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Vulnerable Plaque: Molecular Imaging

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Abstract Despite the remarkable advances in cardiovascular imaging over the last decade, it is still challenging to identify high-risk atherosclerotic plaques prior to onset of major cardiovascular complications. Accumulating knowledge regarding the pathophysiological properties of vulnerable plaque (VP) has driven the development of molecular imaging technologies that target biologic process to characterize vulnerable plaques. Given the importance of VP detection in vivo, molecular imaging has emerged as an attractive diagnostic tool to more accurately estimate the risk of plaque rupture.

Keywords Vulnerable plaque · Molecular imaging · Atherosclerosis · Inflammation · Macrophage

Introduction

The development of medical treatments to stabilize plaque and advance in interventional techniques such as coronary stenting to treat local lesions have dramatically decreased cardiovascular mortality and morbidity [1]. However, plaque destabilization is a complex, multistep process and thus, current imaging techniques focusing on plaque morphology and burden quantification has been insufficient to accurately predict the risk of plaque rupture. As the crucial steps in determining the probability of plaque rupture is not related to plaque size, but to its functional properties [2, 3], novel diagnostic strategies, to estimate plaque biology at a molecular level, is urgently required. This growing demand led us to focus on the molecular imaging to look at the biological behavior of the atheroma beyond structural information using a variety of imaging modalities [4]. By targeting different molecular pathways, the biological characteristics of high-risk plaques are better explained [5]. With encouraging earlier results and new understanding of the biological process regarding high-risk plaques, multimodal imaging strategies that provide more accurate visualization of plaque structure and molecular pathways simultaneously have emerged [6].

This review will discuss the novel and clinically relevant molecular imaging strategies targeting biological pathways with respect to vulnerable plaques in coronary artery. We will also examine novel targeting agents to image vulnerable plaque with an emphasis on currently available noninvasive and invasive molecular imaging modalities.

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Pathophysiology of Vulnerable Plaques and Molecular Imaging

The concept of vulnerable plaque or high-risk plaque was first described [7] as a silent, nonobstructive coronary lesion that suddenly transforms into an obstructive and symptomatic lesion. Nowadays, rupture of VP is widely accepted as major cause of acute coronary events and sudden cardiac deaths [8]. Moreover, vulnerable plaques are not only susceptible to MI events, but more liable to silently generate thrombosis or may rapidly progress to severe stenotic lesions [7]. The features of vulnerable plaques consist of structural characteristics such as the presence of a thin fibrous cap, a large lipids-necrotic core, expansive outward remodeling, neoangiogenesis, and cellular active immunologic response including macrophage and leukocyte infiltrations [7]. Among these features, the thickness of fibrous cap and the size of lipid necrotic core have been suggested as major structural determinants of vulnerable plaque [9]. As stated in this study, the thickness of fibrous cap less than 55 μm is the most important morphologic discriminator of plaque associated with fatal ruptures. Besides fibrous cap thickness, inflammation and large lipid necrotic cores are also

two key features of vulnerable plaque. Particularly, active inflammatory cells degrade the extracellular matrix (ECM) by secreting proteolytic enzymes and thus playing a pivotal role in plaque disruption. Moreover, the immunologic activation by a macrophage subset is associated with complicated process of intra-plaque hemorrhage (IPH) which contributes to plaque vulnerability [10].

However, not all inflamed, thin cap fibroatheromas (TCFAs) will rupture, although many of those plaques do rupture silently. Therefore, to more accurately detect high-risk coronary plaques, molecular imaging has become an important strategy that is urgently needed.

Molecular Imaging Targets for Vulnerable Plaque

Improvement in the ability to detect biological process for atherosclerosis and plaque destabilization is based on potential targets for molecular imaging (Fig. 1, Table 1). The targets described here represent those that have shown potential for clinical application, especially in coronary vessels.

Activation of Endothelial Cell

Endothelial cells cover the luminal vessel surface and are known to act as a dynamic barrier that regulates the molecular constituents and monocyte trafficking between the circulating blood and subendothelial layers. When endothelial cells encounter stimulations implicated in risk factors of atherogenesis such as hypercholesterolemia and hypertension, they promote the uptake of low-density lipoproteins (LDLs) through the endothelium into the subendothelial space [11]. During the process, activated endothelial cells express adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and contribute to lipid deposition. Hence, several approaches to target VCAM-1 with respective molecular imaging moieties have been introduced, such as ultrasound [12], nuclear [13], and magnetic resonance imaging (MRI) [14]. Although they are important research tools for the early atherosclerosis detection and potential drug monitoring, the clinical applications of these approaches for vulnerable plaque imaging are still limited

Inflammation

Inflammation, currently considered as the critical determinant of vulnerable plaques, is a major target for molecular imaging to evaluate the disease progression and the risk of plaque rupture [15]. Histomorphologic studies from patients with culprit plaques who had died suddenly demonstrate that abundant macrophage infiltration in fibrous cap is a main component of vulnerable plaque [9]. In this section, we will describe clinically feasible noninvasive molecular imaging approaches for plaque inflammation. Then, we will shift our focus toward promising intravascular catheter based molecular imaging for plaque inflammation that is able to provide the comprehensive assessment of high-risk coronary plaques.

Positron Emission Tomography

Previous studies with fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) combined multislice computed tomography (CT) revealed the feasibility of macrophage metabolic activity imaging in carotid plaque inflammation [16]. Additionally, a prospective study has reported that ^{18}F-FDG PET/CT was able to estimate the therapeutic efficacy in patients with established atheroma in large vasculature, nicely demonstrating that inflammation (FDG uptake) was significantly reduced from baseline after high-dose statin therapy [17•]. Regarding coronary beds, Rogers et al. have reported that ^{18}F-FDG PET co-registered with CT allows the detection of inflamed plaque in left main or proximal segments of major epicardial coronary arteries (Fig. 2) [18]. However, the resolution of PET is insufficient for tiny plaque in more distal segments. Also, many other cells, including cardiac myocytes, smooth muscle cells, and leukocytes, can also uptake high amounts of glucose, making substantial blurring of specific uptake of FDG in coronary atherosclerotic plaque. Thus, to date, molecular imaging of inflamed coronary atheroma using ^{18}F-FDG PET/CT is still challenging.

Magnetic Resonance Imaging

Several approaches targeting the phagocytic property of macrophages in molecular imaging have been demonstrated. Ultra-small superparamagnetic iron oxide (USPIO) nanoparticles with diameter similar to that of LDL (18 to 25 nm) have been used to detect enhanced activity within macrophage-rich rupture-prone plaques in human [19]. In clinical studies, the USPIO-MRI was able to monitor the therapeutic efficacy of anti-inflammatory interventions (e.g., statin) in carotid atherosclerotic patients [20]. Recently, macrophage-targeted superparamagnetic nanoparticle, MION-47, has demonstrated the feasibility of macrophage imaging in inflamed atheroma of rabbit aorta with using a 3-T high-resolution MRI scanner [21]. After 72 h of MION-47 injection, T2 MRI signal intensity was reduced in atherosclerotic rabbits' aorta, and ex vivo MRI corroborated in vivo findings, supporting the capability of this modality to estimate inflammatory burden in vulnerable plaques. In future, the clinical application of this approach needs to be further tested. However, these studies have focused on the imaging of larger and relatively immobile arteries such as the carotid artery or the aorta.

