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Review

Fluorescence Imaging of Inflammation with Optical Probes

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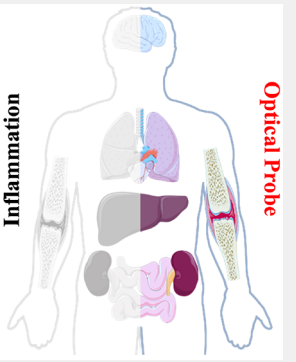
ABSTRACT: Inflammation plays an important role in the occurrence and development of disease; dysregulation of inflammatory progression often leads to disease such as tissue sclerosis, cancers, stroke, etc. Optical imaging technology, due to its higher sensitivity and resolution, can provide finer images for the observation of inflammation. Many optical probes have been developed as contrast agents for optical imaging techniques in different diseases. In this review, we summarize the recent advances of optical probe and imaging methods for imaging inflammation in different organs, such as brain, liver, lung, kidney, intestine, etc. Finally, we discuss the opportunities and challenges of optical probes used in the clinic for inflammation monitoring and prospect their future development in disease detection.



KEYWORDS: *tissue inflammation, inflammation imaging, fluorescence probe, inflammation makers, inflammation related disease, hypoxia imaging, ratiometric imaging, NIR II imaging*

1. INTRODUCTION

Inflammation is widely recognized as the human body's natural protective mechanism against trauma, including processes such as penetrating tissues of microorganisms, commanding and dispatching cells, eliminating microorganisms and infected cells, liquefying surrounding tissues, and healing damaged tissues.[1] In the inflammatory process, inflammation can also lead to tissue damage if accurate destruction and auxiliary repair are not carried out correctly.[2] Persistent tissue damage can further lead to diseases such as tissue sclerosis, cancers, stroke, etc. Thus, the early recognition and determination of the process of inflammation may be important for predicting disease progression and improving effective treatment.

Bioimaging has become an indispensable diagnostic tool for a variety of diseases including inflammation.[3] Many different imaging techniques have been used in the clinic, such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT).[4] They greatly facilitate disease diagnosis, but there are also some disadvantages, for example, the radiation hazards of CT, PET, and SPECT, low spatial resolution of PET, SPECT, and US, and low temporal resolution of MRI.[5] In the past decades, fluorescence imaging has been extensively studied for its noninvasive detection of dynamic biological processes with high sensitivity, low cost, real-time results, and excellent compatibility.[6] Since the advent of optical imaging, molecular dyes have been widely used as probes or contrast agents for optical imaging. Four optical probes have been approved for routine clinical use, and dozens of optical imaging probes are undergoing clinical trials at various stages.[7] Optical probes and optical imaging have made remarkable progress in disease detection, showing outstanding application potential and wide application prospects.

In this review, we focus on imaging inflammation with optical probes in response to different inflammatory markers, including biomarkers and physical markers in different diseases (Figure 1). We also display some optical probes with unique instruments for in vivo imaging in some special diseases. Finally, we discuss the advantages and disadvantages of optical probes and the outlook for their future development.

2. MARKERS IN INFLAMED TISSUE

The inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of infection or injury. Tissue-resident macrophages and mast cells can initially recognize the onset of infection and injury and secrete a variety of inflammatory mediators, including chemokines, cytokines, vasoactive amines, arachidonic acids, and proteolytic cascades.[8] The main and most immediate effect of these mediators is to locally cause the exudation of inflammatory

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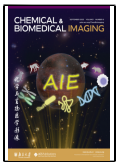

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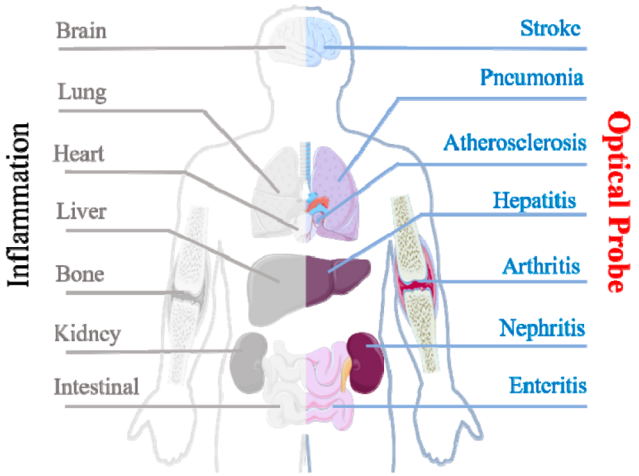

Figure 1. Illustration of the organs that may be inflamed, such as brain, lung, liver, kidney, intestine, bone, and heart. Inflammation in these organs will cause stroke, pneumonia, atherosclerosis, arthritis, and enteritis. Lighting up the inflammation by well designed optical probes with appropriate imaging equipment provides an effective method for real time and dynamic monitoring of the occurrence and development of inflammation.

substances from blood vessels. The activated endothelium of blood vessels allows selective extravasation of neutrophils. When neutrophils reach infected tissue sites, they are activated by cytokines secreted by tissue-resident cells, which release toxic substances to repair tissues, including reactive oxygen species (ROS) and reactive nitrogen species, proteinase 3, cathepsin G, and elastase.[9,10] The cells and cytokines involved in the inflammatory response can be used as biomarkers for optical probe design (Table 1).

Table 1. Markers Used in Designing Optical Probes for Inflammation

markers	responsive moiety	refs
nitric oxide (NO)	*O*-phenylenediamine (OPD)	18, 19
	thiadiazole-5,6-diamine (BPDA)	
hydrogen peroxide (H_2O_2)	spirolactam ring	21, 49
	peroxyoxalate	
hypochlorous acid	*P*-methylthio-phenylene	34, 46, 47
	C═N bond	
	Cy925	
superoxide anion	trifluoromethanesulfonyl	39, 55
	diphenylphosphinyl	
glutathione (GSH)	disulfide bond	40
leukotriene A4 hydrolase (LTA4H)	unnatural amino acid L-AspBzl	26
cathepsin	peptide sequence Cbz-Phe-Lys	86
lipid droplets	bis(trifluoromethyl) phenyl	34, 35
polarity	triphenylamine-thiophene-ethylene	41
viscosity	indole salt	42
temperature	Ag_2S NPs	43
hypoxia	Pd porphyrin	56, 58−64, 68−70
	Ir(III) complex	
pH	imine bond	69, 73, 74
	ionizable group	
	poly(*β*-amino ester/amidoamine)-dodecylamine	

In addition, inflammatory response in the body also triggers temperature elevation in the body and internal organs which is a severe condition with a high rate of morbidity and mortality.[11] Apart from temperature elevation in tissue, oxygen content in the inflammatory tissue is also reduced due to increased cellular metabolic demands and decreased metabolic substrates caused by thrombosis and trauma.[12] Meanwhile, the polarity and viscosity of subcellular organelles also varies in inflammation related cells.[13,14] Changes in the physical microenvironment can also be designed as the response substrate of optical probes for inflammation imaging.

3. INFLAMMATION IN THE BODY

3.1. Inflammation of the Intestine

Intestinal inflammation is a common chronic and recurrent inflammatory disorder of the gastrointestinal tract and one of the most refractory gastrointestinal diseases resulting in abdominal pain, fecal blood, and diarrhea as well as further potential severe complications.[15,16] The cause of intestinal inflammation has not been uniformly concluded to date; early diagnosis and prompt institution of treatment are the cornerstones for improving outcomes.

