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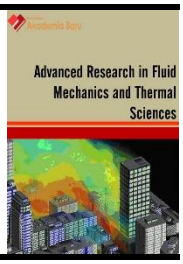

Nanotechnology for Molecular Imaging of Atherosclerosis: Current Design and Approaches

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ABSTRACT

Atherosclerosis complications such as myocardial infarction or stroke is still one of the most critical causes of death worldwide. Advance and innovative diagnostic technologies are urgently required to discover an early stage of the disease, such as plaque instability and thrombosis. A combination of molecular imaging probes based on well-designed nanomaterials with leading-edge imaging methods is currently concreting the direction for novel and distinctive approaches to examine the inflammatory growth in atherosclerosis. Over the past several decades, an exceptional understanding of the biological nature of atherosclerosis provides unique opportunities to better treat atherosclerotic disease with targeted imaging and nanomedicines. Consequently, tremendous development has been initiated in the nanotechnology application; the leading engineering tools working at molecular range, which is designed for diagnostic and therapeutic approach, called theranostic. This review underlying ideas involving the potential and development of molecular imaging technologies that had been invented for studying atherosclerosis. We envisage that many molecular imaging methods will become valuable assistants to the clinical management of targeted treatment in the atherosclerosis disease together with their challenges and future perspective in clinical translation.

1. Introduction

Cardiovascular diseases (CVDs) are the leading causes of death [1]. According to a study by the World Health Organization, the World Heart Federation and the World Stroke Organization stated that from two-thirds of all deaths in the world which belong to non-communicable diseases, almost 50 % are from CVDs alone [2]. Furthermore, the key for overcoming the challenges in the prevention of cardiovascular disease require global solutions that should comprise of economic, political, public health, technological solution, training for health professionals and researchers [2]. Besides, many popular conventional therapeutic CVD treatments, for instance; anti-hyperlipidemic drugs, anti-

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inflammatory drugs, anticoagulant drugs and anti-hypertensive drugs, poorly treat the inflammatory processes during plaque formation [4]. As a result, researchers and clinicians continue to explore other innovative therapeutic methods using nanomedicines-based approaches to help fight the disease with better precision and effect [3].

Zdrojewicz *et al.,* [5] reported that the injection of amino acids 2–26 loaded nanoparticles (Col IV–Ac2-26 NPs) that targeted the collagen IV on atherosclerotic mice revealed their potential in treating chronic atherosclerosis by substantially improved the characteristics of the atherosclerotic plaque, for instance, increased the protective collagen layer that further minimized the plaque necrosis [5]. Another supportive finding revealed that phosphatidylserine-containing nanoparticles combined with curcumin could greatly influence the atherosclerotic plaque stability by efficiently blocked lipid build-up, inhibit pro-inflammatory factor production and encouraged the flow of anti-inflammatory cytokines via greater transcription and secretion levels of anti-inflammatory factors (TGF-β and IL-10) from oxLDL activated macrophages after the uptake of PS-containing curcumin-loaded nanostructured lipid carriers(Cur-mNLCs) [6]. Thus, these nanoparticles can be used to transport active constituents together with anti-inflammatory agents, and significantly treat atherosclerotic lesion [5-8].

Nanotechnologies are defined as current approach in designing, classifying and functionalizing models or tools, by modifying the structures and the molecular sizes at nano-meter level [7]. As nanotechnologies advanced into clinical medicine, novel techniques utilizing nanomedicines to treat cardiovascular diseases has been undertaken to resolve the complex pathogenesis of atherosclerosis [8]. Different imaging technologies are being developed to visualize various features of vessel walls that could indicate the onset of acute atherosclerotic cases. For example, Four-dimensional (4D)-flow magnetic resonance imaging (MRI) offers a detailed study of complex blood circulation in the aortic valve and thoracic aorta with short scanning times, enhanced pre-processing study and visualization equipment, thus promoting its clinical practicality [9].

