Published in final edited form as:

Thromb Haemost. 2011 May 3; 105(5): 828–836. doi:10.1160/TH10-09-0589.

Molecular imaging of macrophage protease activity in cardiovascular inflammation *in vivo*

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Summary

Macrophages contribute pivotally to cardiovascular diseases (CVD), notably to atherosclerosis. Imaging of macrophages *in vivo* could furnish new tools to advance evaluation of disease and therapies. Proteolytic enzymes serve as key effectors of many macrophage contributions to CVD. Therefore, intravital imaging of protease activity could aid evaluation of the progress and outcome of atherosclerosis, aortic aneurysm formation, or rejection of cardiac allografts. Among the large families of proteases, matrix metalloproteinases (MMPs) and cysteinyl cathepsins have garnered the most interest because of their participation in extracellular matrix remodeling. These considerations have spurred the development of dedicated imaging agents for protease activity detection. Activatable fluorescent probes, radiolabeled inhibitors, and nanoparticles are currently under exploration for this purpose. While some agents and technologies may soon see clinical use, others will require further refinement. Imaging of macrophages and protease activity should provide an important adjunct to understanding pathophysiology *in vivo*, evaluating the effects of interventions, and ultimately aiding clinical care.

Introduction

Innovations in imaging continue to revolutionize the practice of medicine by providing exquisite anatomical details that enhance diagnostic capabilities and enable monitoring of therapeutic interventions. In cardiovascular medicine, ultrasound, computed tomography (CT), nuclear, optical, and magnetic resonance imaging (MRI) provide high-resolution anatomic and functional images of the cardiovascular system, and x-ray fluoroscopy guides interventions to treat obstructive vascular disease and cardiac arrhythmias. Although many of these advanced imaging techniques provide excellent spatial resolution, in most cases they simply define anatomy or disease burden and provide physiologic information, rather than disclose information about the basic biological aspects of disease activity or therapies. Newer imaging strategies add to anatomic information by defining the cellular and molecular processes involved in cardiovascular disease (CVD), to improve understanding of the disease process, to obtain prognostic information, and to guide and follow cardiovascular therapies (1).

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Inflammation plays a major role in CVD, and heightened inflammatory responses promote atherosclerosis, arterial thrombosis, vascular aneurysm formation, and rejection of transplanted hearts (2–4). Macrophages — mononuclear phagocytes in tissues derived from monocytes — operate as the major effectors of innate immunity in all of these CVD processes, often in response to signals from adaptive immune cells. Macrophage inflammatory functions that fight infection and promote tissue repair can turn against the host and promote disease progression. Thus, macrophages furnish an attractive target for the development of novel molecular cardiovascular imaging strategies, because these cells figure prominently in CVD, and hence macrophage-based imaging strategies might apply widely across the spectrum of cardiovascular disorders.

Molecular probes for macrophage imaging target several aspects of macrophage cell biology. Cellular probes specific for membrane markers on the cell surface can localize macrophages within tissues, and such surface proteins whose levels increase in stimulated cells can preferentially identify activated cells. Surface targets for macrophage imaging, although not specific for this cell type, include VCAM-1 (Vascular cell adhesion protein-1) (5), $\alpha_v\beta_3$ integrin (6–8), scavenger receptors (9, 10), ICAM-1 (Inter-Cellular Adhesion Molecule-1) (11), and CCR-2 9 (Chemokine (C-C motif) receptor 2) (12). In addition to localization by targeting surface proteins, internalization of probes through phagocytosis by macrophages can also detect such cells preferentially. Several nanoparticle-based, superparamagnetic probes show promise in this regard (13, 14). Stimulated phagocytes elaborate reactive oxygen species that could permit their detection. Macrophage proteases also hold promises as targets for cardiovascular imaging, because these enzymes (matrix metalloproteinases [MMPs] and cysteinyl cathepsins) function as important regulators of CVD processes and may comprise therapeutic targets for modulating disease activity.