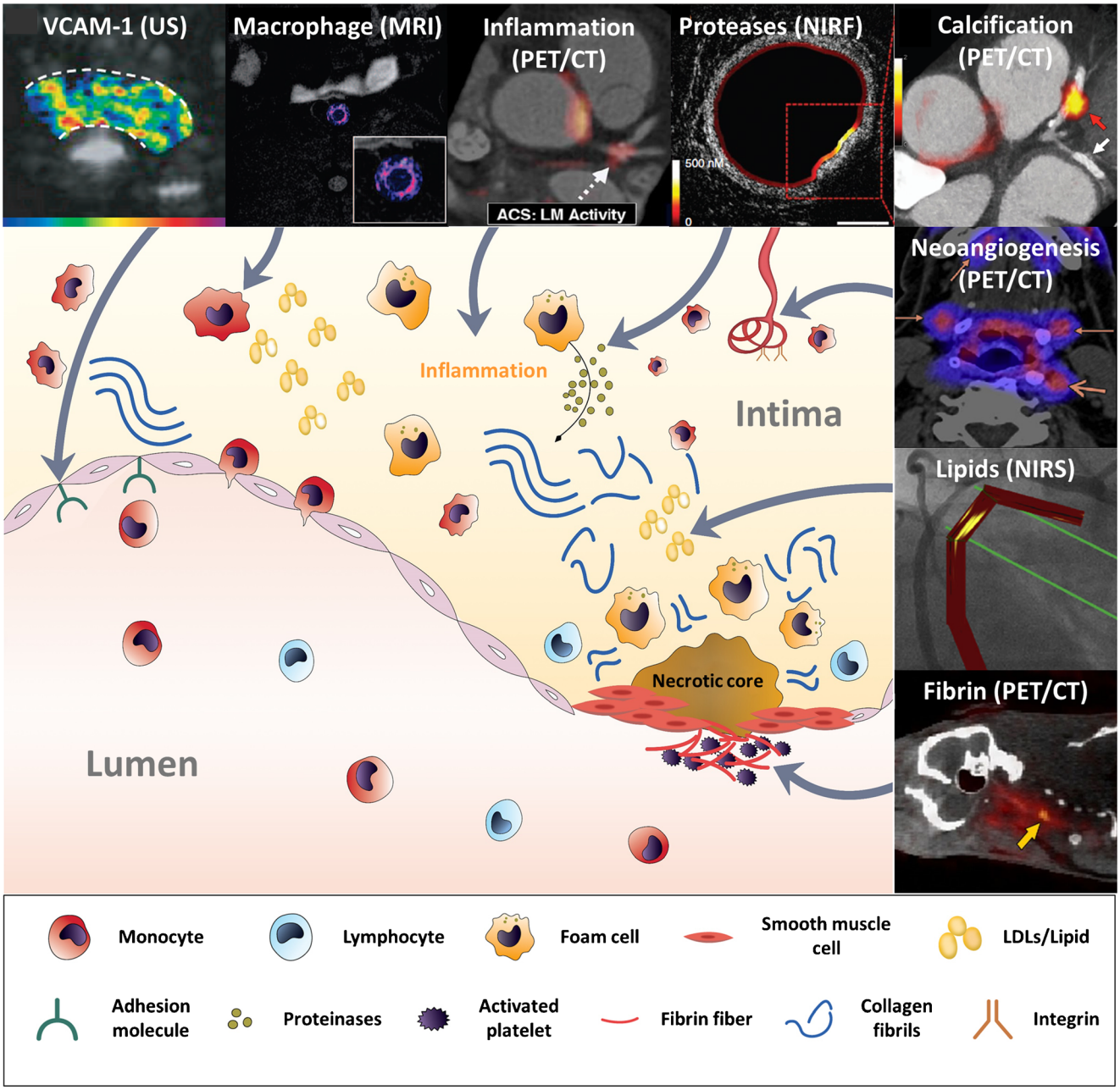

Fig. 1 Selected molecular imaging targets for atherosclerotic vulnerable plaques. The mechanisms of atherosclerotic plaques are shown in the simplified illustration, representing a normal vessel (*left*) to a vulnerable plaque with rupture (*right*). Potential imaging targets for molecular imaging at each process are displayed. VCAM-1, ultrasound image of adhesion molecules with targeted microbubbles in the aortic arch of apoE$^{-/-}$ mouse; adapted with permission from reference [12]. Macrophages, high-resolution MRI (3-T) with macrophage-targeted superparamagnetic nanoparticle (MION-47); adapted with permission from reference [21]. Metabolic activity (Inflammation), PET/CT imaging in a patient with acute coronary syndrome using ^{18}F-FDG; adapted with permission from reference [18]. Proteases, integrated OCT-NIRF image of inflammatory protease activity (Prosense/VM110) in a living rabbit aorta in vivo; adapted with permission from reference [29••]. Calcification, ^{18}F-NaF PET/CT imaging of an anterior non-ST-elevation myocardial infarction patient; adapted with permission from reference [48••]. Neovascularization (Integrins), [^{18}F]Galacto-RGD PET/CT imaging in human atherosclerotic carotid plaques; adapted with permission from reference [44]. Lipids, NIRS image from a culprit coronary vessel in a patient with acute ST-elevation myocardial infarction after restoration of coronary flow by thrombus aspiration; adapted with permission from reference [51]. Thrombosis (Fibrin), PET/CT imaging using a fibrin-binding PET probe (FBP7) in carotid crush injury rat model for intramural thrombosis; adapted with permission from reference [53]

As temporal resolution of MRI is limited, the USPIO-MR imaging of smaller plaques in mobile coronary arteries is still challenging.

Recently, P947, a gadolinium-labeled agent using an antibody against activated matrix metalloproteinases (MMPs) has been investigated and validated in several in vivo studies [22,

Table 1 Major specific molecular targets for plaque vulnerability and related imaging modality

Process	Target	Molecular agent	Imaging modality	Limitations
Endothelial activation and adhesion	Adhesion molecule	VCAM-1 conjugated microbubbles	US	Pre-clinical and resolution problem, technically difficult
Inflammation	Metabolism	18F-FDG	PET/CT	Myocardial uptake, limited specificity
	Phagocytosis	USPIO	MRI	Spatial resolution, challenging in coronary artery
	Macrophage and lipids	ICG	NIRF	Invasive method (intravascular), less specificity of ICG
Matrix remodeling	Cathepsins (B, K)	Prosense/VM110	NIRF	Pre-clinical, low tissue penetration of depth
	MMPs activity	P947	MRI	Pre-clinical, limited specificity of MMP tracers
Apoptosis	Externalized phosphatidylserine	Annexin A	SPECT	Less specificity, lack of structural delineation of SPECT
Neoangiogenesis	Integrin $\alpha_v\beta_3$	Labeled RGD peptidomimetics	MRI, PET/CT	Only clinical feasibility study is present
Calcification	Calcification activity	18F-NaF	PET/CT	Clinical relevance of identify high-risk plaque

23]. In these preclinical studies, P947-enhanced MRI showed excellent morphologic characterization of the plaque with strong signal enhancement. MMPs belong to the superfamily of calcium dependent zinc-endopeptidases. They play key roles in the pathological remodeling and degradation of ECM scaffold contributing to the weakening and thinning of fibrous caps [6]. Hence, molecular imaging of MMP activation in atherosclerotic plaques represents an attractive approach to identify those rupture-prone plaques [24, 25]. Thus, together with morphological characteristics of atherosclerotic lesions identified by MRI, imaging of MMP enzymatic activity may help identify patients with vulnerable plaques. However, as currently existing MMP tracers have a broad range of MMP specificity, it is still challenging to discriminate specific roles of various MMP subsets in plaque progression and destabilization.

Intravascular Integrated Optical Coherence Tomography-Near Infrared Fluorescence Imaging

While molecular imaging of plaque inflammation by FDG-PET/CT or magnetic nanoparticle-enhanced MRI has shown encouraging results with clinical feasibility and advantages as a noninvasive strategy, inherent limitations including the spatiotemporal resolution, and the blurring factors such as continuous cardiac and respiratory motion does not allow accurate estimation of subtle changes in coronary trees. To overcome those limitations, catheter based intravascular molecular imaging strategy has been developed for the detection of high-risk coronary plaques. In the following sections, therefore, we will focus on a novel approach utilizing intravascular molecular imaging to estimate arterial inflammation.

One of the feasible molecular imaging targets is a specific enzyme secreted from macrophages residing in fibrous caps. Proteases degrade the interstitial collagen in plaque's fibrous cap, rendering it more susceptible to rupture. Such weakening of fibrous cap precipitates fatal acute myocardial infarctions [8]. Cathepsins and MMPs are major groups of proteases that play a pivotal role in plaque destabilization. Therefore, these enzyme-activatable imaging agents are attractive for molecular imaging of plaque vulnerability. By conjugation of these enzyme-activatable nanoprobes for near-infrared fluorescence, optical imaging has successfully imaged MMPs and cysteine proteases in murine carotid plaques [26, 27]. NIR fluorochromes are silent state (quenched) at baseline and then become highly fluorescent after a proteolytic cleavage process (de-quenching). These high near infrared fluorescence (NIRF) signals were successfully detected in an invasive NIRF-fiber sensing manner [28].

