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Recent Advances of Radionuclide-based Molecular Imaging of Atherosclerosis

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Abstract

Atherosclerosis is a systemic disease characterized by the development of multifocal plaque lesions within vessel walls and extending into the vascular lumen. The disease takes decades to develop symptomatic lesions, affording opportunities for accurate detection of plaque progression, analysis of risk factors responsible for clinical events, and planning personalized treatment. Of the available molecular imaging modalities, radionuclide-based imaging strategies have been favored due to their sensitivity, quantitative detection and pathways for translational research. This review summarizes recent advances of radiolabeled small molecules, peptides, antibodies and nanoparticles for atherosclerotic plaque imaging during disease progression.

Keywords

Atherosclerosis; molecular imaging; PET; SPECT; radionuclide; peptide; nanoparticle

Introduction

1.1 Biology of atherosclerosis

Atherosclerosis, the leading cause of morbidity and mortality in Westernized societies, is a progressive disease characterized by the development of lipid-rich plaque lesions within vessel walls and extending into the vascular lumen. It is the underlying basis of cardiovascular diseases including myocardial infarction, stroke, and peripheral arterial disease [1]. Several factors, including hypertension, lifestyle choices (smoking, diet, sedentary habits), and genetic predisposition increase the risk of developing atherosclerosis. The disease begins with the accumulation of low-density lipoproteins (LDL) on the arterial wall which triggers an inflammatory process through modifications including oxidation, glycation, acetylation and carbamylation [2]. Tissue undergoing the initiation of atheroma becomes more permeable to leukocytes which are recruited by molecules in the vascular

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endothelium. Adhesion molecules, containing vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1), and selectins, containing P-selectin and E-selectin, are overexpressed by endothelial cells [3]. The activated endothelium produces monocyte chemoattractant protein-1 (MCP-1), a chemokine for leukocyte recruitment, in addition to growth factors such as macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [4]. Reactive oxygen species (ROS), which are produced by activated macrophages, endothelial cells and smooth muscle cells (SMCs), generate the most important modification in LDL - oxidation [5]. Cells of the innate immune system express pattern-recognition receptors (PRRs) such as scavenger receptors, especially CD36 and SRA (scavenger receptor A, CD204) [6]. These are known as endocytic type receptors for recognizing and capturing oxidized LDL (oxLDL), the main process responsible for the formation of foam cells. Signaling receptors are also PRRs playing key roles in the activation of proinflammatory factors [7].

In early atherosclerotic lesions, foam cells containing large amounts of LDL undergo apoptosis and are removed from the lesion. With the progression of the plaque the apoptotic process slows down. Accumulating foam cell deposits in the lesion lead to the formation of a necrotic core [8]. Moreover, neoangiogenic processes in the plaque provide supplemental oxygen while facilitating the mobilization of leukocytes for plaque development. In advanced plaque, these fragile microvessels are prone to break, resulting in thrombus formation which can collaborate with the inflammatory process and inhibit macrophage migration. Interestingly, with the formation of necrotic core and plaque progression, the plaque tends to be hypoxic which correlates with inflammation, angiogenesis and apoptosis [9], and has been demonstrated in a recent study showing that the reversal of hypoxia could decrease necrotic core formation and prevent apoptotic cell accumulation in atherosclerotic plaques in LDLR^{-/-} mice [10].

Adaptive immune response participates in the progression of atherosclerosis through interactions between antigen-presenting cells and T-cells. CD4⁺ T-cell is a subtype found in the atherosclerotic lesion that presents epitopes bound to the major histocompatibility complex class II (MHC II) [11]. These T-cells can also differentiate into proinflammatory T-helper 1 (Th-1) cells which secrete interferon gamma (IFN- γ) to decrease collagen synthesis, narrow the thickness of fibrotic cap, and perpetuate the Th-1 inflammatory response [12]. The antigens exposed by antigen-presenting cells are presented to adaptive immunity cells, activating T-cells and inducing antibody production by B-cells. The association between autoantigens produced during atherogenesis and their respective autoantibodies has been studied as a target for atherosclerosis imaging [13-15].

The stability of atherosclerotic lesions depends on the balance between inflammatory response and those mechanisms that stimulate extracellular matrix synthesis. When the fibrotic cap thins and tensile strength decreases the chance of rupture increases. The mechanism that elicits this clinical consequence involves the process of thrombosis resulting from the extravasation of the necrotic lipid core and subsequent contact with proteins of the coagulation cascade [16]. Important clinical outcomes of thrombosis and disruptions of the plaque include acute myocardial infarction and unstable angina.

