ligand only resulted in a 54% reduction in tracer uptake. Beside imaging AT1R receptors to image the myofibroblast, αVβ3 integrin has also been evaluated using 99mTc–Cy5.5 RGD imaging peptide (CRIP, which carries both the radiolabel and a red fluorescence) in a mouse MI model (Figure 5A). Mice were imaged at 2, 4, and 12 weeks after infarction. Intense uptake was seen in infarct area at 2 weeks and declined over time. However, uptake in remote area increased imaging, but its avidity to the target and feasibility of in vivo imaging suggest its potential applicability in the characterization of deposition of new collagen. Finally, the feasibility of collagen imaging in a mouse model of MI using a peptidomimetic of platelet collagen receptor glycoprotein VI, called collagelin, has been shown (Figure 3B). Biotin-collagelin bound to collagen with a Kd of 10−7 mol/L in vitro. Collagelin labeled with 99mTc and in vivo SPECT and planar imaging showed high tracer uptake in infarcted zones; no radiotracer uptake was seen in unmanipulated mice or in MI mice receiving 99mTc-labeled control peptide. Autoradiography confirmed tracer uptake in regions with collagen deposition. Collagelin imaging was also demonstrated in pulmonary fibrosis.
(albeit statistically not significantly) over time, with uptake at 12 weeks being higher than at 4 weeks, indicating that it might become possible to target remote remodeling. Specificity of the radiotracer was confirmed by using a scrambled version of the RGD peptide. Binding of the peptide to myofibroblasts was confirmed by electron immunoelectron microscopy and immunofluorescence experiments. Radiotracer uptake correlated with the presence of newly formed collagen fibers. In the subsequent study, 99mTc-CRIP imaging was used to evaluate the therapeutic effects of captopril, losartan, and spironolactone, individually or in combination. Radiotracer infarct uptake of 2.3% injected dose per gram of tissue (%ID/g) in untreated animals reduced to 1.7% ID/g by single treatment using any of the 3 drugs and further reduced by a combination of 2 or 3 drugs (1.31% and 1.16% ID/g, respectively). As in the previous study, radiotracer uptake correlated with the extent of myofibroblast accumulation and the presence of newly formed collagen fibers; the treatment did not reduce collagen formation in the scar. Lower radiotracer uptake in infarct area reflected reduced presence of newly formed collagen fibers, which was probably due to increased rate of maturation of collagen. In the remote area, total collagen deposition was reduced. Following these promising initial studies, clinical feasibility of myofibroblast imaging of CRIP without the Cy5.5 label (RIP) was evaluated in 10 patients with MI (Figure 5B). The infarct area was evaluated by myocardial perfusion imaging in the first week, followed by 99mTc-RIP imaging at 3 and 8 weeks. In 7 patients, myocardial uptake of RIP was observed. In these, uptake was usually seen in and around the infarct area. In 2 patients, diffuse uptake was seen. Importantly, scarring at 1 year, as shown by late-enhanced MRI, colocalized with the regions of RIP uptake.
indicating that it might become possible to predict subsequent (ensuing) fibrosis. Before these studies evaluated RGD-driven targeting of αVβ3 integrin to show myofibroblast, RGD tracers had been explored to visualize angiogenesis, because αVβ3 integrin was also expressed on endothelial cells of proliferating vessels. RGD imaging has been performed in animal models and patients with malignant tumors.64,65 RGD-based probes have also been explored for imaging angiogenesis early (1–3 weeks) after MI in rats and dogs.66 Uptake of the radiotracer was seen in regions of perfusion deficit as shown by serial 201Tl imaging. Specificity of the radiotracer was confirmed by using a nonspecific tracer, and radiotracer uptake correlated with αVβ3 expression in the hearts. They demonstrated increased presence of newly formed vessels, but exact mechanism of tracer targeting in the myocardium was not explored. Higuchi et al67 also evaluated 18F-galacto-RGD, a αVβ3-avid PET tracer in a rat MI model. Using PET and autoradiography, they obtained results similar to those with CRIP; uptake peaked between 1 and 3 weeks and declined over time. They showed that increased uptake of galacto-RGD was associated with increased expression of αVβ3 integrin and formation of new vessels; the study did not determine the mechanism of radiotracer targeting. In a multivariate analysis, 18F-galacto-RGD uptake at 1 week post-MI was a significant predictor of increase in MRI-verified left ventricular end-diastolic diameter at 12 weeks.68 The feasibility of clinical use of 18F-galacto-RGD was also demonstrated in 1 patient at 2 weeks post-MI69 (Figure 5C). Uptake was seen in infarct area as demonstrated by 13NH3 perfusion defect and LGE MRI imaging.