Proteases belong to several families of enzymes that catalyze protein breakdown. These enzymes can exert endoproteolytic activity, exopeptidase activity, or both. Approximately 2% to 4% of a typical genome encodes proteolytic enzymes (15). The Degradome database, which does not incorporate protease pseudogenes or sequences derived from retroviruses, lists 569 human proteases and homologs classified into 68 families (16). Proteases fall into six groups, depending on their mechanism of action: serine, threonine, cysteine, aspartate, glutamic acid proteases, and MMPs (also known as matrixins). Mammals seem not to express glutamate proteases.

As uncontrolled proteinase action could prove catastrophic, several levels of regulation govern their expression and activity: regulation of gene expression; activation of precursor zymogens; blockade by endogenous inhibitors; targeting to specific compartments such as lysosomes, mitochondria, or membranes; and post-translational modifications. Proteinases control a variety of cellular and extracellular processes. Within cells, proteinases can regulate gene expression, cell differentiation and proliferation, or cell death, often through limited proteolysis. Outside of cells, proteinases participate in extracellular matrix protein turnover, in digestion of nutrients in the gut, and in host defenses and responses to tissue injury and healing (17, 18).

Molecular imaging in cardiovascular research mostly has focused on two families of proteases, produced abundantly by activated macrophages in inflamed tissues and implicated by considerable evidence in CVD: cysteinyl cathepsins and MMPs (19). This review will summarize the current state of the art of *in vivo* protease activity imaging in this context and highlight some recent alternatives for macrophage molecular imaging.

Imaging strategies and platforms for protease activity

Molecular imaging probes typically exhibit a targeting moiety that provides specificity linked to an imaging constituent (fluorophore, radionuclide, magnetic moiety, scatterer/absorber such as microbubbles, or photon generator such as luciferin) detectable by the imaging platform. In the case of extracellular proteinases, reporting on their activity conveniently does not require delivery of the probes to intracellular compartments.

Protease imaging has used two generic approaches: a) targeting labeled small molecules to specific protein pockets, typically the active site; and b) imaging substrates of proteases, which become activated and detectable upon cleavage. The first approach, commonly used with radionuclides, has wide application in clinical imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) platforms, especially because of their non-invasiveness, high versatility, and high sensitivity. Protease inhibitors (almost exclusively MMP inhibitors) have been labeled with ^{11}C (20, 21) or with ^{18}F (22–24) for PET imaging in cancer. SPECT-labeled MMP inhibitors have undergone investigation in atherosclerosis (25–28), vascular injury (29), and post-infarct remodeling (30). SPECT requires the use of isotopes like $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , and ^{131}I . To define the anatomical origin of the radioactive signals, both of these nuclear imaging platforms often combine with CT or MRI. The major limitations of PET and SPECT include low spatial resolution, exposure to radiation, a limited stoichiometry (one imaging probe per target enzyme), and the inability to differentiate protease-bound from unbound (circulating, sequestered, nonspecifically bound) imaging labels (Fig. 1).

Like PET and SPECT, MRI instrumentation has wide availability in the clinical setting, and offers good spatial resolution and multicontrast capabilities. MRI used in coregistration with PET or fluorescent molecular tomography (FMT) provides fiducial anatomical references. MRI suffers, however, from lower sensitivity compared with radionuclide-based or optical approaches (Fig. 1). MRI technology relies on the interaction of water protons' magnetic moments in tissues with magnetic properties of the surrounding environment. By adding contrast agents that magnetically modify this environment, positive (T1- targeted) and negative (T2-targeted) enhancements can be generated and detected by MRI. The most commonly used untargeted contrast agents are based on gadolinium, which shortens the spin-lattice relaxation time (T1) of nearby water protons. Studies have reported potential systemic toxicity of gadolinium, however, in patients with renal insufficiency (31). Superparamagnetic iron oxide nanoparticles, fluorine (^{19}F)-carrying particles, manganese-based agents, and chemical exchange saturation transfer (CEST) agents are the current alternatives to contrast agents for increasing MRI sensitivity. For protease imaging in particular, studies have reported the development of specific inhibitors associated with magnetic agents (32, 33) and protease-sensitive magnetic nanoparticles (34, 35).