1.2 Challenges in clinical diagnosis

Clinical challenges in CVD include predicting when a plaque may rupture and managing treatment to avoid associated fatal consequences. The need for early diagnosis is critical since atherosclerosis is a silent disease that progresses slowly over years. Factors influencing plaque vulnerability include plaque thickness, composition and degree of inflammation. The amount of cholesterol in the plaque is also important as cholesterol esters may be softer, thus increasing the pressure on the fibrous cap. Additionally, the intense activity of inflammatory cells may increase the temperature of the plaque, thus reducing the rigidity of the lipid core [17].

Currently, imaging techniques for the diagnosis of atherosclerosis include both noninvasive methods [computed tomography (CT), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), positron emission tomography (PET) and fluorescence molecular tomography (FMT)] and invasive methods [intravascular ultrasound (IVUS), optical coherence tomography (OCT)], as summarized in Table 1.

Invasive techniques provide valuable morphological information while affording high-resolution images of the atherosclerotic plaque. Noninvasive imaging platforms such as CT and MRI show high-resolution images but may require the administration of contrast agents due to their low sensitivity. In order to improve MRI images, contrast agents in the form of iron oxide nanoparticles have been developed for directing the localization of activated macrophages, since these cells are able to phagocytose the nanoparticles. Although this approach correlates with inflammatory activity of an atherosclerotic plaque, it does not present sufficient specificity to accurately identify specific biomarkers expressed on the plaque [18]. Nuclear imaging with PET and SPECT can characterize atherosclerotic plaques with the highest sensitivity allowing quantification of the biologic process and tracking of disease progression during treatment. PET and SPECT represent areas of molecular imaging in which relevant biological targets are identified by specific radiotracers. The co-registration with MRI or CT can combine high sensitivity with increased resolution to better determine the plaque activity [19, 20]. Some of these dual-modality imaging techniques are already being used in translational research [21], providing great anatomical detail coupled with information at the cellular and molecular level. Of the above imaging modalities, CT is able to report on the degree of plaque stenosis, and those with high-grade stenosis are certainly at increased risk of interruption of blood flow. However, there is a positive remodeling that occurs in the beginning of plaque formation towards the adventitia rather than the lumen, suggesting that when the stenosis begins, there is already a large and complex atherosclerotic plaque [3]. Studies with human arteries found early stenosis occurs only when the growth of an atherosclerotic plaque reaches 40% of the cross sectional area of the artery and then the growth begins in opposite direction [22]. This data reinforces the need for advances in early diagnosis.

Due to the high sensitivity and specificity, radionuclide imaging techniques are valuable tools in imaging the biological processes during the plaque development, providing information about plaque composition and status of the disease. We discuss here the recent advances in radionuclide-based molecular imaging for PET and SPECT in preclinical studies of atherosclerosis, covering discoveries of potential targets for distinct biological

processes and their imaging by radiolabeled compounds such as peptides, nanoparticles, small molecules and antibodies.

2. Radionuclides

Mode of decay and half-life typically direct the choice of radionuclide for molecular imaging. In general, SPECT probes are labeled with γ -emitting radionuclides as the SPECT camera is coupled to lead collimators which detect γ -rays in different energy levels, whereas PET probes utilize positron emitting radionuclides with detection of γ photons resulting from positron-electron annihilation [21].

Molecular imaging probes need to follow certain criteria in order to achieve the best contrast against normal tissue surrounding the pathological disease. In this context, key characteristics of the imaging agent include high affinity for the molecular target, the ability to overcome biological barriers, low toxicity, efficient clearance *in vivo*, straightforward production [23, 24], and high specific activity for radioactive probes which is defined as radioactivity per mass unit of radionuclide or radiolabeled compound [25]. The number of targets and the affinity of the imaging agent to these targets play an important role in its effectiveness because the presence of non-radioactive components may compete with the radiolabeled compound by binding to the target and decreasing signal uptake in the tissue [26]. Some radiolabeled compounds are already being used in clinical practice, however, most are dedicated to cancer imaging. In contrast, in the field of cardiac research few molecular imaging probes can be considered effective in accurate diagnosis.