**Imaging Matricellular Proteins**

Matricellular proteins, such as tenasin C, SPARC, thrombospondins, and osteopontin,16,17 play an important role in tissue development. Although hardly expressed in normal interstitium, they are strongly upregulated after tissue injury. Involved in inflammatory, angiogenic, and fibrotic responses, their role has been implicated in aging, MI, myocarditis, diabetic cardiomyopathy, and volume and pressure overload states. Antitenasin antibodies by direct90 and pretargeting91 approaches have been used for clinical targeting of tenasin C to localize gliomas.111In-labeled antitenasin C monoclonal antibody Fab’ fragment has also been used to detect experimental

---

**Figure 4.** Targeted micro-SPECT (single photon emission computed tomography)/CT imaging of renin–angiotensin system. A. In vivo 99mTc-lisinopril micro-SPECT/CT images in control rats (A1), angiotensin-converting enzyme (ACE-1)–overexpressing transgenic rats (A2), and ACE-1–overexpressing transgenic rats after cold lisinopril pretreatment (A3). No myocardial uptake is seen in wild-type mice, but intense myocardial radiotracer uptake is seen in ACE-transgenic animals (yellow arrows). Cold lisinopril pretreatment significantly reduced cardiac and pulmonary radiotracer uptake (white arrows). Adapted from Dilsizian et al with permission of the publisher.99 Copyright © 2012, the American College of Cardiology Foundation. B. Intravital microscopy images after administration of green fluorescent angiogenin peptide analog in a control mouse (B1) and in mice at 3 (B2) and 12 (B3) wk post-myocardial infarction (MI). Uptake increases up to 3 wk and decreases thereafter. Adapted from Verjans et al with permission of the publisher.60 Copyright © 2008, American College of Cardiology Foundation. SPECT (C1) and SPECT/CT (C2) imaging after administration of 99mTc-losartan in a control mouse show no myocardial uptake, whereas SPECT (C3) and SPECT/CT (C4) images in a mouse at 4 wk post-MI show intense myocardial uptake (arrows). Adapted from Verjans et al.60 Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
myocarditis in rats, confirmed by 7.5-fold higher cardiac uptake in myocarditis rats. The feasibility of detecting tenascin localization was also demonstrated in cardiac repair after MI using a different antibody Fab fragment labeled with 111In (Figure 3C). Dual-isotope SPECT imaging demonstrated distinct tracer uptake in the infarct as shown by 99mTc-sestamibi; minimal epicardial uptake was seen in sham-operated mice corresponding to slight inflammation caused by thoracotomy. No uptake was seen with control antibody. Uptake of antitenascin C monoclonal antibody fragment labeled with 125I has been evaluated in rat MI models at multiple time points. Tracer uptake in the 20-minute ischemia model peaked at day 3 and declined over time with faint uptake seen at day 7 and suggested that probes such as those directed against tenascin may be used to monitor cardiac repair process, the extent of which depends on the severity of tissue damage. To reduce half-life and increase resolution, 111In-single-chain antitenascin C fragment was produced, and increased uptake in the infarct was demonstrated. Finally, a nanoparticle simultaneously targeting tenascin C, αvβ3 integrin, as well as nucleolin showed increased specificity and signal intensity in various cancer cell lines when compared with single-target probes. A similar multitargeting approach could also be viable in cardiac imaging.