The use of enzyme-activatable selective fluorescent or magnetic substrates takes advantage of the enzyme's catalytic activity. In this approach, each protease can convert many different imaging molecules. This property of labeled substrates provides the advantage of manifold amplification of the signal. One class of such "smart probes," fluorescently-labeled substrates, produces intense fluorescence when cleaved due to separation from moieties that quench their fluorescence when in close proximity. Selectivity for certain enzymes derives from the sequence of the peptide-based substrate. In addition to avoiding radiation exposure, the near-infrared fluorescence (NIRF) imaging spectrum combines low autofluorescence and useful tissue penetration (millimeters to centimeters). Fluorescence signal can be acquired in surface-weighted mode by fluorescent reflectance imaging (FRI) or in tomographic mode (FMT), which employs mathematical algorithms that localize the origin of the fluorescence based on the NIRF diffusion in tissues (36). The tissue penetration of NIRF suffices for

imaging of intact small animals such as mice, but would likely not permit external visualization of deep arteries (> 5–10 cm) in humans with today's technology. To overcome this limitation, a NIRF intravascular catheter approach in development could extend this technique to the imaging of proteinases related to inflammation in atherosclerotic human arteries (37).

Intravital confocal and multiphoton microscopy (IVM) also enables NIRF imaging. Despite limited application in humans due to its invasiveness, IVM provides high spatial resolution and sensitivity in experimental animals (Fig. 1). Multiphoton microscopy presents several advantages over confocal microscopy, including increased imaging depths and reduced out-of-focus photodamage. Multiphoton technology can also detect biological materials such as collagen, microtubules, and muscle myosin without any exogenous targeted agents by second harmonic generation (SHG) imaging.

Protease imaging can also be achieved with targeted microbubbles that permit imaging by contrast-enhanced ultrasound (CEU) (38, 39). Advantages of this approach include the widespread availability of suitable imaging platforms, the lack of ionizing radiation, and non-invasiveness. Disadvantages include lower inherent sensitivity, resolution, and penetration depth. Applied frequencies can currently achieve a resolution of <50 μm at reduced depth but this is not applicable when the structure of interest is located at deeper depths.

MMP imaging

In cardiovascular research and in protease imaging, the MMP collagenases and gelatinases have undergone the most extensive study because of their participation in degradation of macromolecules of the extracellular matrix — processes integrally involved in the remodeling of blood vessels and the myocardium (table I). In the inflamed atheroma and myocardium, proteinase activity increases compared to healthy tissues, in large part due to production by and release from macrophages (19, 40) (Fig. 5, upper panel).

In atherosclerosis, degradation of the collagenous extracellular matrix of the protective fibrous cap covering the lipid core of the plaque can lessen the lesion's biomechanical strength. Rupture of the fibrous cap causes the majority of fatal acute myocardial infarcts by initiating thrombus formation (40). In the infarcting myocardium that is deprived of blood flow, ventricular remodeling may involve the extracellular matrix-degrading activities of MMP-2, MMP-9, and MMP-14 (41, 42). Elastolytic activity, potentially driven in part by MMP-9 and MMP-12, may promote aneurysm formation (43).

Proof-of-concept studies in mice have imaged gelatinase activity (MMP-2 and MMP-9) *in vivo* using an activatable NIRF substrate (44, 45). Confirmed by *ex vivo* FRI and *in situ* zymography, IVM and FMT imaging detected higher MMP and gelatinase activity in macrophage-rich atherosclerotic plaques and in infarct zones (Fig. 2). Recent studies on aneurysm formation also have succeeded in monitoring MMP activity by multimodality imaging, with FMT coregistered with CT to provide anatomical colocalization (46, 47); the intensity of MMP activity correlated with aneurysm development.

