Advances in molecular imaging of atherosclerosis and myocardial infarction: shedding new light on in vivo cardiovascular biology

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Phinikaridou A, Andia ME, Shah AM, Botnar RM. Advances in molecular imaging of atherosclerosis and myocardial infarction: shedding new light on in vivo cardiovascular biology. Am J Physiol Heart Circ Physiol 303: H1397–H1410, 2012. First published October 12, 2012; doi:10.1152/ajpheart.00583.2012.—Molecular imaging of the cardiovascular system heavily relies on the development of new imaging probes and technologies to facilitate visualization of biological processes underlying or preceding disease. Molecular imaging is a highly active research discipline that has seen tremendous growth over the past decade. It has broadened our understanding of oncologic, neurologic, and cardiovascular diseases by providing new insights into the in vivo biology of disease progression and therapeutic interventions. As it allows for the longitudinal evaluation of biological processes, it is ideally suited for monitoring treatment response. In this review, we will concentrate on the major accomplishments and advances in the field of molecular imaging of atherosclerosis and myocardial infarction with a special focus on magnetic resonance imaging.

atherosclerosis; imaging; MRI; myocardial infarction

Imaging of Atherosclerosis

Atherosclerosis-related cardiovascular complications, primarily myocardial infarction (MI) and stroke, are estimated to become the leading cause of death worldwide by the year 2020 (117). As “high-risk vulnerable plaques” are often undetected until they cause life-threatening adverse events, an increasing effort has been made to better understand plaque biology and vulnerability with a special focus on the development of new targeted molecular imaging agents. Such agents may allow in vivo 1) monitoring of plaque progression, 2) detecting of vulnerable plaque, 3) monitoring of the effectiveness of interventions, and 4) guiding of patient-specific medical treatment. Currently, a plethora of targeted imaging agents are available for the visualization of the major biological processes involved in atherosclerosis both for experimental and clinical utilization. Some of these applications are summarized in Fig. 1.

Endothelial permeability and activation. Increased endothelial cell permeability, dysfunction, and expression of surface adhesion molecules (E- and P-selectin) are considered to be the initiating events in atherogenesis. Increased endothelial leakage allows circulating low-density lipoprotein particles (LDL) (73) to passively diffuse into the vessel wall, whereas expression of adhesion molecules is responsible for the receptor-mediated recruitment of leukocytes (30). Additionally, endothelial dysfunction defined as the decrease in the bioavailability of nitric oxide promotes platelet activation and smooth muscle cell (SMC) proliferation (30). Functionally, endothelial dysfunction is associated with a paradoxical vasoconstriction in response to acetylcholine both in humans (98) and apolipoprotein E-deficient (ApoE−/−) mice (7, 28). It precedes angiographic evidence of coronary artery disease (207) and is a predictor of future cardiovascular events (52). Recently, contrast-enhanced magnetic resonance (MR) imaging (MRI) with gadofosveset has been used to image endothelial permeability in an ApoE−/− mouse model of accelerated atherosclerosis (139) (Fig. 1). Gadofosveset is a clinically approved gadolinium-based contrast agent that reversibly binds to serum albumin, resulting in a prolonged vascular presence and a 5–10-fold increase in relaxivity (r1) (16, 92). Gadofosveset uptake was associated with mechanically damaged endothelium in a swine model of coronary injury (138), atherosclerosis-associated endothelial damage in ApoE−/− mice (139) (Fig. 1), and leaky neovessels in human carotid and rabbit aortic plaque (96, 97). Furthermore, Val-His-Ser-Pro-Asn-Lys-Lys-modified magnetofluorescent particles targeting the vascular cell adhesion molecule-1 (VCAM-1) receptor (74, 121) and microparticles of iron oxide...
Fig. 1. Examples of available imaging modalities and targets for atherosclerosis. Endothelium, molecular MRI imaging of increased endothelial permeability in the brachiocephalic artery of apolipoprotein E-deficient (ApoE^{-/-}) mice using gadofosveset and complementary Evan’s blue staining (139). MRI detection of endothelial adhesion molecules (VCAM-1 and P-selectin) in the aortic root of ApoE^{-/-} mice using dual-targeted microparticles of iron oxide (MPIO) (103). Inflammation (macrophages), MRI of macrophage content in the brachiocephalic artery of ApoE^{-/-} mice after administration of very small iron oxide particles (VSOP) using susceptibility gradient mapping (100). MRI of macrophages in the abdominal aorta of ApoE^{-/-} mice using immunonanocles targeting the macrophage scavenger receptor [used with permission (3)]. Lipids, MRI of plaque lipid using recombinant LDL-like gadolinium(Gd)-based nanoparticles in the thoracic aorta of ApoE^{-/-} mice (213) [reproduced by permission of The Royal Society of Chemistry]. MRI of plaque lipid using recombinant HDL-like gadolinium-based nanoparticles in the abdominal aorta of ApoE^{-/-} mice (41). Ex vivo MRI detection of lipids in human endarterectomy plaque using diffusion-weighted imaging (DWI) (143). Enzymes, MRI of myeloperoxidase activity in atherosclerotic plaque of cholesterol-fed rabbits using an enzyme-sensitive gadolinium-based contrast agent, bis-5-hydroxytryptamine (5-HT) -diethylenetriaminepentacetate (DTPA)(Gd) [myeloperoxidase (MPO)(Gd)] (150). Apoptosis, MRI of apoptosis in the abdominal plaque of ApoE^{-/-} mice using annexin A5-functionalized bimodal nanoparticles (196). Neovascularization, MRI of angiogenesis in ApoE^{-/-} mice using a low molecular weight peptidomimetic of Arg-Gly-Asp (mimRGD) grafted to gadolinium diethylenetriaminepenta-acetate (Gd-DTPA-g-mimRGD) (12). Extracellular matrix (elastin), MRI of intraplaque elastin in the brachiocephalic artery of ApoE^{-/-} mice using a low molecular weight gadolinium-based contrast agent (101). Extracellular matrix (collagen), ex vivo MRI detection of collagen-rich fibrous cap in human endarterectomy plaques using magnetization transfer (142). Thrombus, MRI direct thrombus imaging (MRDTI) due to the T1 shortening effect of methemoglobin (71). MRI of thrombus associated with plaque rupture in a rabbit model using a fibrin-binding gadolinium-labeled peptide (EP-1873) (9). All images were reprinted and/or adopted with permission.
drop visible on T2-weighted (T2W) and T2*-weighted (T2*W) MR images 24–48 h after administration. Histological correlation showed colocalization of the magnetic nanoparticles with macrophage-rich regions of the plaque, whereas little uptake was observed in SMCs and endothelial cells. Magnetofluorescent nanoparticles that combine MRI with optical imaging properties by additionally carrying a fluorescent moiety, e.g., near-infrared fluorescent (NIRF), are also available and have been used to quantify the cellular distribution of nanoparticles (70). 64Cu-labeled nanoparticles allowed for PET/CT imaging (127) and crystalline iodinated particles (N1177) for CT imaging (65) of macrophages. Receptor-based macrophage imaging has also been achieved by using gadolinium-loaded micelles targeting the macrophage scavenger receptor (Fig. 1) (3), the cannabinoid receptor, and neutrophil gelatinase-associated lipocalin 2 in murine plaques (3, 116, 183, 184).