**Imaging Tissue Transglutaminase**

Tissue transglutaminases (blood coagulation factor XIII, FXIII), beside its hemostatic role, mediate collagen deposition and crosslinking after tissue damage. Its activity and role in myocardial infarct healing has been well documented using an 111In-labeled FXIII-affinity peptide. The importance of FXIII was underscored by the fact that FXIII−/− and FXIII−/+ mice had 100% mortality <5 days after MI. Although FXIII suppletion...
prevented mortality in FXIII−/− animals, MRI showed worse remodeling and left ventricular ejection fraction decline in these animals. Autoradiography of hearts explanted from animals receiving an 111In-labeled FXIII-sensitive probe in wild-type animals after MI demonstrated increased uptake in infarct area compared with remote area. In FXIII−/− mice, no uptake in infarct area was seen; suppletion of FXIII resulted in the uptake of probe in infarct area. FXIII−/− showed 53% lower collagen 1 in the infarct, and 650% higher MMP-9 activity. A subsequent study, using in vivo 111In-FXIII SPECT imaging,78 demonstrated increased uptake of the probe in infarct area of wild-type mice, but not in FXIII−/− mice; FXIII suppletion increased uptake, reduced MRI-verified remodeling, and improved collagen deposition in infarct area. Anti-FXIII therapy by dalteparin reduced FXIII uptake and resulted in early mortality by cardiac rupture <5 days of the infarct in all animals.

In a recent study, gadolinium-MPO MRI and PET imaging were used to evaluate the efficacy of anti-inflammatory therapy and its effect on matrix crosslinking79 (Figure 3D). After induction of MI, Apo E−/− animals received CCR2 siRNA; this therapy silences the CCR2 chemokine receptor that is responsible for the attraction of inflammatory Ly-6C+ monocytes. Dual-target PET/MRI revealed lower infiltration of MPO-rich leukocytes to the infarct area when compared with saline-treated animals, whereas FXIII activity was not affected; cine MRI showed that therapy reduced left ventricular dilatation and improved left ventricular ejection fraction. Flow cytometry, histology, and gene expression demonstrated reduced monocyte infiltration and enhanced the resolution of inflammation, whereas formation of new vessels, collagen 1 deposition, and presence of myofibroblasts were not affected.