Nanotechnology is a promising method for imaging and therapy of atherosclerosis due to its small size nanoparticles (NPs) [10]. These nanoparticles can be loaded with bio-active compounds such as drugs and contrast agents for targeting, imaging and lesion treatment thereby resulted in accurate diagnosis and treatment of atherosclerosis [11]. The choice of the standard imaging technique, however, varies based on the biological features to be assessed, the readiness of molecular probes, and the procedure's approachability [10]. Table 1 showed several types of nanoparticles that have potential applications for atherosclerosis imaging. Targeted-imaging nanoparticles may also be used to specifically obtain information on the detail development and treatment of the specific lesions [11]. Recently, Schnitzler *et al.,* [12] reported that the use of nanomedicines in drug delivery and tracer imaging improves the sensitivity and effectiveness of these agents intended for atherosclerotic diseases prevention by means of targeting the resident macrophages and activating the endothelial cells. Nanomedicines-based treatment has become an essential strategy for delivering safe and targeted nanoparticles to further reduce the residual risk of cardiovascular events [13].

Table 1
Current research development on imaging-based nanoparticles for atherosclerosis

Nanoparticles and contrast agent	Size (nm)	Imaging	Target	Result	Year [Reference]
Fluorescein isothiocyanate and peptide conjugated mesoporous silicon nanoparticle (FITC-VHP-Fe3O4@SiO2)	50	MRI	VCAM-1	High affinity on the plaque by reducing T2 relaxation time for negative enhancement of T2-weighted images.	2019 [14]
Tetrazine conjugated 68Ga iron oxide nano-radiomaterials (68Ga-NRM-TZ)	15	PET combined with MRI	IgM antibody	Selective accumulation in atherosclerotic plaques, by clearly providing simultaneous PET and T1-MRI fluorescence signals.	2019 [15]
Superparamagnetic iron-oxide nanoparticles conjugated with the fusion-protein (EGFP-EGF1-SPIONs)	9.66 ± 1.05	MRI	Tissue factor	Good integrity and stability via reduced focal negative signals of T2-weighted images	2019 [16]
Curcumin incorporated titanium dioxide nanoparticles (CTNPs) conjugated MCP-1 antibody	29.5	MRI	Aortic region	Improved *in vivo* contrast at aortic regions by reducing longitudinal (T1) and transverse (T2) relaxation times of the water protons.	2020 [17]

*T1 & T2 refer to the relaxation time constant in MRI; where the T1 provides positive contrast effect (gadolinium particles), while T2 provides negative contrast effect (iron oxide particles). The contrast variances between T1 and T2 may enable the plaque segmentation in different constituents.

2. The Emergence of Nanoparticles for Diagnosis and Therapy of Atherosclerosis

Among many of the imaging techniques, high-resolution magnetic resonance imaging (MRI) has started to emerge in the '90s as a valuable imaging modality that offered high soft tissue contrast for characterization of atherosclerotic arteries in a noninvasive and nondestructive way. Taking advantage on the tools, Weissleder *et al.,* [18] developed a promising contrast agent consisted of an ultrasmall superparamagnetic iron oxide (USPIO) that are about 10 nm in particle size that may potentially use as an intravenous contrast agent for the lymphatic system and other organs such as in brain and bone marrow for MRI. The work also reported on the comparison between dextran-coated ultra-small superparamagnetic iron oxide (USPIO) nanoparticles with the superparamagnetic iron-oxide (AMI-25) nanoparticles. The T1 effect of dextran-coated USPIO in rats showed a considerably longer blood half-life than traditional AMI-25, suggesting that the dextran-coated USPIO particles is not immediately detected, opsonized, and phagocytosed by mononuclear phagocytic system (MPS) of the liver and spleen [18]. In addition, the small size and plasma half-life prolongation of the USPIO allows this agent to reach the capillary wall and have a more widespread distribution of tissue, including lymph node and bone marrow uptake by the MPS than the AMI-25 [18]. Thus, utilization of dextran was a good strategy to improve the uptake and the specificity for macrophages in atherosclerotic plaques [18]. Moreover, USPIO was also been applied in a magnetic resonance angiography (MRA) because of the T1 shortening effect and their long intravascular half-life, thus increasing vascular signal intensity and improving the visualization of arteries and veins from the thoracic region [19]. Ruehm *et al.,* [20] investigated the use of USPIO nanoparticles as a marker for atherosclerosis-associated inflammatory changes in the vessel wall in hyperlipidemic rabbits. As a result, USPIOs were phagocytosed by macrophages in a sufficient quantity to cause iron-induced susceptibility effects within the atherosclerotic plaques of hyperlipidemic rabbits. Besides USPIO, a lipid-encapsulated perfluorocarbon emulsion with gadolinium targeted on fibrin, and a

