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Review

Multimodality Molecular Imaging of Cardiovascular Disease Based on Nanoprobes

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Key Words
Molecular imaging • Multimodality • Nanoprobes • Cardiovascular diseases

Abstract
Recently, multimodality molecular imaging has evolved into a fast-growing research field with goals of detecting and measuring biological processes in vivo non-invasively. Researchers have come to realize that the complementary abilities of different imaging modalities over single modality could provide more precisely information for the diagnosis of diseases. At present, nanoparticles-based multimodal imaging probes have received significant attention because of their ease of preparation and straightforward integration of each modality into one entity. More importantly, nanotechnology has an increasing impact on multimodality molecular imaging of cardiovascular diseases, such as atherosclerosis and vulnerable plaque, myocardial infarction, angiogenesis, apoptosis and so on. In this review, we briefly summarize that various nanoprobes are exploited for targeted molecular imaging of cardiovascular diseases, as well as associated multimodality imaging approaches and their applications in the diagnosis and treatment of cardiovascular diseases.

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Introduction

At present, despite the development of various optimal treatments, cardiovascular diseases are still one of the leading cause of death, disability, and healthcare expense in China, which is also the number one killer all over the world [1]. In recent years, there has been a shift in emphasis from treatment of cardiovascular diseases to the primary or secondary prevention, whereby, to some extent, we can improve human health through the preservation of life quality and control the increasing costs of health care [2, 3]. However, this shift puts forward new challenges to basic research as well as to clinical practice, and it further stimulates technological revolution of existing diagnostic procedures.

Molecular imaging has evolved into a fast-growing research field and plays a crucial role in early and accurate diagnosis and therapy of diseases since it can provide us important information such as anatomic, physiologic, molecular information of diseases in living organisms. Tremendous efforts have been spent on the development of highly specific and sensitive molecular probes especially multimodal imaging probes to achieve these aims in recent years. Various types of platforms such as small molecules, polymers and nanomaterials have been actively explored for the fabrication of multimodal imaging probes. Among them, nanoparticles-based systems have received significant attention because of their ease of preparation, straightforward integration of multiple functional moieties into one entity and which is expected to lead major advances in the detection, diagnosis and clinical treatment of cardiovascular diseases [4-8]. Although this field is still in its infancy, molecular imaging based on nanotechnology could have exciting features in multimodality molecular imaging and clinical diagnosis.

Nowadays, lots of literatures report the possibility of multimodality molecular imaging using nanoprobes, which supports the notion that clinical translation of this technology will be feasible in the near future [7, 9, 10]. Hence, the purpose of this review is to provide readers with a current update of multimodality molecular imaging of cardiovascular disease based on nanoprobes, which focus on six important aspects of cardiovascular diseases, including atherosclerosis, thrombosis, myocardial infarction and postinfarction remodeling, angiogenesis, apoptosis and cardiac stem cell-based therapy. In addition, we also discuss strengths and limitations of molecular imaging based on nanotechnology, and the transformation potential of multimodality imaging approaches with nanoprobes in clinic.

Imaging of Atherosclerosis and Vulnerable Plaque

The atherosclerotic instable plaques, which is responsible for many myocardial infarctions and sudden cardiac deaths, is morphologically characterized by a cascade of events including inflammatory activity, ulceration, plaque rupture, plaque erosion, calcified nodule, intraplaque hemorrhage, and intraplaque thrombosis [11, 12]. Currently, it is very hard to identify the atherosclerotic instable plaques of patients, which directly leads to considerable morbidity in clinical practice. X-ray contrast arteriography is the traditional "gold standard" for imaging atherosclerosis. And optical coherence tomography (OCT) is a high-resolution (10–15mm) intracoronary imaging modality that has shown to be able to differentiate the plaque rupture and erosion from unstable plaque *in vivo* [13, 14]. However, these methods could not accurately *in vivo* assess plaque characterization at the molecular level and evaluate the severity of atherosclerotic plaques. To meet this need, different molecular probes based on various nanomaterials have been developed and applied as novel approaches for imaging related biomarkers of atherosclerosis [10, 15, 16].

Activated macrophages can secrete multiple proteolytic enzymes, such as matrix metalloproteinases (MMP) and so on, which play key roles in degrading extracellular matrix and destroying the integrity of fibrous cap. Thus, the macrophage content in atherosclerotic plaques is an important contributor to atherosclerotic plaque instability. For above reasons, it is extremely urgent and necessary to develop effective molecular imaging

