Strategies for visualizing inflammation

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Abstract
As a basic innate immune response to the disordered tissue homeostasis, inflammation is related to the pathogenesis of multiple diseases, including bacterial infections, atherosclerosis, neurodegenerative diseases, and cancers. It is also a pivotal feature of some metabolic disorders such as diabetes and obesity. The visualization of in vivo inflammations can help us to comprehend the pathogenesis of these diseases and develop new solutions to diagnose them. Over the past few decades, a variety of strategies (eg, computed tomography, magnetic resonance imaging [MRI], and ultrasound [US] imaging) have been utilized for visualizing inflammations by imaging the structural changes of inflammatory tissues. Moreover, many recent studies have focused on some probes that can target or localize the inflammatory sites by specific binding to inflammation-related molecules, being internalized by inflammatory cells, or becoming detectable only under inflammatory conditions. These probes can also be applied to visualize inflammations by MRI, positron emission tomography, single-photon emission computed tomography, photoacoustic imaging, optical imaging (eg, fluorescence imaging, bioluminescence imaging, and chemiluminescence imaging), contrast-enhanced US imaging, and the combined use of the abovementioned methods. This review not only summarizes the existing strategies for visualizing inflammations, but also discusses the limitations of the present strategies and the future directions on the development of new strategies for realizing the in vivo inflammation visualization.

KEYWORDS
imaging strategies, immune cells, inflammation imaging, inflammation targeting, inflammatory diseases

1 | INTRODUCTION

Inflammation is a basic immune response of body to the stimuli including allergens, pathogens, damaged cells, and other biological, chemical, or physical factors. These stimulations trigger body’s inflammatory processes, which begin with the release of pro-inflammatory cytokines (eg, interleukin-1 [IL-1], IL-6, and tumor necrosis factor [TNF]) and chemokines (eg, IL-8). Cytokines activate endothelial cells, which causes the increase of vascular permeability and as a result, facilitates immune cells to infiltrate into tissues at the site of inflammation. On the
other hand, chemokines recruit mast cells and leukocytes (eg, neutrophils, monocytes, and eosinophils) to the site of damage.\(^3\) The cytokines in inflammatory microenvironment also mediate the activation of leukocytes and then activated leukocytes phagocytose and eliminate the irritants or causative agents.\(^3\)\(^4\) In the end, the normal inflammation will be resolved with the apoptosis of inflammatory cells, and after that, the tissue will return to homeostasis.\(^3\) The mechanisms that terminate inflammation are related to the release of some anti-inflammatory cytokines.\(^3\) As a part of innate immunity, inflammation plays an important role in host defense, and it also participates in the restoration and regeneration of injured tissues.\(^3\) However, long-standing and nonresolving inflammation is closely associated with many diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), atherosclerosis, asthma, chronic obstructive pulmonary disease, neurodegenerative disease (eg, Alzheimer’s disease and Parkinson’s disease), and cancer.\(^4\)\(^6\) It is also a pivotal feature of some metabolic disorders, including diabetes and obesity.\(^7\)

Therefore, to comprehend the pathogenesis of the abovementioned diseases, reliable and effective strategies for visualizing inflammations are indispensable. They are also useful tools for the diagnosis of these diseases, the discovery and development of new drugs, and the assessment of the therapeutic effects. Over the past few decades, a variety of techniques have been utilized for visualizing inflammations by imaging the anatomical changes of inflammatory tissues, including computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) imaging.\(^8\) Moreover, the development of specific contrast agents enables more reliable and effective imaging. These agents can act as inflammation-targeted probes mainly by specific binding to inflammatory molecules, accumulating at the site of inflammation by virtue of the cellular processes of immune cells, or becoming detectable only after being activated in the inflammatory foci.\(^9\) Thus, the visualization of inflammations can be achieved by using corresponding molecular imaging methods, for instance, MRI, positron emission tomography (PET), single photon emission computed tomography (SPECT), contrast-enhanced US imaging, photoacoustic imaging (PAI), and optical imaging (eg, fluorescence imaging [FLI], bioluminescence imaging [BLI], and chemiluminescence imaging [CLI]).\(^10\) Each imaging modality has its own unique feature and its suitable application situations. In other words, using single imaging modality might be unsatisfactory. Consequently, the hybridization of different imaging modalities is becoming a potential direction, which allows both high-sensitivity molecular and anatomical imaging at the same time.

In this review, we summarize the recent progress in visualizing inflammations via various strategies and techniques, list the applications and limitations of them, and discuss the potential future development directions for realizing better visualization.

2 | STRATEGIES FOR VISUALIZING INFLAMMATIONS

The strategies that can visualize inflammations consist of two aspects: the strategies for localizing inflammations and the imaging techniques for acquiring visible images (Figure 1). Thus, diverse targets from immune cells to enzymes involved in the inflammatory processes have been explored to realize more accurate localization. Besides, various imaging modalities are used to provide structural or functional information, and achieve more effective imaging of inflammations. In this section, we make an overview of the existing strategies for visualizing inflammations in terms of which imaging modalities were used.

2.1 | Optical imaging

2.1.1 | Fluorescence imaging

FLI uses an external light at a specific excitation wavelength to excite fluorophores, followed by the detection of the fluorescence emission from them through a sensitive
imaging system. Exploiting biocompatible probes that can preferentially accumulate at the intended target site and offer a high contrast is the emphasis of relevant studies. To reduce the influence of the tissue autofluorescence and the absorption and scattering of photons in living organisms, fluorescent agents with long emission wavelengths at the near-infrared (NIR) region are usually favored. The fluorescent probes applied to visualize inflammations can be classified by targeting strategies into three classes: active targeting-based probes, activatable probes, and immunocyte-mediated targeting-based probes.

**Active targeting-based probes**

For the selective imaging of inflammations, the most straightforward approach is to label agents that can selectively bind to relevant molecules of inflammation with fluorescent dyes, and the resultant conjugates are referred to as active targeting probes. For instance, it is well established that cyclooxygenase-2 (COX-2) is overexpressed in inflamed tissues and can be used as a molecular target. Therefore, Uddin et al developed a range of fluorescent agents by linking COX-2 inhibitors and diverse fluorescent dyes to realize the selective visualization of inflammations. Licha et al synthesized a polymeric probe by loading an NIR fluorescent dye indocyanine green (ICG) in a dendritic polyglycerol sulfate, which can target L-selectin, an inflammatory factor. The probe achieved acceptable imaging performance in a rat model of RA, enabling the distinguishing of inflamed joints. Similarly, Wu et al modified ICG-liposomes by iRGD (immobilized arginine-glycine-aspartate) peptide to monitor synovitis in mouse models of inflammatory arthritis through targeting integrin ανβ3, which is related to the angiogenesis at the inflammation site.

**Activatable probes**

Active targeting depends on both the specific and stable binding of the agent at the target and the rapid clearance from nontarget tissues and blood circulation. The former requires a long residence time to enhance the agent enrichment in target tissues, whereas the latter needs a short circulation time, causing a contradictory. Thereby, inevitable background fluorescence originated from unbound imaging agents can have a serious impact on the imaging performance. As a result, exploiting activatable fluorescent nanoprobes that can respond to the inflammation microenvironment, in other words, only become detectable after being activated in the inflammation region, is becoming a popular strategy for specific inflammation imaging.