On a functional level, the glucose analog [18F]-fluorodeoxyglucose ([18F]FDG), which competes with glucose for uptake into metabolically active cells, has been used to image inflammatory cell activity noninvasively by PET with or without complementary CT or MRI scans in humans (151, 159) and rabbits (13). Experiments in humans showed that the net [18F]FDG accumulation rate (plaque/integral plasma) in symptomatic lesions was 27% higher than in the contralateral asymptomatic lesions. There was no measurable [18F]FDG uptake in angiographically normal carotid arteries. Autoradiography of excised plaques confirmed accumulation of deoxyglucose in macrophage-rich areas of the plaque.

Imaging plaque lipids is also essential as lipid-rich plaques are more likely to rupture. 99mTc-labeled LDL particles have been used to image subjects with large tendon xanthomas secondary to homozygous familial hypercholesterolemia or sitosterolemia over 20 years ago (47). On the molecular level, gadolinium-loaded LDL-based nanoparticles (GdDO3A-OD-LDL) (Fig. 1) (213) and recombinant HDL-like nanoparticles (Fig. 1) (25, 41) have also been developed to image atherosclerosis in mice. Furthermore, the oxidized LDL receptor (LOX-1) and oxidized plaque LDL particles have been imaged using antibodies that bind to the LOX-1 receptor (94) and oxidation-specific epitopes (11, 186, 191) using single photon emission computed tomography (SPECT), CT, and MRI. Finally, diffusion weighted imaging (DWI) that generates endogenous contrast between plaque components based on the characteristic diffusion coefficients of water molecules has been used to visualize plaque components including lipids both in vivo and ex vivo (Fig. 1) (143, 187).