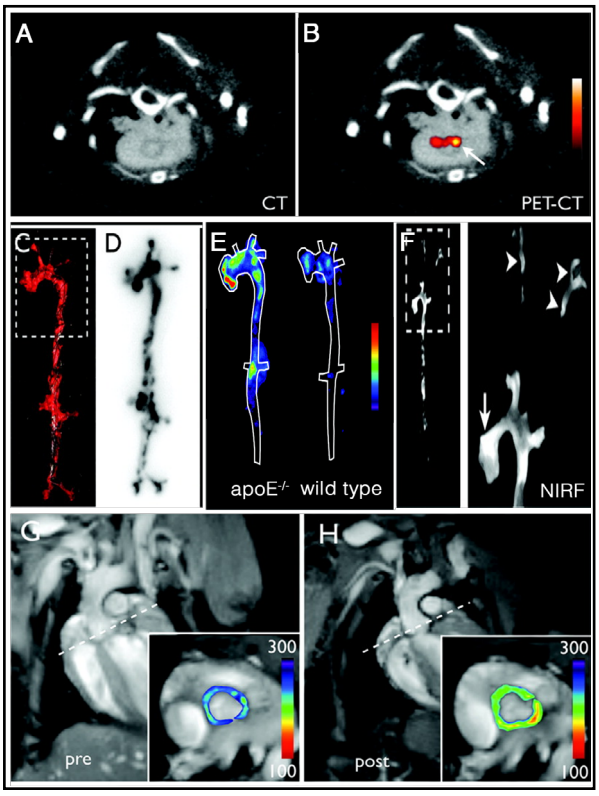

Fig. 1. ^{64}Cu-TNP distributes to atherosclerotic lesions. (A&B) PET/CT shows enhancement of the posterior aortic root (arrow). (C&D) Enface Oil Red O staining of the excised aorta depicts plaque-loaded vessel segments, which co-localize with areas of high ^{64}Cu-TNP uptake on autoradiography. (E) Exposure of excised aortas on the phosphor imager corroborated in vivo imaging findings of high activity in the aortic root and arch of apoE$^{-/-}$ mice. The color legend depicts signal intensity on autoradiography, correlating with activity and ^{64}Cu-TNP accumulation in the tissue. (F) Near-infrared fluorescence reflectance imaging (NIRF) of excised aortas shows accumulation of the probe in plaques residing in the root (arrow), thoracic aorta, and carotid bifurcation (arrowheads), further corroborating the PET signal observed in these vascular territories. *P=0.01. (G&H) Preinjection and postinjection MRIs of the aortic root (inset). The dotted line in the long-axis views demonstrates slice orientation for short-axis root imaging.

technologies, which are capable of identifying macrophages within atherosclerotic plaque and providing valuable information for assessing plaque vulnerability. Recently, Weissleder group have developed a novel multimodal probe to target macrophages biomarker CD68 in atherosclerotic plaques [17]. This novel nanoprobe is composed of dextrinated and DTPA-modified magnetofluorescent 20-nm nanoparticle. A trimodality imaging nanoprobe was synthesized and yielded after labeling with ^{64}Cu and applied for PET, MRI and fluorescence imaging (trireporter nanoprobe [^{64}Cu-TNP]). PET/CT results showed that accumulation of ^{64}Cu-TNP by the aortic root and arch in atherosclerotic arteries model of apoE$^{-/-}$ mice, which was further demonstrated on *in vivo* MRI imaging (Fig. 1). In addition, *in vivo* PET/CT findings were consistent with the results of enface Oil Red O staining, gamma counting and autoradiography *ex vivo*, which showed significant differences between apoE$^{-/-}$ mice and wild-type mice (Fig. 1). Furthermore, in order to promote accumulation of nanoprobe in macrophages in atherosclerotic plaques and provide a better contrast effect, another novel dual-modal contrast agent was synthesized by Gu group, and used for both CT and optical imaging of macrophages within plaques. This novel nanoprobe was composed of a hydrophobic iodinated oil core, containing QDs inside stabilized by PEGylated lipids [18], which was uptake by macrophages in atherosclerotic plaque of rabbit models displayed on clinic CT and fluorescence imaging at the same time (Fig. 2).

Vascular adhesion molecule-1(VCAM-1) is another biomarker of instable plaque, which is up-regulated on the endothelium under inflammatory conditions and plays important role in the development of atherosclerosis [19, 20]. Kelly et al. developed a novel VCAM-1-targeted imaging agent using phage display-derived peptide sequences and multimodal nanoprobe, which were applied in both MRI and fluorescence imaging *in vivo* [21]. Firstly, they synthesized the identified peptide sequence "CVHSPNKKC", which was shown to bind VCAM-1 and to block leukocyte-endothelial interactions. Then, the peptide sequence was attached to CLIO-Cy5.5, a cross-linked iron oxide nanoparticle to synthesize a fluorescent magnetic nanoparticles conjugates. To determine whether VCAM-1 expression of atherosclerotic lesions could be detected with this novel nanoprobe *in vivo*, MRI imaging was

performed in high cholesterol-fed apoE$^{-/-}$ mice. After the intravenous administration of the novel VCAM-1–targeted nanoprobe, the decreasing of extensive signal intensity was found and associated with iron oxide nanoparticle accumulation in atherosclerotic lesions, particularly in the site of aortic arch, which was further verified on MRI and macroscopic fluorescence imaging of the excised aortas *ex vivo*.

