Bioresponsive nanoplatforms for imaging and therapy of cardiovascular diseases

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Abstract
Cardiovascular diseases (CVDs) are still the first cause of death worldwide. Conventional small molecular diagnostic and therapeutic agents generally show limited in vivo detection sensitivity and efficacies, due to their non-targeting distribution and/or low accumulation at diseased sites. New strategies are required to better address the unmet need for the management of CVDs. Bioresponsive nanoplatforms with excellent triggerable release profiles and desirable targeting capability have demonstrated intriguing prospects in detection, prevention, and therapy of CVDs. This review summarizes recent advances in bioresponsive nanoplatforms responsive to biochemical stimuli, such as pH, redox potential, reactive oxygen species, enzymes, and adenosine triphosphate. We highlight the applications of different bioresponsive nanoplatforms for imaging and therapy of typical CVDs, including myocardial infarction, atherosclerosis, thrombosis, ischemic stroke, and heart failure. Finally, existing challenges and future perspectives are also discussed, with respect to the development and translation of bioresponsive nanoplatforms for CVDs.

KEYWORDS
bioresponsive materials, cardiovascular diseases, drug delivery, imaging, nanoparticles

1 INTRODUCTION
Cardiovascular diseases (CVDs) have long been the most common cause of mortality and a major cause of morbidity worldwide.1 Each year, CVDs cause an estimated 17.9 million deaths and result in 31% of all deaths worldwide.2 CVDs are a group of disorders related to the heart and blood vessels, including myocardial infarction (MI), atherosclerosis, restenosis, thrombosis, heart failure (HF), aortic aneurysms, etc. For the treatment of CVDs, many therapeutic agents, such as small molecular drugs, cytokines, and growth factors that act through different mechanisms, are available in the market as traditional formulations. However, poor water solubility,
low pharmacological activity, limited bioavailability, and/or non-targeting capability make them far from satisfactory. On the other hand, various conventional medical imaging modalities have been routinely used for early detection and prognosis of CVDs, such as nuclear magnetic imaging, X-ray angiography, intravascular ultrasound imaging, and optical coherence tomography. Some of these methods, however, are invasive. In addition, for imaging of atherosclerosis that is a major pathological cause of many CVDs, these strategies based on traditional probes cannot accurately evaluate plaque characteristics at the molecular level, and therefore they are unable to assess the stability of atherosclerotic plaques. Molecular imaging has become a rapidly developing research field, since this noninvasive strategy can provide critical molecular and cellular events for early diagnosis, more comprehensive follow-up and assessment of pharmaceutical interventions, and new insight into fundamental mechanisms underlying CVDs. Nevertheless, commercially available imaging probes derived from small molecules typically exhibit rapid elimination as well as relatively low specificity and sensitivity, thereby resulting in unsatisfactory outcomes. Consequently, there are unmet demands for the development of new technologies, strategies, and therapeutic/diagnostic agents to overcome limitations and disadvantages of conventional drugs or probes for CVDs.

In recent years, nanomedicines have received much attention for the management of CVDs. Different types of diagnostic and therapeutic nanomedicines, derived from diverse nanomaterials, have been investigated for imaging and/or therapy of CVDs, such as micelles, liposomes, polymer nanoparticles, and metal nanoparticles. To construct bioresponsive nanoplatforms, different materials with triggerable hydrophobic-hydrophilic changes or responsive hydrolysis/degradation profiles have been developed over the last few decades. We described nanoparticles responsive to pH, redox, ROS, and enzymes in this section, which have been mainly examined for the treatment or imaging of CVDs (Scheme 1).

2.1 pH-responsive nanoparticles

Under pathophysiological conditions, there are distinctly varied pH values in different cellular compartments and tissues. For instance, local acidification is commonly found in the microenvironment of atherosclerotic plaques, thrombus, and cardiac ischemia. Moreover, pH values vary among subcellular organelles. For example, the microcompartments in lysosomes (pH 4.5-5) and endosomes (pH 5.5-6) are moderately acidic, while cytosolic pH is nearly neutral (~pH 7.4). This physiological signal has inspired the development of pH-responsive nanoplatforms that can release drugs or imaging agents in response to pH changes. The protonation of ionizable groups and hydrolysis of acid-labile linkages are most commonly employed strategies to construct pH-responsive nanoplatforms (Table 1).