Enzymatic activity and apoptosis. Matrix metalloproteinases (MMPs) degrade extracellular matrix proteins including elastin and collagen and therefore participate in plaque destabilization (43, 50, 163, 179). MMP expression was shown to be increased in vulnerable atherosclerotic plaque undergoing positive arterial remodeling (136, 156). In vivo SPECT using a 125I- or 123I-radiolabeled agent derived from a compound that binds to the active catalytic site of MMPs (58) demonstrated focal signal enhancement of carotid plaque in atherosclerotic apoE/−/− mice (154). In vivo and ex vivo MRI for the detection of MMP-rich plaques was achieved with the use of a gadolinium-based MMP-sensitive contrast agent (P947) (91). Gadolinium quantification and MRI showed a preferential accumulation of P947 in rabbit atherosclerotic lesions compared with the nontargeted compound Gd-DOTA. Moreover, with the use of human carotid endarterectomy specimens, P947 facilitated discrimination between histologically defined MMP-rich and MMP-poor plaques.

Myeloperoxidase (MPO) is secreted by activated macrophages at multiple stages of plaque development and generates the oxidant species hydrochlorous acid (10, 29, 129, 177, 178) that modifies LDL, activates MMPs, induces endothelial cell apoptosis, inactivates nitric oxide, and triggers tissue factor release. In vivo MR imaging of MPO has been achieved with the development of a gadolinium-based MO sensor bis-5-hydroxypamine-diehydroyetraminepentacacetate (DTPA)(Gd) [MPO(Gd)] in rabbit atherosclerotic plaques (Fig. 1) (144, 150).

Cathepsins expressed by plaque macrophages, endothelial cells, and SMCs can also degrade extracellular matrix proteins via their elastase and/or collagenase properties and promote plaque destabilization (180). In vivo imaging of cathepsin B was achieved with protease-activated NIRF probes (20, 130, 206). NIRF imaging, using this probe, and in combination with MRI showed that murine atherosclerotic lesions were fluorescently labeled 24 h postinjection and the signal colocalized with cathepsin B and macrophage immunopositive regions. Recently, a new two-dimensional intravascular NIRF pullback catheter combined with a cysteine protease-activatable probe (ProSense) provided high-resolution in vivo mapping of arterial inflammation in coronary-sized arteries and revealed increased inflammation in atheromatous plaque and in stent-induced arterial injury (69).

Lastly, cellular apoptosis is also a key feature of plaque progression and stability. Imaging of apoptosis is mainly based on targeting annexin A5 that binds to phosphatidylserine, a membrane phospholipid that becomes externalized on the extracellular leaflet of the cell membrane in apoptotic cells. Apoptosis has been visualized ex vivo with 99mTc-annexin injected in atherosclerotic rabbits (86). Quantitative annexin uptake was 9.3-fold higher in lesion versus nonlesion areas and the uptake paralleled lesion severity and macrophage burden. No correlation was observed with SMCs. In vivo detection of plaque instability using 99mTc-annexin A5 was also shown in patients with carotid-artery atherosclerosis 6 h postinjection of the tracer (77). Higher tracer uptake correlated with histopathological features characteristic of unstable plaque, including substantial macrophage infiltration and intraplaque hemorrhage. Immunohistochemical analysis demonstrated specific binding of annexin A5 to macrophage cell membranes. Recently, MRI and fluorescence imaging of apoptosis has been achieved using annexin A5-functionalized bimodal nanoparticles in murine atherosclerotic plaques (Fig. 1) (196). In vivo T1-weighted (T1W) MRI of the abdominal aorta revealed enhanced uptake of the annexin A5-micelles compared with control-micelles, which corroborated with ex vivo NIRF images of the excised aortas. Confocal laser scanning microscopy demonstrated that the targeted agent was associated with macrophages and apoptotic cells, whereas the nonspecific control agent showed no clear uptake by such cells.

Neovascularization. Neovascularization is also considered a vulnerability component since it can promote intraplaque hemorrhage and cholesterol deposition (113, 201). Dynamic contrast-enhanced MRI using extravascular-nonspecific gadolinium contrast agents, e.g., Gd-DTPA (13, 75, 76) and intravas-
cular agents (B-22956/1, gadofosveset) (26, 95, 97), was shown to correlate with plaque neovascularization.

In addition to VCAM-1, endothelial cells of neovessels express integrin \(\alpha_\beta\) (63). Imaging of plaque neovessels has been achieved with gadolinium-coated perfluorocarbon nanoparticles conjugated with an arginine-glycine-aspartic acid (RGD) peptidomimetic molecule. In vivo MRI experiments performed in cholesterol-fed rabbits with intimal hyperplasia showed signal enhancement after administration of the \(\alpha_\beta\) targeting particles that colocalized with \(\alpha_\beta\) endothelial cells lining the vasa vasorum. The same particles have been used as vehicles for antiangiogenic drug delivery in rabbit aortas (211, 212). A low molecular weight peptidomimetic of Arg-Gly-Asp (mimRGD) grafted to gadolinium diethylenetriaminepentaacetae (Gd-DTPA-g-mimRGD) has also been used to image neovascularization in ApoE\(^{-/-}\) mice (Fig. 1) (12). Additionally, \(\alpha_\beta\) imaging in cancer and MI was also feasible using ultrasound (36), PET (54), SPECT (106), NIRF (90), and MRI (161) agents.