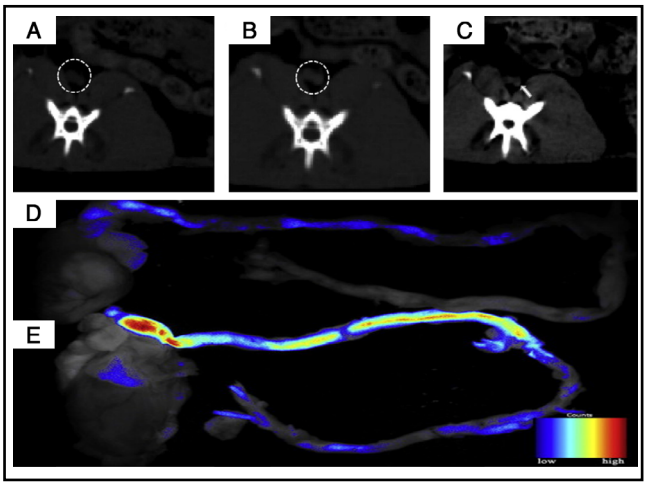

Fig. 2. (A-C) CT images of the abdominal aorta (white circle) of NZW rabbit, the atherosclerotic plaques were indicated by arrows. (A) before; (B) 10 s and (C) 2 h after the injection of QDs-iodinated oil nanoemulsion. Before injection, the atherosclerotic plaques could not be differentiated from the surrounding tissues, whereas a strong enhancement was detected in the plaques 2 h after the injection of the nanoemulsion. (D&E) Fluorescence imaging of excised aortas with QDs-iodinated oil nanoemulsion. The whole aortas were excised (D) without injection of the nanoemulsion; (E) 2 h after the injection. The signal intensity was significantly higher in the atherosclerotic plaques, meaning the accumulation of the nanoemulsion.

In addition to PET, CT, MRI, optical imaging and fluorescence molecular tomography (FMT), as an *in vivo* quantitative imaging modality, can be used to detect and quantitate inflammatory protease activity in atherosclerosis with protease activatable imaging probes [22]. The combination imaging of FMT and CT based on nanotechnology is a robust and observer-independent tool for non-invasive assessment of inflammatory atherosclerosis *in vivo*. Nahrendorf *et al.* reported *in vivo* FMT-CT imaging of the protease activity in atherosclerosis with customized nanosensor [23]. The research disclosed that with the help of FMT-CT, the nanoprobe 'cell anchor' for protease sensors (PS)-40 increased the detecting sensitivity and monitored treatment effects. Surface-enhanced Raman scattering (SERS) is a special vibrational spectroscopy that uses a metal surface to enhance Raman scattering from molecules attached to, or close to, the surface [24, 25]. A wide range of different metals and surfaces can be used to achieve this; however, gold or silver nanoparticles provide an excellent format. McQueenie et al. reported an alternative *in vivo* imaging system using SERS that was targeted to intercellular adhesion molecule 1 [ICAM-1 (CD54)] expression on endothelial cells by antibody conjugation to gold nanotags, allowing to image noninvasively up to 10^{14}-fold higher sensitivity and up to depths of 1-2 cm with high signal specificity than conventional Raman spectroscopy [26].

Recently, combined intravascular ultrasound (IVUS) and intravascular photoacoustic (IVPA) imaging has been demonstrated as a novel imaging modality capable of visualizing both morphology (via IVUS) and cellular/molecular composition (via IVPA) of atherosclerotic plaques. Silica-coated gold nanorods (SiO2AuNR) have been investigated as thermally stable nanosensors which, together with photoacoustic imaging, enable temperature mapping with improved sensitivity over conventional nanorods [27]. In a recent study, Yeager et al. investigated IVUS/IVPA imaging as a modality for detecting and subsequently monitoring local temperature rise during selective laser heating of SiO2AuNR using a single optical fiber for both IVPA imaging and simultaneous tissue heating via continuous wave near-infrared illumination [28]. Briefly, multimodality molecular imaging based on nanoprobe is expected to trigger a revolution in the detection and diagnosis of vulnerable plaques in the coming years.

Imaging of Thrombus

Thrombosis following plaque rupture is recognized as the underlying pathophysiology of most acute coronary syndromes and stroke. Thus, *in vivo* direct visualization of thrombus may be beneficial for the diagnosis and guidance of cardiovascular diseases therapy. Up to now, many different molecular targets, including the activated platelets, fibrinogen, fibrin, coagulation enzymes thrombin and factor XIII, have been exploited and utilized for thrombus imaging. Flacke et al. recently synthesized a novel ultra-small magnetic dual contrast iron oxide nanoprobe functionalized with single-chain antibodies directed against activated platelets for targeting to thrombosis *in vivo* T1 and T2-weighted MRI imaging [29]. Furthermore, in another study, Botnar et al. developed a novel anti-fibrin nanoprobe Gd-DTPA-PE to image the canine thrombi located in the external jugular vein on MRI imaging [30]. The signal of thrombus in dogs was markedly enhanced by this special nanoprobe with 3D T1-weighted, fat-suppressed, fast-gradient echo sequence.