Nitric oxide (NO) is a metastable free radical and plays an important role in regulating various physiological and pathological processes including intestinal inflammation.[17] A donor−acceptor−donor (D−A−D)-type near-infrared fluorescent probe composed of a triazolo-benzo(1,2,5-thiadiazol) moiety and a morpholine group was designed for NO triggered fluorescence turn on based on the mechanism of intramolecular charge transfer by NO. This near-infrared fluorescent probe could image NO in real time in inflammatory bowel disease (IBD) in vivo.[18] Compared to a single emission near-infrared fluorescent probe, a ratiometric imaging probe can quantitatively measure analyte concentration and is not influenced by interference factors. Given this, Wu and co-workers encapsulated a NO/acidity-responsive molecule, dithienopyrrole (DTP)−benzophenone tetracarboxylic dianhydride (BTDA), and a nonresponsive fluorophore DTP−benzobisthiadiazole (BBTD) in amphiphilic triblock copolymer F127 to obtain ratiometric near-infrared fluorescence (NIRF)/photoacoustic imaging (PA) dual-modal nanoprobe RAPNP (Figure 2a). The BTDA moiety in RAPNP could be oxidized by NO in weak acid conditions and shift the absorption and emission wavelength to 720 and 940 nm. Thus, RAPNP enabled ratiometric detection of the endogenous NO in IBD by NIRF/PA dual-modal imaging, and the ratiometric signal was correlated to the NO concentration in IBD (Figure 2b).[19]

Although the most common route of administration of contrast agents is intravenous or topical, oral administration is more convenient for certain pathologies, such as inflammatory diseases of the intestine. Rogler and co-workers found that certain peptides against N-cadherin could specifically bind to inflamed intestinal tissue. To improve the antiprotease hydrolysis of peptides, the sequence of peptides was modified to be a retro-inverso style. Orally administered fluorescence conjugated peptide achieved resistance to gastrointestinal degradation and improved specificity toward colonic inflammation.[20] Shallow depth of tissue penetration, much autofluorescence interference, and low fidelity are common shortcomings, which limited the fluorescence imaging of intestinal inflammation in the short wavelength imaging

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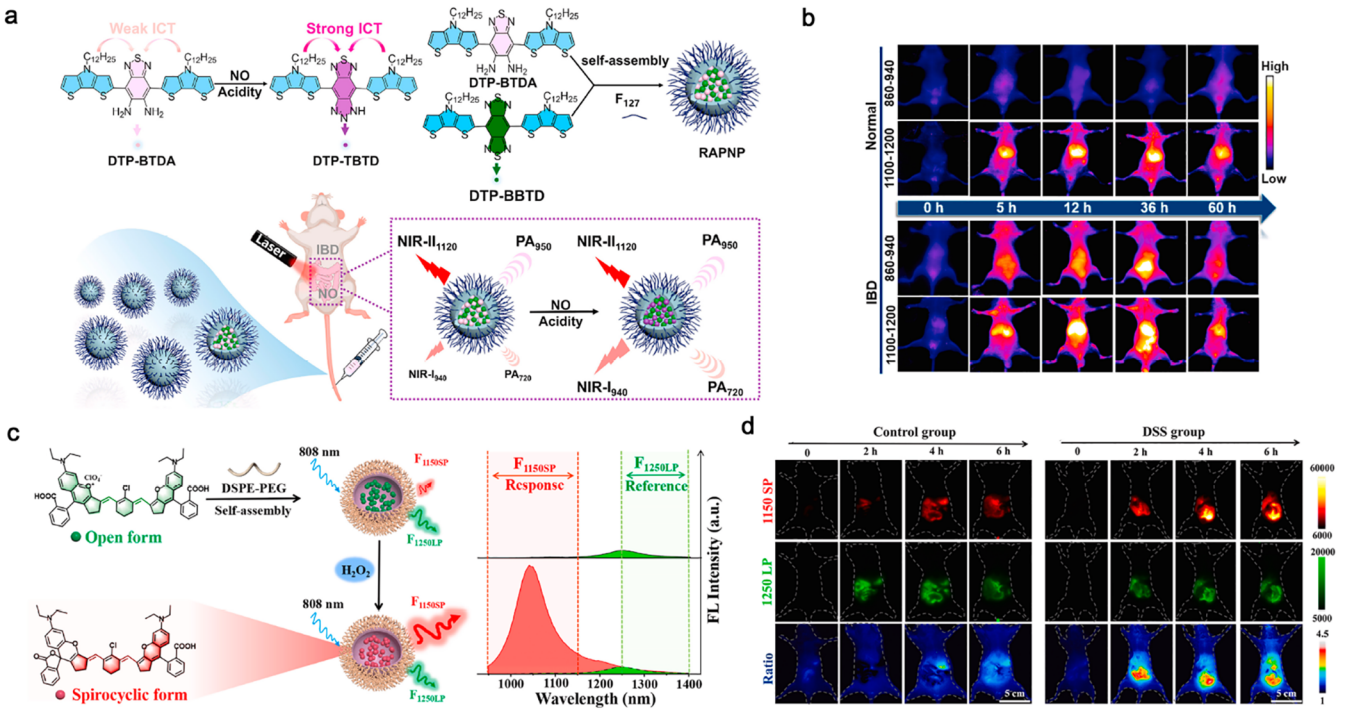

Figure 2. Imaging of the inflammation in the intestine. (a) Illustration of the preparation of the ratiometric probe RAPNP by nanoprecipitation with DTP-BBTD and the fluorescence and the PA signal of RAPNP could be activated in IBD mice. (b) NIRF images of healthy mice and IBD mice at different time points after tail-vein injection of RAPNP demonstrating that RAPNP is able to perform ratiometric imaging of the endogenous NO in IBD mice. Panels a and b reproduced with permission from ref 19. Copyright 2023 Elsevier. (c) Illustration of the design of the oral delivery ratiometric fluorescent probe LC-1250 NP which could react with H_2O_2 and enhance the fluorescence intensity in the wavelength of 1150 nm. (d) NIR-II fluorescence images and ratiometric images in healthy and IBD mice at different time points after oral dosing of LC-1250 NP. The fluorescence ratio of the intestinal tract in the DSS group was enhanced significantly indicating the up-regulated H_2O_2 level in IBD mice. Panels c and d reproduced with permission from ref 21. Copyright 2023 Elsevier.

window (400−900 nm). Therefore, a single-component near-infrared-II (NIR-II) ratiometric molecular nanoprobe (LC-1250 NP) was designed for fluorescence imaging in the second near-infrared window (NIR-II, 1000−1700 nm). LC-1250 NP not only responds to upregulated H_2O_2 in intestinal inflammation through intramolecular cyclization but also maintains fluorescence stability for a long time under acidic/alkaline conditions (Figure 2c,d). After oral delivery to the diseased intestinal tissue, LC-1250 NP presented strong fluorescence emission at 1045 nm while maintaining constant fluorescence intensity at 1250 nm due to the emission tail. Good performance makes LC-1250 NP which is an oral ratiometric NIR-II fluorescent probe a good choice for reliable monitoring of intestinal inflammation in vivo.[21]

3.2. Inflammation of the Lung

Lung inflammation, such as pneumonia, asthma, pulmonary fibrosis, and acute lung injury, is a global health concern. It is also a disease related to the alveoli and respiratory bronchioles caused by pathogens, which induce local and systemic inflammatory responses in the patient.[22] Lung inflammation is also associated with serious complications, including hearing loss, systemic sepsis, meningitis, brain damage, and lung abscess. The mortality rate with lung inflammation is persistently elevated in past decades.[23] Thus, early and accurate diagnosis is essential for treatment of inflammation in the lung.

Integrin receptors which are quiescent on normal alveolar endothelial cells are upregulated in acute lung injury. Targeting pulmonary integrin receptors with RGD peptide enables real-time, noninvasive monitoring of lung inflammation by near-infrared fluorescent probes.[24] Compared with near-infrared fluorescence imaging, two-photon fluorescence imaging technology has the advantages of high penetration depth and low photobleaching and can be used for real-time imaging of lung inflammation.[25] Tang and co-workers synthesized a fluorescent probe (ASPC) that responds to highly expressed LTA_4H in pneumonia tissue. In the absence of LTA_4H, ASPC does not produce fluorescence (Figure 3a).[26] When LTA4H is encountered in the inflamed lungs, the amide bond of ASPC is broken to release fluorescent molecules, generating strong fluorescence. Subsequently, ASPC enables in vivo imaging of lung inflammation in mice using two-photon fluorescence technology. Besides LTA_4H, elevated SO_2 in pneumonia can also be detected by a two-photon near-infrared fluorescent probe (DCQN) in a pneumonia lesion site in deep tissues.[27]

Lung tissue which buried deep under the sternum is difficult to detect by ordinary fluorescent molecules. Therefore, new fluorescence detection methods such as fluorescence molecular tomography (FMT) are used to detect deep tissues in vivo.[28] Ntziachristos and co-workers achieved fluorescent molecular tomography of lung inflammation using a cathepsin-activated near-infrared fluorescent probe and demonstrated that FMT can noninvasively and longitudinally characterize physiological, cellular, and subcellular processes associated with pulmonary inflammatory disease. Further, they combined FMT with X-ray computed tomography (CT) to provide a new imaging method with full coverage at a 360° angle with high fidelity (Figure 3b).[29] After injection of mixed fluorescent molecules