**Extracellular matrix and vascular remodeling.** The balance between the production and degradation of extracellular matrix proteins (collagen, elastin, proteoglycans) is essential for athrosa expansion and stability. Multicontrast in vivo MRI has been used to evaluate the thickness and integrity of the fibrous cap in human carotid disease (53, 109, 217). The recent development of a small molecular weight gadolinium-based elastin-targeting contrast agent enabled in vivo MR imaging of atherosclerosis progression in the brachiocephalic artery of ApoE\(^{-/-}\) mice (Fig. 1) (101) and visualization of vascular remodeling after stent-induced coronary injury in a swine model (202). Furthermore, CNA-35 functionalized nanoparticles have been used to image aortic aneurysm progression (84) and atherosclerosis (195). Ex vivo application of magnetization transfer was shown to discriminate the collagenous fibrous cap and the media from the lipid core and adventitia in human endarterectomy samples (Fig. 1) (133, 142).

The direction of vascular remodeling (expansive versus constrictive) and its association with plaque vulnerability have recently been explored. Traditionally, positive remodeling defined as the increase of the outer vessel wall area was considered advantageous since it prevents luminal obstruction (48). However, additional evidence showed that it is also associated with plaque vulnerability (137) and may lead to clinical sequelae including MI and stroke. In vivo assessment of vascular remodeling in humans has been primarily achieved with intravascular ultrasound and CT (66–68). Importantly, recent prospective studies using intravascular ultrasound and/or CT have identified plaque burden and positive arterial remodeling as a good surrogate marker for high-risk plaques (15, 115, 174). Noninvasive imaging of vascular remodeling by MRI has also been feasible in preclinical models of atherosclerosis (141, 173), patients with coronary (81, 107), and carotid artery disease (5, 194).

**Intraplaque hemorrhage and thrombus.** Intraplaque hemorrhage is a major contributor to plaque vulnerability, and mural thrombosis is the end point of plaque rupture that may initiate myocardial injury due to acute vessel occlusion. Noncontrast enhanced MR imaging of red blood cell-rich thrombus or hemorrhage relies on the changes of \(T_1\) and \(T_2\) relaxation times of different oxygenation states of hemoglobin in erythrocytes. This approach has been used to visualize and estimate the age of hematoma (24, 49), venous thrombosis (112, 145), intraplaque hemorrhage (111), arterial thrombus (27, 200), and intracoronary thrombus (Fig. 1) (71). Detection of thrombus size and protein content has also been achieved by magnetization transfer and diffusion weighted MRI applied ex vivo and in vivo (80, 140, 199, 200). Moreover, the development of targeted contrast agents for fibrin (Fig. 1) (8, 9, 38, 72, 162, 168–171, 175, 210), platelets (72, 85, 155), and \(\alpha_2\)-antiplasmin (108) significantly improved the detection and age characterization of thrombus. Activated platelets have additionally been targeted via their gpIIb/IIIa receptor for noninvasive molecular imaging of intravascular thrombosis (4, 203, 204). These contrasts agents should ideally help to better visualize thrombus, and also differentiate between acute and chronic thrombus and therefore guide further medical interventions.

**Imaging of MI**

Acute MI (AMI) is still one of the main causes of morbidity in Western countries (208). Myocardial ischemia progresses with the duration of coronary occlusion, and the delay in time to reperfusion determines the extent of irreversible necrosis extending from the subendocardial layers toward the epicardium. MRI is an extremely powerful modality for the assessment of function, perfusion, and tissue characterization in AMI in a single examination. In particular, it can detect the location and extent of necrosis (147), infarct size, myocardial edema (myocardium at risk), and microvascular obstruction leading to intramyocardial hemorrhage (44) and assess ventricular volumes and function for the evaluation of postinfarction remodeling (51). In this section we will focus on the use of MRI in the detection of the area at risk, infarct size, and salvaged myocardium.