Imaging Matrix-Degrading Enzymes

MMPs are zinc-dependent proteases80 that play a role in physiological ECM turnover and response to cardiac disease. MMP upregulation during pathological states allows adaptive changes such as infiltration of inflammatory cells, which facilitate removal of cellular debris, migration of cardiomyocytes and myofibroblasts, as well as promote angiogenesis and novel matrix deposition. Although MMP activity can counterbalance the increased ECM deposition, excessive ECM degradation may result in wall thinning, dilatation, and predispose to heart failure, or aneurysm formation and cardiac rupture.18,19 MMPs are inhibited by tissue inhibitors of metalloproteinases 1–4.20 MMP imaging has taken advantage of 2 principles: (1) creating tissue inhibitors of metalloproteinase-like peptides that inhibit either a wide range of MMPs or inhibit a specific MMP; these peptides if appropriately labeled may allow assessment of the magnitude of MMP production, or (2) using a substrate amenable to proteolytic cleavage by MMP, the cleaved reporter molecule accumulates in the tissue and can be detected by imaging. As with many imaging agents, the proof of principle of imaging of MMPs was obtained in tumors.81–87 Substantial studies with MMP imaging have been undertaken in the imaging of atherosclerotic plaques wherein the MMPs have been implicated in plaque instability and fibrous cap thinning. Schäfers et al reported the feasibility of MMP imaging using 123I-labeled broad-spectrum MMP inhibitor (CGS 27023A) in a carotid ligation model of atherosclerosis in ApoE−/− mice.88 This probe was also labeled with C-1181 and F-1889 to allow PET imaging; the results, however, remain preliminary. A broad-spectrum MMP inhibitor has also been labeled with a SPECT tracer (99mTc-RP805). After establishing the specific binding of the probe to MMP, in vivo imaging was performed in a murine model of chronic MI (Figure 6A). Dual-isotope imaging showed increased uptake of the radiotracer in myocardial perfusion defect as shown by 201Tl at 1, 2, and 3 weeks post-MI. Gamma counting confirmed a 5-fold increase in radiotracer uptake, expressed as %ID/g, in the infarct area at 1 week, followed by a declining trend. A 2-fold increase in radiotracer uptake was also observed in the remote area, suggesting remote MMP activation.90 In a subsequent study in a porcine model of MI, cine MRI and dual-isotope SPECT/CT using 201Tl for evaluation of perfusion and RP805 were performed at 1, 2, and 4 weeks post-MI. Tracer retention in the infarct area peaked with a 4-fold increase at 1 week and remained upregulated until 4 weeks post-MI; radiotracer uptake correlated with MMP activity and an MRI-verified increase in left ventricular diastolic diameter.91 99mTc-RP805 has also been successfully used to visualize atherosclerotic plaques and effect of pharmacological and dietary therapy in mouse and rabbit models of atherosclerosis.92–95 Specificity of the tracer and correlation with MMP2 and MMP9 activity and presence of macrophages was shown.92 Also, imaging with
"111In-RP782, a homolog of 99mTc-RP805, has been undertaken in animal models of MI, atherosclerosis, carotid damage, and carotid aneurysms. The feasibility of MRI of MMP using gadolinium-labeled probe (P947) has been demonstrated in rat model of aortic aneurism, mouse and rabbit models of atherosclerosis, and excised human carotid atherosclerotic plaques. This probe binds not only to MMPs but also to other enzymes such as ACE, endothelin-converting enzyme, neutral endopeptidase, and aminopeptidases A and N. In addition to nuclear and magnetic probes, fluorescent peptides have been developed and feasibility of MMP by NIRF imaging evaluated in atherosclerosis. Also, a NIRF probe that is activated through proteolytic cleavage by MMP-2 and MMP-9 was used to evaluate MMP activity after MI. Different groups of mice received the probe at different time points after induction of MI, and MMP activity was evaluated by ex vivo NIRF imaging. The activation of the probe was seen in the infarct area, peaked at 1 week, and remained increased until 4 weeks after MI, which was the latest time point of evaluation. Fluorescence microscopy and flow cytometry demonstrated that MMPs were leukocyte-derived. In a subsequent study, leukocyte infiltration and protease activity after MI were further evaluated (Figure 6B) with 2 different molecular probes: a fluoromagnetic iron oxide nanoparticle (CLIO-VT750), which is ingested by phagocytes, and a fluorescent probe, which is activated by proteolytic activity of cathepsins, mostly type B (prosense-680). Using in vivo fluorescent molecular tomography and coregistration of MRI and FMT imaging, both modalities also demonstrated the accumulation of CLIO-VT750 in hypokinetic infarct area. Using the dual-channel FMT protocol, they demonstrated that cathepsin activity as shown by prosense-680 FMT peaked at day 4 after MI, and phagocytosis as shown by CLIO-VT750 FMT peaked at day 6. Both probes also showed a significantly lower signal in FXIII−/− mice. This paralleled fluorescence microscopy and flow cytometry results showing markedly lower cathepsin B activity and ≈55% lower infiltration of macrophages and neutrophils.