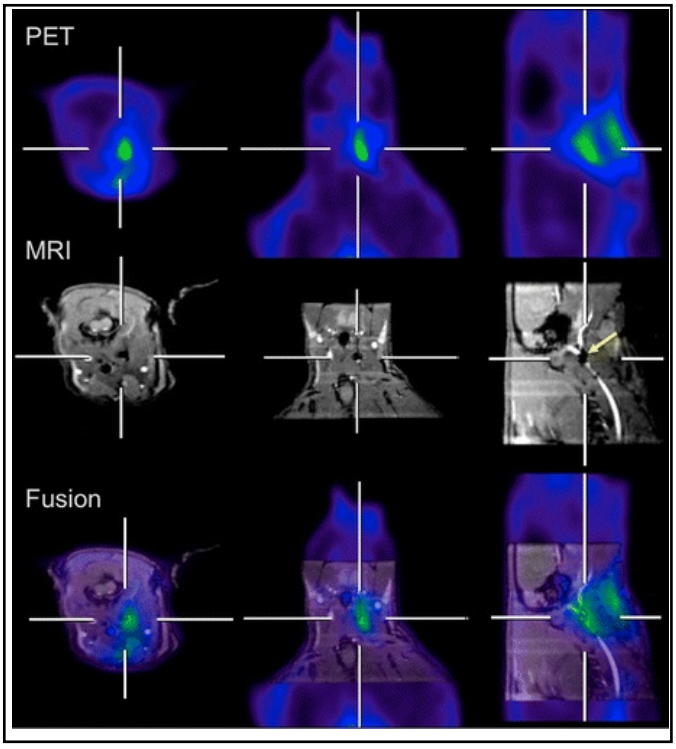

Fig. 3. Hybrid MR-PET imaging of FBP2 in a rat model of arterial thrombosis. The crosshairs show where each plane bisects the site of injury in the right carotid (RC) artery. Top: PET images; middle: Gd-DTPA-BSA enhanced MRI provides positive image contrast to the blood vessels and demonstrate an occlusion in the RC (arrow); bottom: fused MR (grayscale)–PET (color) images showing localization of PET signal to the occlusion in the RC. Additional PET activity is seen superficially at the surgical incision site, presumably due to clotting at the site of tissue injury.

Recently, Kwon et al. developed a freshly thrombin-activatable fluorescent peptide (TAP) incorporated silica-coated gold nanoparticles (TAP-SiO2@AuNPs), which had an average diameter of 39.8 ± 2.55 nm. These probes showed the quenched NIRF signal in aqueous condition, due to the excellent quenching effect of TAP molecules on the silica-gold nanoparticle surface [31]. The prepared TAP-SiO2@AuNPs could be used to directly image thrombus by dual near-infrared fluorescence (NIRF) and micro-CT imaging, wherein TAP molecules were used as targeted thrombin-activatable peptide probes for thrombin-specific NIRF imaging. Ciesienski et al. described the synthesis of three new fibrin-targeted PET probes: FBP1, FBP2 and FBP3, and then using them to image arterial thrombus in the rat models by using *in vivo* PET/MRI. Three fibrin-specific target peptides were labeled with ^{64}Cu after the conjugation with 1, 4,7, 10-tetraaza-cyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA). For MR angiography imaging, the results showed that there was an occlusion in the right carotid artery at the site of crush injury, and this vessel occlusion corresponds to the high radioactivity uptake displayed on PET images [32] (Fig. 3).

Activated platelets rapidly adhere and aggregate at the site of arterial injury, which are also a major focus for modern antithrombotic therapies. An important anti-platelet molecular target is the fibrin receptor integrin $\alpha_{2b}\beta_3$, which mainly mediates platelet aggregation and promotes thrombus propagation. Recent advance of nanotechnology provides a platform to image platelets using non-invasive modalities, such as MRI and so on [33, 34]. Von zur