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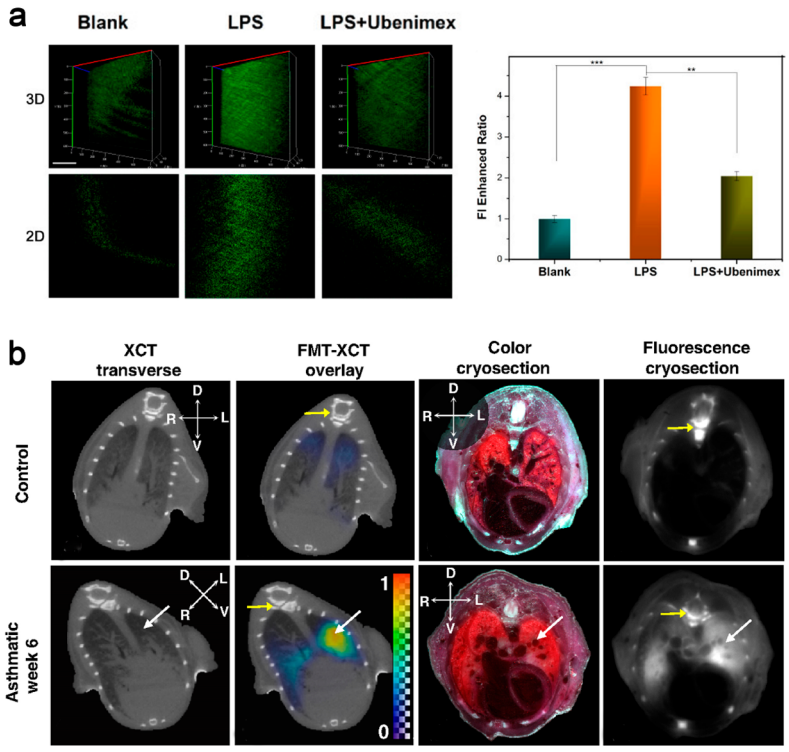

Figure 3. Imaging of inflammation in the lung. (a) Fluorescence imaging of LTA_4H in the mouse model of inflammation after tail-vein injection of ASPC and quantitative intensity analysis from images. Reproduced with permission from ref 26. Copyright 2018 American Chemical Society. (b) Representative slice comparison of FMT-XCT reconstruction and cryoslicing images in healthy and pulmonary eosinophilia mice demonstrated the serious inflammatory lesion in the lung of the asthmatic mouse. Reproduced with permission from ref 29. Copyright 2016 Society of Photo-Optical Instrumentation Engineers.

into the mice with asthma, FMT-CT can measure the activity of pulmonary eosinophilia and matrix metalloproteinases in lung tissue.

3.3. Atherosclerosis

Cardiovascular disease is the leading cause of death in the world; atherosclerosis is considered a chronic, progressive inflammatory disease leading to thrombosis and vascular occlusion and is the main cause of the pathogenesis of myocardial and cerebral infarction.[30] As conventional medical diagnostic tools struggle to detect early atherosclerotic lesions and plaque ruptures, there is a clear need for new diagnostic equipment and imaging agents.

The accumulation of inflammatory cells and their products induces atherosclerosis or maturation of plaques.[31] Thus, targeting inflammatory cells such as dendritic cells allows real-time imaging of atherosclerosis. Scott and co-workers found that assembling poly(ethylene glycol)-*block*-poly(propylene sulfide) (PEG-*b*-PPS) block copolymers into different morphologies, such as micelles and filomicelles, could effectively target splenic dendritic cells and lower uptake by other cells of the mononuclear phagocyte system.[32] In a mouse model of atherosclerosis, the block copolymer micelles showed specific targeting of the plague site. Apart from dendritic cells, atherosclerotic plaques are also characterized by overproduced hypochlorous acid and lipid droplets. Specific detection of these markers of atherosclerosis can improve sensitivity of imaging.[33] Liu and co-workers coupled the highly lipophilic bis(trifluoromethyl)phenyl and *p*-methylthio-phenylene to a boron-dipyrromethene probe.[34] The designed fluorescent probe named iSHERLOCK is able to specifically detect hypochlorous acid and lipid droplets in atherosclerotic plaques (Figure 4a,b). Meanwhile, atherosclerotic lesions in the aortas were precisely profiled by iSHERLOCK in ex vivo imaging.

As targeted near-infrared fluorescence imaging requires ex vivo imaging of atherosclerosis, direct observation of atherosclerosis in blood vessels is still the most effective means. Indocyanine green (ICG) is a food and drug administration (FDA) approved dye for imaging the vascular system at near-infrared (NIR) wavelength.[35] It is also quickly absorbed by lipid-rich plaques and cells in atherosclerotic rabbits, making it a potentially useful plaque-imaging agent. To test whether ICG can be used in patients with clinical atherosclerosis, Jaffer and co-workers imaged atherosclerosis by ICG in vivo with NIRF optical coherence tomography (OCT) in swine.[36] Further, they injected ICG into a patient, and when the carotid plaque was removed, the intra-arterial plaque was imaged using NIRF optical coherence tomography (Figure 4c). The fluorescence of ICG coincided with the position of the carotid plaque demonstrating the effectiveness of ICG in imaging atherosclerosis. Besides NIRF OCT, Ntziachristos and co-workers engineered a 2.9-F rotational, automated pullback 2D imaging catheter optimized for 360° view intravascular NIRF imaging.[37] In conjunction with the cysteine protease-activatable imaging reporter Prosense VM110, the catheter enabled real-time intra-arterial 2D NIRF high-resolution imaging in rabbit aortas with atherosclerosis.

3.4. Hepatic Inflammation

The liver as a main metabolic organ plays important roles in detoxification and waste elimination in the body. Therefore, it is prone to suffering damage or injuries due to chronic

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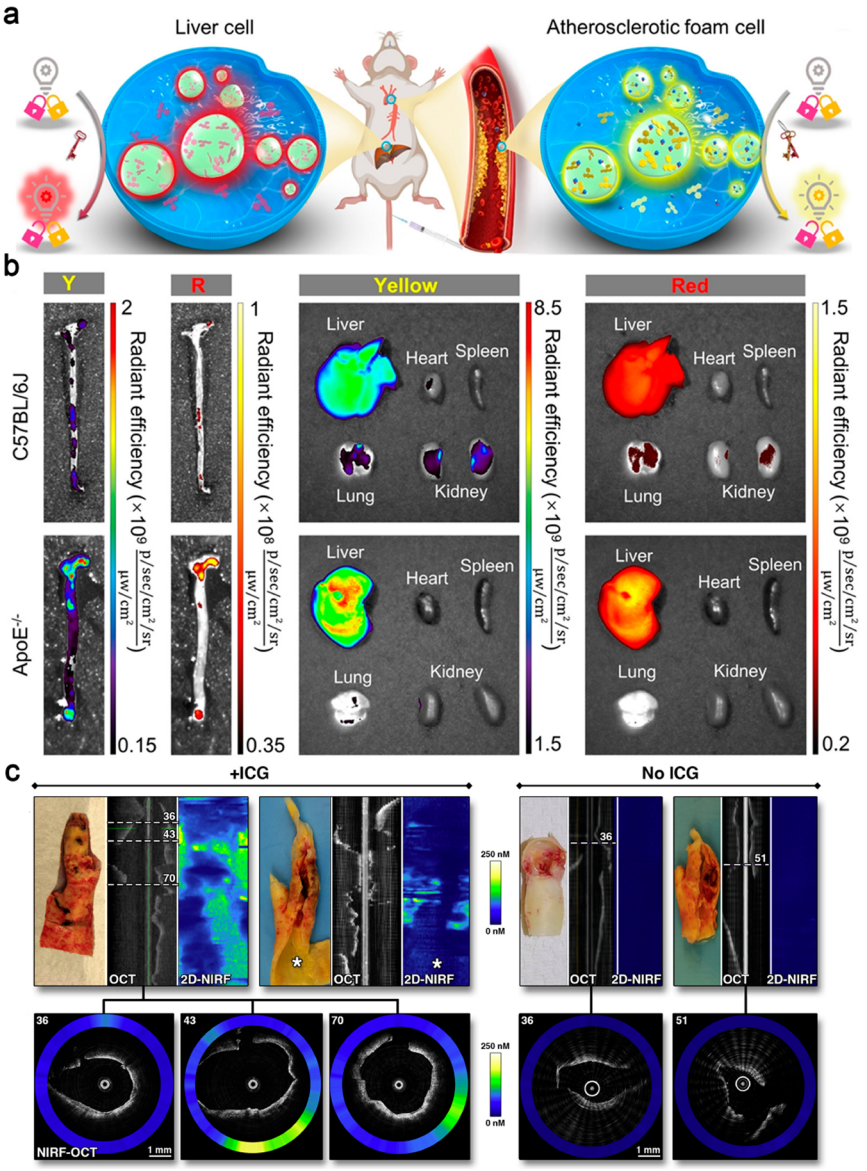

Figure 4. Imaging of atherosclerosis. (a) Illustration of iSHERLOCK for dual responsiveness of lipid accumulation and oxidative stress in the aorta and liver cells. (b) Ex vivo imaging of the aorta and major organs in control and atherosclerotic mice indicated that iSHERLOCK could image atherosclerosis. Panels a and b reproduced with permission from ref 34. Copyright 2022 Wiley-VCH. (c) Representative ex vivo NIRF and OCT imaging of carotid plaques with or without ICG injection in patient. The NIRF and OCT imaging demonstrated that each internal carotid artery plague is localization with elevated ICG signal compared with ICG free patient. Reproduced with permission from ref 36. Copyright 2016 Elsevier.

exposure to drugs, intemperance, viruses, and intestinal microbial metabolites, which often results in liver inflammation as a result of immune response.[38] If left untreated, hepatic inflammation may continue to progress and eventually develop into end-stage liver disease and liver failure.