Despite these encouraging results, NIRF catheter standalone imaging was not capable of visualizing plaque structure. To solve this important limitation, NIRF imaging catheter was fully integrated with optical frequency domain imaging (OFDI, a second-generation optical coherence tomography (OCT)) platform based on a conventional OCT standalone catheter. Using a cathepsin protease activatable NIRF agent (Prosense/VM110), OCT-NIRF catheter imaging was able to provide protease information in vivo in the context of plaque morphology [29••]. In rabbits fed an atherogenic diet with balloon-denudation in aortoiliac arteries, Prosense/VM110 was administered 24 h prior to intravascular imaging. OCT-NIRF imaging successfully visualized both plaque structure and protease molecular imaging. Plaques detected in vivo by OCT colocalized strongly with NIRF signals and immunohistopathology well corroborated in vivo images (Fig. 3). This feasibility study showed promise in allowing us to recognize plaque inflammation in detail. However, this modality has several hurdles to overcome for clinical application such as a slow catheter pullback speed (2.5 mm/s), which is critical for clinical application because arterial balloon occlusion should be required at that speed to eliminate blood in an imaging segment, and also substantial background noise

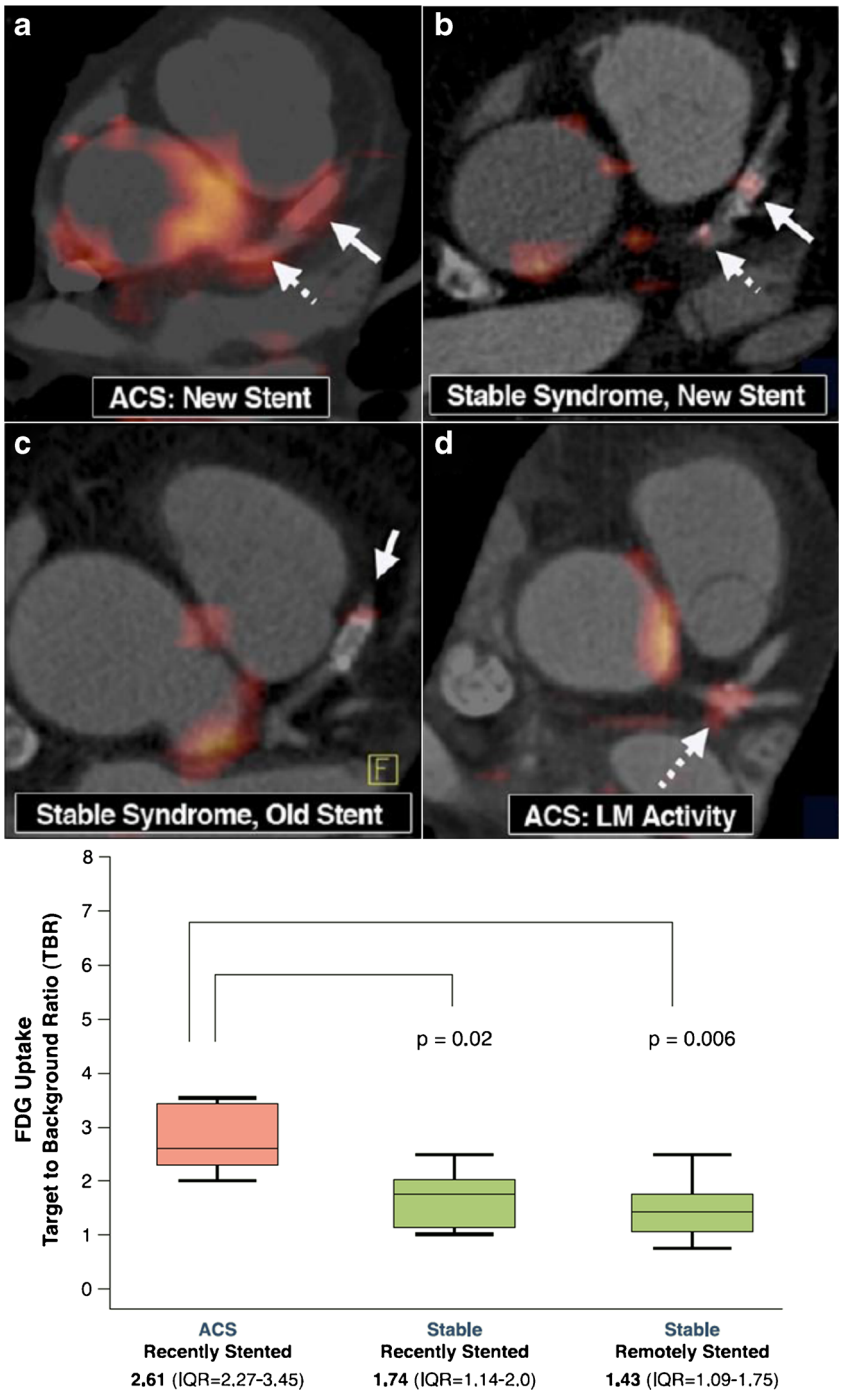

Fig. 2 ^{18}F-FDG PET/CT imaging of inflamed coronary artery in human. **a** Increased ^{18}F-FDG uptake in both left main coronary artery (LMCA) and stented culprit lesion in a patient presenting with acute coronary syndrome (ACS). **b** In stable coronary artery disease (CAD) patient, lesser extent of ^{18}F-FDG uptake was demonstrated in a recently stented lesion and LMCA. **c** Only small amount of ^{18}F-FDG uptake was visible in a stented lesion who have received the procedure several months ago. **d** Elevated ^{18}F-FDG uptake at the lesion of LM trifurcation in an ACS patient. *Lower panel* (box plot) displays comparison result of ^{18}F-FDG uptake in stented lesion among the study patients. The median target-to-background ratio of stented lesion was higher in ACS group than stable angina group (p=0.02) and significantly higher than previous stented stable angina group (p=0.006). Adapted and reproduced with permission from reference [18]

due to the crosstalk between two different properties of the fibers. In addition, Prosense/VM110 is not yet clinically available as a NIRF enhancing probe.

To solve these limitations, second-generation high-speed OCT-NIRF catheter imaging system has been constructed by our group. In this OCT-NIRF system, the pullback speed is much faster up to 20–40 mm/s, which allows to acquire imaging without blood vessel occlusion and also, the crosstalk noise was reduced to a negligible level (Fig. 4) [30••]. Intriguingly, the FDA-approved NIRF emitting indocyanine green (ICG) was successfully applied for lipid-rich inflamed atheroma in coronary-sized vessels. ICG is an amphiphilic NIRF-emitting probe, and well validated for clinical use such as ophthalmologic imaging, and reported to be accumulated in inflamed, lipidic tissues [31, 32]. In the study of Sunki et al., a clinical dose of ICG was injected intravenously into atheromatous rabbits induced by balloon injury and high-cholesterol diet. Twenty minutes after the ICG injection, in vivo OCT-