Generated by the diffusion-controlled reaction of nitric oxide (•NO) and superoxide (O2•) radicals, peroxynitrite (ONOO−), a kind of short-lived reactive oxygen species (ROS), which play a crucial role in the inflammatory processes by interacting with diverse inflammatory factors (eg, IL and inducible nitric oxide synthase [iNOS]), has been well established as an inflammation biomarker. Many peroxynitrite-responsive nanoprobes have been reported. Song et al developed an aggregation-induced emission (AIE) nanoprobe that was responsible for the elevation of the ONOO− level for selective visualization of inflammation in vivo. The nanoprobe, named TPE-IPB-PEG, was composed of an imine-functionalized tetraphenylethene (TPE), a phenylboronate, and a lipid-polyethylene glycol (lipid-PFG) matrix. TPE-IPB (N-(2-(((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)benzylidene)-4-(1,2,2-triphenylvinyl)aniline) was nonfluorescent until encountering with ONOO− at pH 7.4 and it was then converted into TPE-IPH (2-(((4-(1,2,2-triphenylvinyl)phenyl)imino)methyl)phenol), which could form aggregates with yellow fluorescence. With a high detection limit, the nanoprobe can only be activated by the elevated production of ONOO− at the inflammation site but it cannot be activated by the normal generation of ONOO− in other tissues, showing a selective inflammation imaging capacity. Wu et al designed a xanthene fluorescent probe with NIR emission. In this work, the fluorescence of the probe is quenched, and via a nucleophilic addition-elimination pathway, diphenyl phosphinate, the fluorescence quencher of this probe, can be removed by peroxynitrite, hence realizing the imaging of inflammation in response to peroxynitrite.

Besides the high level of peroxynitrite, other inflammatory conditions can also be utilized as a “switch” of fluorescent probes, including elevated levels of enzyme activity (eg, leukotriene A4 hydrolase [LTA4H] and myeloperoxidase [MPO]), other kinds of ROS (eg, hypochlorous acid [HClO] and hydrogen peroxide [H2O2]), and acidic pH.

Moreover, there are other ways to design activatable fluorescent nanoprobes. The probes we mentioned above have no fluorescence or the fluorescence is quenched until interacting with the biological target, and these probes can be referred to as intensity-based systems. Another approach is to construct ratiometric fluorescent systems, that is, the fluorescent probes have innate fluorescence, and when the probes interact with the target, their fluorescence spectra are changed. Thus, by calculating the ratio of the emission intensities of the probes at two different wavelengths, the activated and inactive probes can be differentiated. The ratiometric fluorescent probes can reduce the influence of off-target accumulation by offering a self-calibrated reference signal, enabling the more reliable visualization and quantification of inflammation. Wang et al synthesized a fluorescent probe with a
HClO-responsive emission wavelength of 1550 nm, allowing sensitive and high-resolution FLI of the lymphatic inflammation in vivo.\textsuperscript{21b} The probe incorporated a Er\textsuperscript{3+} -doped down-conversion nanoparticle with multi-wavelength excitation (808 and 980 nm), which was decorated by cyanine7.5 (Cy7.5) fluorophores that could be degraded by HClO for absorption competition on 808-nm excitation energy. By using the emission under 980-nm excitation as a reference, the ratiometric response for HClO could be achieved (Figure 2A). In another example,
Cheng and co-workers designed a novel two-photon ratiometric fluorescent probe (MITO-CC) by building up a FRET system with an energy acceptor that can be quenched after reacting with peroxynitrite.22 MITO-CC used coumarin as the energy donor because of its stability to diverse reactive species and satisfactory optical properties. The energy acceptor was selected from 19 fluorescent dyes for its specific and rapid fluorescence quenching in response to ONOO−. Thus, MITO-CC had a spectral response to ONOO−, enabling the fast and sensitive detection of ONOO− in inflamed tissues. Moreover, Viger et al developed a dually responsive nanoprobe with two emission states.21d In this study, a dextran-based polymer that was responsive to the acidic pH and the increased concentration of H2O2 was utilized to control the release of NIR cyanine dyes (Figure 2B). Once released, these dyes would recover their fluorescence from the low-intensity state due to the aggregation-induced self-quenching, and the spectral profile would be changed as well (Figure 2C). By using a spectrally resolved strategy to isolate the activated signals exclusively in the inflammatory foci, the nanoprobe enabled the rapid in vivo inflammation detection with extremely high target-to-background ratios, which was demonstrated in the inflamed mouse models.

**Immunocyte-mediated targeting-based probes**

During the inflammatory process, immune cells, especially monocytes, neutrophils, and macrophages, are massively recruited to the inflammation tissue. Therefore, immunocyte-mediated targeting by virtue of immune cells or components of immune cells is another emerging approach. For instance, Aizik et al designed a liposomal quantum dot (QD) delivery system, and it was verified that the QD-laden liposomes could passively target the inflammation region through the endocytosis of circulating monocytes.23 The encapsulation of QDs not only realized a selective biodistribution, but also elevated the quantum yield (QY) and prolonged the fluorescence decay lifetime under the quenching conditions of the blood and lysosomes, and most importantly, improved the biocompatibility. Similarly, Yan et al prepared liposomes carrying Cd-free NIR QDs (LipQDs) with no detectable toxicity, which were further decorated with isolated macrophage membranes.24 The resulting LipQDs@M were demonstrated to be competent for the in vivo visualization of postoperative tumor inflamed tissue (Figure 3A).

Moreover, recently, Liu et al used neutrophils as “living” carriers loaded with NIR-II AIE dots to penetrate the blood-brain barrier and accumulate in the brain inflamed region for noninvasive visualization of the deep-seated inflammation (Figures 3B and 3C).10f The AIE dots, self-assembled from lipid-PEG2000 and AIE luminogen that was transformed from an existing photothermal contrast agent through constitutional isomerization, had a high QY and NIR-II emission, which enabled the high-resolution FLI in vivo. Then, the AIE dots were modified by cell-penetrating peptides to facilitate the internalization by neutrophils. After that, the AIE dots were incubated with neutrophils isolated from the murine bone marrow of mice and were then injected into the blood stream, achieving more efficient delivery. In addition, Wu et al presented a creative strategy for visualizing inflammation in atherosclerosis by molecular engineering of inflammation-tropistic M2 macrophage-derived exosomes (M2 Exo).25 According to a previous study, secreted by M2 macrophages, M2 Exo have an innate inflammation-tropism capability due to the existence of multiple chemokine receptors on their surface.26 The authors encapsulated hexyl 5-aminolevulinate hydrochloride (HAL), a precursor of the heme synthesis, into M2 Exo through electroporation. The resultant product (termed HAL@M2 Exo) could accumulate in the inflammation area by the aid of the chemokine receptors present on the HAL@M2 Exo’s surface, and once HAL@M2 Exo were phagocytosed by immune cells, the HAL could be released into the cytoplasm to participate in the intrinsic biosynthesis of heme. Therefore, with red fluorescence emission, protoporphyrin IX, the intermediate of the heme biosynthesis pathway, could be utilized for the FLI of inflammation (Figure 4).