Several publications have reported the generation of specific or broad-range MMP inhibitors (MMPI) labeled by radionuclides for PET/SPECT imaging. With a primary goal of screening of MMPI, broad spectrum hydroxamate pharmacophore MMPI have been coupled with ^{123}I (48), $^{99\text{m}}\text{Tc}$ (30), and ^{18}F (23, 24, 49) for SPECT or PET imaging (Fig. 3). Generation of MMP-2 and MMP-2/MMP-9 inhibitors, coupled with ^{18}F (50) and ^{64}Cu (51) or MMP-14 substrate radiolabeled with $^{99\text{m}}\text{Tc}$, could permit more selective imaging of proteinases (52). Preliminary studies in atheromata, aneurysm, and wire-injured arteries

correlated ^{99m}Tc - or ^{111}In -MMPI signals with macrophages and MMP-2/MMP-9 activity in lesions by multimodal SPECT/CT imaging (25, 26, 53–55).

MRI-dedicated nanosensors for MMP-2/MMP-9 in development use nanoparticles loaded with the MR contrast agent gadolinium. Upon proteolysis, the nanoparticles exhibit highly cationic molecules that trigger attachment and intake into cells (35). Initial reports indicate a potential use for cancer targeting *in vivo*, but no study has yet assessed the utility of such agents in CVD. An MR probe for MMP-9 activity used charged nanoparticles with an iron oxide core coupled with methoxypoly(ethylene glycol) (MPEG) molecules, maintained in a stable state by a domain containing MMP-9 substrate (34). Upon MMP-9 cleavage, the remaining particles aggregated due to magnetic and electrostatic attraction. This study did not report *In vivo* MRI.

Cathepsin imaging

The cysteinyl proteinases included in the broad cathepsin (Cat) category also degrade matrix collagen and elastin (table I). Multiple reports implicate these enzymes in atherogenesis and in aneurysm formation (4, 56, 57). As in the case of MMPs, activatable NIRF probes can visualize cathepsin activity *in vivo*. An early study demonstrated the suitability of a Cat-B-dependent Cy5.5-labeled activatable NIRF probe for atherosclerosis imaging *in vivo*. In atherosclerotic mice after 24 weeks of consuming an atherogenic diet, FMT coregistered with MRI detected a strong signal in aortic plaques. In the same study, aortic aneurysms displayed high Cat-B activity assessed by *ex vivo* FRI. In contrast, arteries of healthy control mice yielded little signal. Histological analysis of the aorta after dissection confirmed a higher expression of Cat-B, colocalized with macrophages within lesions, as compared to healthy arterial tissue (58). A recent study demonstrated by *ex vivo* imaging that a similar probe could assess quantitatively the impact of anti-atherosclerotic treatments, such as statins, that reduced Cat-B activity in macrophage-rich plaques (59).

The potent elastolytic and collagenolytic protease Cat-K (57) that may contribute to plaque destabilization, aneurysm formation, and outward arterial remodeling during atherogenesis or following injury. In a study that used a NIRF imaging agent based on a Cat-K peptide substrate *in vivo* in mice, similar to the study described above involving Cat-B, optical imaging (IVM, *ex vivo* FRI) revealed more than twice as much signal in atherosclerotic mice and in human carotid endarterectomy specimens *ex vivo*, as compared with the control agent. The functional relevance of Cat-K was validated by fluorescent microscopy that colocalized Cat-K NIRF and Cat-K positive macrophages with fragmented elastin fibers within the media of underlying plaques (60).

Activity of the cysteinyl protease Cat-S associates with vascular calcification in mice with experimentally induced chronic renal disease (61). Injection of Cat-S activatable and osteogenesis-targeted imaging agents allowed the colocalization of Cat-S activity and calcification in aortas and aortic valves by IVM and *ex vivo* FRI. Cat-S-deficient mice exhibited less calcification and no Cat-S activity, validating the specificity of the probe and the involvement of the protease in this process.

