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BIOLOGICAL IMAGING OF ATHEROSCLEROSIS: MOVING BEYOND ANATOMY

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Abstract

Biological or molecular imaging is now providing exciting new strategies to study atherosclerosis in both animals and humans. These technologies hold the promise to provide disease-specific, molecular information within the context of a systemic or organ-specific disease *beyond* traditional anatomical-based imaging. By integration of biological, chemical and anatomical imaging knowledge into diagnostic strategies, a more comprehensive and predictive picture of atherosclerosis is likely to emerge. As such, biological imaging is well-positioned to study different stages of atherosclerosis and its treatment, including the sequence of atheroma initiation, progression, and plaque rupture.

In this review we describe the evolving concepts in atherosclerosis imaging with a focus on coronary artery disease, and we provide an overview of recent exciting translational developments in biological imaging. The illuminated examples and discussions will highlight how biological imaging is providing new clinical approaches to identify high-risk plaques, and to streamline the development process of new atherosclerosis therapies.

Keywords

Atherosclerosis; Molecular Imaging; Vascular Biology; Inflammation; Intravascular Imaging; Fluorescence

Evolving concepts in atherosclerosis

Atherosclerosis is a progressive arterial disease characterized by lipid deposition and chronic inflammation. Over decades, atherosclerosis can evolve into obstructive vascular disease, such as coronary artery disease, peripheral artery disease and cerebrovascular disease. Although the mortality rates for coronary heart disease are declining, cardiovascular disease remains the biggest killer worldwide.[1] When considering the aging population, and the growing obesity and diabetes epidemics, substantial numbers of patients are likely to develop complications of atherosclerosis.[2]

Atherogenesis is a systemic immuno-inflammatory driven disease based in the medium- and larger-sized arteries, and usually originates in childhood and adolescence. About two-thirds of subjects will have some degree of plaque development before the age of 35, suggesting that most individuals above 40 years of age in the western world will have some degree of atherosclerosis.[3,4] Atherogenesis involves many processes, such as endothelial cell activation, inflammation and increased lipid deposition, followed by lipid oxidation, macrophage apoptosis, and necrotic core formation, eventually evolving into atheromatous

plaques that can rupture, leading to a thrombotic occlusion of the artery of a specific organ. [5]

Most of our knowledge about structural and biological characteristics of culprit lesions has been obtained from necropsy studies from patients with sudden cardiac death and through experimental studies. In a relatively large autopsy series, three major atherosclerotic plaque morphologies have been found in the human coronary artery to cause acute events: almost two thirds of patients have plaque rupture of a thin-capped fibroatheroma (TCFA) housing a large necrotic core, which has been formed by lipids, macrophage infiltration and intraplaque hemorrhage.[6,7] Importantly, it has been demonstrated that more than one TCFA can occur in the same patient.[8] In the second pathology subtype, around thirty percent of the patients demonstrate plaque erosion, or denudation of the endothelium, accompanied by thrombus formation. The mechanism by which a plaque erodes remains poorly understood, but often affects younger women and smokers. The erosion site usually shows a thrombus that adheres to a proteoglycan-rich layer and smooth muscle cells with minimal inflammation. And lastly, a smaller third lesion group prone to coronary thrombosis is the so-called calcified nodule. This lesion exhibits a calcified plate with superimposed bony nodules that result in discontinuity of the cap and promotes luminal thrombosis.[9,10] Topographically, plaque rupture occurs most frequently in the more proximal portions of the coronary arteries, with plaques arising from lesions less than 50% luminal stenosis, and often demonstrating a high degree of positive or outward remodeling. Plaque erosions, on the other hand, arise from lesions that exhibit more negative remodeling.[11,8,12,13] Another feature of high-risk coronary plaque is the presence of low and oscillating shear stress that influence plaque characteristics and potentially provokes plaque growth and rupture, particularly in the presence of microcalcifications.[14,15]

In recent years, the view that a single vulnerable plaque causes acute vascular events has been challenged based on several observations in coronary artery disease studies. There are several reports finding that plaque rupture and thrombus formation often do not lead to coronary events.[16] When plaques advance beyond 40% luminal narrowing, functional compromise may occur with a series of rupture and healing, as shown in patients who died of non-cardiac causes.[17-20] In another report, it was further demonstrated that only 11% of sudden coronary deaths were caused by “virgin” ruptures.[21]

Since many of the above-mentioned atherosclerotic processes and their location and sequence are only partially known, it remains a challenge to identify lesions before they induce their devastating atherothrombotic events. This is partially due to the limited capabilities of existing imaging modalities, and the complexity of atherothrombosis, a dynamic and unpredictable process. Consequently, there is a need for a clinical, robust, fast, and sensitive set of diagnostic imaging tools that provides new insights into the development of high-risk lesions. Moreover, such imaging tools could provide longitudinal insights into the evolution of atheroma, and the biological effects of putative atherosclerosis therapies.