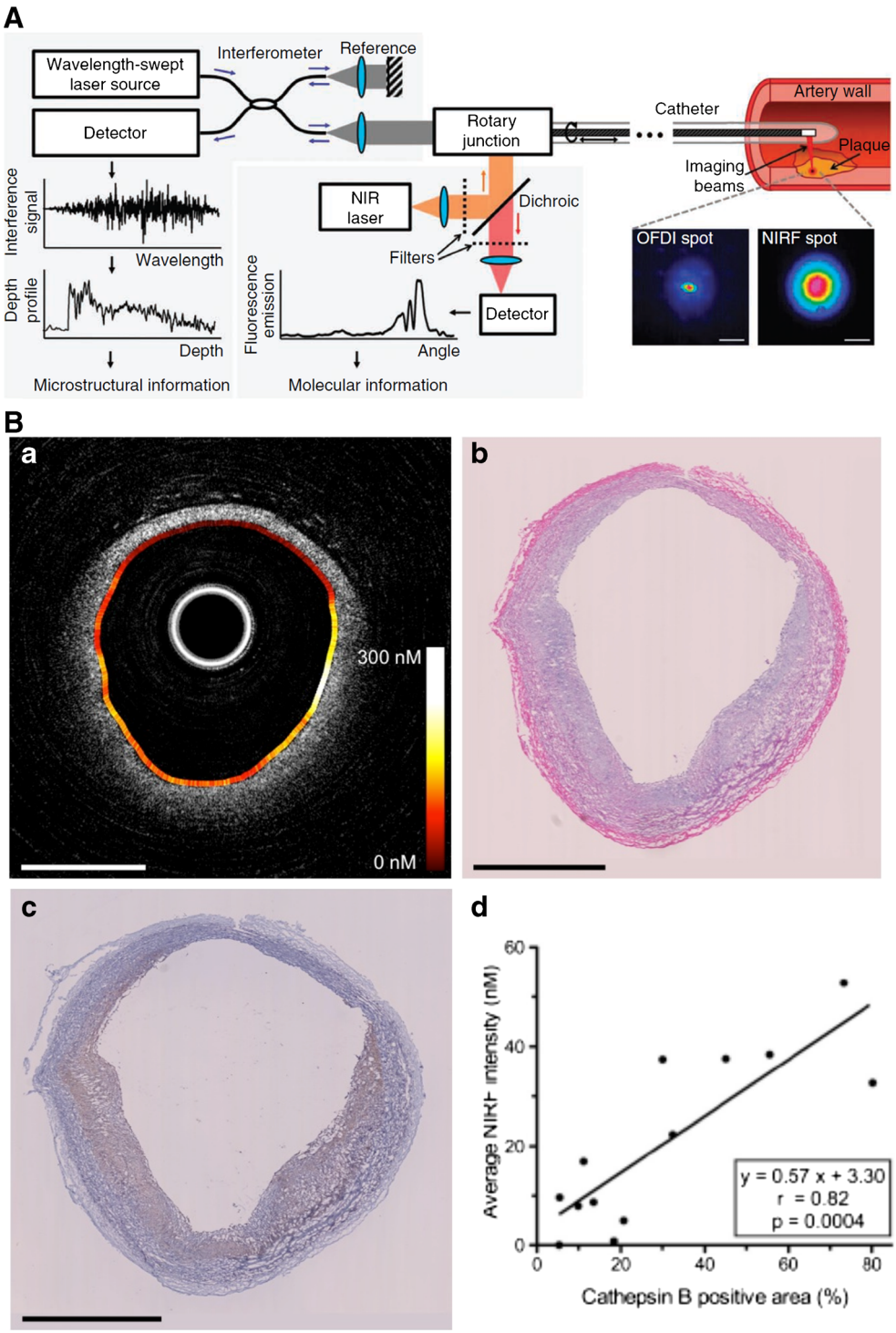

Fig. 3 Intravascular molecular imaging of protease in rabbit plaque using a dual-modality OFDI(OCT)-NIRF imaging system. **A** Schematic of integrated OCT-NIRF system in a single catheter design which enables simultaneous microstructural and molecular imaging of atheroma in vivo. The design of rotary junction based on a double-clad fiber enables dual-modal imaging from two different channels. **B** In vivo OCT-NIRF imaging of atherosclerotic rabbits injected with protease activatable NIRF agent (Prosense/VM110) and corresponding histopathology. **a** Large portion of atherosclerotic region is clearly demonstrated by OCT image (2 to 10 o'clock) with high-NIRF signals, and corresponding with **b** H&E histology and **c** cathepsin B (macrophage) immunostaining. **d** The cathepsin B positive area of plaque and the average NIRF intensity correlated significantly (r=0.82). *Scale bars*, 1 mm. Adapted and reproduced with permission from reference [29••]

NIRF imaging of the aortoiliac vessels was successfully acquired only under contrast flushing through the catheter with a high pullback speed (20 mm/s). The in vivo NIRF signals were intense in the OCT-visualized atheromata of the ICG-injected rabbits. Ex vivo macroscopic fluorescence reflectance imaging signals correlated strongly with the in vivo NIRF signals (Fig. 5). Cellular ICG uptake, correlative fluorescence microscopy, and histopathologic findings corroborated well with the in vivo findings. With all the results, this novel imaging strategy described above is considered as a highly translatable structural-molecular imaging for human vulnerable plaque detection. More recently, the work by our group successfully demonstrated that the high-speed OCT-NIRF with a clinical dosage use of ICG was able to image macrophage

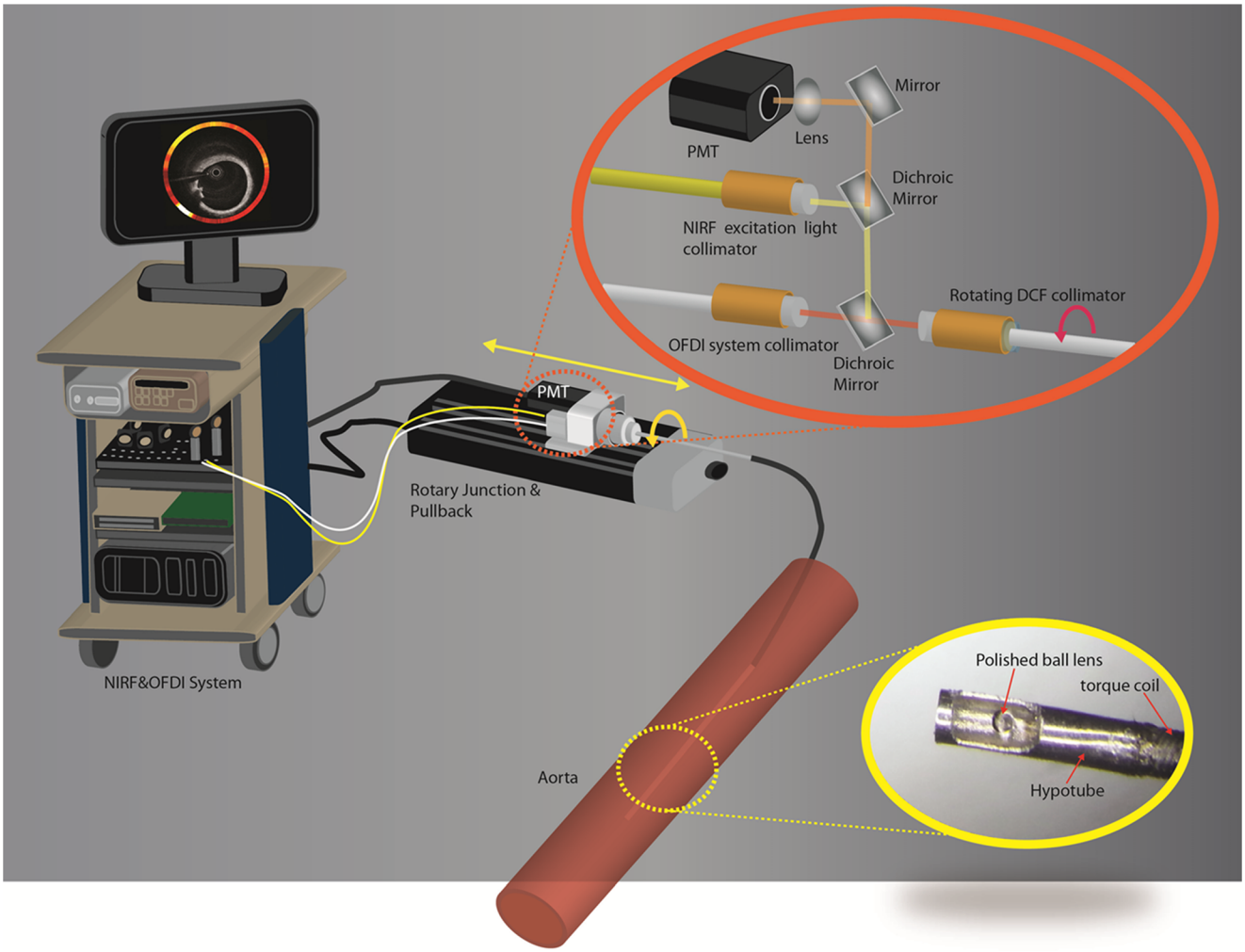

Fig. 4 The schematic of the custom-built second-generation high-speed dual-modal OCT-NIRF system. This catheter system permits stable high-speed rotation (100 revolutions per second (rps)), a rapid pullback speed of 20 mm/s, which is comparable to commercialized OCT systems and allows imaging just under nonocclusive flushing. This ability is essential for clinical application of the system. Background noise due to the use of the double-clad fiber is significantly lower compared to the previous system. Adapted and reproduced with permission from reference [30••]

abundant, lipid-rich plaques in beating coronary arteries of diabetic swine model [33].