peptidomimetic vitronectin antagonist ligand was applied. The studies revealed a dense accumulation of nanoparticles on the targeted sites of fibrin clots [21,22]. These results suggested the potential for sensitive and specific detection of arterial thrombi and the progression of the unstable atherosclerotic plaque [21,22].

Another promising strategy for MRI is the application of annexin A5-functionalized nanoparticle of phosphatidylserine (PS) exposing cells in atherosclerotic lesions was developed [23]. T1-weighted MRI revealed enhanced uptake of the annexin A5-functionalized nanoparticles by PS-expressing cells within atherosclerotic mice lesions in the abdominal aorta [23]. Thus, the annexin A5-conjugated functionalized nanoparticles displayed the capabilities to assess cell types that are considered to contribute to plaque instability and in the evaluation of atherosclerotic lesion phenotype [23]. Wang *et al.,* [24] continued the groundwork of nanoparticles emergence by developing ultralow gadolinium–manganese nanoparticles -(MnOL-Gd NP) for MRI on avβ3-integrin. This MRI agent provided efficient *in vivo* detection of atherosclerotic angiogenesis, with most of the contrast localized within the plaque and media of the diseased aorta [24]. Importantly, this MR molecular imaging agent was readily bio-eliminated *via* the biliary system with no sign of toxicity [24]. Collectively, αvβ3-MnOL-Gd NP offered a practical, safer vehicle for neovascular imaging in atherosclerosis [24].

Besides extensive research on MRI, other imaging techniques such as computed tomography (CT), fluorescence and photoacoustic imaging with or without combination with MRI techniques (e.g., MRI-fluorescence) were also developed for diagnosis and/or mapping of atherosclerosis (Figure 1). For instance, Hyafil *et al.,* [25] developed an iodinated nanoparticulate contrast agent (for instance, N1177) for computed tomography (CT) imaging. A substantial signal enhancement was detected in the plaques after the injection of the iodinated nanoparticulate contrast agent, which was not possible with the iopamidol [25]. Thereby, the N1177 may become an excellent contrast agent for the evaluation of coronary arteries via CT imaging [25]. In the year of 2011, Uchida *et al.,* [26] developed a protein-cage nanoparticle that possesses a macrophage-targeting peptide (LyP-1) and a fluorescence imaging molecule without disrupting the overall structure of a platform protein (MjHsp). Fluorescently labelled LyP-Hsp exhibited specific interaction with macrophages, whereas macrophage-rich carotid lesions in mice showed good fluorescence signal after LyP-Hsp injection [26]. Therefore, demonstrated the potential of LyP-1- conjugated protein cages as a nano delivery of imaging agents for the diagnosis of atherosclerosis [26]. Qin *et al.,* [27] formulated the gold nanorods conjugated with MMP2 antibody (AuNRs-Abs) for atherosclerosis mapping. After labelling with AuNRs-Abs, the distribution of MMP2 antibody of the atherosclerotic plaques was revealed using intravascular photoacoustic imaging (PAI) [27]. The data demonstrated that AuNRs-Abs is a promising probe for intravascular photoacoustic imaging (PAI), which quantitatively detect MMP2 antibody distribution [27].