**Detection of myocardial ischemia: area at risk, infarct size, salvaged myocardium.** The area at risk is defined as the myocardial area related to an occluded coronary artery with complete absence of blood flow, whereas the infarct size represents the subregion within the area at risk that undergoes irreversible damage and progresses to complete tissue necrosis. Subsequently, the salvaged myocardium corresponds to the region of the area at risk that experiences reversible damage. Studies have shown that the duration of ischemia is the major factor determining the fraction of the area at risk that progresses to become irreversibly damaged infarcted region (147, 148). Traditionally, the myocardium at risk has been measured by SPECT, using the injection of a technetium-based tracer (160), and by contrast echocardiography (68). Over the last years, both preclinical and clinical studies have shown that T2W MRI sequences, which are sensitive to the concentration of water-bound protons, can be used to visualize and quantify the area at risk. The high signal intensity on the T2W images is indicative of higher tissue water content and may reflect myocardial inflammation and tissue edema (Fig. 2) (42). In an early experimental study in dogs, the mean \(T_2\) relaxation time for 1-day-old infarcted regions was significantly higher from that of the remote myocardium. Concomitantly, the percent water content of the infarcted area was significantly greater than that of normal regions. Such changes resulted in a linear correlation between the \(T_2\) relaxation time and percent water content (59). Later studies in animal models verified the correlation of the area at risk measured by T2W MRI images...
with corresponding histological sections in swine (46) and canine (2) models of MI. The latter study also demonstrated that the T2 hyperintense area more closely resembled the hypokinetic zone identified on displacement encoding with stimulated echoes systolic strain maps. Interestingly, some of these hypokinetic areas showed a partial recovery of function on follow-up studies of chronic MI. Moreover, quantitative T2 mapping reliably identified myocardial edema without the limitations encountered by T2W short T2 inversion recovery imaging and may therefore be clinically more robust in showing acute ischemic injury (198).

For diagnostic purposes it is essential to accurately identify not only the area at risk but also the actual infarct size and salvaged myocardium to differentiate between irreversible (infarct) and reversible (edema, ischemia) areas of myocardial damage. Longer durations of ischemia generally produce more transmural infarcts as opposed to infarcts with increasing circumferential extent. Thus the transmural extent of the infarction is inversely related to the extent of salvaged myocardium. The extent of the salvaged area at risk contains prognostic information and may serve as a therapeutic target. A better differentiation between the areas of reversible and irreversible damage can be achieved using delayed enhancement imaging with nonspecific gadolinium contrast agents (e.g., Gd-DTPA). Contrast-enhanced images can be acquired early or late after contrast administration.

Early gadolinium enhancement for measuring area at risk. The hyperenhanced region on early gadolinium enhancement (EGE), taken about 2 min after contrast administration, was shown to correlate with the area at risk derived from T2W images in patients with successfully reperfused AMI who underwent an MRI examination within 4 days after the event.
(102). The hyperenhanced region on EGE extended transmurally and was consistently larger than that on late gadolinium enhancement (LGE) MRI. The relative peri-infarct zone was calculated as the difference in hyperenhanced regions between EGE and LGE and normalized to the individual infarct size. The relative peri-infarct zone was inversely correlated with the transmurality of infarction and the time from symptom to reperfusion. Furthermore, a preclinical study in rats (132) showed that the enhanced region overestimated infarct size immediately after the injection of Gd-DTPA (5–7 min), although at 40 min postinjection the area of enhancement decreased and matched the infarct size.

Late gadolinium enhancement for measuring infarct size and salvaged myocardium. Preclinical studies in dogs demonstrated that LGE distinguishes between reversible and irreversible ischemic injury independent of wall motion and infarct age (37, 78). Nonviable infarcted tissue is usually seen as an area of hyperenhancement after contrast injection. This work was further validated when higher gadolinium concentrations were measured in the infarcted area by regional electrolyte concentrations using electron probe X-ray microanalysis (146). Contrast uptake in AMI occurs because of the expansion of the extracellular space due to edema and inflammation and cellular breakdown that allow the contrast agent to enter the intracellular space. The clinical value of LGE is that it) the transmural extent of the infarct on day 3 was shown to be a better predictor of improvement in contractile function than the actual occlusion time in animal models (61) and 2) the transmurality of the infarct was also shown to predict myocardial viability and recovery of contractile function after revascularization in humans (Fig. 2) (22, 79, 157).

Finally, a combination of T2W, LGE, and perfusion MRI imaging has been used. A pig model of reperfused MI showed that first-pass perfusion MRI most accurately reflected the infarct area and that the high signal intensity on T2W images reflects both irreversibly and reversibly injured, but essentially viable, peri-infarct zone (23). An imaging approach combining LGE and T2W MRI in patients with AMI and successful reperfusion 3 ± 3 days after the event showed that the high signal intensity on T2W MRI reflected reversible myocardial damage and LGE reflected irreversibly injured myocardium (42). The combination of T2W MRI and LGE was also used to accurately differentiate acute from chronic MI (1). Although chronic infarcts are relatively acellular, they also enhance with gadolinium agents because of increased extracellular space due to fibrosis and decrease in the washout kinetics of the contrast agent due to the collagenous nature of the remodeled myocardium.