While the OCT-NIRF catheter imaging allows the identification of rupture-prone plaques and holds great promise on invasive plaque imaging to accurately predict coronary plaque events, the mechanistic details that regulate ICG deposition in plaques needs to be further clarified. The lower tissue penetration depth of optical properties of OCT-NIRF imaging remains another hurdle for plaque burden estimation. Regardless of these issues, intravascular OCT-NIRF structural-molecular imaging with ICG is highly translatable and is becoming clinically relevant for intracoronary imaging of plaque vulnerability. This innovative catheter-based approach to visualize plaque inflammation will be clinically tested in near future and open a new avenue for prediction of human coronary events that are responsible for acute coronary syndrome. In addition, this approach will help to better investigate the effect of new pharmaceutical and invasive treatments, and probably accelerate the development of novel therapeutic drugs to stabilize high-risk coronary atheroma.

Apoptosis

Apoptotic cells are established targets for molecular imaging of vulnerable plaques. The gathering of lipids and inflammatory cells in the atherosclerotic lesion, combined with local hypoxia, limited perfusion, and defective efferocytosis, enhances the formation of a necrotic cores in advanced plaques [34, 35]. Annexin A5 is a

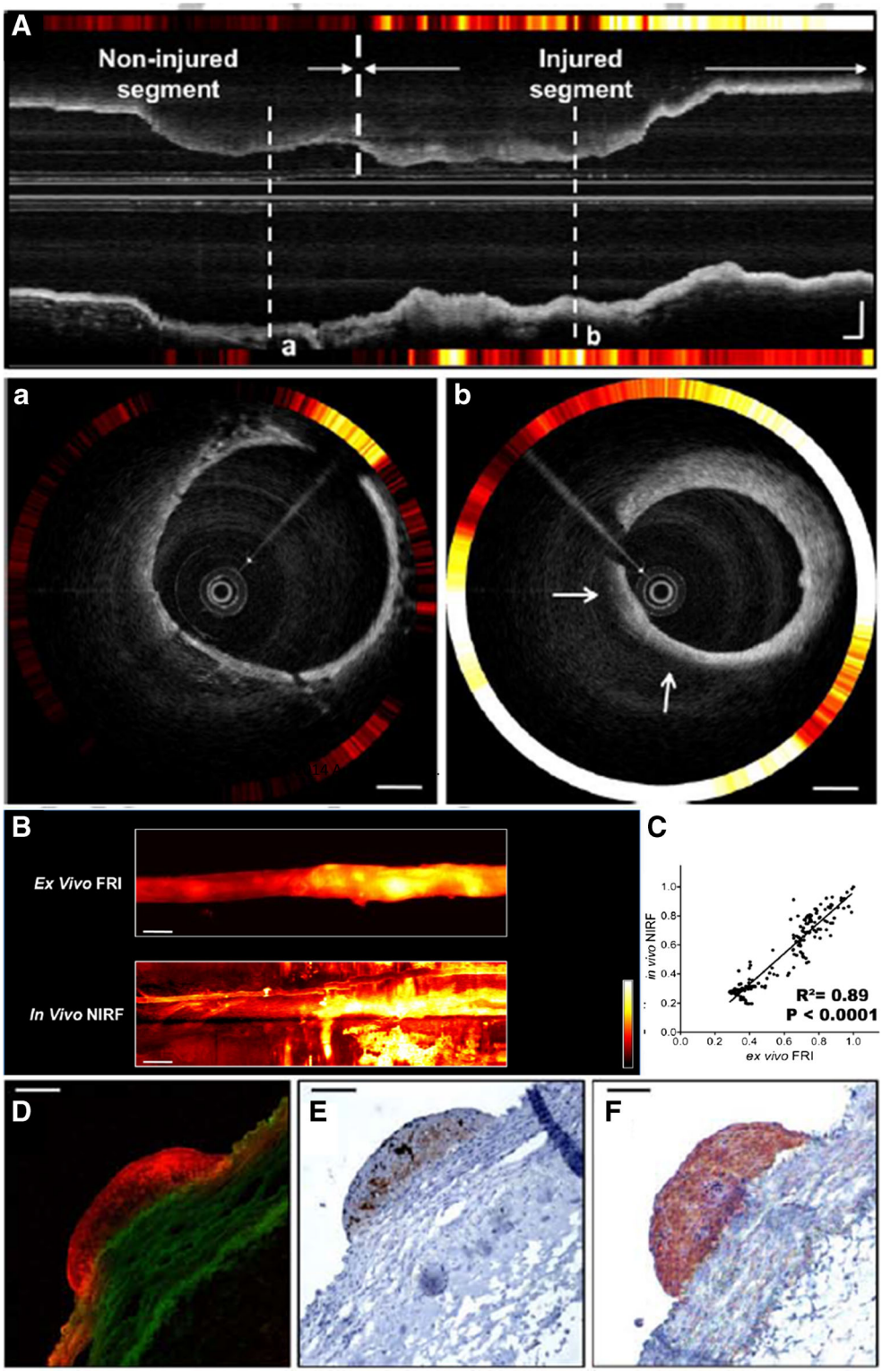

Fig. 5 Fully integrated high-speed intravascular OCT-NIRF imaging from coronary-sized vessels with a NIRF-emitting ICG. **A** In vivo OCT-NIRF longitudinal image and corresponding cross-sectional images of ICG injected atherosclerotic rabbit aorta. Compare to noninjured segment (**a**), the NIRF signal of the lipid-rich inflamed plaque (**b**) identified by OCT shows much higher NIRF signals. *Scale bars*, 1 mm. **B**, **C** Ex vivo fluorescence reflectance images and in vivo NIRF images demonstrate excellent colocalization. *Scale bars*, 5 mm. **D–F** Fluorescence microscope (FM) immunohistopathology corroborated in vivo imagings (*red color*, ICG signal; *green color*, autofluorescence signal) (macrophage, *brown*; lipid, *red staining*). *Scale bars*, 100 μm. Adapted and reproduced with permission from reference [30••]

plasma protein that has a strong affinity for phosphatidylserine expressed on the surface of apoptotic cells. The first small clinical trial to image apoptosis with ^{99m}Tc-radiolabeled annexin A5 was reported [36]. In this pilot study, radiolabled annexin A5 was tested to target macrophage rich plaques with vulnerable morphology in transient ischemic attack patients. However, annexin A5 targets not only apoptotic cells in atheroma