Noninvasive anatomical imaging of atherosclerosis

Angiography is the long-standing, widely adopted gold standard for anatomical imaging that assesses the degree of obstruction due to plaques encroaching onto the lumen. However, angiography cannot measure the degree of plaque burden nor the degree of positive or outward remodeling, features associated with high-risk plaques. Moreover, it is well-known that high-risk atherosclerotic plaques may be present in angiographically normal vessels.[22]

As a result, several new noninvasive and invasive techniques such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging (US), and optical coherence tomography (OCT) have entered the arena of direct vessel wall imaging, focusing on plaque

burden and plaque composition. These modalities were developed to better visualize morphological hallmarks in the vascular wall, looking at signs of plaque vulnerability such as positive remodeling, calcification, plaque hemorrhage, necrotic core size, inflammation, vaso vasorum and fibrous or fatty components, and thickness of the fibrous cap. Additionally, these technologies are now capable of discovering early phases of atherosclerosis and the influence of risk factors on its progression.[23] Plaque erosion has received less attention, mainly due to its relatively unknown pathophysiology and less discriminatory imaging characteristics besides thrombus formation.

Of all the noninvasive non-targeted imaging modalities, MRI has achieved considerable success in the assessment of carotid and aortic plaques. MRI capably identifies different plaque components and size with good reproducibility and fair accuracy, and shows the ability to predict future cerebrovascular events.[24-27] Plaque MRI can also be used to monitor the efficacy of atherosclerosis therapeutics.[28,29] MRI of coronary arteries remains challenging due to factors such as insufficient temporal resolution, motion artifacts and small target size.[30] In addition, its relatively lower inherent sensitivity is a further impediment to biological MRI of coronary plaques.

Multi-detector CT technology has evolved significantly and in a rapid pace in the last ten years. With the invention of multiple detector rows, CT scanners can acquire 256 slices in less than 250ms, resulting in a coronary angiogram in just 15 seconds. Despite high sensitivity and specificity for detection of coronary artery disease, invasive angiography combined with stress testing currently remains the test for patients with a high probability of coronary disease anticipating an intervention. Like angiography, disadvantages of CT are the need for radiation and nephrotoxic iodinated contrast. In addition, CT is limited in determining the severity of stenosis when heavy calcium is present. However, for patients with low to intermediate likelihood of coronary artery disease, CT can be used to reliably exclude clinically significant coronary artery disease as a reliable non-invasive alternative to invasive catheter angiography.[31-33] Recent preliminary investigations have investigated plaque characteristics, showing a suggestion for a relation between plaque characteristics and future cardiovascular events in patients .[34,35]

Ultrasound imaging is a cost-effective, noninvasive and portable imaging modality that has been extensively used to assess carotid arteries for plaque. Plaques that are heterogeneous, lipid-rich, commonly with low calcium content and/or intraplaque hemorrhage, are postulated to be high-risk.[36] Carotid intima-media thickness (CMT) and the carotid plaque, as assessed by ultrasound, have been linked to coronary heart disease and stroke providing a meaningful surrogate endpoint.[37] In addition, the use of gas-filled microspheres have enabled us to assess another feature which has been implied with plaque instability, plaque vaso vasorum or neovascularization.[38,39]

Intravascular anatomical imaging of atherosclerosis

To overcome the limitations of noninvasive imaging, FDA-approved intravascular imaging strategies have been developed that significantly contribute to a more accurate characterization of high-risk plaques. Optical coherence tomography (OCT) utilizes light instead of ultrasound reflection to produce cross-sectional images of the vessel wall from the inside, with an impressive resolution of 10-15 micrometers. OCT however has limited tissue penetration and requires flushing to displace blood, due to the absorption of light by interfering red blood cells. OCT is able to measure lipid-rich cores, and the thickness of the fibrous cap in lesions.[40] Clinically, this technology compares favorably with intravascular ultrasound (IVUS) in illuminating culprit lesion morphology in acute coronary syndromes, and optimizing coronary stent placement, stent endothelial coverage, and stent apposition.