### 2.1.2 Bioluminescence imaging/

**Chemiluminescence imaging**

Bioluminescence is the biological process that generates visible light emission in living organisms.27 As an efficient molecular imaging method, BLI can be applied in the in vivo researches of physiological processes, including inflammation. With the feature of noninvasiveness and low cost, BLI represents a novel approach to visualize inflammatory processes in real time.27

The most well-known bioluminescence process is the oxidation of luciferin catalyzed by firefly luciferase, with the light emission peak at ~562 nm.28 Through genetically engineering animal models to express luciferase reporter gene, the real-time analysis of many inflammatory processes at the molecular level can be fulfilled.29 For example, nuclear factor kappa B (NF-κB) is a transcription factor that controls diverse genes related to inflammation. The inserted luciferase gene is under the control of NF-κB promoter, and thus the expression of NF-κB xB can be represented by the expression of luciferase, which can be reflected by the detectable bioluminescence after the injection of luciferin substrate (Figure 5A).29 In addition to NF-κB, the light-producing transgenic reporter models
of other inflammatory factors, such as IL-1β, interferon-β (IFN-β), and iNOS, have been established as well.30 Furthermore, constructing and transplanting luciferase-expressing immune cells in animal models is also a possible way.31 The luciferase reporter gene technique has been successfully applied to visualize osteoarthritis, inflammatory bowel disease, lung inflammation, and many other inflammatory diseases.29,31,32 However, using transgenic technology usually leads to biosafety concerns, which may thereby limit the applications of this method.

As a result, increasing studies have focused on another bioluminescence process to visualize inflammations by detecting the activity of MPO. MPO is a proinflammatory enzyme that is abundantly expressed in neutrophils, and can also be found in the lysosomes of other leukocytes such as monocytes and macrophages.33 During the inflammatory process, MPO can be secreted by activated neutrophils and macrophages, and is involved in the catalytic production of reactive species (eg, hypochlorous acid, hydroxyl radicals, and tyrosyl radicals) for host defense.33 Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione) is a small-molecule luminescent probe that has been proved to be specific to MPO activity in vivo.34 Thus, the bioluminescence generated by luminol can be used for the...
FIGURE 4 Scheme illustrating (A) the preparation of HAL@M2 Exo, (B) the inflammation-tropism and anti-inflammation effects of HAL@M2 Exo, and (C) the simplified HAL-initiated biosynthesis and metabolism pathway of heme. (A–C) Reprinted with permission.25 Copyright 2019, Wiley-VCH

localization, visualization, and evaluation of inflammation. For example, Gutowski et al established three models of uveitis (a kind of intraocular inflammation) with different causes.35 The luminol-based bioluminescence imaging was performed on each model. Although precise quantitative measurement was not available in this study, significant changes of bioluminescence in different models were detected during the whole inflammatory process. Additionally, stronger signals were observed in acute models rather than chronic models. A similar study conducted by Hofmann et al monitored the inflammatory processes in real time by injecting luminol molecules in three mouse models with diverse dermal inflammations induced by corresponding drugs.36 The results showed that luminol-based BLI achieved satisfactory performance in all the three models. Furthermore, the bioluminescence intensity was responsive to anti-inflammatory intervention, confirming the reliability of this method.

Gao et al developed a biodegradable MPO-responsive luminescent nanoprobe achieving more notable and sustainable bioluminescence signals compared to free luminol in the inflammatory foci (Figure 5B).37 In this case, β-cyclodextrin was activated by 1,10-carbonyldiimidazole, and then conjugated with luminol, forming an amphiphilic unit. Then, the nanoprobe could be obtained via self-assembly of the amphiphile. The authors regarded the enhanced bioluminescence intensity and efficiency as a result of the increased external presentation of luminol on the surface of the nanoparticles, which may promote the bioluminescence process by increasing the probability of molecular collision.

Although the use of luminol has achieved comparatively ideal imaging effect in superficial inflammation models, the performance of luminol-based methods is unsatisfactory in deep tissue inflammation models because of the low tissue penetration of the short-wavelength blue light ($\lambda_{\text{max}} = 425$ nm) emission of luminol. Hence, many researches have tried to red shift the luminol-emitted light to the NIR light with deeper tissue penetration resulting from the reduction of absorption and scattering.10g,38 As early as 2013, Zhang et al proposed that by mixing luminol and Cd-containing NIR QDs together, the blue light emitted by luminol was able to be red shifted by NIR QDs through bioluminescence resonance energy transfer (BRET), thereby realizing the visualization of inflammation in a pulmonary inflammation murine model, as well as in a metastatic tumor model with tumor metastases of diverse depths, demonstrating the feasibility of using this approach to detect and assess deep-seated inflammations in vivo.38a However, the long-term biosafety of the metal-containing QDs has been seriously concerned. Consequently, using materials with
better biocompatibility to red shift luminol-emitted luminescence is greatly desirable. Liu and co-workers developed a nanobubble to accomplish this goal through the integration of BRET and fluorescence resonance energy transfer (FRET). Two lipophilic dyes, DiI and DiD, which have been widely applied in cell membrane staining, were selected to construct the BRET–FRET system (Figure 5C). Being doped in the lipid layers of the nanobubbles, the two dyes acted as an energy transfer relay. The blue luminescence emitted by luminol could excite DiI through the BRET process, followed by the FRET process between DiI and DiD, eventually resulting in red-shifted emission ($\lambda_{\text{max}} = 670$ nm). Compared to free luminol, the nanobubbles led to a 24-fold signal intensity increase in the mouse models of inflammation induced by lipopolysaccharide (LPS), with no observable toxicity. In contrast to the work mentioned above, in the study of Xu et al., luminol was conjugated with a fluorescent acceptor chlorin e6 (Ce6), which achieved a higher efficiency of BRET because of the reduced distance between the donor (ie, luminol) and the acceptor. The conjugate formed by luminol and Ce6 was modified by PEG, forming an amphiphilic polymer. Through the self-assembly of the polymers, a self-illuminating nanoparticle could be synthesized for the sensitive imaging of inflammation. In vivo studies in mice showed that the sensitivity of BLI for acute peritonitis, acute liver injury, and ulcerative colitis was noticeably increased by using the nanoparticles instead of free luminol.

Chemiluminescence (CL) can be simply defined as the chemical reactions that emit light. Actually, bioluminescence is just a special type of CL that occurs in living
organisms. Unlike the aforementioned bioluminescence processes, the catalysis of enzymes is not necessary in CL processes, and some nonenzymatic CL reactions can be used for in vivo inflammation visualization as well. L012 ((8-amino-5-chloro-7-phenylpyrido[3,4-\(d\)]pyridazine-1,4(2H,3H) dione)), a luminol derivative, is an alternative CL agent for visualizing inflammation due to its high response sensitivity to ROS. For example, Lee et al constructed H\(_2\)O\(_2\)-responsive hybrid nanoparticles by linking L012 and PEG to the surface of QDs through the amide bond. Based on the chemiluminescence resonance energy transfer (CRET) between L012–H\(_2\)O\(_2\) and PEGylated QDs, the nanoparticles could change the wavelength of the CL emission generated by the reaction between L012 and H\(_2\)O\(_2\) to the NIR region, facilitating the in vivo visualization of inflammation and other diseases involving the overproduction of H\(_2\)O\(_2\) with enhanced tissue penetration. Besides QDs, polymer dots can also act as CRET energy acceptors. Cai et al prepared catalytic polymer dots with ultrasensitive response to ROS in the presence of L012. The fluorescent conjugated polymers in the polymer dots served as a CRET acceptor of the ROS-responsive CL from L012, whereas hemin in the polymer dots behaved as a catalyst of the CL reaction between L012 and ROS. Further, the in vitro studies and in vivo experiments (on a mouse model of LPS-induced inflammation) demonstrated that the polymer dots possessed enhanced emission intensity and prolonged emission duration in response to ROS.