The detection of liver inflammation can be achieved by using designed fluorescence probes responsive to biomarkers in hepatic inflammation. Su and co-workers reported a hepatocyte-targeted NIRF/PA dual-modal probe (hemicyanine (hCy)-trifluoromethanesulfonyl (Tf)-cholic acid (CA)) for sensitive imaging of upregulated superoxide anion ($O_2^{\bullet-}$) and early diagnosis of hepatic inflammation in vivo.[39] When the probe accumulated in the liver and was exposed to high levels of $O_2^{\bullet-}$, the probe was transformed into the hCy-CA structure with a significant NIRF/PA signal by removing the $O_2^{\bullet-}$-responsive quenching moiety. In addition to superoxide anion, hepatic glutathione (GSH) level is another biomarker in acute hepatitis. A liver-targeted and GSH-responsive trimodal probe (GdNP-Gal) was designed for evaluation of lipopolysaccharide (LPS)-induced acute liver inflammation (Figure 5a).[40] The synthesized GdNP-Gal can be efficiently delivered into the liver and taken up by liver cells through recognition between β-galactose (β-Gal) and the asialoglycoprotein receptor. Finally, GdNP-Gal could image liver inflammation in vivo by integrating molecular coassembly with GSH-driven disassembly.

Apart from biomarkers, the physical markers in inflamed liver are also changed significantly. For example, lipid droplets are significantly less polar in steatohepatitis than in normal livers.[14] Therefore, a fluorescent probe that produces strong fluorescence under low polarity conditions and does not emit fluorescence under high polarity conditions was designed to

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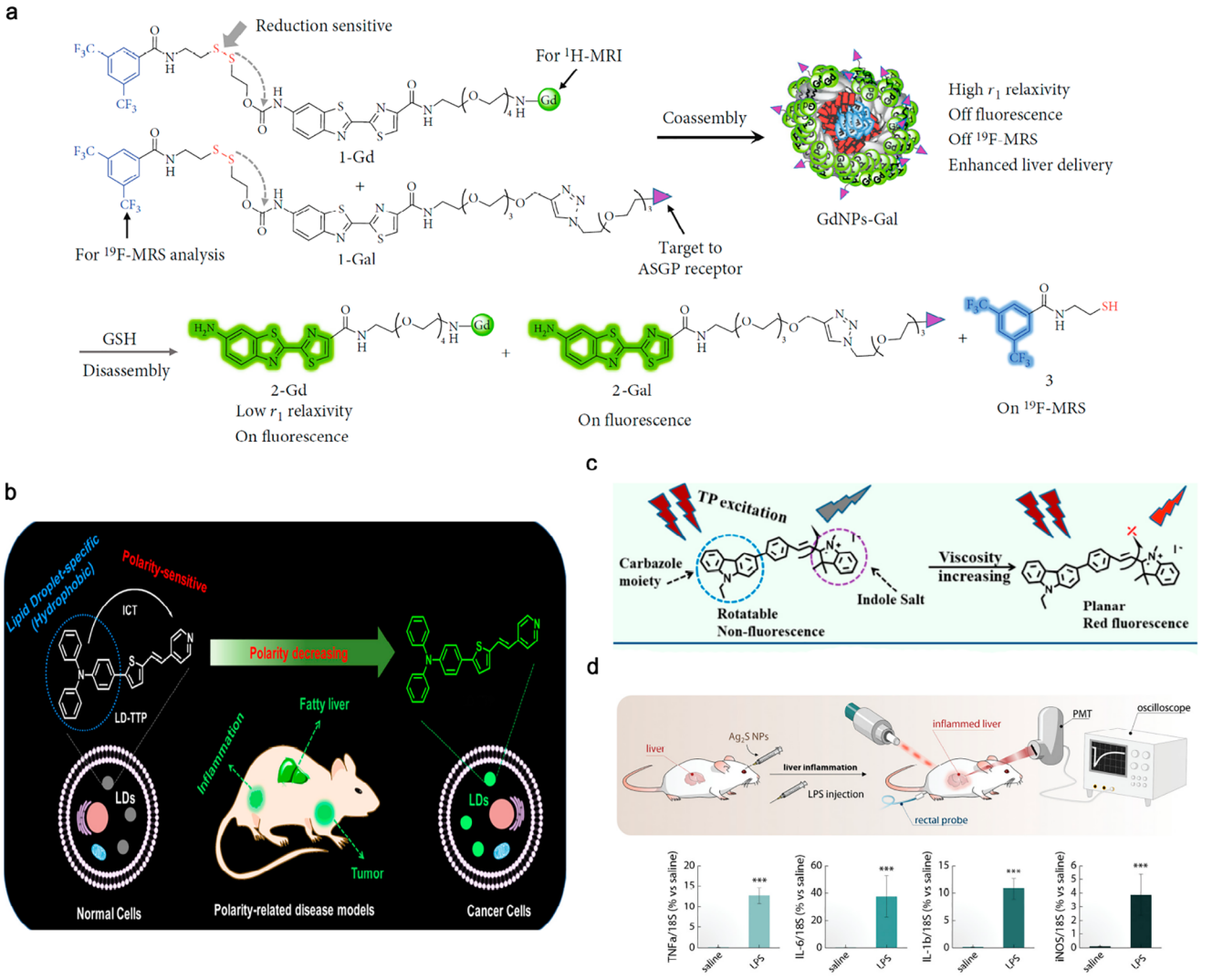

Figure 5. Imaging of inflammation in the liver. (a) Illustration of the design of the liver target and GSH responsive trimodal probe based on the coassembly of 1-Gd and 1-Gal. (b) Design strategy of probe LD-TTP for real time detection of the lipid droplet polarity in steatohepatitis mouse models. Reproduced with permission from ref 41. Copyright 2021 American Chemical Society. (c) Illustration of the design of the Probe CBI-V for monitoring the viscosity of mitochondria and the progress of steatohepatitis in mice. Reproduced with permission from ref 42. Copyright 2019 American Chemical Society. (d) Schematic representation of the experimental procedure designed for real time monitoring of liver temperature during LPS-induced inflammation, and the elevated gene expression of proinflammatory markers in hepatic tissue indicating inflammation in the liver. Reproduced with permission from ref 43. Copyright 2022 Wiley-VCH.

detect the polarity of lipid droplets in steatohepatitis (Figure 5b).[41] The viscosity of mitochondria is also significantly increased in steatohepatitis. Tracking mitochondrial viscosity with a probe that has two-photon excitation and deep red emission could monitor the progress of the steatohepatitis (Figure 5c).[42] Compared with polarity and mitochondrial viscosity changes in inflamed liver, the temperature of liver is one of the first parameters affected by physiological and pathological processes. By utilizing luminescence-lifetime-based nanothermometry, Ximendes and co-workers obtained excellent thermal sensitivity (≈3% °C^{-1} approximately 37 °C) and thermal resolution (less than 0.3 °C) through using Ag_2S nanoparticles as fluorescence lifetime temperature probes (Figure 5d).[43] The Ag_2S nanoparticles could accurately measure the absolute temperature of mouse livers subjected to lipopolysaccharide-induced inflammation and make it possible to monitor inflammation of the liver by temperature.

3.5. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by intense inflammatory and immunological reaction mainly in peripheral joints.[44] Although the anatomical imaging modalities have been improved substantially over the past 10 years, patients and physicians still have to deal with limited information provided by unclear imaging during the progressive course of RA.[45] Molecular imaging in the clinical practice provides a new tool for precise imaging of rheumatoid arthritis.