[41] Notably, the technique has considerably improved in terms of frame rates, pullback speed and resolution, extending into a new technique called optical frequency domain imaging (OFDI). This new technique has an even better ability to characterize initial stages of atherogenesis and composition, and improved ability to assess stent placement and complications.[42]

Intravascular ultrasound (IVUS) is a valuable tool assessing complications of percutaneous coronary intervention (PCI), being able to provide important information during percutaneous interventions, but also on important plaque parameters in carotid and coronary artery, respectively, such as vessel size and necrotic core dimensions, but also intraplaque hemorrhage and vasa vasorum.[43] However, despite its superior depth penetration, the limited 100-200 micrometer resolution of ultrasound limits its value in accurately detecting specific plaque components. Moreover, high echo-reflection of calcified and dense fibrotic tissue remain a problem for determination of plaque composition.[44]

For areas of low echo-reflection, ultrasonic analysis techniques such as known as Virtual Histology (VH-IVUS) can now provide detailed intravascular assessment of plaque composition. This technology utilizes radiofrequency spectral analysis of ultrasound backscatter signals to identify different plaque components, identifying the necrotic core and fibrous tissue in in high-risk lesions. [45] The international multi-center PROSPECT study utilized this technique to investigate three vessel non-culprit lesions in 697 patients with acute coronary syndromes, and comprises the largest prospective natural history study of coronary plaque progression. The authors identified multiple features of non-culprit plaques associated with major adverse events, including plaque burden, VH-IVUS-derived thin-cap fibroatheroma, and a small minimal lumen area, consistent with results from pathologic studies.[46] Although this study is unique and has provided great insight into the natural history of coronary non-culprit plaques, the relatively low predictive accuracy motivates the development of more predictive tools for vascular events. This framework has motivated the development of biological/molecular imaging approaches to address this unmet need.

Biological imaging of atherosclerosis

In recent years, traditional imaging has moved beyond its anatomical boundary to further our understanding of lesion biology. Although plaque morphology has been extensively studied, and the PROSPECT trial improved our understanding of atherogenesis, structural imaging is insufficient as a strategy to accurately predict vascular events.[46] Moreover, we know relatively little of the temporal relation of the vascular events and thrombus maturation and molecular and cellular changes that are involved. These limitations warrant the move beyond anatomical imaging in order to attempt to identify high-risk patients at high-risk lesions.

Biological, or molecular imaging of atherosclerosis is a rapidly developing field in the last decade with the promise to give us a better understanding of the disease beyond its anatomy, and possesses great potential to impact therapeutic strategies.[47-49] Numerous noninvasive but also intravascular approaches have been tested for biological imaging of the different stages and molecular processes of atherosclerosis, but only few have made the translation into the clinic thus far.

Clinical progress

Several noninvasive but also intravascular approaches have been proposed for biological imaging, each having their drawbacks and advantages. The future of biological imaging will most probably favor a combination of imaging modalities, allowing comprehensive assessment of plaque burden, composition, chemistry, and biology. This integrative

approach offers the best chance to add predictive power over conventional risk prediction schemes such as the Framingham risk score or coronary artery calcium scoring. For larger arterial beds such as the carotid artery, noninvasive hybrid technology, such as PET or SPECT combined with CT is already widely available, and more recently PET/MR are emerging as an integrated option.[50]

For biological imaging of patients with atherosclerosis, most work has focused on imaging of plaque inflammation, as inflammation is a driving force in atherogenesis and plaque complication. There are two major imaging platforms that have been tested clinically in patients, predominantly in the larger arteries, with some recent progress into the coronary arteries, albeit at lower resolution. The first approach employs ultrasmall superparamagnetic iron oxide particles (USPIO), ~30nm hydrodynamic nanoparticles that impart strong $R_2 > R_1$ effects that can be detected by MRI. The second approach utilizes ^{18}F -fluorodeoxyglucose (^{18}F -FDG), a metabolic substrate that is phosphorylated and trapped within cells, allowing imaging of metabolic activity as a surrogate for plaque inflammation, and can be detected by positron emission tomography (PET). Two other novel PET imaging strategies, ^{11}C -PK11195 imaging of macrophages, and ^{18}F -NaF imaging of osteogenesis, are detecting new aspects of vascular biology, the latter also emerging into imaging of coronary arteries.

Imaging large arteries: Plaque biology

USPIO imaging - MRI of plaque macrophages

Magnetic resonance USPIO imaging is a technique that uses iron-based blood pool agents with a strong $T_2 > T_1$ shortening effect, thus typically inducing signal loss on T_2 -weighted MRI (Figure 1).[51] Phagocytosis of iron nanoparticles occurs primarily by macrophages, with experimental data showing a small contribution by endothelial cells and smooth muscle cells[52], and therefore USPIO-enhanced MRI reports on carotid plaque inflammation. Subsequently, the prospective molecular MRI ATHEROMA study was performed in 40 patients receiving either high-dose or low-dose statin therapy, and showed that high-dose statin therapy better suppressed plaque inflammation than low-dose statin therapy, and utilized serial USPIO MRI to make this observation in living patients. Notably, the study did not show a clear link between inflammation and the degree of stenosis.[51] A limitation of the USPIO technique is that the technique is semiquantitative and that it still an investigational diagnostic agent without FDA or EMA approval. Moreover artifacts, adjacent lymphatic tissue uptake and a long contrast half-life may cause misinterpretation of targeted USPIO uptake.[53,54]