**Future Perspectives**

ECM imaging using gadolinium-enhanced MRI has become clinically available, and volume assessment using T1 mapping is finding more frequent use for staging disease and determining the effects of (novel) therapies in clinical and preclinical setting. Molecular imaging is likely to add the understanding of pathophysiological mechanisms and insight in the rate of disease progression.
Several probes designed for molecular imaging have been used, and an extensive preclinical and preliminary clinical experience has been acquired with RGD probes $^{99m}$Tc-CRIP and $^{18}$F-galacto-RGD. MMP probe RP-805, and collagen-targeted probe EP-3533. Various additional targets would be worthy of investigation. For instance, the components of RAS and related signaling pathways could be of considerable interest. Notably, the cell-secreted enzyme chymase seems to be a major contributor to cardiac angiotensin II production and is, therefore, a worthy target. Also, galectin-3, which is thought to be a bridging factor between inflammation and fibrosis and a commonly studied circulating biomarker in patients with heart failure, holds promise.

Because of their role in myocardial healing and remodeling responses, matricellular proteins may help increase the understanding of the mechanisms of fibrosis in various disease processes. A SPARC-targeted peptide has been labeled with a nanoparticle that was also coupled with a fluorophore. The affinity for SPARC was confirmed in cancer cell lines, and the feasibility of optical imaging of prostate cancer and lung metastases was demonstrated in murine models. Also, thrombospondin receptor analogs labeled with $^{99m}$Tc have been used to visualize thromboembolism, and these or similar probes may be used to visualize cardiac thrombospondin upregulation. Although no imaging studies have been conducted, cytokines including tumor necrosis factor-$\alpha$, interleukin (IL)-1, IL-6, IL-18, and interferon-$\gamma$ also seem to be tempting targets. On one hand, their upregulation in response to infectious and injurious triggers is beneficial to conquer the insulting agent and required to initiate the healing response, but on the other hand, prolonged overexpression has been linked with worsening of ventricular function in heart disease.

The convergence of disciplines in medicine, molecular and cellular biology, and computer technology will likely translate into the development of better diagnostic and management strategies. It is important that we strive to understand the pathogenesis of disorder, the identification of worthy targets, which are prominently overexpressed in the diseased tissue, should provide a basis for hotspot imaging. Molecular imaging is a unique research discipline with the potential to detect disease in preclinical and early stages, and the only feasible strategy that may noninvasively, quantitatively, and repetitively image biological processes at both cellular and subcellular levels within a living organism. Molecular targeting provides better understanding of the disease process and subcellular pathogenetic processes and unravels more targets worthy of imaging. Molecular imaging is not limited by imaging modality, and once the target and targeting agent is identified, an appropriate reporting tracer can be selected. It is mandatory that an effort be made to express the conceptual premises and practical execution in a simplified manner so as to stimulate close collaboration among clinicians, biologists, and imagers alike. Molecular imaging teaches us there are plenty targets in health and disease, each with a story to tell; imaging and imagers are the storytellers.

Conclusions
Myocardial fibrosis is a complex process in which a wide variety of ECM components play a regulatory role. Targeted imaging techniques to visualize these components may serve as research tools yielding further insights in the mechanisms of fibrosis. Also, they could aid the development of therapies modulating fibrotic response. Lastly, imaging of key players in the process could predict which patients will develop fibrosis and cardiac functional deterioration, thereby guiding therapy decision making. Future studies will explore more targets and clinically evaluate the most promising tools.

Sources of Funding
Supported by National Institutes of Health/National Heart, Lung, and Blood Institute R01 HL 078681 and R01 HL 68657 grants to J.N., and European Union INHERITANCE project 241924, Health-2009-2.4.2-3, and RF Italian Ministry of Health 2010 to E.A.

Disclosures
None.

References


Molecular Imaging of the Cardiac Extracellular Matrix
Hans J. de Haas, Eloisa Arbustini, Valentin Fuster, Christopher M. Kramer and Jagat Narula

Circ Res. 2014;114:903-915
doi: 10.1161/CIRCRESAHA.113.302680
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
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/114/5/903

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

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

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