MRI versus SPECT imaging of MI. The main advantage of MRI LGE is its spatial resolution of 1 to 2 mm (in plane) contrary to about 2–4 mm with PET scans. Similarly to SPECT, PET images are also less sensitive in identifying subendocardial infarcts compared with LGE MRI (83, 89).

Targeted molecular imaging of MI. Although anatomic, functional, perfusion, and infarct size imaging has been validated and the prognostic value of these measurements is widely accepted, the application of molecular imaging using targeted contrast agents may enable the identification of pathologically altered biological processes that would allow early intervention before the disease progresses to the symptomatic stage. In the next section we will focus on the biological processes involved in MI and postinfarct remodeling and discuss the availability of molecular targets allowing their visualization. Representative imaging examples of these biological processes are summarized in Fig. 2.

Apoptosis and necrosis in MI. Hours after the onset of ischemia, cardiomyocyte death occurs via two distinct pathways: apoptosis and necrosis. Although LGE MRI provides an accurate measure of infarct size, it does not differentiate between these biological processes. It is evident that cells that show early features of an apoptotic program may have the potential to be salvaged if they could be detected early. Similarly to atherosclerosis, imaging of apoptosis in MI is mainly based on targeting either annexin A5 or exposed DNA fragments. Imaging of annexin A5 and/or differentiation of apoptosis from necrosis has been achieved with 99Tc-SPECT (40, 62, 153) fluorescence imaging (34) and MRI (165, 166). The spatial and temporal presence of macrophages in the infarcted region has been imaged with different modalities. Magneto-fluorescent cross-linked iron-oxide nanoparticles (CLIO-Cy5.5) injected intravenously 2 days after experimental MI were detected 2 days later in the infarcted region of the heart using T2*W MRI (Fig. 2) (165). Recently, a DNA-binding Gd chelate for the detection of cell death by MRI has also been developed (45).

Inflammation and wound healing in MI. Activation of the vascular endothelium is crucial for leucocyte recruitment. PET/CT imaging of VCAM-1 expressed on endothelial cells has been achieved using a tetrameric peptide 18F-4V (Fig. 2) (122). The infarcted myocardium showed a strong signal in the PET image delayed hyperenhancement after iodine injection in the CT image and a high signal on autoradiography. Monocytes recruited to the injured myocardium (tissue monocytes/macrophages) from the spleen and bone marrow reservoirs play a central role in infarct healing (123). In a mouse model of MI, it has been shown that the recruitment involves a biphasic process in which Ly6C<sup>high</sup> monocytes dominate the first 4 days after the event to remove cellular debris, whereas Ly6C<sup>low</sup> appear between 5 and 10 days to promote tissue repair and resolution of inflammation (126). Insufficient or excessive recruitment of monocytes into the infarcted area has been shown to impair wound healing in preclinical (135) and clinical studies (192). <sup>1</sup>H<sup>19</sup>F MRI at 9.4 T revealed a time-dependent infiltration of injected biochemically inert nanomulsions of perfluorocarbons into the border zone of infarcted areas in murine injury models, and histology demonstrated a colocalization of perfluorocarbons with cells of the monocyte/macrophage system (Fig. 2) (39). Myocardial inflammation was also visualized with 18FDG-PET (Fig. 2) (6, 93). The time course of 18FDG-PET signal showed significantly increased uptake in the infarcts of mice on day 5 after...
coronary ligation (standardized uptake value 2.7 ± 0.1) when compared with myocardium in control mice (1.3 ± 0.2; P < 0.01). On day 14, the 18F-FDG signal approached control values (MI, 1.6 ± 0.1; and remote, 1.4 ± 0.1). Interestingly, there were differences between the activity in noninfarcted remote myocardium on day 5 after MI and control hearts (percentage injected dose/gram of tissue: remote, 8.9 ± 0.5; and control, 5.1 ± 1.0; P < 0.05). The MRI component of hybrid data sets enabled the precise definition of the infarct area and analysis of anatomic and functional parameters (93). In addition to imaging resident macrophages using iron particles by T2*W MRI (Fig. 2), other studies showed the feasibility of tracking the distribution of iron-labeled macrophages by injecting the contrast agent before MI and then imaging the animals post-MI (110, 216). These studies demonstrated that preloaded macrophages infiltrate in the infarcted myocardium up to day 4 post-MI, consistent with histological studies validating the presence of CD68 macrophages. However, microparticles of iron oxide showed persistent localization in the infarct even 14 days post-MI, whereas histological studies showed a progressive decrease of macrophage content 4 days post-MI (215). A combination of fluorescence molecular tomography (FMT-CT) and physiological MRI imaging was used to specifically image Ly6C<sup>+</sup> monocytes and their role in infarct healing (135).