2.1.1 pH-responsive nanoplatforms based on the protonation strategy

Polymers with ionizable functional groups may protonate in an acidic environment and undergo conformational and/or solubility changes, followed by erosion and payload release at the diseased site. In this regard,
| Types of nanoplatforms                      | Responsive moieties | Nanoparticles                                      | Applications       | Diseases                  | Evaluation models                                      | References |
|-------------------------------------------|---------------------|----------------------------------------------------|--------------------|---------------------------|-------------------------------------------------------|------------|
| Nanoplatforms based on the acid-induced protonation strategy | Secondary amine    | PEG-b-PMNT nanoparticles                          | Therapy            | Ischemic stroke           | In vivo in mice                                       | 37         |
|                                            | Carboxylic acid     | Alginate-cisplatin nanogels                        | Imaging and therapy| Atherosclerosis           | In vitro in J774A.1 cells                             | 39         |
|                                            | β-amino ester       | Fe₃O₄-PEG-PAE micelles                            | Imaging            | Ischemic stroke           | In vivo in rats                                       | 175        |
|                                            | Butylamine          | Fe₃O₄-mPEG-b-(DPA-DE)LG micelles                   | Imaging            | Ischemic stroke           | In vitro in HepG2 cells and in vivo in rats           | 40         |
|                                            |                     |                                                    |                    |                           |                                                       |            |
| Nanoplatforms based on acid-cleavable materials | Acetals             | Ac-CD nanoparticles                               | Therapy            | Atherosclerosis and restenosis | In vitro in RAW264.7 cells, HUVECs, and MOVAS; in vivo in mice and rats | 44–46      |
|                                            |                     | AcDex microparticles and nanoparticles            | Therapy            | Myocardial infarction     | In vitro in rat aortic smooth muscle cells, RAW264.7 cells, KG-1 cells, and human/murine monocytes; in vivo in rats | 114,115    |
|                                            |                     |                                                    |                    |                           |                                                       |            |
|                                            | Ketals              | PCADK microparticles                              | Therapy            | Myocardial infarction     | In vitro in RAW264.7 cells and in vivo in mice        | 48         |
|                                            |                     | PK3 nanoparticles                                 | Therapy            | Myocardial infarction     | In vitro in RAW264.7 cells and in vivo in mice        | 49         |
|                                            | Hydrazones          | [18F]FMK-p(HPMA-co-MHP) nanoprobe                  | Imaging            | Tumor and cardiovascular disease | In vitro in human SQ20B head and neck carcinoma cells | 50         |
|                                            | Imines              | PEG-Diosgenin prodrug                             | Therapy            | Thrombosis                | In vitro in human kidney HK-2 cells and human hepatocyte LO2 cells; in vivo in mice and rats | 140        |

Abbreviations: [18F]FMK-p(HPMA-co-MHP), fluorine-18 labeled fluoromisonidazole ketone-poly(N-(2-hydroxypropyl) methacrylamide)-co- methacryl amidopropan-2-yl 2-(4-(3-hydrazineyl-3-oxopropyl)-1H-1,2,3-triazol-1-yl)propanoate; PAE, poly(β-amino ester); PCADK, poly(cyclohexane-1,4-diy acetone dimethylene ketal); PEG, poly(ethylene glycol).
Nagasaki’s group constructed pH-sensitive nanoparticles (t-PA@iRNP) for neuroprotection and ischemic stroke treatment. This self-assembled nanoplatform is consisted of anionic poly(acrylic acid), a redox polymer of poly(ethylene glycol)-b-poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene] (PEG-b-PMNT) covalently conjugated with 4-amino-TEMPO (an ROS scavenger), and tissue plasminogen activator (t-PA). The mentioned nanoparticles can be collapsed in the acidic ischemic region and release t-PA, due to strong protonation and resultant charge repulsion of the poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene (PMNT) segment. Volumetric swelling facilitated release of t-PA into the thrombus region and initiated thrombolysis, concomitant with exposure of nitroxide radicals capable of eliminating excessive ROS after reperfusion. Ex vivo clot lysis experiments revealed negligible clot lysis by t-PA@iRNP at pH 7.4, while its clot lysis activity remarkably increased at pH 6.2, confirming the pH-responsive bioactivity of t-PA@iRNP. After intravenous (i.v.) administration, t-PA@iRNP significantly improved neurological impairment and decreased cerebral infarct size in mice with ischemic stroke, via thrombolytic and antioxidative effects in the ischemic area. Recently, Bunnett et al. synthesized diblock copolymers with a hydrophobic block of P(DIPMA-co-DEGMA) and a hydrophilic segment of P(PEGMA-co-DMAEMA) to prepare pH-sensitive core-shell nanoparticles. After entering cells by dynamin- and clathrin-dependent endocytosis, tertiary amine moieties of DIPMA (pKa = 6.1) were protonized, leading to charge repulsion and disassembly in mildly acidic endosomes as well as controlled release of the loaded NK1R antagonist aprepitant. Considering the pivotal role of macrophages in the development of atherosclerosis, Hong et al. designed alginate-derived and pH-triggerable nanogels as a theranostic nanoplatform for imaging and therapy of proliferating macrophages in the atherosclerotic area. Iminodiacetic acid and a near-infrared (NIR) fluorophore TTO655 were conjugated to the backbone of alginate, and an antiproliferative drug cisplatin was loaded, which simultaneously acted as a crosslinker to construct a nanogel TANgel. In this case, iminodiacetic
acid was employed to improve cisplatin-binding ability, while pH-sensitivity of TANgel was attributed to carboxylic acids of alginate. Nearly complete cisplatin release was achieved at acidic pH within 2 days, while an extremely slow release profile was observed at pH 7.4. In contrast to behaviors in normal human endothelial cells (i.e., HDMVECn cells), the nanogel was preferably internalized by J774A.1 macrophages, which is beneficial for specific NIR fluorescence imaging and therapy of macrophage-associated diseases. Also, pH-responsive polymers based on 2-(dibutylamino)ethylamine or polypyrrole were synthesized for activatable fluorescence imaging of proliferating macrophages or the cerebral ischemic area.40,41