Early diagnosis and precise imaging of RA can be achieved by detecting the biomarkers in arthritis sites, for example, hypochlorous acid and caspase-1. Based on the $HOCl^-$ triggered C−N double bond cleavage reaction, a "turn on" near-infrared fluorescent probe was designed for real time monitoring of the treatment response of RA (Figure 6a).[46] Similarly, caspase-1-responsive biosensors WEHD-hCy and YVAD-hCy were synthesized through the amidation of

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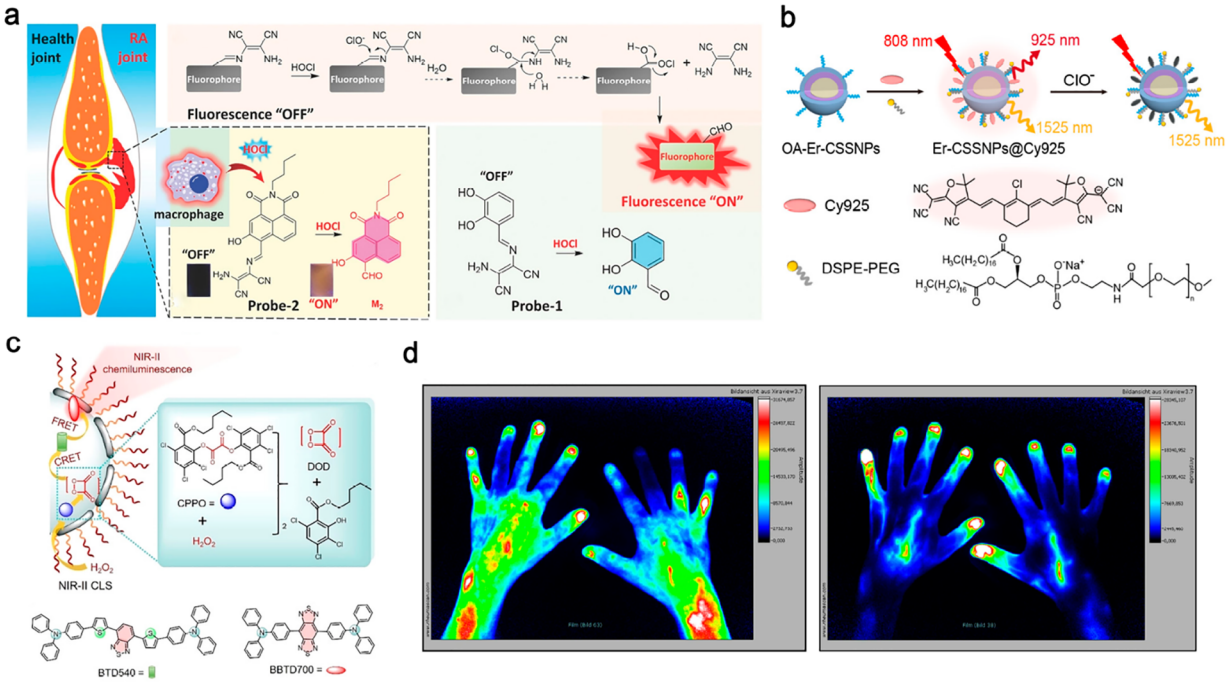

Figure 6. Imaging of rheumatoid arthritis. (a) Illustration of the sensing mechanism and the chemical structure of the "turn on" near-infrared fluorescent probe for monitoring of HOCl-mediated RA. Reproduced with permission from ref 46. Copyright 2018 Wiley-VCH. (b) Illustration of the design strategy of the "turn-off" ratiometric deep-infrared probe for monitoring RA. Reproduced with permission from ref 48. Copyright 2019 American Chemical Society. (c) Illustration of the mechanism for generating NIR-II chemiluminescence sensor emission in the presence of H_2O_2. Reproduced with permission from ref 50. Copyright 2020 Wiley-VCH. (d) Fluorescence optical imaging illustrations of a patient's hands after 52 weeks of treatment showing no increased signal intensities in wrists. Reproduced with permission from ref 51. Copyright 2022 Springer Nature.

caspase-1 cleaved peptide sequences and hemicyanine.[47] The fluorescence signal of the WEHD-hCy and YVAD-hCy could be activated in the presence of caspase-1 for monitoring the inflammasome activation in acute arthritis. Another "turn-off" ratiometric deep-infrared probe based on the cyanine dye (Cy935) is designed to monitor changes in concentration of hypochlorous acid at the site of arthritis (Figure 6b).[48]

To improve the signal-to-noise ratio (SNR) and sensitivity at large imaging depth, Ding and co-workers synthesized a new NIR-II conjugated polymer nanoprobe for NIR-II photoacoustic imaging guided therapy of RA.[49] The synthesized nanoprobe could effectively noninvasively diagnosis RA joint tissue with a high signal-to-noise ratio (SNR) of 35.8 dB in 3D PA tomography images. Apart from fluorescent probes, chemiluminescence that eliminates the demand of excitation light source can also image RA in the second near-infrared window. Zhang and co-workers reported a NIR-II chemiluminescence sensor (NIR-II CLS) based on the peroxyoxalate chemiluminescence system (Figure 6c).[50] The reported sensor can be selectively activated by H_2O_2 under physiological conditions with long duration and deeper penetration depth, allowing for in vivo detection of the arthritic region of the mice.

To monitor the therapeutic response of RA using fluorescence optical imaging, a clinical study using ICG as a contrast agent showed that fluorescence optical imaging technology could provide fast and simple detection in the early stages of anti-TNFα treatment of arthritis compared with clinical outcome parameters, such as DAS28 (CRP), tender joint count (TJC-28), and swollen joint count (Figure 6d).[51] This research is a step forward to the clinic application of optical probes.

3.6. Kidney Injury

Kidney diseases can remarkably impair quality of life and entail expensive treatment cost. Clinical methods for detecting kidney injury include blood biochemical markers and imaging techniques.[52] The early diagnosis of kidney diseases has encountered great difficulties. Therefore, the development of methods for early diagnosis has great clinical significance.

Tabata and co-workers designed an RGD-modified recombinant-gelatin (R-Gel) decorated with a fluorescent molecule (Figure 7a).[53] Following the intravenous administration of fluorescently labeled R-Gel in three different renal disease animal models, R-Gel accumulated well in the inflammation site compared with normal sites. To further improve the spatial resolution and imaging depth of intravital imaging of kidney, Zhang and co-workers developed two kinds of photoacoustic gold nanoparticle (GNP)-based bioprobes with different sizes for noninvasive detection of the early phase of kidney injury by photoacoustic/computed tomography imaging.[54] Delivering bioprobes by tail vein injection in model mice, they found that the probe with 5.5 nm size could be detected in the bladder in the model group with kidney injury and confirmed by computed tomography imaging.

As the kidney is susceptible to injury, acute kidney injury (AKI) remains a critical concern in drug development and clinical care.[55] Given this issue, Pu and co-workers developed a range of hemicyanine-based near-infrared activatable dyes for real-time monitoring and urinalysis in acute kidney injury.[56] They designed different hemicyanine probes targeted to superoxide anion to detect oxidative stress, *N*-acetyl-β-D-glucosaminidase to detect lysosomal damage, and caspase-3 to detect apoptosis in acute kidney injury. All three probes enable real-time imaging of acute kidney injury which is earlier than