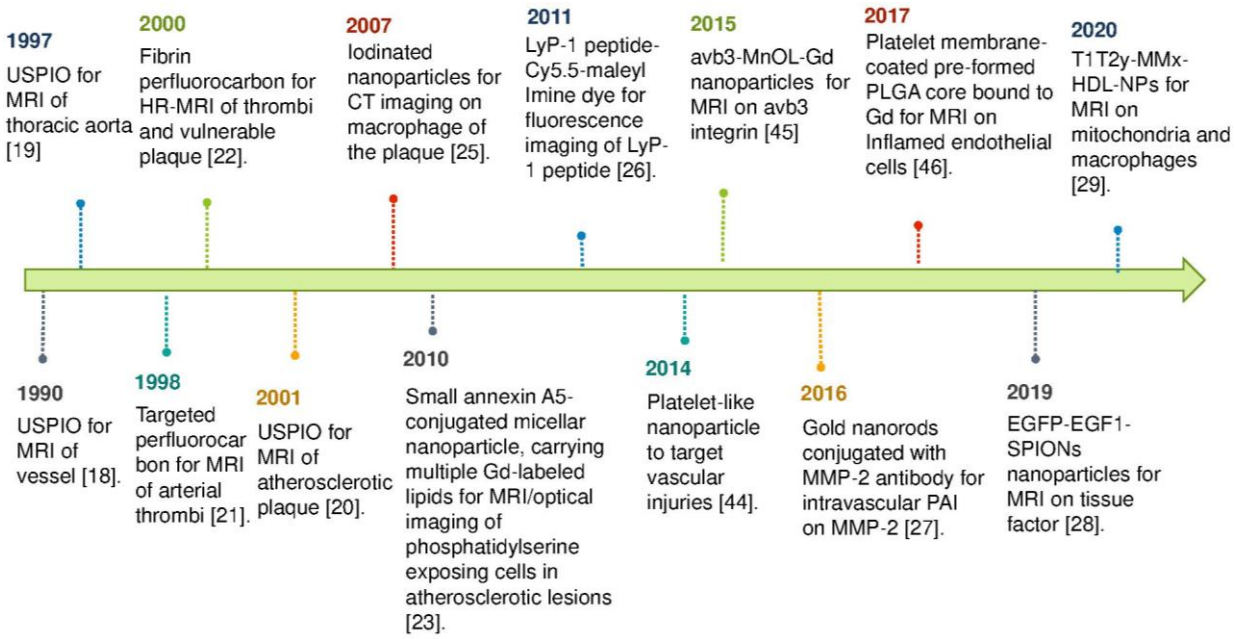

Fig. 1. Nanoparticles development for atherosclerosis imaging techniques

Recent investigations by Wei *et al.,* [28] explored the conjugation of the 'fusion protein' enhanced green fluorescent protein with the first epidermal growth factor domain' (EGFP-EGF1) and superparamagnetic iron oxide nanoparticles (EGFP-EGF1- SPIONs) for molecular imaging of tissue factor (TF)-positive atherosclerotic plaques. It was observed that EGFP-EGF1-SPIONs provided an excellent contrast effects, thus improve the accuracy and great cytocompatibility in the detection of atherosclerotic plaques *in vivo* using MRI [28]. In short, EGFP-EGF1-SPION is a promising TF-targeting nanoprobe in detecting atherosclerotic plaques, which may improve the diagnosis of cardiovascular events for the comprehensive evaluation of atherosclerosis [28]. In more recent year, dual-targeted nanoparticles (NPs) with encapsulated iron oxide nanoparticle and magnetic resonance imaging (MRI) contrast agent were designated and synthesized by Banik *et al.,* [29] to perform the double duty of diagnosis and therapy in atherosclerosis treatment regime. The optimized composition of the dual-targeted NP was found to reduce lipid levels in the body significantly without provoking any significant immunogenic effect inside the body [29]. This finding revealed the invention and optimization of a versatile NP platform, which can be further utilized as theranostic applications for the simultaneous diagnostic and therapy of atherosclerosis [29].