2.1.2 | pH-responsive nanoparticles based on acid-cleavable materials

Nanoparticles derived from acid-cleavable materials will be collapsed and eventually disassociated in acidic microenvironments owing to the hydrolysis of pH-responsive linkages. The frequently examined pH-labile linkages mainly include acetal/ketal, hydrazones, ortho esters, and silyl ether (Table 2).42 Our group synthesized a library of biodegradable and acid-sensitive acetalated cyclodextrin (CD) materials via kinetically controlled acetonation.43 The pH-labile hydrolysis rate of thus obtained CD materials may be tailored by regulating the acetalation time. Thorough hydrolysis of acetalated β-CD-derived nanoparticles was observed within 24 h at pH 5, leading to discharge of free β-CD and other water-soluble molecules. Moreover, in vitro and in vivo safety tests revealed good biocompatibility of acetalated CD materials. By loading different therapeutic agents, the established acetalated CD nanoparticles can be applied for the treatment of atherosclerosis,44,45 restenosis,46 and tumor.47

Davis’ group synthesized poly(cyclohexane-1,4-diyl acetone dimethylene ketal) (PCADK) by an acetal exchange reaction of 2,2-dimethoxypropane and 1,4-cyclohexanedimethanol. Under acidic conditions, PCADK may degrade into excretable products, that is, acetone and 1,4-cyclohexanedimethanol, without exacerbating existing inflammation. Thus developed polyketal microparticles/nanoparticles loaded with a p38-inhibitor or Nox2-siRNA showed promising efficacy in animal models of MI.48,49 In other cases, linear poly(N-(2-(hydroxypropyl)methacrylamide)) was conjugated with a ketone analogue of fluorine-18-labeled fluoromisonidazole ([18F]FMISO) via acyl hydrazone linkages to enhance the signal-to-noise ratio of hypoxia imaging via positron emission tomography (PET).50 Of note, whereas materials derived from acetics or ketals, with outstanding pH-sensitivity, can be easily synthesized, their in vivo safety profiles remain to be thoroughly assessed in different animal models.

2.2 | Reduction-responsive nanoplatforms

Reduction is another targetable characteristic in the microenvironment of CVDs. The well-studied reduction-responsive linkages are disulfide and diselenide bonds (Table 2), which can be easily broken by glutathione (GSH), thereby causing the degradation of carriers. Nevertheless, reduction-responsive nanoplatforms have been mainly investigated in tumor imaging and therapy, and only a few of them have been evaluated for the management of CVDs. Both GSH and ROS levels are relatively high in specific subcellular compartments and at diseased sites of CVDs, which are typical factors to form redox environments.51–53 Taking advantage of this feature, some reduction-responsive nanoplatforms have been constructed for targeted cargo delivery and/or enhanced diagnosis of CVDs.