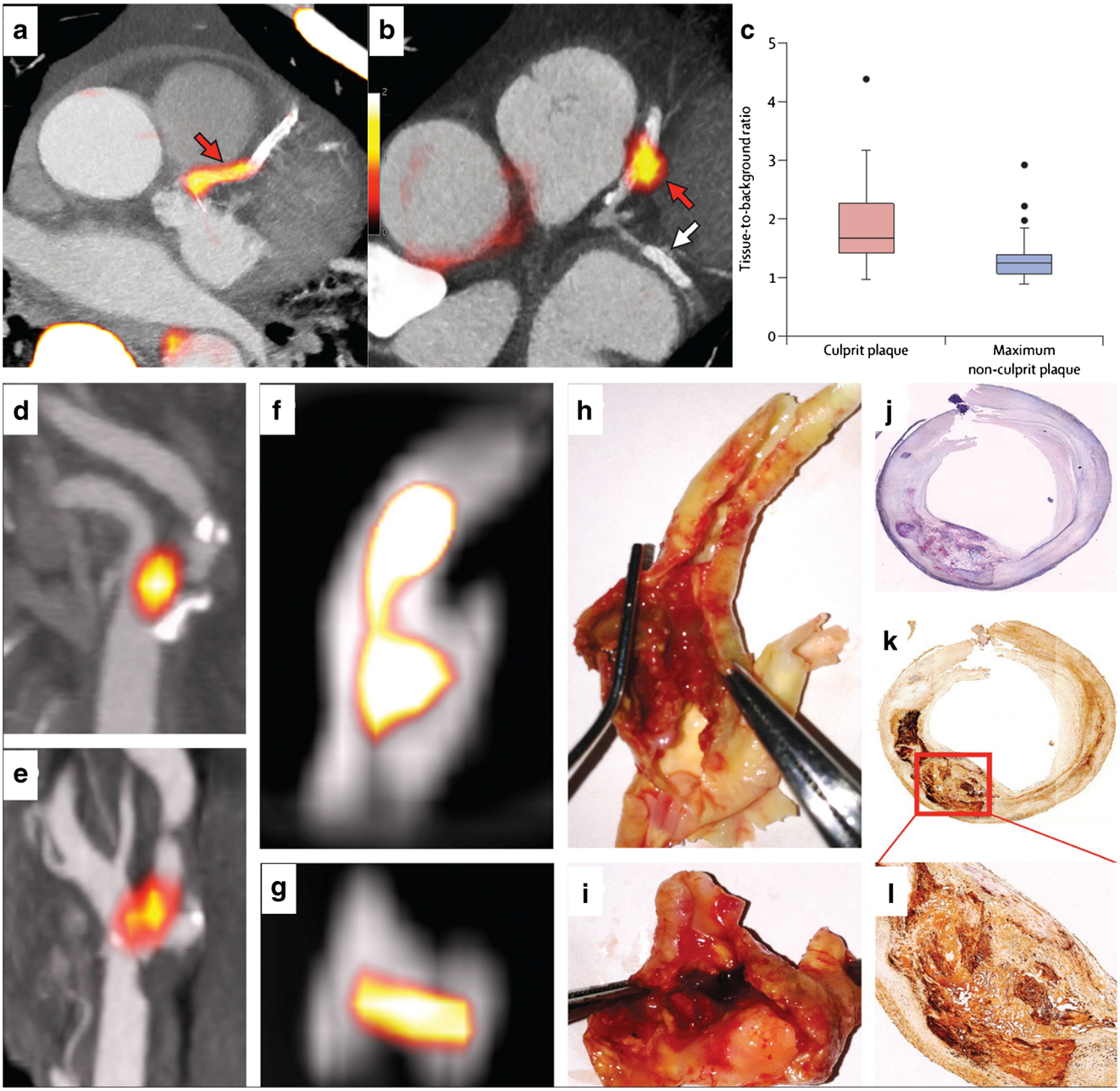

Fig. 6 ^{18}F-NaF PET/CT imaging in patients with myocardial infarction (MI) and carotid plaque rupture. **a** Intense ^{18}F-NaF uptake depicted in a patient with acute ST-elevation MI in a culprit lesion. **b** Anterior MI patient who were stented in both culprit and nonculprit lesion at admission. Notably, ^{18}F-NaF uptake is only increased in the culprit lesion after coronary intervention on PET/CT (*red arrow*). **c** The box plot depicts increased ^{18}F-NaF uptake in culprit plaques as compared with nonculprit lesions. ^{18}F-NaF PET/CT imaging of human carotid artery in vivo (**d**, **e**) and ex vivo (**f**, **G**). ^{18}F-NaF was highly uptaken in the site of plaque rupture (*yellow-orange*). The PET signals colocalized with thrombus on excised carotid endarterectomy specimens (**h**, **i**) **j** Immunohistochemistry of the ^{18}F-NaF-positive area shows large necrotic core and increased calcification activity (**k** 4×, **l** 10×, tissue nonspecific alkaline phosphatase). Adapted and reproduced with permission from reference [48••]

but also nonapoptotic macrophages [37] and platelets [38], which may limit its potential usage in vulnerable plaque imaging. More specific approaches, including the detection of caspase activity, have been investigated to overcome the limitation of annexin A5 targeting molecular imaging [39]. While PET imaging of caspase activity has been utilized for in vivo detection in mouse models of human colorectal cancer [40], caspase-

targeted molecular imaging has not been studied in atherosclerotic plaques.

Neoangiogenesis

Plaque neovascularization related to hypoxia is a histologic hallmark of vulnerable plaques. Microvessels within evolving plaques may cause intraplaque hemorrhage and thus, trigger inflammatory cell migration and plaque expansion through the accumulation of foam cells, erythrocytes, and macrophages [41]. As the integrin $\alpha_v\beta_3$ is a key mediator of neoangiogenesis in atheromatous plaques, it may represent as a molecular imaging target for neovascularization. Particularly, the tri-peptide "R-G-D (arginine-glycine-aspartate)" has a high affinity for integrin $\alpha_v\beta_3$ and is utilized as a primary binding motif in integrin-targeting imaging agents. Using these labeled RGD peptidomimetics, several preclinical studies have been performed for molecular imaging of neovascularization in plaques [42]. Winter et al. reported that in vivo imaging of neovascularization using MRI targeting $\alpha_v\beta_3$ was feasible to estimate rabbit aortic plaque [43]. Recently, Ambros et al. reported that PET/CT imaging of $\alpha_v\beta_3$ expression in human carotid plaques seems promising [44]. Whether this strategy will be effective in detecting vulnerable plaques remains to be further determined.

Calcification

Calcification is one of the main features of atherosclerosis. Coronary calcium scoring by CT scans has been used for many years as a clinical marker of atherosclerotic plaque burden and a predictor of future adverse cardiovascular events. Microcalcification, including speckled or fragmented calcification may mechanically destabilize the plaque and make it susceptible to micro-fracture and prone to rupture [45]. Considering that conventional imaging is not sufficient to clearly estimate microcalcification, molecular imaging approach is expected to provide a new insight to understand the interplay between microcalcification and plaque instability. In Aikawa's molecular imaging work, osteogenesis could be linked to inflammation in early stage of atherosclerosis [46]. Based on this concept, osteoblastic activity imaging has been tested in human atheromata using a PET tracer ^{18}F-sodium fluoride [47]. More recently, PET imaging of calcification was nicely demonstrated in human coronary arteries for in vivo estimation of high-risk coronary plaques [48••]. Nikhil et al. demonstrated that ^{18}F-NaF PET/CT may identify ruptured plaques or even unruptured vulnerable plaques in patients with ACS (Fig. 6). With these results, ^{18}F-NaF PET appears to be promising as a noninvasive coronary molecular imaging for detection of high-risk patients.

Lipid Core

Near-infrared spectroscopy (NIRS) is a new intravascular imaging technique that identifies the chemical signature pattern of the lipid component by near-infrared lights [49], which recently received US FDA approval [50]. NIRS has been extensively evaluated these days particularly in human coronary fields [51]. While NIRS itself cannot provide information about the lumen or plaque character, fusion of IVUS and NIRS imaging has been proposed. Oemrawsingh et al. recently reported the results of ATHEROREMO-NIRS study [52•], which was the first observational, and prospective study designed to assess the prognostic values of lipid volume detected by NIRS in coronary beds. They found that the presence of NIRS-detected lipid-rich plaque was significantly associated with adverse cardiovascular events. However, NIRS imaging is unable to precisely localize lipid distribution within plaques. In addition, NIRS cannot detect the infiltration of inflammatory cells which is a hallmark of vulnerable plaques. Molecular imaging targeting inflammation will be able to cover this information gap.

Conclusions

There is wide evidence supporting the role of molecular imaging in detecting vulnerable plaques both noninvasively and invasively. In PET imaging utilizing inflammation-sensitive metabolic reporters, most clinical trials have focused on large vessels, such as carotid artery and aortic plaques. However, the small size of coronary plaque, continuous motion, and potential myocardial uptake of tracers are known as major barriers to coronary inflammation imaging. In contrast, ^{18}F-NaF PET imaging seems to be appealing in coronary field and needs to be further validated in a large, prospective clinical study.