2.1. PET

In contrast to other imaging modalities, PET has the advantages of high sensitivity and quantification at the region of interest. Basically, upon decay of a PET radionuclide, a positron migrates a small distance until meeting a surrounding electron, suffering an annihilation event in which both are destroyed. Their masses are converted into two photons of γ -radiation that move in opposite directions forming an angle of 180° between their paths, which is then detected by pairs of oppositely disposed detectors in the PET scanner. The sensitivity of detection in concentration ranges from nanomolar (10^{-9}) to picomolar (10^{-12}) and permits quantitative imaging of the signal [27]. Typical PET radionuclides used in imaging applications are listed in Table 2.

2.2. SPECT

SPECT is able to detect γ photons by utilizing a gamma camera and a lead or tungsten collimator that physically selects photons by defining the incidence angle of γ -rays. The acceptable range of energy for SPECT cameras is between 90 and 300 keV but the ideal γ -ray energy is 150 keV for which value the SPECT instrumentation has been optimized. Unlike PET imaging which is unable to distinguish between radionuclides, SPECT can identify the different energy emissions in one image, thus allowing the tracking of multiple biological pathways simultaneously [28]. Furthermore, the relatively long half-lives of some SPECT radionuclides permit more elaborate syntheses of radiotracers and allow delivery to distant imaging sites. ^{99m}Tc eluted from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator is the most widely used

SPECT radionuclides in clinical applications [29, 30]. Commonly used SPECT radionuclides are summarized in Table 3.

3. Biological processes

3.1. Radiotracers imaging early stage atherosclerotic lesions

As discussed earlier, some of the first molecules expressed by endothelial cells in atherosclerotic lesion formation are adhesion molecules such as VCAM-1 [31, 32]. Of the agents developed for VCAM-1 imaging, nanobodies are a new class of probe which carry the smallest functional unit of a whole antibody for antigen recognition. They are highly versatile, combining the specificity of an antibody with rapid clearance, making them ideal imaging agents. In a recent study, up to 10 anti-VCAM-1 nanobodies were screened for VCAM-1 imaging and the candidate agent was radiolabeled with ^{99m}Tc to detect the (^{99m}Tc -cAbVCAM1-5) atherosclerotic lesions [33]. SPECT imaging showed sensitive and specific detection of VCAM-1 with high lesion-to-blood (4.32 ± 0.48) and lesion-to-heart (8.30 ± 1.11) ratios (fig.1A). The *ex vivo* autoradiography also confirmed the localization of tracer in the aorta and the up-regulation of VCAM-1 was verified by immunohistochemistry.

Owing to the straightforward preparation and fast *in vivo* clearance, peptides have been extensively explored in atherosclerosis molecular imaging, especially in cases of cell surface receptor recognition. During plaque development, major histocompatibility complex 1 (MHC-I) expressed on leukocytes is actively involved in immune response [34, 35] Thus, out of 20 peptides (B2702p1 to B2702p20) derived from MHC-1, B2702p1 showed the best binding affinity and specificity in detecting plaque. In the carotid region of ApoE^{-/-} mice, ^{99m}Tc radiolabeled B2702p showed specific accumulation in the left carotid artery and 3.4 fold increase in contrast to the blood pool retention, indicating targeting specificity (fig. 1B). During the plaque progression, HDL (high-density lipoprotein), especially apolipoprotein A-I (apo A-I), plays an important function in the reverse transport of cholesterol from cholesterol-rich cells to the liver, making it an interesting target for atherosclerosis imaging. Thus, a FAMP peptide (Fukuoka University apo A-I Mimetic Peptide) was radiolabeled with ^{68}Ga for *in vivo* atherosclerosis imaging in a myocardial infarction-prone Watanabe heritable hyperlipidemic rabbit model (WHHL-MI) and demonstrated favorable pharmacokinetics and specific accumulation in the plaque (fig. 1C) [36]. Further study should focus on biological assays to confirm the expression levels of HDL during disease progression and its correlation to PET signals.

Another interesting target is the folate receptor present in activated macrophages [37]. Compared to uptake in the aortic arch of ApoE^{-/-} mice fed with normal chow, the accumulation of ^{99m}Tc -EC20 in ApoE^{-/-} mice fed a high fat diet showed 70% higher signal as detected by γ -scintigraphy imaging, which was also confirmed by γ -counting of the dissected aorta. Flow cytometry analysis showed 33% of macrophages were positive for folate receptors in mice fed a high fat diet in contrast to the 11% positivity in mice fed with normal chow.