Tang et al. designed redox-sensitive photoacoustic imaging nanoprobes based on two kinds of NIR fluorescent agents selective for GSH and hydrogen peroxide (H2O2), for detection of the redox status in atherosclerotic plaques.54 Specifically, two fluorescence probes, that is, Mito-NIRHP for H2O2 and Cy-3-NO2 for GSH, were co-assembled with bovine serum albumin (BSA) via hydrophobic interactions to form a BSA-Cy-Mito photoacoustic nanoprobe. This GSH/H2O2-responsive photoacoustic nanoprobe showed high stability and good biocompatibility in vitro. Photoacoustic signals of the nanoprobe showed significant enhancement as the H2O2 and GSH concentrations increased. Kim et al. developed reduction-responsive poly(oligo-L-arginine) (rsPOLA)-derived nanoparticles to specifically deliver endothelial nitric oxide synthase (eNOS) Deoxyribonucleic acid (DNA) into atherosclerotic lesions.55 rsPOLA was synthesized by oxidative polymerization of Cys-L9R-Cys (L9R, a peptide with the sequence of Leu-Val-Arg-D-Cys-Gly-Lys-His-Ser-Arg) in DMSO-containing phosphate buffer solution. The size of rsPOLA nanoparticles displayed time-dependent changes in the existence of β-mercaptoethanol that can reduce disulfide bonds in nanoplexes. The reductive environment inside endothelial cells also led to disruption of disulfide bonds in reduction-sensitive rsPOLA chains and release of eNOS DNA in vivo. Treatment with eNOS/rsPOLA nanoplexes effectively reduced the levels of typical inflammatory mediators, including monocyte chemotactic protein (MCP)-1, vascular cell adhesion molecule-1, interleukins, and tumor necrosis
TABLE 2 A brief summary of chemical structures of different bioresponsive linkages

| Types of bioresponsive linkages | Linkages | Chemical structures |
|---------------------------------|----------|--------------------|
| pH-responsive                   | Acetal/ketal | ![Acetal/ketal structure](image) |
|                                 | Hydrazine | ![Hydrazine structure](image) |
|                                 | Silyl ether | ![Silyl ether structure](image) |
|                                 | Ortho ester | ![Ortho ester structure](image) |
| Redox-responsive                | Disulfide | ![Disulfide structure](image) |
|                                 | Diselenide | ![Diselenide structure](image) |
| ROS-responsive                  | Thioketal | ![Thioketal structure](image) |
|                                 | Phenylboronic acid/eater | ![Phenylboronic acid/eater structure](image) |
|                                 | Proline oligomer | ![Proline oligomer structure](image) |
|                                 | Polyoxalate | ![Polyoxalate structure](image) |

Abbreviation: ROS, reactive oxygen species.

factor (TNF-α) in activated SVEC4-10 endothelial cells. Correspondingly, systemic injection of eNOS/rsPOLA nanoplexes afforded a 30% reduction in the lesion area of the aortic sinus in comparison to that of the control group. In another case, Fullerton et al. compared in vitro drug delivery capability of poly(lactide-co-glycolide) (PLGA)-block-PEG nanoparticles with two reduction-responsive disulfide-backboned polymeric nanoparticles encapsulating GW-3965, an activator of liver X-receptor (LXR).56 In this study, polyesters RR1 (with a dithiodibutyric acid backbone) and RR2 (bearing a dodecanedioic acid backbone) were synthesized by polymerization of diol groups and diacids, followed by conjugation with PEG. Using a nanoprecipitation/self-assembly method, three different nanotherapies containing GW-3965 were obtained. In vitro studies examined in primary murine bone marrow-derived macrophages showed that therapy with the RR1-based nanotherapy increased the expression of ATP-binding cassette transporter A1 (ABCA1), a LXR target gene, suggesting that reduction-responsive nanoparticles are promising for site-specific drug delivery in CVDs.