In proof-of-concept studies, a broad cathepsin-activatable probe has sensed proteolytic potential *in vivo* in rabbit atherosclerotic lesions by an intravascular approach to overcome the limited-depth penetration of NIRF that precludes its clinical use for interrogation of deep arteries in humans (62). The same protease probe has visualized cathepsin activity experimentally in cardiac allografts undergoing rejection, and in myocardial infarcts. Seven days post-transplantation of histo-incompatible hearts without immunosuppression, myocardial graft rejection associated with accumulation of macrophages that produced active cathepsins, as visualized by FMT *in vivo* and FRI *ex vivo* by co-injecting the

cathepsin probe and macrophage-targeted magneto-fluorescent nanoparticles. Macrophage recruitment was also imaged *in vivo* by MRI (13, 32, 33). In other experiments, co-injection of these two imaging agents four days after coronary ligation in mice demonstrated the active recruitment of macrophages and increased cathepsin activity in healing zones of the ischemic heart, as compared to healthy myocardium (14).

Cat-D is a lysosomal aspartyl protease that might play an important role in some cancers and in Alzheimer disease. Preliminary studies reported the development of Cat-D NIRF- and MRI-dedicated probes (63, 64).

Other targets for cardiovascular imaging of macrophage activity

In addition to MMP and cathepsin proteases, molecular imaging may prove useful for more extensively visualizing activity of macrophages via other enzymes or molecules that are especially relevant in CVD.

The enzyme myeloperoxidase (MPO) has received considerable interest as a biomarker for inflammation in atherosclerotic plaques and for future acute coronary events. Patients with stroke have elevated plasma MPO concentrations (65), and MPO levels seem to predict major cardiac events in patients presenting with acute chest pain (66) and in apparently healthy individuals (67). Although generally most abundant in granulocytes, within atheromata, MPO derives mainly from macrophages and macrophage-derived foam cells. MPO activity results in the generation of reactive oxygen species (ROS) — notably, hypochlorous acid (HOCl) — that may contribute to local alterations in arterial biology. In infarcting myocardium, MPO may also impede the healing remodeling process (68). A high level of MPO in plasma predicted an increase in five-year mortality for patients with acute MI (69). To assess MPO activity *in vivo*, a novel MPO sensor based on gadolinium permits detection of MPO by MRI (70). Studies in rabbits and mice reported high MPO activity, reflected by hyper-Zenhancement in diseased aortas and in infarct zones, when conventional gadolinium contrast would have subsided (Fig. 4)(68, 71, 72).

Because macrophages play a key role in inflammatory CVD, they have received much attention as a target for molecular imaging. Iodinated, fluorescent, and/or magnetic nanoparticles based on an iron-oxide core phagocytosed by macrophages in plaques and in injured myocardium, can be imaged by optical or MRI or CT approaches (13, 14, 73–75). ^{18}F -fluorodeoxyglucose (^{18}F FDG) can track glucose uptake in macrophages. FDG signals colocalize with macrophage-rich inflamed sites in atheromata, as assessed in humans and in mice by SPECT/CT (76–78). Uptake of ^{11}C -choline or specific targeting imaging agents ($^{99\text{m}}\text{Tc}$ -anti-RAGE antibody, ^{18}F -galacto-RGD for $\alpha_v\beta_3$ integrin targeting, $^{99\text{m}}\text{Tc}$ -Annexin A5, ^{18}F -radioligand for a mitochondrial benzodiazepine receptor) has undergone testing in experimental models of atherosclerosis (7, 79–83).