3. Imaging Modalities in Atherosclerosis

Accurate and reliable diagnosis of vulnerable atherosclerotic plaques before clinical manifestations is a prerequisite to identify high-risk patients and tailored therapy. In conjunction with present imaging advances that principally target to evaluate structural constituent of atherosclerosis, for instance; fibrous cap thickness and the size of the lipid core (Figure 2), the molecular and cellular imaging were also designed to visualize biological features of atherosclerotic plaques such as inflammation, angiogenesis, and matrix degradation in the living organism [28,29,30-37]. Although the rupture-prone plaque components are detectable by various imaging technologies, it remains challenging to differentiate between erosion-prone plaque and a stable plaque by imaging. This section review on the design and applications of different imaging platforms (Table 2), such as; computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET).

Table 2
Alternative 'target-specific' contrast agents used for atherosclerosis plaque characterizations in different types of imaging platforms

Imaging	Nanoplatforms	Targeted molecule	Outcomes	Year [Reference]
MRI	Peptide conjugated amphiphilic molecules containing gadolinium (CREKA-DSPE-PEG2000-DTPA-Gd)	Fibrin	CREKA-DSPE-PEG2000-DTPA-Gd exhibited positive fluorescence signal of the fibrin, by increasing T1 rate.	2016 [38]
	Gadolinium-bis-5-hydroxytriptamide-diethylenetriamine pentaacetic acid (bis-5HT-DTPA-Gd)	Enzyme myeloperoxidase (MPO)	Bis-5HT-DTPA-Gd demonstrated sustained plaque enhancement on T1-weighted ($T1_w$) imaging, thus emphasizing the potential for atherosclerotic plaque detection	2018 [39]
	Superparamagnetic iron-oxide nanoparticles (SPIONs)	Atheroma plaque	SPIONs showed reasonable signals of the atheroma plaque, by reducing T2-weighted ($T2_w$) magnitude images, thus could be interesting for development of imaging tracer for atherosclerosis	2017 [40]
	Superparamagnetic iron-oxide nanoparticles conjugated with the fusion protein (EGFP-EGF-SPIONs)	Tissue factor	EGFP-EGF-SPIONs exhibited accumulation in the plaque, by resulting in plaque images enhancement contrast of $T2_w$ MRI.	2019 [28]
PET	^{89}Zirconium conjugated hyaluronan nanoparticles (^{89}Zr-HA-NPs)	Hyaluronan (HA)	^{89}Zr-HA-NPs revealed PET signal hotspot of the abdominal aorta, by demonstrating high aortic PET signal localized in macrophages-rich areas.	2017 [41]
CT	11- mercaptoundecanoic acid coated gold nanoparticles (11-MUDA-AuNP)	Macrophage	11-MUDA-AuNP exhibited in monocytes of the atherosclerotic plaque, indicating the feasibility of tracking labelled monocytes with CT	2016 [42]
SPECT/ CT	Technetium-99m radiation-labelled natural H-ferritin nanocage (99mTc-HFn)	Transferrin receptor 1	99mTc-HFn demonstrated intense focal radiotracer uptake in the aortas, for good localization and detection of macrophage-rich plaques	2018 [43]