Intravascular molecular imaging has shown a substantial advancement in the past decade, accelerating the ability to image coronary plaque inflammation at a high resolution level. However, to date, the majority of the data came from preclinical studies. Moreover, it is obvious that intravascular standalone molecular imaging is much more challenging to accurately detect vulnerable plaques. Conceptually, multimodal structural-molecular imaging, combination of two separate imaging strategies, could be an ideal tool to offer comprehensive information regarding plaque vulnerability. From this viewpoint, recent advancement on fully integration of NIRF molecular imaging with OCT structural imaging has many advantages to provide structural detail and molecular information as well. This innovative imaging strategy appears to be promising but should require FDA approval for clinical application. Fortunately, recent success for ICG application as a clinically relevant NIRF imaging agent sheds light on this

issue and, accordingly, intravascular OCT-NIRF imaging of vulnerable plaques may offer an avenue for personalized medicine in patients with coronary disease in future.

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Compliance with Ethics Guidelines

Conflict of Interest S Lee and JW Kim both declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Executive summary: heart disease and stroke statistics–2013 update: a report from the American Heart Association. Circulation. 2013;127(1):143–52. doi:10.1161/CIR.0b013e318282ab8f.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685–95. doi:10.1056/NEJMra043430.
3. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473(7347):317–25.
4. Sanz J, Fayad ZA. Imaging of atherosclerotic cardiovascular disease. Nature. 2008;451(7181):953–7. doi:10.1038/nature06803.
5. Kusters DH, Tegtmeier J, Schurgers LJ, Reutelingsperger CP. Molecular imaging to identify the vulnerable plaque—from basic research to clinical practice. Mol Imaging Biol. 2012;14(5):523–33. doi:10.1007/s11307-012-0586-7.
6. Silvestre-Roig C, de Winther MP, Weber C, Daemen MJ, Lutgens E, Soehnlein O. Atherosclerotic plaque destabilization: mechanisms, models, and therapeutic strategies. Circ Res. 2014;114(1):214–26. doi:10.1161/CIRCRESAHA.114.302355.
7. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation. 2003;108(14):1664–72. doi:10.1161/01.cir.0000087480.94275.97.
8. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. The New England journal of medicine. 2013;368(21):2004–13. doi:10.1056/NEJMra1216063
9. Narula J, Nakano M, Virmani R, Kolodgie FD, Petersen R, Newcomb R, et al. Histopathologic characteristics of atherosclerotic coronary disease and implications of the findings for the invasive and noninvasive detection of vulnerable plaques. J Am Coll Cardiol. 2013;61(10):1041–51. doi:10.1016/j.jacc.2012.10.054.
10. Derksen WJ, Peeters W, van Lammeren GW, Tersteeg C, de Vries JP, de Kleijn DP, et al. Different stages of intraplaque hemorrhage are associated with different plaque phenotypes: a large histopathological study in 794 carotid and 276 femoral endarterectomy specimens. Atherosclerosis. 2011;218(2):369–77. doi:10.1016/j.atherosclerosis.2011.07.104.
11. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med. 1989;320(14):915–24. doi:10.1056/nejm198904063201407.
12. Kaufmann BA, Sanders JM, Davis C, Xie A, Aldred P, Sarembock IJ, et al. Molecular imaging of inflammation in atherosclerosis with targeted ultrasound detection of vascular cell adhesion molecule-1. Circulation. 2007;116(3):276–84. doi:10.1161/CIRCULATIONAHA.106.684738.
13. Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, Figueiredo JL, et al. 18 F-4V for PET-CT imaging of VCAM-1 expression in atherosclerosis. J Am Coll Cardiol Img. 2009;2(10):1213–22. doi:10.1016/j.jcmg.2009.04.016.
14. McAteer MA, Schneider JE, Ali ZA, Warrick N, Bursill CA, von Zur Muhlen C, et al. Magnetic resonance imaging of endothelial adhesion molecules in mouse atherosclerosis using dual-targeted microparticles of iron oxide. Arterioscler Thromb Vasc Biol. 2008;28(1):77–83. doi:10.1161/ATVBAHA.107.145466.
15. Mulder WJ, Jaffer FA, Fayad ZA, Nahrendorf M. Imaging and nanomedicine in inflammatory atherosclerosis. Sci Transl Med. 2014;6(239):239sr1. doi:10.1126/scitranslmed.3005101.
16. Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, et al. In vivo 18 F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol. 2006;48(9):1818–24. doi:10.1016/j.jacc.2006.05.076.
17.• Tawakol A, Fayad ZA, Mogg R, Alon A, Klimas MT, Dansky H, et al. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. J Am Coll Cardiol. 2013;62(10):909–17. doi:10.1016/j.jacc.2013.04.066. **This article demonstrates the feasibility of therapeutic monitoring for individual patients by using a noninvasive PET/CT imaging with FDG probe**.
18. Rogers IS, Nasir K, Figueroa AL, Cury RC, Hoffmann U, Vermylen DA, et al. Feasibility of FDG imaging of the coronary arteries: comparison between acute coronary syndrome and stable angina. J Am Coll Cardiol Img. 2010;3(4):388–97. doi:10.1016/j.jcmg.2010.01.004.
19. Kooi ME, Cappendijk VC, Cleutjens KB, Kessels AG, Kitslaar PJ, Borgers M, et al. Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging. Circulation. 2003;107(19):2453–8. doi:10.1161/01.cir.0000068315.98705.cc.
20. Tang TY, Howarth SP, Miller SR, Graves MJ, Patterson AJ, UK-I JM, et al. The ATHEROMA (Atorvastatin Therapy: Effects on Reduction of Macrophage Activity) Study. Evaluation using ultrasmall superparamagnetic iron oxide-enhanced magnetic resonance imaging in carotid disease. J Am Coll Cardiol. 2009;53(22):2039–50. doi:10.1016/j.jacc.2009.03.018.
21. Morishige K, Kacher DF, Libby P, Josephson L, Ganz P, Weissleder R, et al. High-resolution magnetic resonance imaging enhanced with superparamagnetic nanoparticles measures macrophage burden in atherosclerosis. Circulation. 2010;122(17):1707–15. doi:10.1161/CIRCULATIONAHA.109.891804.
22. Lancelot E, Amirbekian V, Brigger I, Raynaud JS, Ballet S, David C, et al. Evaluation of matrix metalloproteinases in atherosclerosis using a novel noninvasive imaging approach. Arterioscler Thromb Vasc Biol. 2008;28(3):425–32. doi:10.1161/ATVBAHA.107.149666.
23. Hyafil F, Vucic E, Cornily JC, Sharma R, Amirbekian V, Blackwell F, et al. Monitoring of arterial wall remodelling in atherosclerotic

rabbits with a magnetic resonance imaging contrast agent binding to matrix metalloproteinases. Eur Heart J. 2011;32(12):1561–71. doi:10.1093/eurheartj/ehq413.