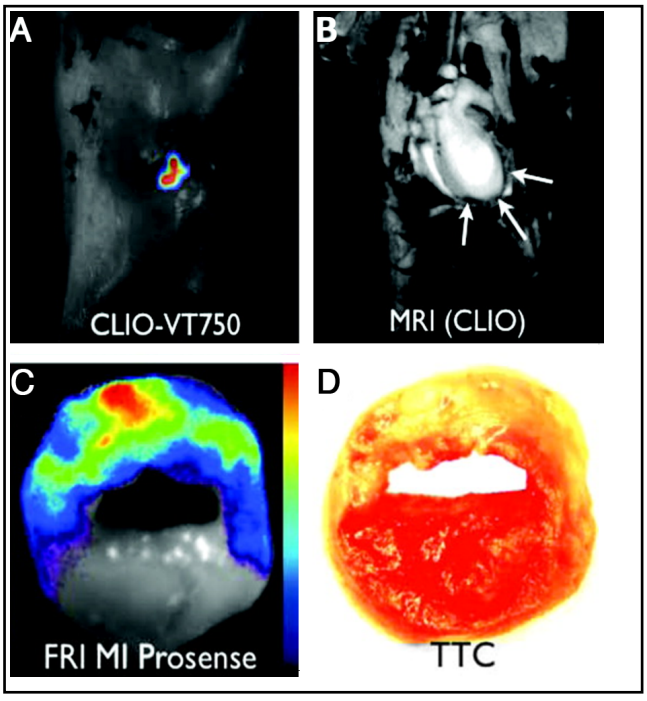

Fig. 4. FMT signal from myocardial infarction colocalizes with MRI and autopsy. (A) Coronal FMT image in the 750 nm channel acquired 4 days after induction of MI and 24 hour after injection of CLIO-VT750 demonstrates strong fluorescence in the cardiac region. (B) T2-weighted MR imaging performed directly after FMT imaging demonstrates uptake of the magneto-optical nanoprobe CLIO-VT750 into the hypokinetic infarct. The arrows indicate regions with negative signal enhancement, the hallmark of iron oxide nanoparticles in MR imaging. The hybrid magneto-optical nanoprobe enables co-registration with FMT image confirming that the fluorescence signal in FMT originates from the infarct. (C) Fluorescence reflectance image of excised heart 4 days after MI. The fusion of fluorescence image with the white light image shows prosense activation mainly in the thin infarct scar (top). Almost no signal is observed in the remote myocardium in the septum and right ventricle (bottom). The infarct can be readily identified as the unstained, pale area in the TTC stain (D).

Muhlen et al. designed a imaging agent consisting of anti-ligand-induced binding sites (anti-LIBS) single-chain antibodies conjugated to microparticles of iron oxide (MPIO), which was explored to target activated platelets using *in vivo* T2-weighted MRI [33]. However, for *in vivo* optical/near-infrared fluorescence imaging offers utility in noninvasive imaging, intravital microscopy as well as high-resolution intravascular molecular imaging catheters compatible with human coronary arteries, few fibrin-targeted molecular imaging agents are available. To this end, McCarthy et al. developed and synthesized a multimodal thrombus-targeted nanoprobe, which exhibited either covalent or noncovalent binding to thrombi [35]. And the functionalization of fluorescently labeled cross-linked iron oxide (CLIO) nanoparticles with targeting peptides for fibrin (GPRPP-CLIO and GPSPP-CLIO) or FXIIIa (CLIO-FXII) yielded thrombus-targeted agents detectable by both MRI and optical imaging modalities. The results showed that the fibrin and FXIII-targeted agents were preferentially accumulated by thrombi and they generated higher signal-to-background ratios (SBR) relative to those of their control agents, respectively.

In another study, Amy M. Wen group developed and investigated bioengineered nanoprobes for identifying thrombus formation; the design parameters of nanoparticle shape and surface chemistry, i.e. incorporation of fibrin-binding peptides CREKA and GPRPP, were investigated. Two nanoparticle platforms based on plant viruses were studied-icosahedral cowpea mosaic virus (CPMV) and elongated rod-shaped tobacco mosaic virus (TMV) [36]. These particles were loaded to carry contrast agents for dual-modality magnetic resonance (MR) and optical imaging, and both modalities demonstrated specificity of fibrin binding *in vitro* with the presence of targeting peptides [36]. The results from this study demonstrated that nanoparticle played a critical role in particle deposition at the site of vascular injury. And shaping nanotechnologies opens a new door for the development of novel targeted diagnostic and therapeutic strategies for thrombosis. In summary, the combination of diagnosis and treatment via nanoparticles platform would allow quick action in the management of the disease before, if necessary, additional follow-up personalized therapy based on the results from imaging.