Conclusion: Limits and future promises

Non-invasive molecular *in vivo* imaging, as an emerging technology, is a reality for several disease states but still requires refinement to progress into the clinical setting. In addition to the technological challenge for further increasing spatial resolution and sensitivity of the imaging hardware, a particular effort to improve or develop new imaging agents is crucial. For example, particle size and electrical charge are of great importance for the behavior of the agents *in vivo*, and therefore need to be carefully established. Moreover, future clinical applications for molecular imaging will require optimization of specificity, brightness (tissue penetration), and development of non-toxic agents.

Despite these current technological and cost-related limits, molecular imaging in general, and protease activity in particular, should contribute to biomedicine in the near future.

Detection of asymptomatic disease, and the prediction of severe complications, remains elusive to current diagnostic tools for many vascular disorders. Intravital imaging of protease activity promises to extend beyond anatomy and disclose biological aspects of the progress of atherosclerosis, myocardial infarct healing, heart transplant rejection, or aortic aneurysms. Moreover, novel therapeutic strategies in CVD should emerge from molecular imaging — including non-invasive imaging markers for prevention and personalized medicine, interventional imaging combined with treatment in the acute phase, and molecular assessment of therapeutic efficacy during treatment.

Preclinical studies in mice and rabbits have demonstrated how close we are to translating molecular imaging tools to the clinic. Imaging agents specifically targeting MMPs, cysteinyl cathepsins, or other enzymes implicated in CVD pathogenesis have demonstrated compatibility with established imaging platforms (PET, SPECT, MRI). The promising field of optical imaging will require special efforts in hardware to detect non-invasively small accumulations of active “smart probes” in inflamed or diseased tissues. Nonetheless, studies using MMPs and cathepsin-activatable probes have achieved proof of concept of the relevance and value of functional intravital imaging.

In addition to more reliable diagnosis and potential prediction of the outcome of a disease, molecular imaging for protease activity should also serve as a tremendous tool for development, evaluation, and dose ranging of novel therapeutics *in vivo*. Moreover, the coupling of imaging and therapeutic interventions with multifunctional nanoparticles opens the possibility of “theranostic” approaches. This concept involves targeting therapeutic drugs in particles carrying imaging moieties, for selective drug delivery to diseased tissues and simultaneous monitoring of not only targeting, but also possibly efficacy. In an illustration of such an approach, agents targeting scavenger receptor 1A on macrophages can detect MMP-9 activity (84). Multi-modality imaging — for example, tri-functional agents combining magnetic, optical, and radionuclide imaging functionality — promises to expand the gamut of molecular imaging (75, 85–87). Such multimodal imaging agents must contain an adequate ratio of the various contrast agents for matching the relative sensitivity of the applied imaging modalities. This particular requirement may lead to the development of chemical constructs to create multiple binding sites of contrast agents, from which overall charge and size remain compatible with penetration into the targeted tissues (88).

Multimodal imaging strategies could also combine non-invasive and high-resolution technologies (e.g., confocal or multiphoton microscopy) to study protease activity *in vivo*, in interstitial as well as in intracellular milieu, to define their roles better and to characterize more precisely the behavior of imaging agents in the field of CVD. Current NIRF contrast agents, for example, may be applicable to combined FMT and (optical parametric oscillator-equipped) two-photon microscopy, where two-photon microscopy contributes both sub cellular resolution and imaging of intrinsic emission derived from extra cellular matrix components.

Protease imaging — and more broadly, molecular imaging — not only applies to CVD, but also to other diseases such as arthritis (89), asthma (90), and cancer (91–93). Non-invasive intravital assessment of protease presence and activity will likely be feasible first by nuclear imaging in clinical trials, because of picomolar dosing and its widespread use in humans. The advantages of activatable probes over labeled inhibitors and the advent of multimodal imaging should increase efforts to introduce optical imaging into the clinic. The pace of progress in the development of novel molecular probes and imaging platforms during recent

years highlights the promise of enabling advances in research and clinical applications through probing physiopathologic processes and assessment of specific molecular processes in intact subjects *in vivo*.