Moreover, the peroxalate chemiluminescence (POCL) system has been widely employed for in vivo inflammation imaging through visualizing the H\(_2\)O\(_2\) levels. Such systems have peroxalate fuels, which can be selectively oxidized by H\(_2\)O\(_2\), and the resulting 1,2-dioxetanediol intermediate can further ignite the nearby emitters to generate luminescence. The luminescent emitters generally possess NIR emission with deep tissue penetration. The most commonly used emitters are NIR fluorescent dyes. Other emitters have also been reported, such as AIE dots and carbon nanodots (CDs). For instance, Shen et al bridged NIR emissive CDs and CPPO (bis(2,4,5-trichloro-6-carbopentoxynaphenyl) oxalate) in the presence of an amphiphilic triblock copolymer PEG-b-PPG-b-PEG (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)), forming activatable H\(_2\)O\(_2\) probes with a low detection limit and a broad linear range. Specifically, citric acid and urea were dissolved in N,N-diethylformamide for preparing the CDs by the solvothermal method. The CDs were further modified to be hydrophobic by octadecylamine, and assembled with PEG-b-PPG-b-PEG and CPPO to form complexes termed P-CDs, which were demonstrated to be promising sensors for inflammation imaging in a mouse model of peritonitis. Without the indispensable external excitation in FLI, BLI and CLI can largely avoid the noise from the light source and the autofluorescence interference from the tissue. Moreover, the luminescence can only be detected after the activation of specific targets. Therefore, BLI and CLI possess a high signal-to-noise ratio, and might be more competent approaches for inflammation visualization.

### 2.2 Nuclear medicine imaging

Nuclear medicine imaging refers to imaging techniques depending on the detection of gamma rays, mainly including PET and SPECT. Both of them involve the administration of radiopharmaceuticals that integrate radionuclides and biologically active molecules related to specific biological processes, thereby allowing the visualization and evaluation of these processes in vivo. PET is different from SPECT in that it administers positron-emitting radionuclides (eg, carbon-11, nitrogen-13, and fluorine-18), and once the positrons interact with electrons, two collinear gamma rays in opposite directions will be produced. In contrast, SPECT uses radionuclides (eg, technetium-99 m, indium-111, and iodine-123) that directly emit high-energy photons (gamma rays), which have a lower level of emitted energy and longer half-lives. The gamma rays are detected by a rotating gamma camera, and can then be used for tomographic image reconstruction. With the sensitivity up to nanomolar level for SPECT and picomolar level for PET, PET and SPECT are the most sensitive in vivo imaging modalities, and serve as powerful tools for the visualization of functional information, which is valuable for the early diagnosis and assessment of diverse diseases. However, the spatial resolutions of PET and SPECT are relatively low, and they perform poorly in the acquirement of anatomical information. Therefore, PET and SPECT are always combined with X-ray CT in a single PET/CT or SPECT/CT imaging system, enabling the acquirement of both functional information and co-localized anatomical information in a single examination. In addition, the structural information obtained from CT can be further used to improve the accuracy of functional images through scatter and attenuation corrections.

Nuclear medicine imaging has found widespread applications in the visualization and evaluation of various inflammatory processes, including sarcoidosis, vasculitis, myocardial infarction (MI), inflammatory bowel disease, neurodegenerative diseases, and chronic autoimmune diseases. Several techniques based on radiopharmaceuticals, such as gallium-67 (\(^{67}\)Ga) citrate imaging, technetium-99 m (\(^{99}\)mTc) methylene diphosphonate imaging, \(^{99}\)mTc hexylmethylpropylene amine oxime or
Inflammation imaging with Positron Emission Tomography (PET) using 18F-FDG has been widely used in clinics. 18F-FDG PET is based on the uptake of 18F-fluorodeoxyglucose (FDG) in cells, which is then phosphorylated by hexokinase to 18F-FDG-6-phosphate, resulting in intracellular accumulation. The cellular uptake level of 18F-FDG is associated with the number of glucose transporters and the cellular metabolic rate, which are both upregulated in activated inflammatory cells, indicating that 18F-FDG PET is an attractive way to assess inflammation. Moreover, it has been found that glucose transporters have an increased affinity for deoxyglucose, owing to the existence of growth factors and cytokines in the inflammatory regions. However, the specificity of 18F-FDG is unsatisfied, because other cells, such as myocytes, may also have a high degree of glucose metabolism. In addition, it is difficult to differentiate inflammation from tumors because the glucose metabolism of malignant cells in tumor tissue is elevated as well. In fact, 18F-FDG PET is also used for detecting tumors. Hence, for more specific detection and assessment of inflammation, various imaging tracers that target diverse biomarkers have been developed.

During an inflammatory process, the cell adhesion molecules that mediate the migration of immune cells are overexpressed on endothelial cells and lymphocytes resulting from the production of cytokines by activated immune cells, and thus they have become promising targets for the selective imaging of inflammation. For example, Bala et al developed a new PET agent for visualizing plaque inflammation in atherosclerosis by radiolabeling the nanobody against vascular cell adhesion molecule-1 (VCAM-1) with 18F, referred to as 18F-FB-cAbVCAM-1. The authors used ApoE-deficient (ApoE–/–) mice as a murine atherosclerotic model. The results showed that the accumulation of the tracer was remarkably higher in the ApoE–/– mice compared to that in the control mice, demonstrating its potential for the noninvasive PET imaging of atherosclerotic plaque inflammation.

The surface markers on immune cells (e.g., macrophage mannose receptor [MMR], somatostatin receptor [SSTR], cluster of differentiation 4 [CD4], and chemokine receptors) are attractive targets as well, and there have been numerous relevant studies for imaging different inflammatory processes, including but not limited to atherosclerosis, RA, and IBD. For example, Put et al utilized 99mTc-labeled nanobodies specific to MMR for visualizing and quantifying joint inflammation in a mouse model of RA induced by collagen. The in vivo SPECT/CT images were obtained from both asymptomatic (Figure 6A) and symptomatic (Figure 6B) mice after intravenous injection of anti-MMR or control nanobodies (BCII10). The remarkable signals were seen in the arthritic paws of the symptomatic mice injected with the 99mTc-labeled antibody of MMR, rather than in the paws of the asymptomatic mice or the symptomatic mice injected with control nanobodies, demonstrating the specificity of the MMR signal in inflamed joints. To be noted, the MMR signals were not only found in the arthritic paws of symptomatic mice, but also in the paws without arthritic
Magnetic resonance imaging

Asymptomatic symptoms, which may suggest the potential of detecting early pathophysiological changes before the onset of apparent clinical symptoms by this strategy.

Additionally, inflammation-related cytokines and enzymes, such as TNF-α, COX-2, and MPO, have also been exploited as molecular targets for PET and SPECT. Moreover, besides the antibodies that can serve as the targeting components, other molecules with high affinity for the corresponding targets can also be used. For instance, Uddin et al constructed new SPECT tracers for inflammation by converting celecoxib into its isomeric iodo-123I analogues, which are the selective inhibitors of COX-2. A higher enrichment of the tracer was detected in the inflamed rat paws than in the contralateral normal paws. Likewise, Albadawi and co-workers confirmed that radiolabeled MPO sensor (111In)-bis-5-HT-DTPA (indium 111-bis-5-hydroxytryptamide-tetraazacyclododecanediethyleneletriaminepentaaacetic acid) could be used for the SPECT/CT imaging of early inflammation in the spinal cord after thoracic aortic ischemia/reperfusion in a mouse model, and may thus have the clinical translation potential to identify patients with the risk of developing neurologic deficit after thoracic aortic reconstruction interventions and enable preventive therapies. However, limited by its poor capability of crossing the blood-brain barrier, bis-5-HT-DTPA cannot be applied to image brain inflammation. Wang et al developed a novel MPO-activatable PET radioprobe (18F-MAPP) that could cross the intact blood-brain barrier. Through being oxidized by MPO, 18F-MAPP could bind to proteins, realizing accumulation at the site of inflammation. With high detection specificity to MPO activity, 18F-MAPP has shown promise for the early detection of inflammation and the monitoring of inflammatory diseases.