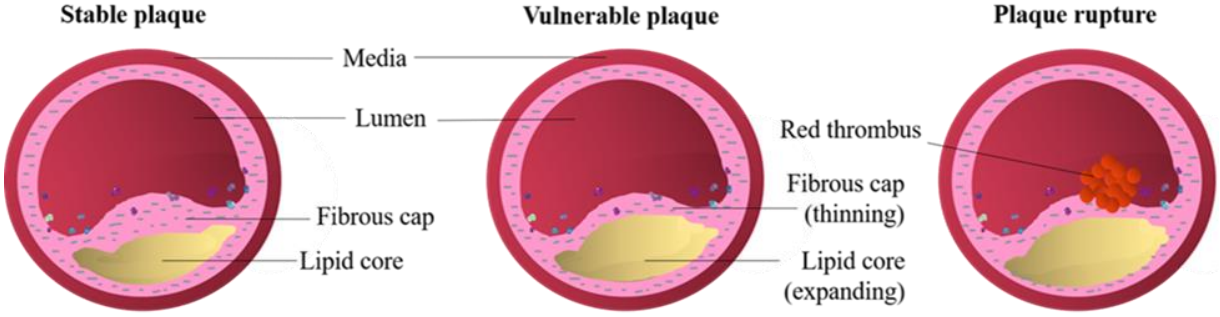

Fig. 2. Illustration of coronary thrombosis mechanism, where the formation of atherosclerosis plaques from stable to vulnerable was occurred. The thin fibrous caps are at risk of rupture

3.1 Computed Tomography (CT)

Computed tomography (CT) is a convenient tool in diagnosing and evaluating the complexity of atherosclerosis throughout the entire vascular regions. CT may provide details on plaque configuration and abnormality, as well as the occurrence of vascular remodelling [47,48]. Its substantial impact on the diagnosis of subclinical atherosclerosis is in the identification and measurement of coronary calcium [49]. CT dominates an excellent temporal and spatial resolution, which can be further enhanced via multi-detector spiral computed tomography and the simultaneous utilization of iodinated contrast medium. This methodology allows the non-invasive detection of plaque locality and at the estimated magnitude [50]. The overall phenotypic category of plaques, together with the comparison among them, may also be acquired by intracoronary ultrasound [51]. Additionally, through engaging multi-colour (or spectral) CT, the composites incorporating tissues such as calcium deposits in atherosclerotic lesion can be analysed using different contrast vehicles, such as gold-labelled and iodine-labelled probe imaging. Thus, permitting instantaneous imaging of various atherosclerotic plaque mechanisms [52].

3.2 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) offers incredibly accurate data on plaque magnitude, the occurrence of inflammation, and the state of the fibrous stratum [53,54]. MRI is possibly the most favourable approach for molecular imaging in atherosclerosis, because of the exceptional spatial (number of pixels utilized) and temporal (distance of time between the acquisitions of two images of the same area collected) resolution without demanding the management of ionizing radioactive nucleotides [55]. Additionally, MRI can also suggest the phenomenon of constructive vascular restoration, which holds the benefit of not utilize radiation exposure, thus providing an advantage to its usefulness in patient's convenient upon medical treatment [56].

To defeat the disadvantage of MRI that shows inadequate sensitivity during imaging, the application of paramagnetic vehicles such as gadolinium chelates and superparamagnetic iron oxide nanoparticles (SPIONs) contrast medium have been engaged to non-invasively image the arterial diseases [57]. In principle, these ultra-small paramagnetic iron oxide nanoparticles (USPIOs; 10–50nm in diameter) permit extravasation into vascular tissues allowing hypothetical assessment of atherosclerotic lesions [57]. This methodology has been applied for quantifying plaque macrophage load via MRI signal depletion as a result of phagocytic absorption of USPIOs in rabbits and in humans, where MRI signal concentrations associated with macrophage-rich unstable plaques [20,58]. However, mechanical problems correlated with vessel wall imaging using USPIOs indicates of low signal-to-noise ratio, reduced the specificity to visibly differentiate signal concerning anatomical sites, prolonged imaging periods and the complication in standardizing settings between scans [20].

In contrast, another research of USPIOs, involving the usage of peptides (VCAM-1) targeted nanoparticles for an *in vivo* study showed dissimilarity results to USPIOs. These nanoparticles were taken up by inflamed endothelium in accumulation to macrophages and vascular smooth muscle cells, permitting the discovery of fatty-streak lesions in MRI and optical imaging of juvenile hyperlipidemic Apo-lipoprotein E mice. Thus, enlightened the capability of this technique for primary intervention and monitoring of atherosclerosis [59].