2.3 ROS-responsive nanoparticles

ROS play a pivotal role in normal cardiovascular physiology and signaling processes, such as phenotypic regulation, migration, adhesion, and proliferation of vascular smooth muscle cells (VSMCs), endothelial cells, and fibroblasts. Abnormally up-regulated ROS can influence signaling pathways, contribute to autophagy, endoplasmic reticulum stress, and cell apoptosis, and ultimately lead to CVDs, such as MI, atherosclerosis, abdominal aortic aneurysms (AAA), arterial restenosis, and HF. In this regard, ROS-responsive nanoplatforms demonstrate great potential for imaging and therapy of CVDs (Table 3).
| Types of nanoplatforms | Responsive linkages | Nanocarriers | Applications | Diseases | Evaluation models | References |
|------------------------|---------------------|--------------|--------------|----------|------------------|------------|
| Nanoplatforms based on ROS-cleavable materials | Thioketal | PUTK nanofiber | Therapy | Myocardial infarction | In vitro in RAW264.7 cells and in vivo in mice | 61 |
| | HA-TK-Ce6 nanosensor | Therapy | Atherosclerosis | In vitro in RAW264.7 cells and in vivo in mice | 62 |
| | HASF micelles | Imaging and therapy | Atherosclerosis | In vitro in RAW264.7 cells and in vivo in rats | 63 |
| Phenylboronic ester | | OxβCD nanoparticles | Therapy | Atherosclerosis, restenosis, and abdominal aortic aneurysms | In vitro in RAW264.7 cells and VSMCs; in vivo in mice and rats | 44,46,68 |
| | | Nanoplatforms based on ROS-induced hydrophobic-hydrophilic transition | | | | |
| Polyoxyalate | Thioether | PEG-PPS micelle | Therapy | Atherosclerosis | In vitro in RAW264.7 cells and in vivo in mice | 79,80 |
| | | PEGDA-EDT nanofiber | Therapy | Thrombosis | In vitro in erythrocytes, murine fibroblast cells, and HUVECs; in vivo in rats | 79,80 |

Abbreviations: B-PDEA, poly[(2-acryloyl)ethyl(p-boronic acid benzyl)triethylammonium bromide]; COS, chitosan oligosaccharide; CPLB, CREKA-PEG-LysB; FBM, fluorescence dye-conjugated boronated maltodextrin; FTIAN, a fibrin-targeted imaging and antithrombotic nanomedicine prepared from near-infrared (NIR) fluorescent dye-conjugated boronate antioxidant polymers and fibrin-targeting lipopeptides; HA, hyaluronic acid; HASF, a dual ROS-sensitive and CD44 receptor-targeting amphiphilic carrier material (oligomeric hyaluronic acid-[2-[(propylene-2,2-diyl)bis(thio)]diacetylhydroxymethylferrocene]); HPOX, hydroxybenzyl alcohol (HBA) incorporating copolyoxalate; I/R, ischemia/reperfusion; MSNs, mesoporous silica nanoparticles; PEGDA-EDT, a poly(ethylene glycol)-based β-thioetherester copolymer; PEG, poly(ethylene glycol); PHB-dextran, boronic ester-conjugated dextran; PPS, poly(propylene sulfide); PUTK, polyurethane containing thioket; PVAX, polyoxalate containing vanillyl alcohol; TPP, two-photon fluorophore conjugated with prednisolone.
2.3.1 ROS-responsive nanoparticles based on ROS-cleavable materials

ROS-responsive nanoplatforms applied in CVDs are generally prepared using oxidation-degradable materials containing ROS-labile groups such as thioketal, phenylboronic acids/esters, polyoxalates, and proline oligomers (Table 2). Thioketal bonds can be selectively cleaved by 
H2O2 and superoxide, followed by oxidation into ketones and organic thiols (or disulfide).

In view of this distinct feature, Gao et al. developed an ROS-labile polyurethane with thioketal linkages (PUTK), which was synthesized via the reaction of 1,6-hexamethylene diisocyanate, polycaprolactone diol (PCL-diol), and a thioketal linker. ROS-responsive fibrous patches were further prepared using PUTK for triggerable release of glucocorticoid and antioxidant HBA. By chemically conjugating different phenylboronic acid pinacol esters (PBAPs) onto β-CD, a biocompatible cyclic oligosaccharide, a set of ROS-sensitive β-CD (OxbCD) biomaterials were obtained. Notably, H2O2-sensitivity and scavenging capability of OxbCD materials can be easily regulated by the type of PBAP groups, the linkage type, and the number of PBAP units conjugated on β-CD. OxbCDs were further processed into well-defined nanoparticles via a simple nanoprecipitation/self-assembly technique. The established OxbCD nanoparticles containing different therapeutic agents were applied for the treatment of inflammatory bowel disease, atherosclerosis, restenosis, and AAA. Lu et al. synthesized an amphiphilic polymer containing a hydrophilic block PEG and phenylboronic ester-modified polylysine, which can be assembled into ROS-responsive micelles. Targeting efficiency of thus obtained micellar nanoparticles to the ischemic regions was further improved by decorating with a fibrin-binding pentapeptide CREKA. Rapamycin (RAP)-loaded micelles (defined as CREKA-PEG-LysB (CPLB)) prepared by this ROS-responsive material demonstrated prominent H2O2-dependent release curves and ROS-scavenging ability. The micelles notably accumulated in the injured brain and co-localized with CD31, indicating the distribution of thrombus-binding CPLB within the blood vessels. Also, CPLB exhibited significant neuroprotective effects in an oxygen-glucose deprivation/reoxygenation model of SH-SY5Y neuronal cells and also demonstrated successful neuroprotection and improved blood perfusion in a murine model of ischemic stroke. Similarly, an ROS-sensitive material poly[(2-acryloyl)ethyl(p-boronic acid benzyl)diethylammonium bromide] (B-PDEA) was synthesized and examined as a polymeric vector that could mediate effective gene transfection in neural stem cells (NSCs), thereby showing desirable efficacy in the treatment of ischemic stroke.