2.3 Magnetic resonance imaging

MRI creates images through detecting the signals generated by the relaxation of the deflected spinning nuclei in high-intensity magnetic fields. Thus, in addition to the magnetic field generator, an MRI instrument has the following two components: an electromagnetic wave transmitter and receiver to deflect nuclei and receive signals, respectively. Unlike nuclear medicine methods and CT, the radiation utilized by MRI is in the radiofrequency range that is harmless to human body, which represents a major advantage over other imaging modalities in clinical use. Generally, MRI obtains the structural information of target tissues. Therefore, MRI is widely used for diagnosing and assessing diseases related to anatomical deviations, involving inflammation. Furthermore, the occurrence of molecular contrast agents has made molecular MRI available, which offers functional information for more accurate inflammation visualization. By changing the relaxation rate of the susceptible nuclei in local tissues, contrast agents can reduce the imaging time and enhance the clarity and contrast of images. Gadolinium (Gd)-based complexes (T1 agent) and iron oxide-based nanoparticles (T2 agent) are the most common MRI contrast agents in lab studies and clinical examinations. Similar to the probes for FLI, MRI contrast agents for inflammation visualization can also be divided into three classes according to the targeting strategies.

2.3.1 Active targeting-based contrast agents/probes

The conjugation of contrast agents with targeting ligands is a mainstream approach to develop molecular agents for visualizing inflammation. For example, Pagoto et al constructed a nanomicelle self-assembled via amphiphilic Gd-complex (Gd-DOTAMA(C18)2), DSPE-PEG2000-OMe, and VCAM-1 targeting peptide-grafted DSPE-PEG2000. The conjugation of contrast agents with targeting ligands can also be divided into three classes according to the targeting strategies.

The Chan group monitored the endothelial cell inflammation through the biotinylated antibodies of P-selectin and VCAM-1, and streptavidin-labeled superparamagnetic particles of iron oxide (SPIO) in a TNF-α-induced ex vivo vascular inflammation model. The feasibility of employing VCAM-1 and P-selectin as the molecular targets of MRI was confirmed in this work. A similar study also led by Chan realized the in vivo MRI detection of inflammation in atherosclerosis using the same targeting strategy. Different from the above research, microparticles of iron oxide (MPIO) with higher iron oxide content were used instead of SPIO for a better magnetic resonance contrast effect. Surface modified by anti-VCAM-1 and anti-P-selectin antibodies, the dually targetable MPIO (Dual-MPIO) showed a swift and stable binding to the intravascular target, and the unbound particles could be rapidly cleared from the blood circulation, which provided an efficient and safe approach for assessing the degree of inflammation in atherosclerotic plaques (Figure 7B).
2.3.2 | Activatable contrast agents

The activatable contrast agents for MRI have variable magnetic resonance properties modulated by specific biochemical targets, thereby enabling the visualization of biochemical changes and the selective and sensitive detection of particular biochemical targets with high signal-to-background ratios.\(^63\)\(^64\) Regarding inflammation, the ROS level is the most commonly used target. Wang and co-workers synthesized a redox-active Fe complex, Fe-PyC3A, which could be used as an activatable MRI contrast agent for inflammation visualization.\(^65\) Fe-PyC3A could rapidly switch between the two oxidation states, Fe\(^{2+}\)-PyC3A and Fe\(^{3+}\)-PyC3A, accompanied with a significant change in magnitude relaxivity. Fe\(^{3+}\)-PyC3A possessed a low relaxivity, and was a more stable form under...
normal metabolism states. The elevated level of ROS at the site of inflammation could oxidize Fe$^{2+}$-PyC3A to Fe$^{3+}$-PyC3A, which had one order of magnitude higher relaxivity and could produce strong MRI signals. The authors demonstrated the feasibility of using Fe-PyC3A for detecting the oxidative stress in a mouse model of acute pancreatitis induced by caerulein/LPS, and a remarkable signal enhancement was detected in the inflamed pancreatic tissue, whereas negligible signal enhancement was observed in the normal pancreatic tissue. Recently, Zhou et al designed nanovesicles (termed IO-Gd NVs) that could serve as an activatable contrast agent for detecting inflammation-caused ROS generation by MRI. The NVs were fabricated by the self-assembly of Gd complexes (DOTA-Gd), iron oxide nanoparticles (IO NPs), and triblock poly(ethylene glycol)-poly(propylene sulfide)-poly(ethylene glycol)-amine (PEG-PPS-PEG-NH$_2$) polymers (Figure 8). Among the three components, the DOTA-Gd and IO NPs were $T_1$ enhancer (E) and $T_2$ quencher (Q), respectively. The triblock PEG-PPS-PEG-NH$_2$ polymers, which can be converted from a hydrophobic state to a hydrophilic state through the oxidation of thioethers to sulfones, were utilized for modulating the Q−E distance. Once being oxidized by ROS, the polymers would swell, and thereby the IO-Gd NVs would disassemble, further resulting in the increase of Q−E distance. Moreover, the $T_2$ effect of the quencher was decreased upon disassembly because of the dispersed magnetic field coupling effect. The increased Q−E distance and the decreased quencher’s $T_2$ effect finally led to the activation of the $T_1$ MRI signals. The authors further demonstrated that the IO-Gd NVs could be used for the early stratification of radiotherapy responses by evaluating the radiation-induced acute inflammation.