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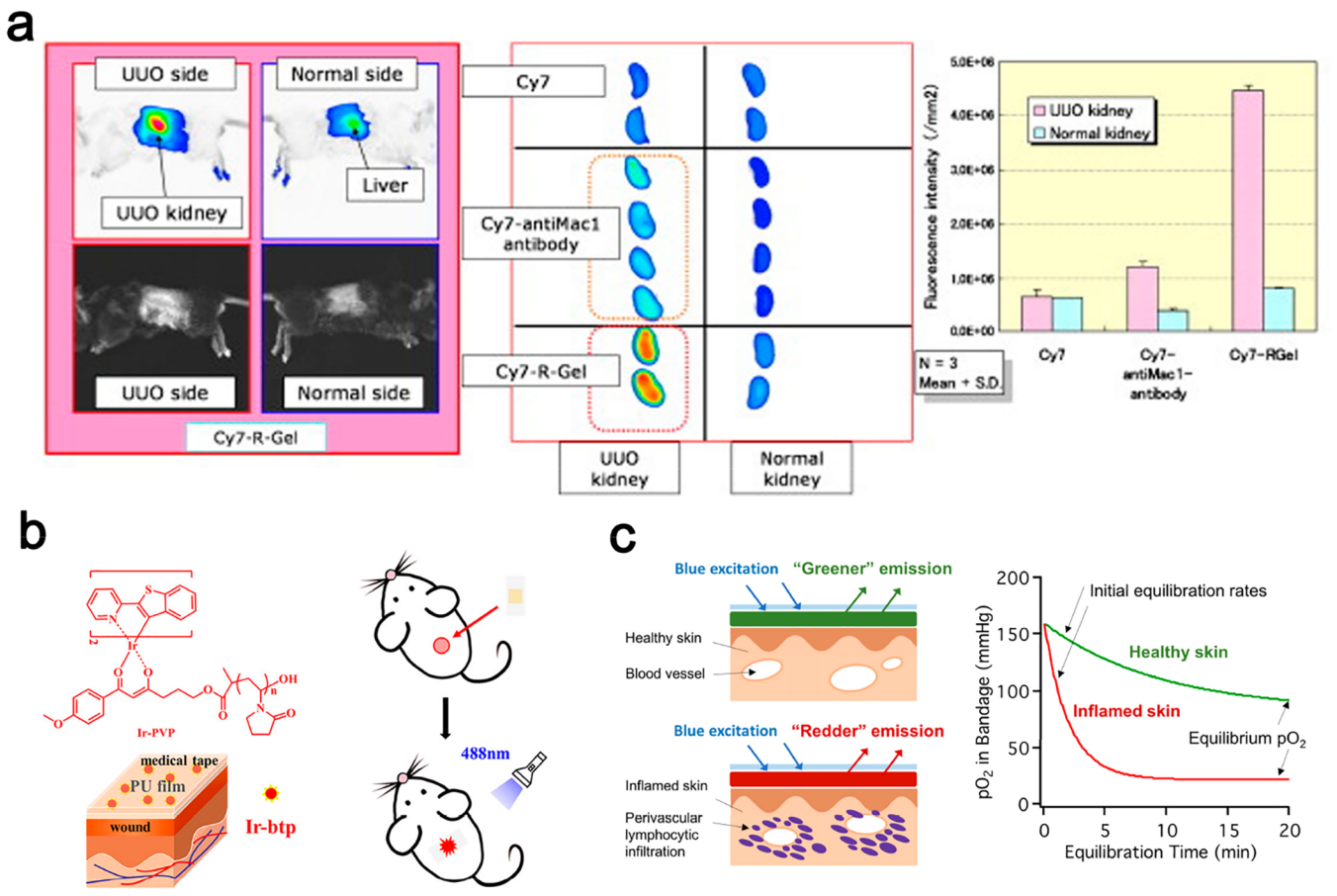

Figure 7. Imaging of kidney disease and skin inflammation. (a) Fluorescence imaging of normal and diseased kidneys with interstitial nephritis. Compared with Cy7 labeled mac1 antibody, Cy7 labeled RGD gel accumulated much more in diseased kidneys in the unilateral ureteral obstruction model. Reproduced with permission from ref 53. Copyright 2010 Elsevier. (b) Illustration of the chemical structure of the hydrophobic small-molecular probe (Ir-btp) and integration of the probe into a polyurethane thin film for monitoring the inflammation of chronic wounds in a mouse model. Reproduced with permission from ref 59. Copyright 2019 American Chemical Society. (c) Illustration of the oxygen-sensing liquid anoxic bandage applied to skin as a liquid and drying into a solid thin film. When the bandage is applied to inflamed skin where oxygen is consumed by perivascular-infiltrated lymphocytes, the bandage shows a lower equilibrium pO_2 and faster initial equilibrium rate.

existing clinical probes in mouse models and the probes might improve AKI management.

3.7. Skin Inflammation

Accurate measurement of tissue oxygen content is decisive for diagnostic applications in burns, limb injuries, and surgical procedures,[57,58] while medical devices used to measure oxygen content require complex electronic and/or chemical techniques that often render the device immobile and expensive.

Our group developed a hypoxia-responsive small molecule probe (Ir-btp) and a water-soluble polymer probe (IrPVP), which both can effectively image inflammation in mice and distinguish healthy areas from inflamed areas in lipopolysaccharide (LPS)-induced mouse models.[59] At the same time, we also doped Ir-btp into a polyurethane film to create a smart bandage for wound inflammation and hypoxia imaging, which can effectively identify the development of inflammation in mouse wound models (Figure 7b). To explore whether iridium complex probes can be used for imaging chronic inflammatory wounds. We synthesized novel hypoxia-responsive probes based on iridium complexes, Ir-fliq and Ir-fliq-PVP. The presence of isoquinoline and fluorene moieties in Ir-fliq red-shifts the emission wavelength of the probe while reducing intermolecular packing and inhibiting self-quenching. We further cross-linked the Ir-fliq with carboxymethyl chitosan (CMCS) and sodium alginate (SA) to form a CSGI hydrogel. In chronic wound models in diabetic mice, CSGI hydrogel enabled the long-term and real-time observation of the hypoxia process in the healing of chronic wounds.[60] Real-time observation of the oxygen content of immune cells at the single-cell level can provide an intuitive way to observe the development of inflammation. Recently, our group designed a ratiometric phosphorescent probe, Ir-GFP, for single-cell detection of macrophage oxygen concentration. After incubation of RAW 264.7 cells with Ir-GFP in a microwells, the ratiometric imaging of the GFP and iridium complex could distinguish hypoxic and normoxic wells with different numbers of immune cells.[58]

Similarly, Evans and co-workers prepared a liquid anoxic bandage through mixing Pd porphyrin hypoxic probe and liquid bandage that could be applied on the skin (Figure 7c).[61] After the inflammatory injection, the bandage can track the increase, smoothing, and decrease in oxygen consumption at the site of injury over a 7-week period and identify inflammation caused by injection at different depths below the surface of the skin. These probes are designed to provide long-term observation of the dynamic inflammatory processes and healing. Evans and co-workers also report a prototype of a

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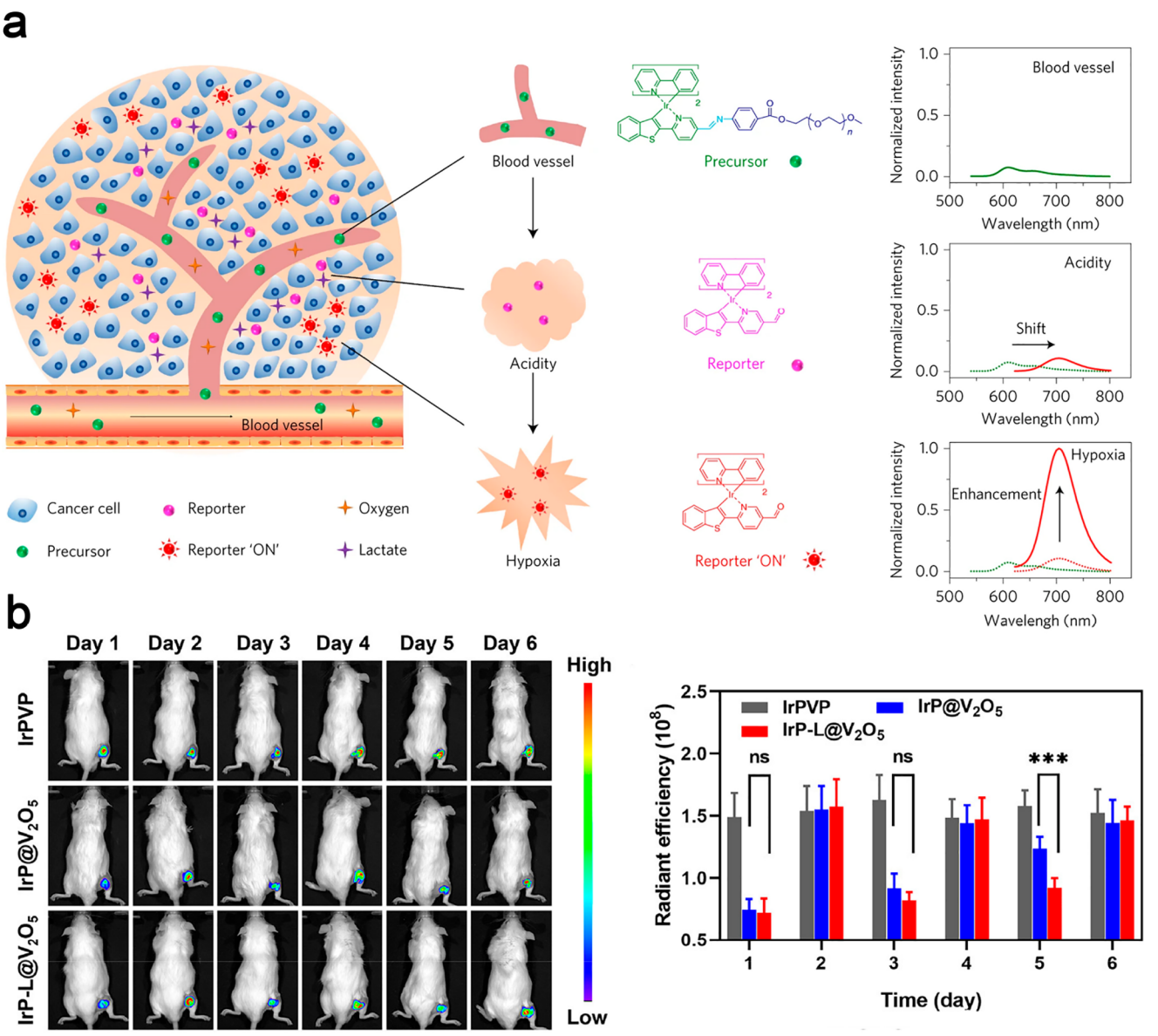