**Enzymatic activity in MI.** Monocytes/macrophages are active cells secreting a variety of enzymes and cytokines. MPO is an inflammatory enzyme abundantly expressed in neutrophils that induces production of hypochlorous acid (HOCI), which is cytotoxic. MPO is released post-MI (126), and although it does not significantly affect tissue necrosis, it has a profound adverse effect on left ventricular remodeling and function (197). MPO in MI has been imaged using an activable MPO-Gd contrast agent (Fig. 2) (21, 124) that is first radicalized by MPO and then either spontaneously oligomerizes or binds to matrix proteins, all leading to enhanced r1 relaxivity and delayed washout kinetics. In a serial imaging study, MPO activity in the myocardium peaked 2 days after coronary ligation in mice. Finally, a fluorogenic sensor for monitoring peroxynitrite (ONOO<sup>−</sup>) and MPO-mediated hypochlorous acid (HOCI/OCl<sup>−</sup>) production has been developed and used for ex vivo imaging of reactive oxygen/nitrogen species in myocardial ischemia (134). Another enzyme family actively involved in myocardial healing is MMPs that are capable of degrading all types of extracellular matrix proteins. In myocardial ischemia, increased MMP activity may lead to infarct expansion that contributes to ventricular rupture and dilation. Imaging of MMPs and cathepsin activity (ProSense) has been achieved with SPECT using a 99mTc-labeled radiotracer (99mTc-RP805) (176) and fluorescent modalities (Fig. 2) (19, 93, 125).

**Neovascularization in MI.** Angiogenesis occurs naturally after MI to restore perfusion with oxygen and nutrients to the ischemic myocardium. Several imaging modalities have been developed to image angiogenesis mainly by targeting the α<sub>3</sub>β<sub>3</sub> integrin, expressed on the cell surface of proliferating endothelial cells and the vascular endothelial growth factor (VEGF). Serial in vivo dual-isotope SPECT imaging with an 111In-labeled α<sub>3</sub>β<sub>3</sub>-targeted agent demonstrated focal radiotracer uptake in hypoperfused regions of canine myocardium where angiogenesis was stimulated. There was a fourfold increase in myocardial radiotracer uptake in the infarcted region associated with histological evidence of angiogenesis and increased expression of the α<sub>3</sub>β<sub>3</sub> integrin (106). A novel compound called regioselectivity addressable functionalized template-RGD (RAFT-RGD) is composed of four cyclo-RGD<sub>F</sub> sequences tethered on a cyclodecapeptide that specifically binds to the α<sub>3</sub>β<sub>3</sub> integrin and is conformationalized with the receptor, suggesting increased affinity of the peptide scaffold for the α<sub>3</sub>β<sub>3</sub> integrin. The infarcted area was readily visible in vivo in a rat model of reperfused MI by SPECT with the 99mTc-RAFT-RGD compound but not with the negative control 99mTc-RAFT-RAD (Fig. 2) (32). Moreover, a PET (18F-Galaktos-RGD) agent, carrying the cyclic Arg-Gly-Asp (cRGD) peptidic sequence specifically binds to the α<sub>3</sub>β<sub>3</sub>-targeting agent, was used to assess the time course of post-MI angiogenesis in rats (60). In this model, focal accumulation in the infarcted area started at day 3, peaked between 1 and 3 wk, and decreased to baseline at 6 mo after reperfusion (60). The feasibility for translating the preclinical work in the clinical setting was demonstrated when the same agent was later used in a patient 2 wk after MI and primary coronary intervention (99). Another PET tracer (64Cu-DOTA-VEGF<sub>121</sub>) showed minimal baseline myocardial uptake that increased significantly at day 3 after MI and remained elevated for 2 wk post-MI, after which it returned to baseline levels (Fig. 2) (149). Finally, cyclic Asn-Gly-Arg (cNGR)-labeled paramagnetic quantum dots (pQDs) have been developed for MRI imaging of angiogenesis (131). The tripeptide cNGR homes specifically to CD13, an aminopeptidase that is strongly upregulated during myocardial angiogenesis. Injection of cNGR-pQDs resulted in a strong negative contrast that was located mainly in the infarcted myocardium of mice 7 days after surgery and up to 2 h after intravenous contrast agent administration. This negative contrast was significantly less in MI mice injected with unlabeled pQDs and in sham-operated mice injected with cNGR-pQDs. Validation with ex vivo two-photon laser scanning microscopy revealed a strong colocalization of cNGR-pQDs with vascular endothelial cells.