Chemokine receptors such as CCR5 hold potential as biomarkers to determine plaque progression and activity. In a vascular injury accelerated ApoE^{-/-} mouse atherosclerosis

model, a CCR5 binding peptide D-Ala₁-peptide T-amide (DAPTA) labeled with ⁶⁴Cu showed specific plaque accumulation. With the conjugation of DAPTA peptide onto a multivalent comb-like nanoparticle (⁶⁴Cu-DOTA-DAPTA-comb) with well-controlled structure to extend blood circulation, the imaging specificity and targeting efficiency were significantly improved compared to DAPTA peptide tracer alone. At the injured artery, targeted ⁶⁴Cu-DOTA-DAPTA-comb demonstrated specific accumulation and binding to the CCR5 receptor. The quantification of PET images showed the targeted ⁶⁴Cu-DOTA-DAPTA-comb had more than 3 times higher uptake in the plaque relative to the non-targeted ⁶⁴Cu-DOTA-comb, indicating the potential of this targeted nanoprobe for plaque detection (fig. 1D) [38].

Of the targets evaluated for atherosclerosis imaging, the selectin family (P-selectin and E-selectin) has been widely studied due to their role in promoting arterial inflammation. Through radiolabeling with ⁶⁴Cu, the aortic uptake of ⁶⁴Cu-anti-P-selectin mAb in LDLR^{-/-} mice fed with high-cholesterol diet for 12 weeks was 6 fold higher than the uptake obtained in chow-diet mice. PET/CT imaging clearly showed the specific accumulation of this tracer at the aortic arch (fig. 2A) [39]. The role of oxLDL in the formation of foam cells is well established and oxidation-specific epitopes have been explored as biomarkers of disease risk and for therapeutic treatments. Malondialdehyde 2 monoclonal antibody (MDA2 mAb) radiolabeled with ¹²⁵I targeting MDA-LDL, LDL oxidized *in vitro* by malondialdehyde (MDA), was studied by gamma camera imaging in a rabbit atherosclerosis model. In contrast to control rabbits, the uptake of ¹²⁵I-MDA2 mAb in the aorta of WHHL rabbits was 17.4 fold higher, indicating the potential of this agent for plaque detection [40]. In another study, lectin-like oxidized LDL receptor 1 (LOX-1), which is overexpressed by activated endothelial cells and macrophages in the early stage of atherosclerosis development, was imaged with ^{99m}Tc-LOX-1-mAb. Compared to control rabbit, ^{99m}Tc-LOX-1-mAb planar images showed specific and higher accumulation in the abdominal aorta of WHHL-MI rabbit, which was also more than the data acquired with ^{99m}Tc-IgG2 control radiotracer (fig. 2B) [41].

As atherosclerosis develops, chondroitin sulfate on proteoglycans is produced and interacts with LDL, leading to the retention and accumulation of LDL in the arterial wall. Thus, an antibody-based tracer of ^{99m}Tc-chP3R99 mAb targeting chondroitin sulfate was developed for atherosclerosis imaging in a lipofundin-induced New Zealand White (NZW) rabbit atherosclerotic model using immunoscintigram planar imaging (fig. 2C) [42]. Compared to non-atherosclerotic rabbits, the uptake of ^{99m}Tc-chP3R99 mAb at the atherosclerotic lesion was 3.9 fold higher. However, the decay half-life of ^{99m}Tc does not match the long blood retention of chP3R99 mAb. Further studies may need to focus on the use of antibody fragments or peptides.

3.2. Radiotracers imaging the progression of atherosclerosis

Metalloproteinases (MMPs) are enzymes that degrade protein constituents of the extracellular matrix. Their activity is strongly induced by inflammatory cells during atherogenesis. More specifically, RP782 is a small molecule able to identify MMP activation epitopes. When radiolabeled with ¹¹¹In (fig. 3A), evaluation of the effects of diet

on ApoE^{-/-} mice was possible, correlating macrophage density found on atherosclerotic plaques with uptake signal [43]. Most macrophage cells were found in the atherosclerotic plaques of animals on a prolonged high fat diet (> 3 months). Moreover, ApoE^{-/-} mice fed a high fat diet for 2 to 3 months followed by a chow diet for 1 month presented reduced relative plaque area by 30%.