Figure 8. Imaging of cancer. (a) Schematic of the design strategy of the dual-stimuli signal-amplification process of the hypoxia probe. When extravasated from tumor blood vessels, precursors are converted into reporters, accompanied by emission wavelength shifts into the near-infrared range. On subsequent exposure to hypoxia, the reporter's near-infrared emission is "turned on", which results in an enhanced signal intensity in tumor. Reproduced with permission from ref 70. Copyright 2017 Springer Nature. (b) NIR imaging of the hypoxia level of tumors after different treatments on each day demonstrating that IrPVP-based nanoparticles could dynamically monitor changes in tumor oxygen content. Reproduced with permission from ref 71. Copyright 2020 American Chemical Society.

wireless wearable device for skin oxygen monitoring that uses metalloporphyrin as an oxygen monitoring probe and embeds it in a highly breathable oxygen sensing membrane.[62] The device measures the tissue oxygen content on the skin surface by detecting the lifetime and intensity of phosphorescence, providing stable and reliable measurement data even under conditions of temperature and humidity changes. In the porcine ischemia model, the wearable device is highly sensitive to changes in tissue oxygen content within the physiological range when limb perfusion is reduced.

Flaps are common in plastic surgery and are used to reconstruct large tissue defects in situations such as trauma or cancer.[63] However, most tissue oximeters used to monitor postoperative flap ischemia are complex and cumbersome wired devices that hinder direct observation of the post-operative flap.[64] In view of this, Evans and co-workers further mixed the internal standard molecular fluorescein and Pd porphyrin in a liquid bandage. The bandage was then coated on the flap of the patient after flap surgery and isolated from air by a transparent dressing.[65] The change in tissue oxygen content measured by the bandage matched the oxygen saturation measured by the oximeter, demonstrating the reliability and accuracy of the anoxic bandage. Besides, the liquid bandage requires only a traditional digital single-lens reflex (DSLR) camera to monitor changes in oxygen levels in skin tissue in real time for more than 10 days, laying the foundation for simple and fast oxygen monitoring technology in other tissues in the future.

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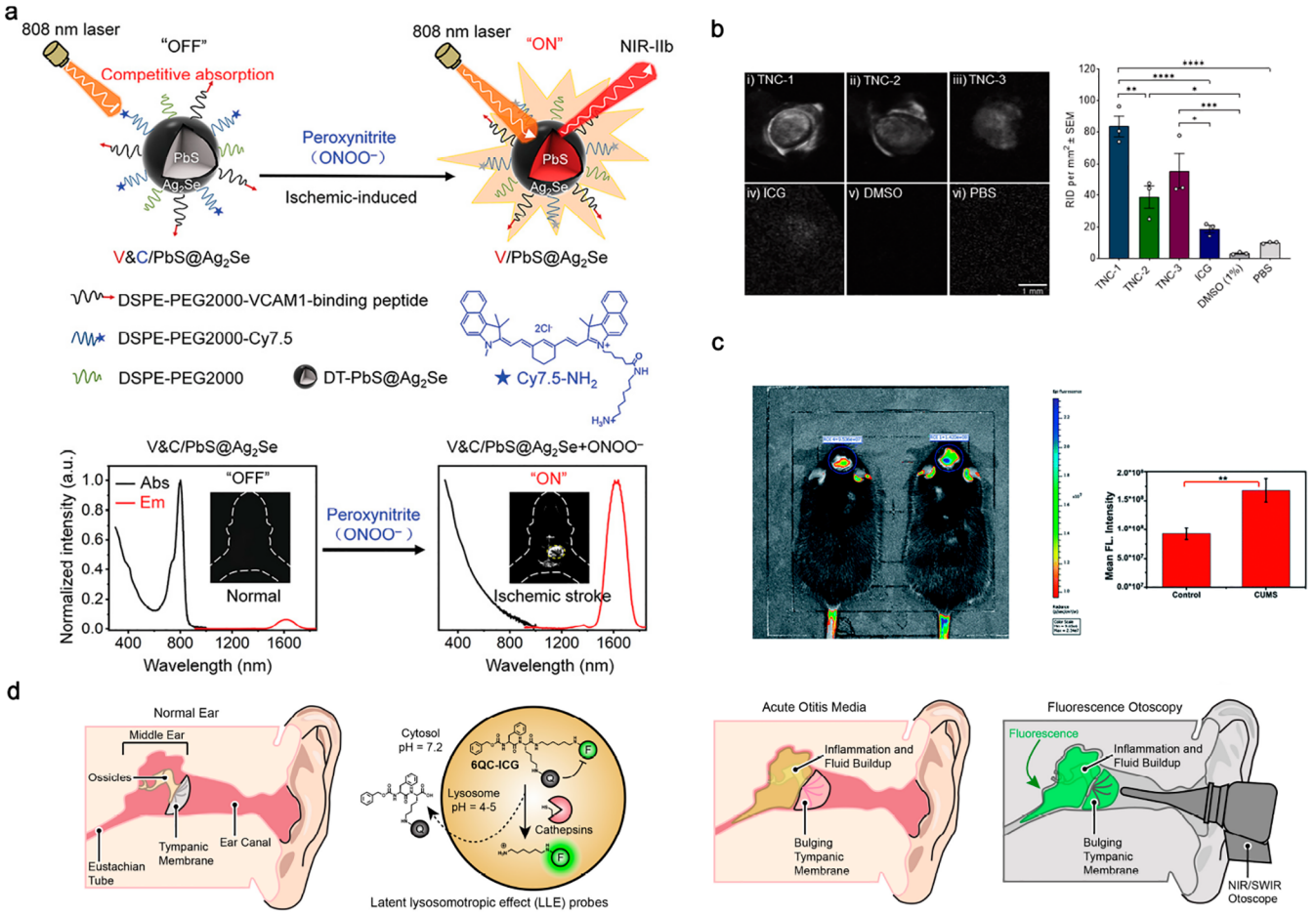

Figure 9. Imaging of inflammation in stroke, eyes, depression, and otitis media. (a) Schematic illustration of the design of the near-infrared nanoprobe which is activated in the presence of $ONOO^-$ and detection of $ONOO^-$ in mice with ischemic stroke. Reproduced with permission from ref 76. Copyright 2021 Wiley-VCH. (b) Clinical ICG angiography imaging of porcine eyes following injection of different treatments into the anterior chamber. The quantification of relative integrated density for each treatment group demonstrated that the synthesized NIR cyanine dyes could characterize inflammation in eyes. Reproduced with permission from ref 79. Copyright 2022 Elsevier. (c) NIR fluorescence imaging and quantitative intensity of ACy7 in control mice (left) and mice with depression-like behaviors (right) revealing that ACy7 probe could image O_3 in the brain of mice. Reproduced with permission from ref 83. Copyright 2019 Royal Society of Chemistry. (d). Illustration of the structure of normal ear and ear with acute otitis media and the chemical structure of the probe 6QC. When the probe enters the lysosome, the latent lysosomotropic effect will result in fluorophore retention in the lysosome upon cleavage by cysteine cathepsins and detection by fluorescence otoscopy. Reproduced with permission from ref 87. Copyright 2020 American Chemical Society.