2.3.3 Immunocyte-mediated targeting-based contrast agents

Another targeting strategy for contrast agents is being internalized by inflammatory cells to achieve their enrichment at the inflammation sites. Ultrasmall superparamagnetic particles of iron oxide (USPIO) can reach inflammatory lesions either through leaky endothelium alone or by being phagocytized by macrophages. Moreover, the iron oxide can ultimately be degraded by lysosomes, and the product can be utilized for cellular syntheses that need iron ions. Thereby, due to their high sensitivity and biocompatibility, USPIO have been widely studied and applied for inflammation imaging, especially in cardiovascular diseases. Strirrat et al visualized inflammation in patients after MI by using USPIO-enhanced MRI, providing a noninvasive way for detecting and assessing inflammation of myocardial tissues. Combined with more prolonged monitoring of myocardial edema, it may be valuable for the diagnosis, risk stratification, and prognosis evaluation of the inflammatory diseases in the heart. Additionally, a study reported by Smits et al evaluated the MRI contrast effect of the same kind of USPIO (ferumoxytol) in inflammatory atherosclerotic plaques. During clinical trials, enhanced signals were detected 72 h after the injection of ferumoxytol in the atherosclerotic plaques, rather than in the carotid arterial wall, which proved the feasibility of using ferumoxytol as a contrast agent to quantify arterial wall inflammation. Similarly, there are also researches that achieve inflammation detection by using other immunocyte-mediated targeting agents. For instance, Shi et al proposed a novel approach to detect carotid inflammatory plaques using a type of superparamagnetic nanoparticles (SNPs) synthesized by themselves.
obtain the SNPs, Fe₃O₄ nanoparticles with hydrophilicity and high magnetism were coated with organosilica first, and then modified by PEG, which improved the biocompatibility of the particles. The SNPs retained the strong magnetism of Fe₃O₄ nanoparticles, and could thereby be influenced by an external magnetic field. The authors designed a magnetic collar to retard the flow of SNPs in the superficial common carotid arteries, facilitating immune cells to capture the SNPs and thus realizing the increased enrichment of the SNPs at the inflammation site to enhance MRI. Lewis et al utilized hyperpolarized [¹³C] MRI to visualize local cardiac inflammatory response after MI. Hyperpolarization techniques can greatly improve the magnetic resonance (MR) signal quality, and thus, other nuclei except protons, such as [¹³C] and [¹⁵N], can be used for in vivo imaging. The authors reported that hyperpolarized MRI of [¹⁻¹³C]pyruvate could reflect the metabolic reprogramming toward glycolysis in activated leukocytes, which is closely related to the production of proinflammatory cytokines, and the signals of the resulting downstream metabolic product [¹⁻¹³C]lactate could be used to assess inflammation. The strong [¹⁻¹³C]lactate signals were successfully detected in the animal experiments on rats and pigs, and in the in vitro experiments on macrophage-like cell lines, indicating the feasibility and potential of this approach in clinical inflammation assessment.

### 2.3.4 Combination of different contrast agents

Besides, studies that combine different contrast agents for visualizing inflammation have been reported as well. For instance, recently, in a mouse model, Hoffmann et al realized the visualization of inflammation in cerebral malaria by the combined use of Gd complexes and cross-linked iron oxide nanoparticles (CLIO-NPs). For targeting inflammation, the authors utilized not only the internalization of iron oxide nanoparticles by immunocytes, which mainly showed the inflammation in the microvasculature, but also the overexpression of MPO at the inflammation site to reflect the parenchymal and intraventricular inflammation. As mentioned above, MPO is a biomarker of inflammation, and thus the authors used MPO-Gd (bis-hydroxy-tryptamide-diethylenetriaminepentaacetate gadolinium), a specific and sensitive MRI probe that can be activated by MPO, to fulfill the molecular MRI-based visualization of inflammation. With the combined use of MPO-Gd and CLIO-NPs, the vascular inflammation and structural damage of the blood-brain barrier and blood-cerebrospinal fluid barrier could be linked, which contributed to the assessment of diseases.

### 2.4 Ultrasound imaging

US imaging creates images based on the differences of acoustic impedance between different tissue types and structures. Similar to electrical impedance, acoustic impedance reflects the obstruction of medium on the sound wave propagation, which depends on medium elastic modulus and density. The core device of US imaging system is US transducer. It can produce source sound waves as well as detect the reflected echo signals that can be further processed into visible images. As the sound waves generated by the transducer travel through the tissue of interest, they will reflect off surfaces of discontinuity in density. With the advantages of low cost, high portability, high imaging depth, lack of ionizing irradiation, and high spatial and temporal resolution, US imaging has obtained extensive applications in the biomedical field. However, the intensity of the reflected echo signal is determined by the mismatch of acoustic impedance, which is not significant between tissues, especially in soft tissues, and this limits the applications of US imaging. The emergence of US contrast agents (UCAs) with a large difference in acoustic impedance compared to tissues offers a feasible tool to overcome this limitation. Typically, UCAs are microbubbles with a gaseous core that can lead to a high echogenic response, and as a result, a high image contrast can be obtained. Additionally, through surface modification, UCAs can be further utilized for realizing molecular imaging.

Detecting the structural changes induced by inflammation, mainly in vasculature, is a traditional method to visualize inflammation by US imaging, and has been used in clinic. Nevertheless, the new attempts using this traditional approach have not been stopped in recent years. For example, compared to power Doppler, superb microvascular imaging, a novel Doppler technology developed by Toshiba Medical Systems, has an improved resolution and sensitivity for imaging vascularity, enabling the visualization of low-grade inflammation that cannot be previously detected.

Targeted contrast-enhanced molecular US imaging is an emerging strategy for evaluating inflammatory processes at the molecular level. The inflammation-targeted UCAs are decorated with binding ligands that can recognize molecules involved in inflammatory processes, thereby enhancing the retention of UCAs at inflammation sites, providing enhanced US signals. UCAs that can target endothelial cell adhesion molecules (eg, P-selectin and VCAM-1) have been widely reported. Moreover, Moccetti et al assessed a series of microbubble UCAs conjugated with small-peptide targeting ligands against P-selectin, VCAM-1, oxidized low-density
lipoprotein cholesterol receptor-1 (LOX-1), and von Willebrand factor (vWF). Among these modified UCAs, the VCAM-1- and vWF-targetable ones showed a better contrast effect in mouse models, and the histological analyses performed on the human carotid endarterectomy samples also indicated the higher expression of these two targets than the other ones, demonstrating the feasibility of using these targeted agents for US molecular imaging of inflammatory plaques in advanced atherosclerosis.

Besides the active-targeting microbubbles, the gas-generating systems that can be activated in pathological regions, also known as activatable UCAs, have recently been employed for the US molecular imaging of inflammation. Specifically, activatable UCAs are initially not echogenic until they are activated by the reaction with the specific phenotypes in inflamed tissues to generate gas and coalesce into echogenic microbubbles. For example, Heo et al developed a H$_2$O$_2$-responsive CO$_2$-generating system (USNG) as a UCA based on a peroxamide nanogel (Figure 9A). Peroxamide and H$_2$O$_2$ could undergo the well-known POCL reaction, accompanied by the generation of CO$_2$ molecules. In this study, the nanoscale crosslinking of the polymeric aliphatic amines with oxalyl chloride offered a high concentration of peroxamide, and...
Meanwhile, the residual uncrosslinked amines could act as the in situ alkaline catalysts for POCL reactions, realizing the simultaneous high resistance to hydrolysis and reactivity to H$_2$O$_2$. By further formulating the crosslinked aliphatic peroxamide into a water-dispersible nanogel colloid to form a nanoscale catalytic reactor for POCL reactions and a gas reservoir capable of inflation into a stable microbubble, the H$_2$O$_2$-responsive enhancement of the US contrast effect for the diagnostic imaging of inflammatory diseases could be achieved (Figure 9B).

### 2.5 Photoacoustic imaging

PAI, also known as optoacoustic or thermoacoustic imaging, integrates optical excitation with acoustic detection for simultaneously realizing the high spatial resolution and high contrast imaging in deep tissues. When a pulsed laser irradiates chromophores, it will induce a thermoelastic expansion, followed by the emission of ultrasonic waves, which can be utilized to build up reconstructed images. On one hand, compared to optical imaging, PAI possesses a better resolution in deep tissues because the scattering of US waves in biological tissues is much weaker than light waves. On the other hand, in contrast to a US image, the contrast of a photoacoustic (PA) image depends on the optical properties of the target, especially the optical absorption, which have a larger difference between the different types of tissues than acoustic impedance. Moreover, the magnitude of the involved acoustic pressures in PAI is usually several orders of magnitude lower than in US imaging, indicating a better safety of in vivo detection. Therefore, PAI has an appealing application prospect in the biomedical field. The common endogenous chromophores for PAI include hemoglobin, melanin, water, and lipids. In particular, the strong absorption of hemoglobin enables the high-contrast visualization of microvasculature, and the differences in the optical absorption between oxygenated and deoxygenated states of hemoglobin can provide further functional information about blood oxygenation. In addition to the endogenous chromophores, diverse contrast agents have been used to carry out the selective PAI of a variety of diseases, including inflammation. Through linking the targeting components to the PA contrast agents, such as metallic nanorods and fluorescent dyes, the active probes can be constructed. With the excellent NIR absorption characteristic, gold nanorods (AuNRs) have been conjugated with the antibody of ICAM-1 (intracellular cell adhesion molecule 1) for visualizing the early inflammatory response by Kim et al. The upregulated expression of ICAM-1 during the inflammatory process makes it a potential target of inflammation. Other targets have also been exploited. For example, Qin et al developed an efficacious PA probe by conjugating AuNRs with the antibody of matrix metalloproteinase-2 (MMP-2), which is expressed in the inflammatory atherosclerotic plaques at an elevated level.