When a cell becomes apoptotic, phosphatidylserine (PS) present in the cell membrane is exposed. Because a massive presence of apoptotic macrophages could cause plaque rupture, Annexin V, a molecule with an affinity to PS in nanomole concentrations, was studied as a ^{99m}Tc-Annexin V tracer to monitor different stages of atherosclerosis in LDLR^{-/-} and ApoE^{-/-} mice on high cholesterol and chow diet [44]. SPECT/CT imaging and aorta autoradiography showed highest tracer uptake in cholesterol-diet ApoE^{-/-} mice ($0.88 \pm 0.27\% \text{ID/g}$), followed by chow-diet ApoE^{-/-} mice ($0.60 \pm 0.16\% \text{ID/g}$) and cholesterol-diet LDLR^{-/-} mice ($0.59 \pm 0.14\% \text{ID/g}$) (fig. 3B). Immunohistochemistry demonstrated that apoptosis was correlated to macrophage cell number and Annexin V uptake. These results suggested that radiotracer uptake may detect different stages of disease, presenting an option for monitoring therapeutic intervention.

Macrophage-induced lesion inflammation has been studied as a means to follow disease progression. The molecule diadenosine-5',5'''-P¹,P⁴-tetraphosphate (Ap₄A) and its analogue P²,P³-monochloromethylene diadenosine-5',5'''-P¹,P⁴-tetraphosphate (AppCHClppA) are competitive inhibitors of platelet aggregation. ¹⁸F-AppCHFppA was investigated for imaging inflamed atherosclerotic plaques in male NZW rabbits (fig. 3D) [45]. Uptake correlated to the presence of macrophages by histological examinations ($r=0.87$, $P=0.0001$). ¹⁸F-AppCHFppA shows potential for use in *in vivo* quantification of atherosclerosis progression.

A strategy based on CD68-Fc soluble protein which targets oxLDL foam cells was investigated [46]. ApoE^{-/-} mice fed a high-fat diet for 12 weeks were analyzed for detection of atherosclerotic lesion by ⁶⁴Cu-CD68-Fc-NOTA [46]. Standardized uptake value (SUV) quantification by PET/CT showed higher values in the aortic arch of ApoE^{-/-} mice ($10.5 \pm 1.5 \text{ Bq/cm}^3$) than wild type (WT) mice ($2.1 \pm 0.3 \text{ Bq/cm}^3$) (fig. 3D). In addition, higher R₁ relaxation rates by MRI imaging using gadolinium-based elastin-specific contrast agent in ApoE^{-/-} mice ($1.47 \pm 0.06 \text{ s}^{-1}$) compared to WT mice ($0.92 \pm 0.05 \text{ s}^{-1}$) were observed, demonstrating increased vessel wall remodeling and strong correlation data ($r=0.946$; $P=0.042$), which suggests the potential of ⁶⁴Cu-CD68-Fc-NOTA as a useful tool for cardiovascular risk stratification and for monitoring treatment effects from statin therapy.

3.3. Radiotracers imaging the complications of atherosclerosis

The efficacy of treatment and healing following myocardial infarction in ApoE^{-/-} mice was evaluated by using ¹⁸F-FXIII (coagulation factor) and myeloperoxidase coupled with gadolinium (MPO-Gd) (fig. 4A) [47]. PET/MRI imaging could monitor inflammation resolution which was useful in evaluating the nanoparticle-guided iRNA silencing of CCR2, wherein monocyte migration to inflamed sites was inhibited.

Hypoxia imaging was reported in an advanced atherosclerosis rabbit model since this pathogenic process triggers inflammation and angiogenesis, two critical factors for the disease [48]. This study made comparisons between PET imaging of atherosclerosis using ^{18}F -FMISO and ^{18}F -FDG by evaluating radiotracer uptake during disease progression until advanced lesions were obtained (fig. 4B). ^{18}F -FMISO showed higher signal (SUV mean of 0.20 ± 0.03 vs 0.10 ± 0.01 in healthy animals) with uptake increasing over the time of atherogenic diet. ^{18}F -FMISO and ^{18}F -FDG signals in the abdominal aorta were poorly correlated, suggesting the uptake of these two radiotracers does not share the same biological process.

Apoptotic cells present in plaques are recognized and phagocytized by macrophages through the surface presentation of phosphatidylserine (PS). ^{111}In radiolabeled liposome nanoparticles ranging from 100-200 nm were used as carriers of PS in a study designed to image the presence of vulnerable plaques [49]. Uptake of ^{111}In -PS200 was greater than that of ^{111}In -PS100 *in vivo* (fig. 4C), however, both particles showed high liver accumulation. The authors suggested that PEGylation of the liposomes may increase blood retention and decrease uptake by the liver.