Besides USPIOS, micro-particles consisted of iron oxide (MPIO) were suggested to have additional benefits in MRI imaging with their greater size (0.9–8μm). Unlike nano-particles iron oxide, which offered passive accumulation via macrophage uptake in atherosclerosis lesion, the larger particles size of MPIO has restricted them to localize at the endothelial surfaces that is suitable for

endovascular molecular specificity [60]. The superior iron content brought by the MPIOs has also enhanced the signal recognition through high target-to-background ratio, thus allowed quick imaging of vascular inflammation with an exceptional level of specificity [60].

3.3 Positron Emission Tomography (PET)

Positron emission tomography (PET) is constructed based on the discovery of extraordinary-energy photon pairs formed throughout annihilation impacts between positron and electron, after which 3D pictures are reassembled by computer analysis [61]. PET contrast agents comprising of positron-emitting radionuclides, such as fluorine-18 (18F), copper-64 (64Cu), and gallium-68 (68Ga) were manufactured from a cyclotron nearby the PET imaging capacity and chemically combined into the assembly of PET probes, thus applied instantly to prevent substantial decay with unlimited depth of penetration and excellent sensitivity [61,62]. Nevertheless, PET is a relatively expensive approach and has restricted accessibility because of the requirement of nearby cyclotron [62]. The main restriction of PET imaging is its low spatial resolution and shortage of anatomic reference frame [62]. The low spatial resolution can be improved by optical fluorescence imaging (OFI)/PET imaging, whereas additional CT or MRI imaging (PET/CT or PET/MRI) can overcome the limitation in anatomic reference frame [62].

3.4 Single-Photon Emission Computed Tomography (SPECT)

Single-photon emission computed tomography (SPECT) provides longer half-life of radionuclides than PET radionuclides, enabling the non-specific signal to be biologically washed out within the imaging time frame, leaving a cleaner signal of interest. Thus, makes SPECT imaging more practically available for imaging with longer radiosynthesis times and more affordable in price [63]. SPECT possessed comparable strengths and limitations with PET, which incorporate boundless depth penetration, great sensitivity to some extent, low spatial resolution and minimal 3D anatomical data [64]. Although SPECT is fundamentally less sensitive than PET, the shorter half-life of the PET radionuclides limits the accessibility of PET imaging thus, makes PET imaging more challenging [63].

Oxidized LDL-receptor-1 (LOX-1) is recognized as the primary receptor for oxidized LDL that has been associated with atherosclerosis-stimulating components and is considered to perform a central role in atherosclerosis [65]. An *in vivo* SPECT/CT imaging showed that the probe preferably bound to the region with vulnerable plaque features. Thus, this flexible multimodal imaging approach could be useful to identify characteristics of vulnerable plaque in high-risk atherosclerotic patients [66].

3.5 Near Infrared Fluorescence (NIRF) Imaging

Application of fluorescent imaging techniques in the NIR region has gained significant attention in the 21st century [67,68]. A satisfactory NIRF image requires excellent NIR coloration with superior image properties [69]. The ideal NIR imaging best work in the NIR spectrum (600–900 nm) due to a minimal interference of the fluorescence, a sensitive and non-invasive effect of imaging, and an improved tissue depth penetration [70,72]. The success of functionalized MRI-NIRF imaging was obtained by the conjugation of profilin-1 antibodies with β-cyclodextrin magnetic nanoparticles (PFN1-CD-MNPs) loaded NIRF cyanine dye, that showed significantly high fluorescence signals in the aorta, leading to better atherosclerotic plaque targeted-imaging [73]. Another research on MRI-NIRF imaging of versatile ultra-small super-paramagnetic iron oxide (VUSPIO) similarly demonstrated

excellent fluorescence signal in the plaque site, indicating potential of functional VUSPIO as a biocompatible and atheroma-specific diagnostic agent [85].