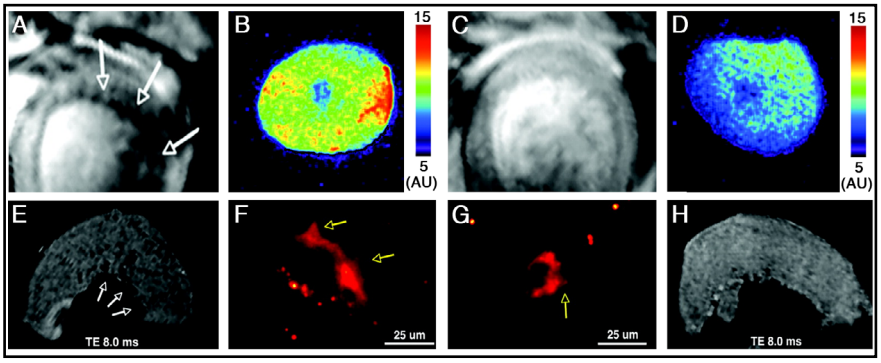

Fig. 5. Molecular MRI and FRI of cardiomyocyte apoptosis in vivo. (A) T2-weighted image in the mouse (injected with AnxCLIO-Cy5.5) at a TE of 8 ms. (B) FRI of mice injected with 3 mg Fe/kg of AnxCLIO-Cy5.5. (C) T2-weighted image in a mouse injected with CLIO-Cy5.5 at a TE of 8 ms. (D) FRI of mice injected with 3 mg Fe/kg of the control probe CLIO-Cy5.5. (E) T2-weighted ex vivo MRI in a mouse injected with AnxCLIO-Cy5.5 at TEs of 8 ms. (F&G) Fluorescence microscopy of the myocardium (magnification ×400) in a mouse injected with AnxCLIO-Cy5.5 shows the agent bound to the cell membrane of CMs in their long (F) and short (G) axes. (H) No significant evidence of probe accumulation was seen in the mice injected with CLIO-Cy5.5 by either fluorescence microscopy or ex vivo MRI at TEs of 8.0 ms.

Imaging of Myocardial Infarction and Postinfarction Remodeling

Myocardial infarction is a major cause of death and disability in the world. Molecular imaging of special receptors [37], apoptosis [38], and transplanted stem cells [39, 40] in the infarcted myocardium has been previously performed *in vivo* with the use of single imaging model based on nanoprobes. Nowadays, with the development of nanotechnology, the combination of MRI with fluorescent imaging is found to be highly promising and desirable in detection and diagnosis of myocardial infarction *in vivo*. MRI possesses high spatial resolution, excellent soft tissue contrast, and ability to characterize myocardial contraction and function, meanwhile fluorescence imaging can offer sensitive detection of fluorochromes at picomole amounts and allow imaging of several biological processes to be performed simultaneously with high throughput [41].

Recently, the magneto-fluorescent nanoprobe CLIO-Cy5.5 has been widely used in imaging tissue inflammation in atherosclerosis [42] , cardiac transplant rejection and diabetic insulitis [43], which could provide dual magnetic and fluorescence readouts of macrophage infiltration. Magneto-fluorescent nanoprobes are avidly accumulated by macrophages, which make them to be ideal agents to image myocardial macrophage infiltration in postinfarction remodeling [44]. Sosnovik et al. combined MRI imaging modality and FMT, which could also be used to image the macrophages in infarcted myocardium using CLIO-Cy5.5 magneto-fluorescent nanoprobe [44]. MRI of the infarcted mice consistently showed the presence of akinesis of the anterior, lateral and inferolateral walls of the left ventricle and left ventricular dilatation. The presence of negative contrast enhancement could be clearly seen in the hypocontractile areas of myocardium, which was same with the accumulation of the magnetic nanoparticle. The accumulation of CLIO-Cy5.5 was also found in the infarcted myocardium, which could be obviously detected using fluorescence imaging *in vivo*. And FMT imaging further proved the accumulation of the nanoprobe in the ischemia area of the infarcted mice but not in the hearts of the sham-operated mice [44].

In recent years, the growing evidence has showed that myocardial infarction triggers a local and systemic inflammatory response characterized by recruitment of macrophages and neutrophils into the ischemic myocardium, which significantly has impacts on cardiac tissue repair. Proper understanding of the macrophage response is essential to limiting injury post-MI. Yet, efficient tools to noninvasively and serially detect cellular and molecular functions in postinfarct inflammation are lacking. In a recent study, Ralph Weissleder group developed

a novel magneto-fluorescent nanoprobe (CLIO-VT75) for imaging of phagocyte recruitment in infracted heart mice [45]. The infracted heart mice were administered CLIO-VT750, and 4 days later, robust fluorescence in the heart region showed on FMT imaging (Fig. 4). In order to validate the source of the FMT signal, they subjected the same mice to MR T2 weighted imaging, which displayed the decreasing signal in the hypokinetic, apical infarct region, and indicated uptake of CLIO-VT750 by the scar (Fig. 4) [45]. The results of *in vivo* FMT findings were consistent with *ex vivo* fluorescence reflectance images from excised myocardial rings (Fig. 4). And Ni et al. generated a ^{64}Cu labeled folate-conjugated porphyrin nanoparticle suitable for multimodal non-invasive active macrophage tracking post-myocardial infarction (MI) [46]. In conclusion, multimodal molecular imaging tools based on nanotechnology may enable researchers to noninvasively image myocardial macrophage infiltration in the infracted myocardium in both the research and clinical settings.