Acknowledgments

We thank our colleagues affiliated with the Donald W. Reynolds Clinical Cardiovascular Research Center at Harvard Medical School, who contributed to our efforts to develop molecular imaging of atherosclerosis and cardiovascular diseases. We also acknowledge the National Institutes of Health (R01-HL080472), the Translational Program of Excellence in Nanotechnology (TPEN) (U01 HL080731), and the American Heart Association for their support.

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Figure 1.

Properties of imaging systems.

Evaluation of clinical and preclinical imaging modalities (SPECT, PET, MRI, FMT, IVM, and CEU), regarding their respective properties: penetration depth, spatial resolution, sensitivity, associated risk (of the related imaging agents), and cost. Imaging moieties/agents associated with the respective systems are indicated.

Figure 2.

Fluorescence imaging of protease activity in mouse CVD.

A. Intravital confocal microscopy on carotid artery exposed in an atherosclerotic mouse (apoE-deficient mice fed an atherogenic diet for 3 months). Fluorescence from macrophages (fluorescent nanoparticles) and MMP-13 activity (activatable probe) colocalized in plaque (visible under white light). B.(Top panel) FMT 7 days post-isograft or post-allograft transplantation (infrarenal location). The fluorescence signal from cathepsins activity sensor indicated ischemia-reperfusion injury (isograft, left) and reflected the alloimmune response (allograft, right). Quantification (C) and validation of *in vivo* imaging by *ex vivo* FRI on excised isografts (B, left) and allografts (B, right). Adapted from Christen et al., 2009 (13).

Figure 3.

MMPs imaging *in vivo* in atherosclerotic plaques by SPECT.

Sagittal (left) and frontal (right) projections of SPECT and CT imaging 4 hours after radiotracer administration (^{99m}Tc -MMP inhibitor). Images were obtained from a healthy rabbit (regular diet; no atherosclerotic lesions), and an atherosclerotic rabbit fed an atherogenic diet for 4 months. Decreased radiotracer uptake was observed when atherosclerotic rabbits on similar diets were treated with fluvastatin for four months.

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Figure 4. MPO activity following myocardial infarction (MI) by MRI. A. and B. Strong and persistent hyper-enhancement was detected in the infarct zone (left ventricular free wall, indicated by arrows) after injection of MPO-gadolinium (MPO-Gd) 2 days post-MI. As conventional gadolinium (Gd-DTPA) was washed out 2 hours post-injection, the infarcted left ventricle was still brightly enhanced in the MPO-Gd group. C. Contrast-to-noise ratio (CNR) quantification indicated higher values and persistent uptake after MPO-Gd versus Gd-DTPA. Adapted from Nahrendorf et al., 2008 (65).

Figure 5. Macrophage protease activity imaging in CVD. (Upper panel) Impact of protease activity on extracellular matrix turnover in atherosclerosis, aneurysm, heart transplant rejection, and myocardial infarction. (Lower panel) Current imaging agents and strategies for protease activity imaging *in vivo*.

Table I**Macrophage Protease Imaging Targets**

Protease Class	Activity Targeted	Active Site or Substrate Imaging Agents*
<i>Matrix Metalloproteinases</i>		
Broad MMPs	elastolytic, collagenolytic, gelatinolytic	Pet/SPECT
MMP-2	elastolytic, gelatinolytic	Pet/SPECT, MRI, Fluorescent
MMP-9	elastolytic, gelatinolytic	Pet/SPECT, MRI, Fluorescent
MMP-14	collagenolytic	Pet/SPECT
<i>Cathepsins</i>		
Broad Cat	elastolytic, collagenolytic,	Fluorescent
Cat-B	elastolytic, collagenolytic	Fluorescent
Cat-K	elastolytic, collagenolytic	Fluorescent
Cat-S	elastolytic, collagenolytic	Fluorescent
Cat-D	lysosomal protease activity	MRI, Fluorescent

* Several active site imaging agents also inhibit protease function.