^{18}F -FDG – imaging plaque glucose metabolism

Imaging plaque glucose metabolism via ^{18}F -FDG is widely utilized clinically for detecting cancer (progression) and myocardial viability. It is a widespread imaging modality that examines the entire patient in less than 1 hour, and combines biological and anatomical imaging via PET/CT. While relatively costly, its proven clinical record has allowed ^{18}F -FDG to expand into investigation of metabolic activity, as a surrogate of plaque inflammation or plaque hypoxia[55] in atherosclerotic arteries.[56-58] An observational ^{18}F -FDG PET study involving 932 patients demonstrated that significant carotid uptake was a 4-fold stronger predictor for future cardiovascular events compared to calcification.[59] Similarly, this was substantiated other studies, showing an association between statins and consequently significant fewer PET positive plaques (Figure 2A).[60,61] A recent study accentuates the potential of ^{18}F -FDG in assisting drug discovery in assessing drug efficacy and safety in a relatively small group of patients, investigating a new investigative drug, dalcetrapib, demonstrating that there is no evidence of pathological effects on large arteries. [29] ^{18}F -FDG PET is therefore a promising noninvasive tool for functional imaging of

inflammation and is of considerable potential in drug discovery and translation. However, there are still many challenges remaining. ^{18}F -FDG has inherent weaknesses, since metabolic activity is not only limited to high-risk plaques, but also to surrounding tissue with elevated metabolic activity. In addition, the radiation exposure with PET and CT is nontrivial. In addition to cost and the limited half-life of radioisotopes, other limitations of FDG PET include variable uptake due to certain medications, stress and/or fasting. Finally, the relatively low spatial resolution (3 mm isotropic) strongly limits the possibility of comprehensive coronary arterial biological imaging.

^{11}C -PK11195 - imaging inflammation

Another PET agent termed ^{11}C -PK11195, a ligand of the translocator protein (TSPO) that is highly expressed in activated mononuclear lineage cells (e.g. macrophages) has been clinically investigated for detection of vascular inflammation in large vessels.[62] In a study of carotid plaques inflammation, ^{11}C -PK11195 was i.v. injected into 9 symptomatic and 23 asymptomatic patients with carotid stenoses of similar severity ($77 \pm 16\%$ vs. $68 \pm 16\%$ stenosis, respectively, $P = 0.18$). In the nine symptomatic patients with TIA/stroke with an ipsilateral event to the carotid stenosis, ^{11}C -PK11195 uptake illuminated areas of inflammation with high target-to-background (TBR) ratios (Figure 2B). Using ^{11}C -PK11195 uptake and lower CT attenuation as an outcome in ipsilateral plaques allowed for identification of recently symptomatic patients with high-risk plaques.[63] More studies will need to be conducted to establish the true meaning of its targeting, since in a study in a mouse model of atherosclerosis, ^{11}C -PK11195 demonstrated similar uptake in non-inflamed plaques.[64] In addition, studies of ^{11}C -PK11195 will need to determine what advantages are realized over the more established ^{18}F -FDG approach described above.

^{18}F -NaF - imaging osteogenic activity

A third promising PET-tracer, ^{18}F -sodium fluoride (^{18}F -NaF), has entered the cardiovascular research arena after being developed 50 years ago for clinical imaging of bone disease. This marker does not target inflammation, but reports on osteogenic activity and remodeling in diseases such as Paget's disease, osteoblastic tumors and metastatic bone disease. Its kinetics and mechanism of uptake are well established.[65] Initial trials were performed in cancer patients for screening of bone metastases. However, several recent observations lead to the suggestion that ^{18}F -NaF uptake represents ongoing mineral deposition or active calcification in arteries, a finding that has been substantiated by optical molecular imaging of plaque osteogenic activity in mice.[66] This finding was corroborated by the correlation of increased vascular uptake to traditional risk factors.[67,68] These studies served as hypothesis-generating, with the potential for ^{18}F -NaF imaging to improve the prognostic capability of calcium imaging, and the potential to refine drug assessment and discovery.

Imaging coronary arteries: Plaque biology

^{18}F -FDG – imaging plaque glucose metabolism/inflammation/hypoxia

Coronary artery disease is a major worldwide cause of cardiovascular events, and dedicated coronary imaging approaches are likely to provide greater insights and preventative opportunities to prevent myocardial infarction and sudden cardiac death. Imaging the smaller coronary arteries however remains a significant challenge, due to multi-organ motion artifacts, and its demanding requirements for excellent spatial and temporal resolution. However, there are preliminary reports demonstrating the potential for noninvasive coronary plaque imaging in proximal coronary arteries using ^{18}F -FDG imaging strategies. The physiological glucose background uptake of the myocardium in these coronary imaging studies was minimized by consumption of a high-lipid, low glucose diet

followed by fasting of the patients (Figure 3).[69-71] While insights into the left main and proximal coronary beds may be possible, the lower resolution of PET coupled with myocardial background makes comprehensive coronary artery imaging less successful, and many coronary segments may be uninterpretable clinically.[72] In addition to the above-mentioned limitations, coronary ^{18}F -FDG imaging faces the challenges of motion and size in the coronary artery. Consequently, standardized imaging protocols are clinically not yet available and need to be developed with attention to optimize gating strategies that do not significantly influence sensitivity. However, these results remain exciting and promising in this early stage of development, since they form the first clinical noninvasive biological imaging strategy of millimeter-sized coronary arteries.