3.8. Cancer

Inflammation and hypoxia are closely related in cancers, and both of them have significantly oxygen consumption function. Thus, it is difficult to identify them in cancers. Tumor hypoxia is caused by damage to the vascular system and insufficient oxygen delivery due to rapid consumption of oxygen by cancer cells. Hypoxia has also been shown to be associated with tumor resistance to chemotherapy, radiotherapy, and photodynamic therapy.[66−68] Thus, hypoxia is also an ideal biomarker for monitoring tumor progression and response to treatment.

Based on the nature of the hypoxia response of iridium complexes, our group has developed a near-infrared optical imaging probe, IrPVP, with high specificity for the hypoxic tumor microenvironment.[69] This oxygen-sensitive, near-infrared luminescent, water-soluble phosphorescent macromolecular probe not only detects the hypoxic microenvironment of various cancer models, including metastatic tumors, in vivo but also detects a small number of viable cancer cells prior to tumor formation based on increased oxygen consumption during cancer cell proliferation. In order to further improve the sensitivity of this probe, our group designed a macromolecular optical probe that continuously responds to acidification and hypoxia (Figure 8a).[70] Different from the traditional switching probe design principle, this probe uses the movement of the probe signal wavelength caused by the change of the biological environment of the lesion to achieve the amplification of the probe signal. Two-step amplification of tumor microenvironment signals can be effectively achieved through a continuous response to wavelength shift and phosphorescence intensity enhancement. Results from mouse tumor models showed that this two-step continuous response macromolecular optical probe exhibited an order of magnitude higher signal-to-noise ratio in tumor imaging than conventional one-step response optical probes. In a mouse model of tumor liver metastasis, the probe was able to detect tiny tumor metastases smaller than 1 mm in size with high sensitivity. In order to realize the real-time monitoring of the change of oxygen content of tumor tissue, we further used IrPVP to monitor the change of oxygen content during fractioned photodynamic therapy (Figure 8b).

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The results show that the our IrPVP can effectively detect the dynamic change of oxygen content of tumor tissues, which provides a new tool for the dynamic detection of tumor evolution.[71]

3.9. Other Diseases

In addition to the above-mentioned disease inflammation, optical probes can also image some special diseases, such as stroke, otitis media, depression, and eye inflammation.

Stroke is a sudden onset disease of the human brain, which has become the second-leading global cause of death after ischemic heart disease. Nowadays, it is especially important to find a rapid, simple, and nontoxic targeted imaging reagent for early stroke diagnosis.[72] Nanoparticles that use physiological stimulation to improve imaging sensitivity can be used as an innovative diagnostic system for early stroke diagnosis. For example, ROS-sensitive gold nanoprobes (HHAuNPs) are designed to perform near-infrared imaging of ROS levels in ischemic brains.[73] Lee et al. successfully designed several pH-responsive nanoprobes.[74,75] These probes utilize the acidic environment in cerebral ischemia to enable pH-triggered MR or NIRF imaging, thereby targeting and imaging cerebral ischemic regions. Peroxynitrite ($ONOO^-$) is another biomarker of ischemic stroke. In previous study, a near-infrared nanoprobe (PbS@Ag2Se QD) was designed that exhibited fluorescence quenching under normal conditions[76] but was activated in the presence of $ONOO^-$ and illuminated ischemic lesions, making for early diagnosis of stroke possible (Figure 9a).

Intraocular inflammation is a pathological marker of many vision-threatening intraocular inflammatory diseases, collectively referred to as "uveitis" in clinic. Uveitis is a disease mediated primarily by the level of intraocular T cell recruitment and activation, which corresponds to the severity of the disease.[77,78] Hill and co-workers synthesized three triazole NIR cyanine dyes based on the *N*-triazole tricarbocyanine core with or without the inclusion of water-solubilizing carboxylic acid groups at different positions of the scaffold (Figure 9b).[79] The synthesized dyes could effectively detect and characterize activated T-cell activity within the eye inflammation.

Depression is a mental illness that affects people around the world, leading to high mortality and disability rates.[80] Inflammatory mediators such as ozone (O_3) play an important role in the development of depression, and an immunoinflammatory reaction may be an essential cause of the poor efficacy of conventional antidepressant treatments.[81,82] Tang and co-workers developed a near-infrared (NIR) fluorescent probe (ACy7) for the direct visualization of O_3 in mice brains (Figure 9c).[83] The specific cycloaddition reaction between O_3 and the terminal double bond of the butenyl group in ACy7 results in NIR fluorescence emission of the heptamethine cyanine. ACy7 produced strong fluorescence in the brains of depressed mice demonstrating oxidative stress contributing to depression.

Otitis media (OM) or middle ear infections remain one of the most common infections in children worldwide.[84] Traditional otoscopy cannot see the sample of tympanic membrane or middle ear fluid, leading to low diagnostic certainty of OM.[85] Based on the characteristics of high expression of cathepsin in the process of otitis media,[86] Valdez and co-workers designed a biosensor, 6QC-ICG, that can detect the degree of otitis media inflammation by conjugating a fluorescent quenching agent to ICG through a short peptide that can be cleaved by cathepsin (Figure 9d).[87] Using a preclinical model and custom-built short-wave infrared otomicroscope, they successfully distinguished inflamed ears from normal ears with 6QC-ICG.

4. CONCLUSION AND OUTLOOK

Inflammation plays an important role in the occurrence and development of the disease, and it is often appeared when tissues protect themselves. Therefore, real-time detection and dynamic observation of inflammation can provide a reliable basis for understanding and dissecting the occurrence and development of diseases. Optical imaging technology, due to its higher sensitivity and resolution, can provide finer images for the observation of inflammation and molecular events. Meanwhile, there are still some limitations of optical imaging in inflammation. Hence, more efforts are needed to meet the needs of inflammation monitoring before optical imaging technology is widely used in the clinic.

First, tissue scattering and dissipation of light affect the imaging depth of optical probes. New technologies such as fluorescence molecular tomography can image small animals' whole-body and 3D image inflammatory tissues, which partially solves the problem of light penetration depth, but its imaging resolution needs to be further improved. At the same time, some researchers have proposed the use of short-wave infrared lasers or long-wave chemiluminescence to solve this problem. Indeed, short-wave infrared light has a higher penetration depth and a better signal-to-noise ratio than near-infrared light.

Second, most of the existing optical imaging techniques focus on two-dimensional imaging, and rarely achieve three-dimensional imaging. However, if the depth of light penetration is correlated with changes in fluorescence intensity or wavelength, it may be possible to design an optical probe that detects different depths of inflammation for 3D imaging of inflammatory tissue. Imaging of the superficial tissue avoids the problem of depth of penetration of optical probes. Some existing optical probes can indeed enable in situ imaging of skin inflammation. However, the development of new simple and convenient imaging devices or chromogenic materials, such as smart bandages or patches that can detect inflammation in real time, can provide new tools for real-time visual detection of epidermal inflammation.

Due to the high sensitivity of optical probes, the use of optical probes for in vitro detection of inflammation is another promising application direction. According to the biomarkers of different inflammatory tissues, such as alanine aminotransferase in the liver, serum creatinine in the kidney, and inflammatory related factors, optical probe response to specific inflammatory tissue can be designed for portable detection equipment. The use of this device allows people to check their physical condition anytime and anywhere.

Moreover, image processing technology is also important. Optical image processing is a technology that uses optical means and equipment to complete simulation computing processing and image information transmission. In order to objectively measure the imaging quality of systems by characterizing the optical transfer function, spectral analysis and filtering are an important area of research. The use of advanced image processing technology can provide a clearer and more detailed image for the observation of inflammation.

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In general, optical probes have their own advantages and disadvantages for inflammation imaging, and the overall design of optical probes is not complicated and difficult. However, it is still challenging to develop new imaging methods by addressing the disadvantages of optical probes or to magnify the advantages of optical probes and apply them in new scenes.

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Notes

The authors declare no competing financial interest.

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VOCABULARY

bioimaging	methods that noninvasively visualize biological processes in real time and aim to interfere as little as possible with life processes
hypoxia	pathological process in which abnormal changes in the metabolism, function, and morphological structure of tissues occur due to insufficient oxygen supply or oxygen use disorders of tissues.
photodynamic therapy (PDT)	a type of treatment that uses light along with chemicals known as photosensitizers to kill cancer cells
fluorescence	luminescence that is caused by the absorption of radiation at one wavelength followed by nearly immediate reradiation usually at a different wavelength that ceases almost at once when the incident radiation is stopped
phosphorescence	luminescence that is caused by the absorption of radiation (such as light or electrons) and continues for a noticeable time after the radiation has stopped

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