The expression of MMPs has also been studied to evaluate rupture risk because their intense activity can destabilize atherosclerotic plaques. Membrane type 1 matrix metalloproteinase (MT1-MMP) was evaluated as a target for advanced stages of atherosclerosis by nuclear imaging using anti-MT1-MMP monoclonal antibody radiolabeled with $^{99\text{m}}\text{Tc}$ [15]. $^{99\text{m}}\text{Tc}$ -MT1-MMP mAb had 3.9-6.6 fold higher uptake in WHHL-MI atherosclerotic rabbits as compared to control models. MT1-MMP expression levels correlated closely with tracer uptake. Also, according to atherosclerotic lesion stage classification by histological analysis, $^{99\text{m}}\text{Tc}$ -MT1-MMP showed higher uptake in atheromatous lesion (stage IV), which presents vulnerable lesion characteristics such as a large lipid necrotic core and many macrophage-derived foam cells.

A wide range of possible targets and imaging agents were studied but in preclinical settings the challenge remains finding appropriate animal models of plaque rupture. This is still a matter of much discussion among researchers in the field, considering the general animal utilized in experiments does not have propensity to events like myocardial infarction found in humans.

4. Multimodality probes for atherosclerosis imaging

Multimodality imaging is a fast-growing area because these imaging platforms can provide both functional and anatomic information to better characterize the progression and activity of plaque. Of the various molecules developed for multimodality imaging of plaque, nanoparticles have directed the advancement in this field, thus boosting the development of new hybrid systems for clinical use. Nanoparticles targeting monocytes and macrophages can characterize the degree of plaque inflammation. Trireporter nanoparticle (TNP) derivatized to DTPA for ^{64}Cu radiolabeling and to a dextran-coated VT680 infrared fluorochrome for macrophage targeting was evaluated [50]. Biodistribution indicated higher uptake in aortas (260%) and carotid arteries (392%) in apoE^{-/-} mice compared to wild type

animals. Uptake of ^{64}Cu -TNP was observed by PET/CT and MRI images (fig. 5A) and colocalization was demonstrated by autoradiography and Oil Red-O staining of excised aortas. Tissues from the aortic arch, root and carotid arteries were strongly positive by *ex vivo* fluorescence imaging. In another application, 13 nm dextran nanoparticles (DNP) were labeled with ^{89}Zr and Vivotag-680 (VT680) fluorochrome to investigate atherosclerotic apoE^{-/-} mice using PET imaging and flow cytometry, respectively [51]. In these mice, higher PET signal was found in the aortic arch which correlated to excised aortas in autoradiography (fig. 5B). Additionally, the radiotracer was able to monitor treatment with siRNA-CCR2 which was characterized by decreased PET signal and inflammatory gene expression.

LyP-1, a cyclic peptide that binds to overexpressed p32 protein on the surface of macrophages, was radiolabeled with ^{64}Cu to evaluate its potential for atherosclerotic molecular imaging and labeled with fluorescent probes (FAM or 6-BAT) for optical imaging [52]. This study compared the uptake of (LyP-1)₄-dendrimer- ^{64}Cu with control (ARAL)₄-dendrimer. The dynamics of the tracer demonstrated higher uptake and accumulation in the aortic arch of apoE^{-/-} mice by PET/CT (fig. 5C), biodistribution analysis and fluorescence imaging of the aortic atherosclerotic plaques with (LyP-1)₄-dendrimer-FAM.

Nevertheless, concerns about nanoparticle clearance properties are great because of their potential toxicity. Efforts to improve this characteristic, such as using renal clearable nanostructures, have been undertaken [53]. Currently, PET/CT and SPECT/CT configurations have been commonly used for preclinical setting and new hybrid systems are currently in evaluation for assembly. Furthermore, effort has been devoted to study the data from PET, SPECT, fluorescence and MRI images by utilizing landmarks in the aorta regions in order to find correlations between these modalities, taking in to consideration the benefits offered by each [54].

5. Conclusions and prospective

Despite the immense volume of papers published in the area of atherosclerosis imaging, there are still many challenges and issues to be clarified due to the complexity of the disease through formation, evolution and rupture of the plaque. Of the various imaging platforms, small molecules and peptides have proven to be valuable tools for atherosclerosis detection due to their specificity and high binding affinity. However, fast *in vivo* pharmacokinetics and clearance may limit their capability to deliver high target-to-background contrast ratio. Despite the high binding specificity to targets expressed on the plaque, antibody-based tracers also face the challenge of generating high contrast ratio for accurate and sensitive detection of atherosclerotic lesions due to their extended blood circulation. Interestingly, engineered antibody fragments may have the potential for improved imaging owing to their optimized pharmacokinetics. Nanoparticles, due to their improved binding affinity and tunable pharmacokinetics, have shown great potential for plaque detection. However, *in vivo* clearance and toxicity need further confirmation.