Similar to fluorescent probes, in addition to active targeting, several activatable PA probes in response to the oxidative stress during the inflammation process have been reported. Chen et al developed a nanoprobe (Lipo@HRP&ABTS) for the in vivo real-time PAI of H$_2$O$_2$, which offered a useful tool to visualize H$_2$O$_2$-related inflammation induced by bacterial infection or LPS. The authors integrated horseradish peroxidase (HRP) and the substrate of it, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), into liposomes, and thereby once the probe was activated by H$_2$O$_2$, HRP would catalyze the conversion of the colorless substrate to an oxidized form that possesses strong NIR absorption, realizing a sensitive and specific PA-detectable response to H$_2$O$_2$ (Figures 9C and 9D). Similarly, Gao et al introduced a self-assembled nanoprobe with a specific PA response to glutathione (GSH)/H$_2$O$_2$, enabling the in vivo visualization of the redox state associated with inflammation (Figure 10A).

To construct the redox-responsive PA nanoprobe, two types of NIR fluorescent probes whose absorption depends on H$_2$O$_2$ and GSH at 680 and 765 nm, respectively, were packaged by bovine serum albumin using a self-assembly method, which could both prolong the blood circulation time and enhance the biocompatibility of the nanoprobe. The detection capability for the inflammatory process was confirmed in the macrophage model and the atherosclerotic mouse model, and could be further applied to evaluate the vulnerability of atherosclerotic plaques (Figures 10B and 10C).

Nevertheless, the current clinical translation of PAI faces a huge challenge. To obtain high-resolution images, most illumination light sources are class-IV lasers, which can provide sufficient light energy by short light pulses. However, their high cost, large space occupation, and low mobility limit the clinical applications of PAI. To solve the abovementioned issues, some researches utilized light emitting diodes as a possible alternative light source in the PAI system, which produced enough contrast by signal averaging over a large number of pulses, and have been successfully applied in the inflammation imaging.

### 2.6 Hybrid imaging

In fact, each imaging modality has its limitations. Typically, the imaging techniques that can provide detailed anatomical information have poor performance on the acquisition of functional information, and vice versa.
FIGURE 10  (A) Scheme showing the preparation of the redox-responsive nanoprobes and the mechanism for assessing the atherosclerotic plaque vulnerability through the in vivo PAI of GSH/H$_2$O$_2$. (B) Representative PA images of the atherosclerotic plaques in ApoE$^{-/-}$ mice at different stages (4, 8, 12, and 16 weeks on high-fat diet). The images were obtained 2 h postinjection of the nanoprobes. Scavenger: Catalase (for H$_2$O$_2$) and buthionine sulfoximine (for GSH). (C) Normalized PA signal intensities derived from the data in (B). (A–C) Reprinted with permission. Copyright 2019, American Chemical Society

Therefore, hybrid imaging with the ability of offering complementary synergistic information simultaneously has become a popular approach in biomedical imaging.

PET is always combined with a structural imaging modality. Other than the abovementioned PET/CT, the fusion of PET and MRI is now increasingly adopted for imaging inflammation, with a lower required radiation dose. A potential application of PET/MRI is cardiac inflammation imaging, predominantly by utilizing inflammatory cells that can respond to sterile injury as a target.$^{90}$ For instance, Lee et al reported that the combination of $^{18}$F-FDG PET and MRI could realize a synergy in imaging myocardial inflammation after MI, on condition that the glucose usage of myocytes was suppressed.$^{91}$ Furthermore, there have been clinical cases that performed $^{18}$F-FDG PET/MRI to evaluate cardiac inflammation.$^{92}$ Besides, attempts of using PET/MRI for assessing other inflammatory diseases have been made, such as atherosclerosis and brain epilepsy.$^{93}$ For a more detailed overview of PET/MRI in inflammation imaging, readers may refer to the recent review published by Sollini et al.$^{94}$

Other than PET, the combination of FLI and MRI has been widely reported.$^{95}$ Denis and co-workers developed the use of long-circulating magnetofluorescent nanoparticles for visualizing the microvascular leakage of leukocytes, which is a biomarker of pancreatic islet inflammation, and the nanoparticles could be further utilized to evaluate the onset and development of insulitis in mouse models of type 1 diabetes.$^{96}$ The nanoparticle was composed of an SPIO core and a dextran coating conjugated
TABLE 1  Some present strategies for the construction of the animal models bearing inflammatory diseases

| Animal model | Intervention | Inflammatory disease | Ref. |
|--------------|--------------|----------------------|------|
| BALB/c mice  | LPS injection| Peritonitis           | 45c  |
|              | Caerulein/LPS injection | Lung Inflammation     | 21a  |
|              | Acetaminophen injection | Arthritis            | 76   |
|              | Zymosan injection | Acute liver injury    | 38b  |
|              | Carrageenan injection | Paw inflammation     | 56b  |
|              | TPA administration | Ear inflammation     | 56a  |
|              | Imiquimod administration | Psoriasis          | 36   |
|              | MRSA inoculation | Bacterial infection   | 19   |
|              | DSS feeding | Colitis               | 55d  |
| C57BL/6 mice | K/BxN mice serum injection | Arthritis        | 21d  |
|              | P. berghei ANKA parasite injection | Cerebral malaria   | 73a  |
|              | LPS injection | Leg inflammation      | 59   |
|              | Mycobacterium tuberculosis H37Ra antigen | Uveitis          | 35   |
| CD-1 mice    | Oxazolone administration | Dermatitis         | 36   |
|              | Bleomycin administration | Scleroderma       | 36   |
|              | Collagen injection | Arthritis            | 55a  |
| DBA/1 mice   | Kilham rat virus injection | Diabetes          | 78b  |
|              | High-fat diet feeding | Atherosclerosis     | 88   |

Abbreviations: BBDR, BioBreeding Diabetes Resistant; DSS, Dextran sulfate sodium; MRSA, methicillin-resistant Staphylococcus aureus; TPA, 12-O-tetradecanoylphorbol-13-acetate.

*In incomplete Freund’s adjuvant.

with a fluochrome, permitting a real-time dual-modality imaging (MRI and FLI) in vivo. Burckman et al constructed a novel dual-modality imaging platform by integrating tobacco mosaic viruses as carriers, fluorescent dyes for FLI, chelated Gd ions for MRI, and oligopeptides specific to the VCAM-1 receptor for targeting inflammation. It was demonstrated that this platform could deliver imaging agents with high efficiency and realize sensitive molecular imaging of inflammatory atherosclerotic plaques.