^{18}F -NaF - imaging osteogenic activity

In an observational substudy of an aortic valve calcification imaging study in 119 patients, the feasibility and repeatability was demonstrated for ^{18}F -NaF uptake in coronary arteries with a low inter-observer variability.[73] Higher activity was seen in patients with more atherosclerotic burden and coronary uptake was detectable in the absence of myocardial uptake (Figure 3). Patients with increased ^{18}F -NaF uptake were predominantly male, older, had lower HDL levels and had a calcium score that correlated to ^{18}F -NaF uptake. However, 40% of patients with *normal* uptake had calcium scores above 1000, suggesting that active osteogenesis detected by NaF is different than bulk calcification detected by CT. Furthermore, patients with increased ^{18}F -NaF uptake were significantly more likely to have coronary artery disease (60% vs. 26%), anginal symptoms (38% vs. 11%), prior major adverse cardiac events (45% vs. 23%) and an increased cardiovascular risk factor burden. Important next steps are prospective studies, histological studies combined with imaging that will further elucidate and validate the true meaning of the tracer uptake. Results from ^{18}F -FDG coronary imaging in this study were less promising, with a poor reproducibility and quantification not being possible in almost half of the coronary arteries that were assessed. However, similar to ^{11}C -PK11195, in order to become more widely utilized, ^{18}F -NaF will need to show predictive or management-based improvements over ^{18}F -FDG.

Preclinical progress

A multitude of biological mechanisms have been shown to be promising imaging targets that go beyond the scope of this article.[48,74] The targets are most importantly linked with inflammation, such as endothelial activation, angiogenesis, MMP expression, apoptosis and protease activity (Table 1). Several targets have potential to become FDA approved and are clinically relevant, such as RGD peptides for imaging angiogenesis[75], and another promising agent includes indocyanine green, which is already FDA approved.[76] Investigation of promising agents in the preclinical arena allows target validation and understanding of pharmacokinetics, as well as new insights in the atheroma biology. However, animal models do not fully recapitulate human coronary artery disease, and therefore a strong focus in the field is on developing imaging approaches with high translatability to the human coronary arteries.

Intravascular biological imaging: near-infrared fluorescence (NIRF)

Of particular interest are high-resolution intravascular biological imaging strategies that currently are being developed based on fluorescence molecular imaging. Fluorescence-based intravascular imaging utilizes light in the near-infrared spectrum through flexible optical fibers to detect imaging agents in the vascular wall. The major advantage of this part of the light spectrum is two-fold, taking advantage of a reduction of autofluorescence *and* markedly reduced blood attenuation of light by red blood cells, both of which are far less in

the NIR compared to the visible light range. While intravascular procedures requires invasive methods, intravascular optical catheters overcome earlier key limitations of noninvasive strategies, and offer superior resolution, sensitivity, and potential for detecting multiple targets simultaneously in real-time, without the use of radiation.[76-80]

In 2008 we reported a novel first-generation one-dimensional near-infrared fluorescence (NIRF) sensing catheter, and showed the ability to detect protease activity in atheroma in rabbit iliac arteries in vivo, in concert with a cathepsin-activatable NIRF molecular imaging beacon (Prosense/VM110).[77] NIRF signal detection was feasible without the need for flushing of blood nor artery occlusion. However, this first generation catheter was not capable of full 360-degree imaging. Subsequently we developed its successor with additional capabilities, such as a 360 degree evaluation of the vessel wall and with an automated pull back with a z-resolution of 1.0 mm at a catheter-to-target distance of 2.0 mm.[81] After phantom experiments, we tested whether this novel catheter could successfully image 2D inflammatory cathepsin protease activity in rabbit atherosclerosis, again in concert with the agent Prosense/VM110. We observed significantly higher signal-to-noise and target-to-background ratios in atheromata compared to animals that were not injected with this NIRF probe; ex vivo measurements corroborated the in vivo findings. Interestingly, NIRF signal was appreciably detected in more advanced atherosclerotic lesions, suggesting a higher degree of inflammation. A limitation of this catheter was the need for separate anatomical intravascular imaging, in this case via intravascular ultrasound (IVUS). This limitation also precluded distance-based correction of the NIRF signal, which limited quantification ability.