24. Deguchi JO, Aikawa M, Tung CH, Aikawa E, Kim DE, Ntziachristos V, et al. Inflammation in atherosclerosis: visualizing matrix metalloproteinase action in macrophages in vivo. Circulation. 2006;114(1):55–62. doi:10.1161/CIRCULATIONAHA.106.619056.
25. Razavian M, Tavakoli S, Zhang J, Nie L, Dobrucki LW, Sinusas AJ, et al. Atherosclerosis plaque heterogeneity and response to therapy detected by in vivo molecular imaging of matrix metalloproteinase activation. J Nucl Med. 2011;52(11):1795–802. doi:10.2967/jnumed.111.092379.
26. Chen J, Tung CH, Allport JR, Chen S, Weissleder R, Huang PL. Near-infrared fluorescent imaging of matrix metalloproteinase activity after myocardial infarction. Circulation. 2005;111(14):1800–5. doi:10.1161/01.cir.0000160936.91849.9f.
27. Jaffer FA, Kim DE, Quinti L, Tung CH, Aikawa E, Pande AN, et al. Optical visualization of cathepsin K activity in atherosclerosis with a novel, protease-activatable fluorescence sensor. Circulation. 2007;115(17):2292–8. doi:10.1161/circulationaha.106.660340.
28. Jaffer FA, Vinegoni C, John MC, Aikawa E, Gold HK, Finn AV, et al. Real-time catheter molecular sensing of inflammation in proteolytically active atherosclerosis. Circulation. 2008;118(18):1802–9. doi:10.1161/circulationaha.108.785881.
29.•• Yoo H, Kim JW, Shishkov M, Namati E, Morse T, Shubochkin R, et al. Intra-arterial catheter for simultaneous microstructural and molecular imaging in vivo. Nat Med. 2011;17(12):1680–4. doi:10.1038/nm.2555. **This is the first study of catheter-based, dual-modality intravascular OFDI/NIRF imaging in vivo to identify high-risk plaques**.
30.•• Lee S, Lee MW, Cho HS, Song JW, Nam HS, Oh DJ, et al. Fully integrated high-speed intravascular optical coherence tomography/near-infrared fluorescence structural/molecular imaging in vivo using a clinically available near-infrared fluorescence-emitting indocyanine green to detect inflamed lipid-rich atheromata in coronary-sized vessels. Circ Cardiovasc Interv. 2014;7(4):560–9. doi:10.1161/circinterventions.114.001498. **This study demonstrates important progress in intravascular structural/molecular imaging of high-risk atheroma in coronary-sized vessels in vivo using a clinically available NIRF-emiiting ICG by utilizing an advanced OCT-NIRF system**.
31. Werner SG, Langer HE, Schott P, Bahner M, Schwenke C, Lind-Albrecht G, et al. Indocyanine green-enhanced fluorescence optical imaging in patients with early and very early arthritis: a comparative study with magnetic resonance imaging. Arthritis Rheum. 2013;65(12):3036–44. doi:10.1002/art.38175.
32. Vinegoni C, Botnaru I, Aikawa E, Calfon MA, Iwamoto Y, Folco EJ, et al. Indocyanine green enables near-infrared fluorescence imaging of lipid-rich, inflamed atherosclerotic plaques. Sci Transl Med. 2011;3(84):84ra45. doi:10.1126/scitranslmed.3001577.
33. Kim S, Lee MW, Cho HS, Song JW, Lee S, Oh DJ, et al. Abstract 17216: Intracoronary Dual-modal OCT/NIRF Structural-Molecular Imaging with a Clinical Dose of Indocyanine Green (ICG) for Detection of High-risk Coronary Plaques in Diabetic Swine Model. Circulation. 2014;130 Suppl 2:A17216.
34. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol. 2010;10(1):36–46. doi:10.1038/nri2675.
35. Wang JC, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ Res. 2012;111(2):245–59. doi:10.1161/circresaha.111.261388.
36. Kietselaer BL, Reutelingsperger CP, Heidendal GA, Daemen MJ, Mess WH, Hofstra L, et al. Noninvasive detection of plaque instability with use of radiolabeled annexin A5 in patients with carotid-artery atherosclerosis. N Engl J Med. 2004;350(14):1472–3. doi:10.1056/nejm200404013501425.
37. Laufer EM, Winkens MH, Narula J, Hofstra L. Molecular imaging of macrophage cell death for the assessment of plaque vulnerability. Arterioscler Thromb Vasc Biol. 2009;29(7):1031–8. doi:10.1161/ATVBAHA.108.165522.
38. Ramstrom S, O'Neill S, Dunne E, Kenny D. Annexin V binding to platelets is agonist, time and temperature dependent. Platelets. 2010;21(4):289–96. doi:10.3109/09537101003660564.
39. Edgington LE, Berger AB, Blum G, Albrow VE, Paulick MG, Lineberry N, et al. Noninvasive optical imaging of apoptosis by caspase-targeted activity-based probes. Nat Med. 2009;15(8):967–73. doi:10.1038/nm.1938.
40. Hight MR, Cheung YY, Nickels ML, Dawson ES, Zhao P, Saleh S, et al. A peptide-based positron emission tomography probe for in vivo detection of caspase activity in apoptotic cells. Clin Cancer Res. 2014;20(8):2126–35. doi:10.1158/1078-0432.CCR-13-2444.
41. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol. 2005;25(10):2054–61. doi:10.1161/01.ATV.0000178991.71605.18.
42. Su H, Gorodny N, Gomez LF, Gangadharmath UB, Mu F, Chen G, et al. Atherosclerotic plaque uptake of a novel integrin tracer (1)(8)F-Flotegatide in a mouse model of atherosclerosis. J Nucl Cardiol. 2014;21(3):553–62. doi:10.1007/s12350-014-9879-3.
43. Winter PM, Morawski AM, Caruthers SD, Fuhrhop RW, Zhang H, Williams TA, et al. Molecular imaging of angiogenesis in early-stage atherosclerosis with alpha(v)beta3-integrin-targeted nanoparticles. Circulation. 2003;108(18):2270–4. doi:10.1161/01.cir.0000093185.16083.95.
44. Beer AJ, Pelisek J, Heider P, Saraste A, Reeps C, Metz S, et al. PET/CT imaging of integrin alphavbeta3 expression in human carotid atherosclerosis. J Am Coll Cardiol Img. 2014;7(2):178–87. doi:10.1016/j.jcmg.2013.12.003.
45. Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed? Arterioscler Thromb Vasc Biol. 2014;34(4):724–36. doi:10.1161/atvbaha.113.302642.
46. Aikawa E, Nahrendorf M, Figueiredo JL, Swirski FK, Shtatland T, Kohler RH, et al. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging in vivo. Circulation. 2007;116(24):2841–50. doi:10.1161/circulationaha.107.732867.
47. Dweck MR, Chow MW, Joshi NV, Williams MC, Jones C, Fletcher AM, et al. Coronary arterial 18 F-sodium fluoride uptake: a novel marker of plaque biology. J Am Coll Cardiol. 2012;59(17):1539–48. doi:10.1016/j.jacc.2011.12.037.
48.•• Joshi NV, Vesey AT, Williams MC, Shah ASV, Calvert PA, Craighead FHM, et al. 18F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial. Lancet. 2014;383(9918):705–13. doi:10.1016/s0140-6736(13)61754-7. *This is the first noninvasive molecular imaging method using 18F-NaF PET/CT for detecting high-risk plaques and localizing ruptured plaques as well*.
49. Caplan JD, Waxman S, Nesto RW, Muller JE. Near-infrared spectroscopy for the detection of vulnerable coronary artery plaques. J Am Coll Cardiol. 2006;47(8 Suppl):C92–6. doi:10.1016/j.jacc.2005.12.045.
50. Jang IK. Near infrared spectroscopy: another toy or indispensible diagnostic tool? Circ Cardiovasc Interv. 2012;5(1):10–1. doi:10.1161/circinterventions.111.967935.
51. Madder RD, Goldstein JA, Madden SP, Puri R, Wolski K, Hendricks M, et al. Detection by near-infrared spectroscopy of

large lipid core plaques at culprit sites in patients with acute ST-segment elevation myocardial infarction. JACC Cardiovasc Interv. 2013;6(8):838–46. doi:10.1016/j.jcin.2013.04.012.

52.• Oemrawsingh RM, Cheng JM, Garcia-Garcia HM, van Geuns RJ, de Boer SP, Simsek C, et al. Near-infrared spectroscopy predicts cardiovascular outcome in patients with coronary artery disease. J Am Coll Cardiol. 2014;64(23):2510–8. doi:10.1016/j.jacc.2014.07.998. **First prospective study assess the long-term prognostic value of intracoronary NIRS in coronary disease patients**.

53. Ay I, Blasi F, Rietz TA, Rotile NJ, Kura S, Brownell AL, et al. In vivo molecular imaging of thrombosis and thrombolysis using a fibrin-binding positron emission tomographic probe. Circ Cardiovasc Imaging. 2014;7(4):697–705. doi:10.1161/circimaging.113.001806.