Imaging of Angiogenesis

Angiogenesis is a complex biologic process, which represents the sprouting of new capillaries from existing microvasculature. It is a critical feature of plaque development in atherosclerosis [47] and might play a key role in many cardiovascular diseases such as myocardial infarction [48], cardiac hypertrophy and so on [49]. During the formation of new capillaries, various cell interactions produce a series of biological targets for developing angiogenesis diagnostic agents and for assessing the efficacy of antiangiogenic therapy, including vascular endothelial growth factor (EGFR) [50], vascular integrin $\alpha_v\beta_3$ [51] and so on. Accordingly, recent studies proposed and developed a series of paramagnetic nanoprobes for specifically binding to $\alpha_v\beta_3$-integrins [52] or EGFR [53] and permitting noninvasive molecular imaging of angiogenesis. For example, Winter et al. synthesized a novel αvβ3-integrin-targeted, paramagnetic nanoprobe, which could be used to detect the angiogenesis of the early-stage atherosclerosis through MRI [54]. The nanoprobe was accumulated in the fat-fed rabbit aortic wall on MRI imaging after the injection of contrast agents, indicating the presence of targeted nanoprobe bound to $\alpha_v\beta_3$-integrin epitopes on the neovasculature.

With the development of new synthetic strategies in nanotechnology, nanoparticles applied in the multimodality molecular imaging have significantly increased. It is due to the inherent advantages offered by these materials, such as a substantial loading capacity to increase diagnostic sensitivity and targeting efficiency, which contributes to understand cardiovascular diseases on the basis of a systematic level. Natriuretic peptides, as family of heart- and vessel-derived hormones, play important roles in cardiovascular homeostasis via interacting with their corresponding natriuretic peptide receptors (NPRs). It is literatured that atrial natriuretic peptide and C-type natriuretic peptide can suppress signaling of vascular endothelial growth factor (VEGF) to inhibit angiogenesis [55], which have been broadly investigated for their potential therapeutic effect [56]. To this end, Michael and his co-workers recently reported that small-molecule ^{64}Cu-labeled C-type atrial natriuretic factor (CANF) was an applicable and promising probe for PET imaging of NPR-C in atherosclerosis model [57]. Moreover, they developed and synthesized a multifunctional CANF-conjugated comblike nanoprobe, which was used to image NPR-C receptors during angiogenesis in a mouse model of hind limb ischemia with the use of micro PET/CT scan [58]. PET/CT imaging showed that an increasing accumulation of radiotracer by the lesion site of the ischemic limb was observed at 1 h and 24 h post-injection of ^{64}Cu-DOTA-CANF-comb nanoprobe. The results of immunohistochemistry *ex vitro* also confirmed the presence of NPR-C receptors [58].

MRI can provide representing anatomical information, while the sensitivity is very limited for analysis of molecular events. However, PET has high sensitivity and allows quantitative measurements, albeit with relatively lower spatial resolution. So, using hybrid imaging methods allows combination of the benefits of different imaging platforms. Recently, Su et al. explored a special dual-modality nanoparticle, which could be used to

non-invasively image angiogenesis in atherosclerotic plaque through magnetic resonance imaging (MRI) and positron emission tomography (PET) by using GEBP11 peptide targeted magnetic iron oxide nanoparticles in a rabbit model of atherosclerosis [59]. In this study, this dual-modality imaging probe was constructed by coupling 2, 3-dimercaptosuccinnic acid-coated paramagnetic nanoparticles (DMSA-MNPs) and the PET 68Ga chelator 1, 4,7-triazacyclononane-N, N', N''-triacetic acid (NOTA) to GEBP11 peptide [59]. Despite these encouraging results, there are still some limitations in this research. Further work should be underway to provide a detailed structural understanding of the interaction between GEBP11 peptide and its ligand. And whether the probe of NGD-MNPs could be used as a therapeutic intervention vector still requires further investigation.

Imaging of Apoptosis

Apoptosis is a tightly regulated, energy-requiring process in which a programmed sequence of events leads to the death of cells without releasing harmful substances into the surrounding area [60]. Apoptosis also plays a crucial role in human cardiovascular diseases, including atherosclerosis [61, 62], myocardial infarction [63], and heart failure [64]. The identification of annexin V as a high-affinity ligand to apoptotic cells presented a natural opportunity to apply molecular imaging technology in imaging apoptosis *in vivo.* Similar to their application in other aspects of cardiovascular diseases, nano-contrast agents could also provide an increasing contribution to the field of apoptosis through multimodality imaging.