Recently, a third generation high-speed, hybrid imaging catheter was combined with microstructural imaging using OFDI and NIRF signal, offering exact co-registration of NIRF fluorescence with high-resolution OCT anatomical images.[80] Using this catheter, in addition to fluorescence, various components can be detected using post-processing algorithms that can distinguish the optical “signatures” of stents, thrombus, lipid and calcium, similar to IVUS-VH. Integrated NIRF-OFDI allowed biological imaging of fibrin deposition on coronary stents implanted into rabbits, as well as inflammation with atheroma. The ability to simultaneously map the catheter position via OFDI also allowed true quantification of NIRF signal via correction of the catheter-to-wall difference. Of note, due to the need for OFDI imaging, intravascular NIRF-OFDI was performed with balloon occlusion and saline-flushing. This necessity served to further improve the SNR of the NIRF images. The overall catheter platform is 2.4F and based on a clinical prototype, and therefore highly translatable to the human coronary artery.

New atherosclerosis-targeted biological imaging agents—As Prosense VM110 is a useful but not clinically available agent, we investigated whether alternative NIRF biological agents could be harnessed for atherosclerosis imaging. After carefully reviewing the optical literature, we investigated the FDA-approved fluorochrome indocyanine green (ICG), an imaging agent that has been used in clinic for many years for cardiac output measurement and retinal angiography. In a rabbit model of inflamed atheroma after aortic balloon injury, ICG was found to bind atheroma rapidly, co-localizing with lipid-rich lesions and RAM-11 positive macrophages.[82] The intravascular sensing catheter detected a strong ICG signal in aortic plaques within 20 minutes after injection, demonstrating potential for point-of-care clinical intravascular imaging of atherosclerosis (Figure 4). Moreover, human endarterectomy specimens incubated with ICG ex vivo demonstrated ICG uptake that colocalized with CD68+ macrophage accumulation and Oil Red O-positive lipid.[76] Biological imaging using NIRF combined with OFDI offers detection of in vivo biological processes with a possibility for longitudinal, nondestructive imaging, opening an wide array of investigational possibilities. To translate this technology into clinic, more NIRF imaging

agents are needed that specifically investigate vascular pathophysiology and other important fields such as stent injury, restenosis and thrombosis.

Potential relevance and future of biological imaging in clinical management

Biological or molecular imaging can be defined as the in vivo characterisation and measurement of molecular and cellular processes within living organisms. Diagnostic strategies based on imaging the biology of atherosclerosis offer new insights in the atheroma pathophysiology, drug development, and risk prediction. Biological imaging, in particular combined with other modalities, is positioned to provide new insights into the natural history of high-risk plaques and has the potential to visualize the atherosclerotic plaque in an early stage, and also its progression or resolution. Accordingly, it can provide early surrogate endpoints for rapid drug development or phenotype specific treatment.

We have presented the current developments in detection of atherosclerotic disease biology with a focus on clinical translational concepts. The PROSPECT trial has demonstrated that anatomical/compositional imaging, does not accurately predict which non-culprit plaques will cause recurrent major adverse cardiovascular events.[46] Consequently, the concept of imaging key biological processes offers a transformative approach to strengthen the role of invasive imaging in coronary risk prediction and to streamline the process of atherosclerosis pharmacotherapy clinical trials. For example, the discussed biological imaging strategies could provide new surrogate biomarker endpoints for the assessment of natural history studies and/or new atherosclerosis therapies, as a prelude to costlier and more time-intensive phase III clinical trials that require clinical endpoints such as death or myocardial infarction.

Several biological imaging applications have been assessed clinically, but it is evident that more research needs to be performed to prove their value and cost-effectiveness in clinic. The main impediments for molecular imaging biomarkers are the challenging production potentially involving radioactivity, labeling, cost and regulatory issues.[83] Like many accomplished techniques, significant challenges must be overcome before routine applications are available in the clinic. Hybrid imaging (anatomical/biological/chemical) readouts in a single system are inherently attractive for amassing comprehensive knowledge of coronary arterial phenomenon in a time-efficient and cost-effective manner. So-called triple catheters (OCT + NIR spectroscopy + NIRF) are undergoing active engineering designs. In the near-term, obtaining FDA-approval for intravascular NIRF-OFDI catheters is top priority, followed by evaluation of FDA-approved or testable targeted fluorescent compounds, including ICG. Recent developments in molecular cancer imaging[84] are also expected to increase the availability of fluorochromes that can be useful for atherosclerosis imaging. Overall the next five years appear extremely “bright” for NIRF imaging of coronary arterial biology -- a wealth of information awaits.

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FIGURE 1. Imaging of carotid plaque macrophages using USPIO MRI

(A,B) T2-weighted imaging at carotid bifurcation of a patient receiving a low dose of 10mg atorvastatin, before (A) and after (B) infusion of USPIO at baseline, after 6 and 12 weeks. Pre-USPIO imaging (A) remains very similar at all 3 time points suggesting elimination of USPIO by the plaque. This study shows that multiple time points can be used to assess USPIO uptake in response to an intervention. (B) USPIO uptake in the plaque can be appreciated at baseline, 6 and 12 weeks after injection (yellow arrowheads).