**Extracellular matrix in MI.** The extracellular matrix plays a fundamental and increasingly more appreciated role in AMI and infarct healing (33). During the inflammatory phase (rods, 1–48 h; and larger animals, 1 h–4 days), cytokines and adhesion molecules become upregulated leading to leukocyte recruitment to the infarct zone. Simultaneously, protease activation increases resulting in degradation of matrix proteins and generation of low molecular weight fragments that exert potent proinflammatory effects. Later on, as the acute inflammatory response subsides, mesenchymal cells infiltrate the infarct marking the transition to the proliferative phase (rods, 48 h–5 days; and larger animals, 4–14 days) when myofibroblasts secret extracellular matrix and angiogenesis occurs. During the maturation phase (rods, 5–28 days; and larger animals, 14 days–2 mon), the cellularity of the infarct decreases and lysyl-oxidase becomes upregulated inducing matrix cross-linking and formation of a dense collagen-based scar. Formation of a stable cross-linked extracellular matrix may shield the myofibroblasts from mechanical tension promoting quiescence. Conversely, overabundant collagen is also harmful because it hinders diastolic function and predisposes to arrhythmia. A type I collagen-specific peptide was identified using phage display and subsequently modified to improve affinity for collagen (17). The peptide conjugated to gadolinium (EP-3533) enabled in vivo molecular MR imaging of...
fluorescent in a murine model of healed postinfarction myocardial scarring (Fig. 2) (56) and perfusion in a swine model (172). In the murine MI model, dynamic T1W MRI revealed that the washout time for EP-3533 was significantly longer (195 min) than those for Gd-DTPA (25 min) in regions of scarring. The area of enhancement correlated with the histological presence of collagen. The gadolinium concentration in scar was twofold higher compared with that of remote myocardium 50 min after EP-3533 injection. Another collagen specific peptide (collagelin) has been identified and used for SPECT imaging in a rat model of healed MI. Scintigraphic imaging following injection of 99mTc-labeled-collagelin showed that uptake of the probe occurred in rats with MI but not in controls (118).

Newly synthesized collagen fibers in the ischemic myocardium become cross-linked into interconnected matrix. In addition to lysyl-oxidase, tissue and plasma transglutaminase (FXIII) are involved in this process (120). The in vivo enzyme activity of FXIII within the infarct was assessed using an 111indium-labeled affinity peptide (111In-DOTA-FXIII) (Fig. 2) (119, 193). FXIII recognizes the probe as a substrate and cross-links it to extracellular matrix proteins, leading to local entrapment of 111In-DOTA-FXIII in the healing infarct. Decreased FXIII SPECT signal enhancement in the infarct was associated with increased left ventricular dilation post-MI. Conversely, FXIII-treated mice showed increased FXIII activity and a faster resolution of the neutrophil response, enhanced macrophage recruitment, increased collagen content, and augmented angiogenesis in the healing infarct. Finally, an MRI elastin-specific contrast agent showed increased deposition of elastin 7 days post-MI and enabled visualization of myocardial remodeling in a mouse model of coronary ligation (209). The CNR peaked at 45 min postinjection, and it was significantly higher than for Gd-DTPA at 30 min.

Changes in myocardial microstructure are important components of the tissue response to infarction but are difficult to resolve with current imaging techniques. A novel technique, diffusion spectrum MRI tractography (DSI tractography), has been shown to resolve three-dimensional myofiber architecture. Meshlike networks of orthogonal myofibers in infarcted myocardium may resist mechanical remodeling but also probably increase the risk for lethal reentrant arrhythmias (167).

**Outlook.** Extensive progress in the field of molecular imaging of atherosclerosis and MI has enabled the in vivo detection of several biological processes involved in disease progression and resolution and monitoring of interventions both in the preclinical and clinical setting using MRI and other imaging modalities. Developments of novel imaging agents with improved signal detection properties, sensitivity, specificity, and favorable pharmacokinetics together with improvements in MRI acquisition and image processing protocols have elucidated disease biology and therapeutic mechanisms and shed more light on the longitudinal processes of atherosclerotic plaque progression and instability, myocardial infarct healing, regeneration, and repair. Translation of these new imaging methods in the clinical arena is a very challenging aspect of molecular imaging, as it requires clinical trials with histological and clinical end points that demonstrate utility of the complimentary information to that already clinically available. However, if successful, it may revolutionize medicine as it may allow more accurate disease assessment and personalized care in patients with atherosclerosis or after MI.

## DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS
A.P. prepared figures; A.P., M.E.A., A.M.S., and R.B. drafted manuscript; A.P. edited and revised manuscript; A.P. and R.B. approved final version of manuscript.

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