In addition to MRI, US imaging can serve as a structural imaging method. The combination of US imaging and other functional imaging modalities has also been used for visualizing inflammation. For instance, the abovementioned nanobubbles designed by Liu et al could not only serve as a BLI probe for inflammation, but also act as a US contrast agent, enabling the BLI- and US imaging-based visualization of tissue structure and vasculature in the inflammation region. There are also dual-modality imaging approaches combining US imaging and FLI. For example, Glimm et al reported the possibility of using FLI and US imaging to visualize the severity and distribution of inflammation in wrist and finger joints, offering a potential method to determine and differentiate the inflammatory status in patients with rheumatoid arthritis compared to osteoarthritis. Moreover, for visualizing synovitis, combining PAI and US imaging is another feasible way, because PAI can provide functional information about the content and oxygen saturation of hemoglobin, which are at abnormal levels in inflamed synovium.

3  CONCLUSIONS AND PERSPECTIVES

Inflammation is intimately related to the pathogenesis of various diseases, and thus, to develop more efficient therapies for treating inflammation, it is definitely essential to improve the comprehension of the inflammatory processes. For the preclinical investigations of various inflammatory diseases, a host of intervention methods have been adopted to establish the corresponding animal models (as summarized in Table 1), enabling the in vivo demonstration of the effectiveness of various developed imaging reagents. On the other hand, the strategies for visualizing inflammations can serve as a powerful tool for the relevant theoretical/fundamental researches, and can also be applied in disease diagnosis, therapy response monitoring,
TABLE 2  Typical inflammation targeting strategies and examples of the corresponding imaging modalities for visualization of inflammation

| Target type                          | Target         | Targeting approach | Imaging modality | Ref. |
|--------------------------------------|----------------|--------------------|------------------|------|
| Cell-adhesion molecules              | VCAM-1         | Active             | PET              | 54   |
|                                      |                |                    | MRI              | 59   |
|                                      |                |                    | US               | 79d  |
|                                      | ICAM-1         | Active             | PAI              | 85   |
|                                      | P-selectin     | Active             | MRI              | 62   |
|                                      |                |                    | US               | 79a  |
|                                      | L-selectin     | Active             | FLI              | 15   |
|                                      | Integrin       | Active             | FLI              | 16   |
| Cytokines                            | TNF-α          | Active             | PET              | 56a  |
| Surface markers on immune cells      | MMR            | Active             | SPECT            | 55a  |
|                                      | SSTR           | Active             | PET              | 55b  |
|                                      | CD4            | Active             | PET              | 55c  |
|                                      | Chemokine receptors | Active | PET | 55d  |
| Enzymes                              | COX-2          | Active             | FLI              | 14   |
|                                      |                |                    | SPECT            | 56b  |
|                                      | MMP-2          | Active             | PAI              | 87   |
|                                      | MPO            | Responsive          | SPECT            | 56c  |
|                                      |                |                    | PET              | 56d  |
|                                      |                |                    | MRI              | 73a  |
|                                      |                |                    | BLI              | 38b  |
| Redox state                          | LTA₄H          | Responsive          | FLI              | 21a  |
|                                      | H₂O₂           | Responsive          | FLI              | 21c  |
|                                      |                |                    | CLI              | 44   |
|                                      |                |                    | MRI              | 65   |
|                                      |                |                    | US               | 76   |
|                                      |                |                    | PAI              | 10e  |
|                                      | ONOO⁻          | Responsive          | FLI              | 19   |
|                                      | HClO           | Responsive          | FLI              | 21b  |
| Immune cell behavior                 | Glucose metabolism | Immunocyte-mediated | PET            | 50d  |
|                                      |                |                    | MRI              | 71   |
|                                      | Phagocytosis    | Immunocyte-mediated | FLI              | 24   |
|                                      |                |                    | MRI              | 68   |

and prognosis evaluation in clinic. Hence, in this review, we have also summarized the current targeting strategies and imaging modalities for inflammations (Table 2).

To achieve more ideal visualizations of inflammations, the development of new imaging strategies for acquiring images of higher quality is required. So far, luminol and its derivatives have been extensively used to visualize inflammation. Besides the development of BRET or CRET systems to red shift the blue light emitted by luminol/luminol derivatives to the NIR light to ensure deep tissue penetration, the exploration of novel inflammation-related bioluminescence/CL processes with long-wavelength emission might be another potential way. For the same reason, new fluorescent agents with emission in the NIR region for the FLI of inflammations are highly desired. Likewise, developing PA contrast agents with longer absorption wavelengths is promising to realize inflammation visualization in deep tissues. With regard to the nuclear medicine imaging, developing new radiopharmaceuticals that have suitable physical and biological half-lives is a possible way to improve the imaging performance, decrease the dose of radiation, and improve the biosafety of the imaging probes. As for MRI, novel contrast agents with enhanced relaxivity might be able to provide an increased imaging contrast for inflammations. Similarly, microbubbles with better stability and acoustic performance
will be beneficial for obtaining higher quality US images. Furthermore, the improvement of imaging instruments will also facilitate the facile and clear visualization of inflammation. The imaging systems with lower cost, better portability and operability, and higher resolution are expected. Last but not least, the combination of different imaging modalities is a popular trend, which can simultaneously offer complementary information of the inflammations. In addition to the existing dual-modal imaging techniques such as SPECT/CT, PET/CT, and PET/MRI, new dual-modal or multimodal imaging strategies are needed to achieve more comprehensive visualization of inflammations.

From another perspective, the strategies for visualizing inflammations can also be divided into three categories in terms of targeting approaches, that is, active targeting, responsive targeting, and immunocyte-mediated targeting. Active targeting refers to the use of probes that can specifically bind to some inflammation-related biomarkers. In this regard, various targets have been discovered and utilized, including cell-adhesion molecules, the surface markers on immune cells, pro-inflammatory cytokines, and enzymes (Table 2). Responsive targeting utilizes activatable probes that produce particular signals after being activated in the inflammatory environment. Currently, the activatable probes that can respond to abnormal redox state and enzymatic activity have been widely reported (Table 2). Immunocyte-mediated targeting achieves the localization of inflammation by virtue of the inflammation-tropism of immune cells. The imaging agents are internalized by immune cells through cellular processes, such as metabolism and phagocytosis, and thereby can accumulate in the inflammation region through the migration and recruitment of immune cells. Using the components of immune cells to construct carriers for loading imaging agents can also achieve this purpose.

Despite the remarkable achievements already made, the existing strategies for targeting inflammation are still not ideal. The specificity of most of the currently used strategies is still not satisfactory. In particular, distinguishing inflammation from tumor is the most prominent challenge, because they have similar features, such as unnatural redox state, pH, and metabolic rate. Therefore, the exploitation of more specific biomarkers or novel probes with higher selectivity for the existing inflammation targets will become the future research focus. Additionally, multi-targeted probes that aim at multiple biomarkers will also contribute to the enhanced visualization of inflammation. Moreover, as a way to decrease the background signals originating from the off-target imaging agents, developing more sensitive and specific activatable probes that are responsive to the inflammatory environ-

ment will become an attractive direction. In addition, with a deeper insight of the behaviors of immune cells, better immunocyte-mediated targeting strategies might be developed. Finally, more efforts should be made to examine the necessity of developing the new and unexplored combinations of the various imaging modalities developed. Overall, the advancements of targeting and imaging strategies will expand the role of inflammation visualization in preclinical and clinical studies.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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