(C) T2-weighted imaging of a left common carotid artery of a patient receiving a high dose of 80mg atorvastatin after infusion of USPIO at baseline, after 6 and 12 weeks. USPIO uptake can be appreciated in the plaque at baseline (yellow arrowhead). Plaque enhancement can be seen at 6 weeks and 12 weeks (blue arrowheads), indicating minimal or no USPIO uptake, and suggesting a predominant T1 effect and minimal inflammation. Pre-USPIO imaging (not shown) was again similar at all time-points.

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FIGURE 2. Imaging of carotid plaque inflammation using PET imaging

(A) ^{18}F -FDG-uptake in atherosclerotic plaques of patients treated with dietary management without or with 3 months of treatment with simvastatin. 1st row, diet had minimal effect on ^{18}F -FDG uptake (arrows) in the aortic arch and carotid arteries. 2nd row, ^{18}F -FDG uptake was attenuated by simvastatin treatment. 3rd row, co-registered images show no uptake of ^{18}F -FDG after 3 months of treatment with statin.

(B) ^{11}C -PK11195 macrophage-sensor uptake on PET, co-registered with CT in a symptomatic (Patient A) and an asymptomatic (Patient B) patient. In Patient A, areas of low CT attenuation (23HU) can be seen (arrows, column 1) near regions with increased ^{11}C -

PK11195 uptake (block arrows, column 2). In Patient B, plaque CT attenuation was higher (76HU) indicating a predominance of fibrotic tissue (column 3), without any ^{11}C -PK11195 uptake (column 4).

HU = Hounsfield Unit, TOF = time of flight; CE-T1W = contrast-enhanced T1-weighted, PDW = proton density-weighted, T2W= T2-weighted. Reproduced with permission [61], [63] respectively.

FIGURE 3. Biological imaging of coronary arteries - emerging noninvasive options

(top panels) ^{18}F -FDG co-registered with CT images demonstrating F-FDG uptake in stents in the left main coronary artery (LM) in different patients. Interrupted arrows show lesions within the LM. (A) Increased uptake in both LM and stent in culprit lesions in a patient with acute coronary syndrome (ACS). (B) More modest uptake in a recently stented coronary lesion in a patient with a stable coronary syndrome (some F-FDG uptake in a mixed LM plaque) (C) Modest F-FDG uptake several months after stenting LM lesion. (D) F-FDG uptake at LM trifurcation in an ACS patient. The box plot graph shows a comparison of another part of the study that compared TBR in the ascending aorta of ACS patients versus stable angina patients. The median TBR in aorta and LM was significantly higher in ACS patients compared to stable patients. Horizontal lines are medians, ends of boxes represent upper and lower quartiles, vertical lines represent the full range of data.

(bottom panels) ^{18}F -NaF activity in coronary arteries of different patients co-registered with CT images. (A,B) Patients with no coronary calcium (A) and extensive calcification

(B) in the left anterior descending artery (LAD), show no focal ^{18}F -NaF uptake. (C) Clear focal ^{18}F -NaF uptake is seen in the calcified part of the proximal LAD of this patient. (D) Increased focal uptake of ^{18}F -NaF adjacent to the calcified mid-LAD. (E) Patient with recent non-ST-segment elevation myocardial infarction with intense focal uptake of the proximal right coronary artery, being the culprit on the basis of the electrocardiogram and ulcerated plaque appearance on coronary angiography (F).

The graph demonstrates that 10-Year Framingham risk prediction scores were significantly higher in those with increased ^{18}F -NaF uptake in terms of Framingham CVD ($p = 0.033$), CHD ($p = 0.049$), CVD death ($p = 0.011$), and CHD death ($p = 0.024$), compared to when there was no ^{18}F -NaF uptake.

Error bars denote the SD of the mean. ACS = acute coronary syndrome; CT = computed tomography; F-FDG = fluorodeoxyglucose; LM = left main coronary artery; PET = positron emission tomography. ^{18}F -NaF = ^{18}F -sodium fluoride; CHD = coronary heart disease; CVD = cardiovascular disease. Reproduced with permission [69,73]

FIGURE 4. Intravascular near-infrared fluorescence imaging of inflammation and fibrin deposition in coronary-sized arteries

(A) In vivo biological and molecular imaging of inflamed atheroma. (a) angiographic appearance of radiopaque tip of NIRF catheter positioned just proximal to the iliac bifurcation (with inset high magnification image in dotted yellow lines), enabling coregistration with intravascular ultrasound (IVUS). (b) Angiogram of atherosclerotic aorta, with tip of the NIRF catheter at pullback initiation. (c) Longitudinal IVUS imaging of abdominal aortoiliac region with plaque zones (P1,P2). (d) NIRF pullback images, aligned with angiogram and IVUS, demonstrating NIRF signal in small volume plaques, in >3.0mm diameter arteries. Corresponding 1-dimensional plot of the angle-averaged NIRF intensity pull-back below. (e) Longitudinal superimposed fusion images of NIRF and IVUS (yellow/white = strongest NIRF signal intensity, red/black = lowest NIRF signal intensity). (f,g) High magnification of plaque zones P1 and P2, showing minimally stenotic plaques. (h,i) Axial IVUS images of P1 and P2.