Owing to the longitudinal nature of the inflammation process numerous atherosclerosis biomarkers have been identified and imaged. However, the connection between the

expression of these targets and progression or severity of disease warrants further investigation, which will significantly affect the development of prognostic imaging agents to detect plaque activity and prone-to-rupture risk.

Taken together, radionuclide-based imaging agents have been widely used for pre-clinical research and show promise. However, there is a long way to go to develop prognostic agents to sensitively and specifically detect atherosclerotic plaque, track the progression and predict the rupture.

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Figure 1.

Representative images detecting early stage atherosclerosis. (A) SPECT/CT imaging showed uptake of ^{99m}Tc-cAbVCAM1-5 in the aortic arch of C57Bl/6J and apoE^{-/-} mice in coronal views, demonstrating higher signal as compared to non-specific control nanobody ^{99m}Tc-cAbBcl110. (B) Planar images after injection of ^{99m}Tc-B2702p1, ^{99m}Tc-B2702p1 mismatch and ^{99m}Tc-B2702p with higher left carotid uptake indicated by white arrow. (C) ⁶⁸Ga-DOTA-FAMP peptide uptake in PET imaging of Japanese white (JW) control rabbit and WHHL-MI, without and with stent implanted presenting high uptake by atherosclerosis model which was more significant with the presence of the stent. (D) PET/CT images of ⁶⁴Cu-DOTA-DAPTA-comb detecting plaque at right femoral arteries of apoE^{-/-} mice 1, 4, 24 and 48 hours post injection, demonstrating increasing uptake over time. Images modified with permission [33, 36, 38, 85].

Figure 2.

Representative images using antibodies as radiotracers for atherosclerosis imaging. (A) *In vivo* PET/CT imaging in LDLR^{-/-} mice injected with ^{64}Cu -DOTA-anti-P-selectin mAb, demonstrating signal uptake in the brachiocephalic artery and aortic arch, as indicated by arrowhead and arrow, respectively. (B) Planar images after injection ^{99m}Tc -LOX-1-mAb compared to ^{99m}Tc -IgG2a in WHHL-MI and control rabbits. White arrows indicate aorta, K is kidney, L is liver, S is spleen. (C) Noninvasive planar images with injection of ^{99m}Tc -chP3R99 mAb by atherosclerotic (left image) and healthy (center image) rabbits, demonstrating the accumulation in the carotid indicated by arrowheads, compared to the injection of control radiotracer ^{99m}Tc -chT3 mAb (right image). Modified with permission [39, 41, 42].

Figure 3.

Representative images determining the progression of atherosclerosis. (A) Transversal SPECT/CT imaging using ¹¹¹In-RP782 radiotracer in apoE^{-/-} mice with aorta uptake indicated by red circles and the inferior vena cava circled in yellow. (B) *In vivo* SPECT/CT and *ex vivo* autoradiography images of the aorta after injection of ^{99m}Tc-annexin A5 in the control (left image) and LDLR^{-/-} (right image) mice with the highest uptake evident in the atherosclerotic model. (C) PET/CT imaging in an atherosclerotic rabbit injected with ¹⁸F-AppCHFppA by sagittal sections in the upper row and transversal sections in the lower row, with arrows indicating the lumen by CT and arterial wall by PET imaging. (E) Higher uptake in aortic arch of apoE^{-/-} compared to wild type mice that received ⁶⁴Cu-CD68-Fc-NOTA by PET/CT images with the SUV data quantification from the images. Modified with permission [43-46].

Figure 4.

Radiotracers imaging atherosclerotic plaque complication. (A) PET/MRI imaging after injection of ^{18}F -FXIII/myeloperoxidase-MPO-Gd in the myocardial infarction model WT mice, with the arrows indicating border regions of infarct, with increased signal by MRI image (center) and no uptake of ^{18}F -FXIII/MPO-Gd observed in control mice. (B) Uptake of ^{18}F -FMISO for hypoxia imaging in atherosclerotic rabbits with 8 months (induction) and 16 months of atherogenic diet (progression) by PET/CT imaging, demonstrating higher signal compared to control rabbits. The green line is related to axial views captured in bottom of the image and green arrows are showing abdominal aorta. Comparisons between FDG and FMISO uptake specificity by PET/CT imaging were made, indicated by green arrows. (C) *In vivo* SPECT/CT imaging in the aorta (arrows) of WHHL rabbits injected with ^{111}In -PS100 and ^{111}In -PS200. Images modified with permission [47-49].