In recent studies, apoptosis of vascular smooth muscle cells has been identified to be crucial in the pathological process of atherosclerosis. Several research groups have successfully imaged cell apoptosis in atherosclerotic plaques *in vivo* [65-67]. Van Tilborg et al. developed and applied a small micellar fluorescent annexin A5- functionalized nanoprobe, which carried multiple Gd-labeled lipids and fluorescent lipids for both noninvasive MRI and fluorescence imaging [68]. They found the distributed hyperintense areas throughout plaque-rich regions of the abdominal aorta on *in vivo* MRI images at 24 h after the administration of annexin A5-functionalized micelles, which was further confirmed by *ex vivo* near-infrared fluorescence images of excised whole aortas. Anthony and co-workers synthesized a novel annexin-labeled nanoprobe (AnxCLIO-Cy5.5) to quantitatively and specifically image the cardiomyocyte apoptosis in mouse heart failure models [69]. Their findings demonstrated that AnxCLIO-Cy5.5 can noninvasively image cardiomyocyte apoptosis in mouse heart failure on molecular T2-weighted MRI. *Ex vivo* fluorescence microscope and MRI analyses of the excised hearts were further in agreement with *in vivo* findings (Fig. 5). Moreover, Chen et al. designed and produced another AnxCLIO-Cy5.5 nanotracer which was consisted of annexin V conjugated to the magneto-fluorescent nanoprobe CLIO-Cy5.5 [70]. AnxCLIO-Cy5.5 could protect apoptotic cells by stabilizing their cell membranes, and this nanoprobe had the potential to become a theranostic agent for cell apoptosis, which could be capable of both identifying and salvaging early apoptotic cells in heart diseases, such as ischemia-reperfusion, heart failure and so on [70]. More recently, Sosnovik et al. simultaneously applied AnxCLIO-Cy5.5 and a fluorescently labeled small gadolinium chelate (gadolinium-DTPA-NBD) to detect the cell apoptosis and necrosis of infracted mouse heart [71]. They observed the presence of AnxCLIO-Cy5.5 in the midmyocardium and absence of AnxCLIO-Cy5.5 in segments of myocardium with normal contraction on *in vivo* T2-weighted MRI. In addition, the research results also showed that delayed-enhancement of Gd-DTPA-NBD was detected in areas of severe myocardial infarction, particularly in the subendocardium. The extent of delayed-enhancement was usually mild at the midventricular level, progressively strong in the apex of heart. Immunohistochemistry for Gd-DTPA-NBD further confirmed the *in vivo* delayed-enhancement findings [71]. However, further studies will be needed to determine the mechanisms underlying this spatial pattern of apoptosis, and whether it is replicated in larger animals and humans.

In vivo Tracking in Cardiac Stem Cell-Based Therapy

The therapy based on stem cells has heralded a promising and novel therapeutic strategy for recovery of the damaged myocardium [72-74]. To monitor and evaluate the survival proliferation and migration of stem cells after *in vivo* administration, molecular imaging techniques have been widely proposed and applied for basic research, preclinical and clinical studies [75, 76]. However, untill now, the current imaging modalities are unable to meet all requirements at the same time. The development of nanotechnology offers the new opportunity for labeling, tracking and monitoring stem cells *in vivo* [75, 77, 78] . For example, Chapon et al. monitored the iron-oxide nanoparticle-labelled adult rat bone marrow-derived stem cells by MRI and judged their effect in host cardiac tissue using 2-deoxy-2-[F-18] fluoro-D-glucose-PET (FDG-PET). In their study, with the help of combination of MRI and PET examinations, a protocol for labeling, subsequent longitudinal imaging of transplanted stem cells and assessing their functional efficacy *in vivo* was reported and studied [79]. Shi *et al.* developed bi-functional anionic Eu^{3+}-doped Gd_2O_3 hybrid nanoparticles as a novel dual-modal contrast agent for both luminescent and MRI T1-weighted imaging, which could also be used for imaging stem cell [80].

In addition to PET and MRI, ultrasound-guided photoacoustic (US/PA) imaging technology may have great potential for *in vivo* continuously monitoring of stem cells behavior and neoangiogenesis promoted by stem cells [81, 82]. Recently, Nam et al. successfully monitored mesenchymal stem cells (MSCs) labeled with gold nanotracers (Au NTs) based on US/PA imaging [83]. The results demonstrated that due to noninvasive, quantitative, sensitive and continuous assessment of stem cell behaviors with high spatial and temporal resolutions at sufficient depths, Au NTs labeling of MSCs and US/PA imaging modality could be a greatly promising method in stem cell imaging. And Zhang group recently also introduced a new class of contrast agents based on gold nanocages (AuNCs) with hollow interiors and porous walls to label human mesenchymal stem cells (hMSCs) for both *in vitro* and *in vivo* tracking using two-photon microscopy and photoacoustic microscopy [84]. Moreover, L.M. Ricles group developed a dual gold nanoparticle system which was capable of monitoring both delivered stem cells and infiltrating macrophages using *in vivo* ultrasound and photoacoustic imaging [85]. In sum, these works have important implications for cell tracking and monitoring cell-based therapies in cardiovascular diseases fields. However, current techniques based on nanoparticle-labeling, such as fluorescence microscopy, magnetic resonance imaging, and micro-computed tomography, are plagued by limitations including relatively low sensitivity or penetration depth, involvement of ionizing irradiation, and potential cytotoxicity of the nanoparticles.

Conclusion

In conclusion, the application of nanotechnology triggers a revolution in the diagnosis and management of cardiovascular diseases. Advances in the design and synthesis of nanoparticles will offer the ability to produce novel multimodality imaging probes, which could target and reflect more pathophysiological details in cardiovascular diseases, including atherosclerosis, thrombosis, myocardial infarction and stem cell biology. In near future, it is expected that molecular imaging probes based on nanotechnology will ultimately translated to the diagnosis and treatment of human cardiovascular disease in clinic.

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Disclosure Statement

The authors declare no conflict of interests.

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