3.5.1 Indocyanine Green (ICG)

There are several NIRF dyes used for diagnosing and imaging atherosclerotic plaque known as cyanines. They are considered as preferable candidates due to improve physical properties and great-scale synthesis availability, whilst the most prominent of all is the indocyanine green (ICG) [74,76-78,86].

Iron oxide nanoparticles combined with indocyanine green (IONP-ICG) showed strong and intense fluorescence signals of arteriosclerotic plaques, thus considered to be the most effective dye for arteriosclerotic plaque visualization [79]. Likewise, ICG and the modified sirtuin 1 activator with a peptide targeting osteopontin (ICG / SRT@HSA-pept) exhibited strong fluorescence signals in the *in vivo* atherosclerotic lesions, allowing NIRF dye to be used clinically for cardiovascular disease nanotherapeutics [80]. Another interesting research was reported on the use of indocyanine green (ICG) loaded into a 90 nm contrast model, which developed using hemoglobin-depleted mice erythrocyte-ghosts known as near-infrared erythrocyte-derived transducers (NETs) [81]. NET was designed to specifically identify the locations of atherosclerotic lesions and occlusion, by detecting the fluorescence signal from NETs accumulation in inflammatory cells like macrophages. Using combined photoacoustic (PA) and fluorescence imaging tools, more findings to accurately distinguish symptomatic from asymptomatic coronary arteries in the future clinical detection could be achieved [81].

3.5.2 Other cyanine-based dye

Recent analysis on the development of extra domain B of fibronectin-specific nanoparticles (FN-EDB-specific NPs) discovered cyanine accumulation in plaque-containing aortas, suggesting high NIRF intensity. This finding has suggested the possible opportunities for an improved diagnosis and therapy in high-risk atherosclerotic patients [82]. On the other hand, cyanine 5 (Cy5) monocyte-targeting iron oxide magnetic nanoparticles (MNPs) demonstrated a possible targeting of NIRF dye for an early stage of atherosclerosis progression by exhibiting longer atherosclerosis plaque retention time in the aorta with substantial fluorescence signals [83].

In conclusion, NIRF is an effective imaging technique designed for envisioning both the high- and low-risk atherosclerotic plaque. Most of the NIRF studies have primarily exploited an FDA-approved NIRF dye of indocyanine green (ICG) to diagnose the unstable plaque permeability [84]. Clinical trials are required to properly evaluate the versatility of intravascular NIRF imaging using ICG along with several other novel NIRF dyes to better explain vulnerable plaque pathology, predict the outcomes and encourage customized pharmacotherapy of atherosclerotic plaques [84].

4. Conclusions

To date, advance research in diagnosing and assessing atherosclerosis are the outcome of integrated fields between cardiovascular medicine, immunology, medical imaging, bioengineering, and biochemical engineering. Although the application of the widespread imaging technique such as MRI is yet to be recommended for routine assessment in the clinics, this non-invasive approach in characterizing atherosclerosis such as evaluating plaque structure and measuring inflammatory cells and progression is considered promising. The diagnostic supremacy of existing and evolving

atherosclerosis imaging procedures will certainly encourage our strategy to patient care and assist determination towards cardiovascular study in establishment to greater comprehend disease systems and assess recent personalized medications. These objectives can be achieved throughout 1) better understanding of the complexity emphasizing the pathogenesis in atherosclerosis, 2) improve early detection of individual atherosclerosis risk and 3) assist in developing new targeted-atherosclerosis therapies via the aids of imaging modalities including PET, SPECT and/or NIRF. Beside developing expertise and invention, additional research in patient-specific multimodal imaging approaches can also be personalized to disclose molecular signals with anatomic accuracy (such as assimilating information on plaque arrangement and local hemodynamic), cost-effectiveness and the consequence to patient's compliance.

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