Figure 5.

Studies involving multimodal imaging agents and new hybrid systems. (A) PET/CT imaging with ^{64}Cu -TNP with uptake in aortic arch demonstrated by arrow, MRI images showing signal in the aortic root pre and post-injection, respectively and versatility of the tracer showed by *ex vivo* near-infrared fluorescence reflectance imaging (NIRF) of the aorta with signal observed in the root (arrow) and carotid bifurcation (arrowhead). (B) Uptake of ^{89}Zr -DNP in atherosclerotic plaque of aortic root from apoE^{-/-} mice. Hybrid PET/MRI (top) and MRI (bottom) images demonstrating the signal from radiotracer and Oil-Red O staining and autoradiography of aorta correlating uptake in PET/MRI imaging. (C) PET/CT images in axial views demonstrating uptake of (LyP-1)₄-dendrimer-6-BAT- ^{64}Cu and (ARAL)₄-dendrimer-6-BAT- ^{64}Cu in the aortic root (AR) and descending aorta (DA). Modified with permission [50-52].

Table 1
Comparison of imaging modalities for atherosclerosis [55, 56]

Modality	Advantages	Disadvantages
SPECT	High sensitivity (10^{-14} – fmol) Signal quantification	Low spatial resolution (1-2mm) Radiation exposure
PET	High sensitivity (10^{-15} – fmol) Signal quantification Use of short half-life radionuclides	Low spatial resolution (1-2mm) Radiation exposure
CT	High spatial resolution (50-200 μ m)	Low sensitivity (10^{-6} - μ mol) Radiation exposure
MRI	High spatial resolution (10-100 μ m)	Low sensitivity (10^{-9} – nmol) Use of contrast agents
IVUS	Differentiation between layers in the arterial wall High axial resolution ($\sim 100\mu$ m) Details about plaque composition	Invasive method by catheter
OCT	Higher resolution than IVUS ($\sim 10\mu$ m)	Invasive method by catheter Requires saline flushing of the lumen Limited depth of view
FMT	Versatility of use	Limited depth of view for infrared probes

Table 2
Characteristics of Selected PET Radionuclides and Applications [57-60]

Nuclide	Half-life	Decay (%)	Maximum β^+ energy	γ Energy (keV)	Nuclear reaction	Application
^{11}C	20.4 min	β^+ (99.8) EC (0.2)	960 keV	511	$^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$	[61, 62]
^{18}F	109.8 min	β^+ (97) EC (3)	635 keV	511	$^{18}\text{O}(\text{p}, \text{n})^{18}\text{F}$	[32, 63-66]
^{64}Cu	12.7 h	β^+ (18) β^- (37) EC (45)	653 keV	511	$^{64}\text{Ni}(\text{p}, \text{n})^{64}\text{Cu}$	[38, 50, 67, 68]
^{68}Ga	67.7 min	β^+ (90) EC (10)	1.9 MeV	511	$^{68}\text{Ge}/^{68}\text{Ga}$ Generator	[69-71]
^{89}Zr	78.5 h	β^+ (22.7) EC (77)	2.8 MeV	511 909	$^{89}\text{Y}(\text{p}, \text{n})^{89}\text{Zr}$	[51, 72-75]
^{124}I	4.2 d	β^+ (23) EC (77)	2.2 MeV	511 603 723	$^{124}\text{Te}(\text{p}, \text{n})^{124}\text{I}$ $^{124}\text{Te}(\text{d}, 2\text{n})^{124}\text{I}$	[76-78]

Table 3
Characteristics of Selected SPECT Radionuclides and Applications [57, 79]

Nuclide	Half-life	Decay (%)	γ Energy (keV)	Nuclear reaction	Application
^{123}I	13.2 h	EC (100)	159	$^{124}\text{Te} (p, 2n) ^{123}\text{I}$	[31, 80]
^{111}In	67.3 h	EC (100)	171.3 245	$^{111}\text{Cd} (p, n) ^{111}\text{In}$ $^{112}\text{Cd} (p, 2n) ^{111}\text{In}$	[49, 81-83]
$^{99\text{m}}\text{Tc}$	6.0 h	IT (100)	140.5	$^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ Generator	[84-87]