(B) In vivo, dual-modality OFDI-NIRF images in an atherosclerotic rabbit model atherosclerosis representing microstructure and inflammatory enzyme activity. (a) OFDI cross-sectional image showing normal artery wall as well as a focal, raised, lipid-containing (L) atherosclerotic lesion at 3–6 o'clock (arrowheads). (b) The OFDI-NIRF fusion image and (c) expanded view of (b) shows a strong NIRF signal, reflecting inflammatory protease activity in an on OFDI normal-appearing section of the plaque. On expanded view a stronger activity on 4 o'clock portion (green asterisk) can be appreciated, compared to adjacent regions (blue asterisk). (d) RAM-11 staining demonstrates dense accumulation of macrophages in the NIRF positive region. (e) Fluorescence microscopy of protease activity-induced NIR fluorescence (red) and autofluorescence (green), confirming strong NIRF signal at the luminal surface of the plaque in a similar way as the in vivo NIRF signal. (f) Immunoreactive cathepsin B positivity in macrophage-rich plaque region. (g–i) Expanded views of d–f, respectively. RAM-11 staining did not appear to vary significantly across the plaque. Heterogeneous cathepsin B activity (i) showed a similar pattern in the plaque as on fluorescence microscopy (h) with a higher signal at 4 o'clock (green asterisk) compared with that at 5 o'clock (blue asterisk). *Scale bars, 500 μm (a,b,d–f) and 200 μm (c,g–i).*

(C) Dual-modality OFDI-NIRF images of an iliac artery of a rabbit with an implanted near-infrared fluorescent fibrin-coated stent, in vivo. Images show three-dimensional rendering images of the stented right iliac artery of a living rabbit. Structural components were segmented and color-coded in OFDI images for clear visualization. Pink-artery wall; white-stent; purple-thrombus; yellow-fluorescent fibrin. Scale bars, 500 μm . Reproduced with permission [81],[80].

Table 1
Partial list of molecular beacons for biological imaging of atherosclerosis

Molecular Process	Contrast agents	Modality
ENDOTHELIAL ACTIVATION		
Van Willebrand factor,	VCAM nanoparticle	MRI-NIRF, PET
VCAM-1, ICAM, Selectins	Antibody-microbubble conjugates	Ultrasound
	Micron particles of iron oxide (MPIO)	MRI
INFLAMMATION/METABOLISM		
Macrophage activity	¹⁸ F-FDG	PET
	HDL-gold nanoparticle	CT
Protease activity	ProSense, MMPsense	NIRF
Chemotactic peptides	^{99m} Tc-MCP-1	SPECT
Osteogenic activity	¹⁸ F-NaF	PET
	OsteoSense	NIRF
Myeloperoxidase (MPO) activity	MPO sensors	MRI, SPECT
CELL DEATH		
Phosphatidylserine expression	^{99m} Tc-Annexin A5	SPECT
	Annexin A5-CLIO (crosslinked iron oxide)	MRI/NIRF
ANGIOGENESIS		
3 integrins	^{99m} Tc-NC100692	SPECT
	RGD mimetic Gd-DTPA	MRI
VEGF	^{99m} Tc-VEGF	SPECT
EXTRACELLULAR MATRIX		
Matrix components	Gadofluorine	MRI
LIPOPROTEINS		
Lipids	Indocyanine Green	NIRF
Low-density lipoprotein	^{99m} Tc-MDA2	SPECT
High-density lipoprotein	HDL nanoparticle	MRI
Oxidized Low density lipoprotein	Gd nanoparticles	MRI
THROMBOSIS		
Fibrin	EP-2104R	MRI
	FTP11-Cy7	NIRF
Iib 3 integrins (platelet)	^{99m} Tc-apcitide	SPECT
Platelets	IR-786	NIRF

VCAM=Vascular cell adhesion molecule, ICAM=Intercellular adhesion molecule, MCP=monocyte chemoattractant protein, FTP=fibrin-targeted peptide, VEGF=vascular endothelial growth factor, MRI=magnetic resonance imaging, NIRF=near-infrared fluorescence, SPECT=single-photon emission computed tomography, PET=positron emission tomography