Polyoxalates are also a type of ROS-labile materials that were initially developed for imaging of H2O2 produced in a lipopolysaccharide-induced peritonitis model, due to high specificity, sensitivity, and deep-tissue imaging capability of polyoxalate-derived self-luminescent probes. Kang’s group reported novel diagnostic and therapeutic polyoxalate nanoparticles based on a polyoxalate copolymer (HPOX). HPOX incorporating an anti-inflammatory and antioxidant HBA would degrade completely into three biocompatible products, that is, HBA, cyclohexanediol, and CO2 in the presence of H2O2. HPOX nanoparticles exhibited desirable effectiveness for imaging H2O2 and targeted drug delivery in murine models of hindlimb and myocardial ischemia/reperfusion (I/R) injury. Also, HPOX nanoparticles showed efficient angiogenic efficacy in a murine ischemic hindlimb model. Likewise, an H2O2-sensitive nanoplatform prepared by polyoxalate containing vanillyl alcohol (PVAX) was constructed for targeted treatment of hindlimb and liver I/R injury and HF. Most recently, our group synthesized a vitamin E-derived peroxalate compound (OVE). By combining with different fluorophores, OVE nanoparticles can generate ROS-responsive long persistent luminescence with tunable emission wavelengths from blue to the NIR light. This type of self-luminescent nanoprobes can be used for in vitro imaging of activated inflammatory cells (such as neutrophils and macrophages) and real-time in vivo imaging of inflammation and disease progression of different inflammatory diseases and tumor with overexpressed ROS in mouse models. Importantly, preliminary in vivo toxicity tests revealed excellent biocompatibility of OVE nanoparticles.

Among different ROS-responsive nanoparticles above mentioned, those based on thioketal-containing materials exhibit desirable ROS-sensitivity only at high levels of H2O2. For nanoparticles derived from ROS-responsive materials containing boronic esters and polyoxalates, they generally show good H2O2-sensitivity even at low levels of H2O2, while the structure-sensitivity relationship of the used materials needs to be carefully elucidated by in-depth studies. Also, the biocompatibility of hydrolyzed products of different ROS-responsive materials should be evaluated for future translation. In addition, the relatively complex structure of some ROS-responsive nanoparticles is disadvantageous for clinical applications.
2.3.2 | ROS-responsive nanoparticles based on materials with ROS-induced hydrophobic-hydrophilic transition

Since hydrophobic sulfides undergo structural transitions to hydrophilic sulfoxides or sulfones upon oxidation, organic sulfides are commonly employed to formulate ROS-sensitive materials based on a solubility switching strategy. For example, Duaval and coworkers synthesized an ROS-responsive material poly(propylene sulfide) (PPS) via ring-opening polymerization of propylene sulfide, which was further produced into microparticles containing an antioxidant and anti-inflammatory drug curcumin via an oil-in-water emulsion solvent evaporation method. Such microspheres could ROS-triggerably release curcumin molecules, notably reduce intracellular ROS levels, and effectively inhibit MCP-1 expression. After local injection, curcumin-PPS microspheres retained in the ischemic hindlimb muscle, remarkably increased hemoglobin oxygen saturation, improved blood perfusion in the ischemic limb, and demonstrated remarkable efficacy in diabetic peripheral arterial disease. In addition, oxidation-responsive micelles were developed through self-assembly of block copolymers containing PPS and PEG. The obtained micelles effectively alleviated oxidative stress and inflammation, thereby affording notable therapeutic effects in atherosclerosis.

2.4 | Enzyme-responsive nanoplatfroms

Enzymes are of great importance for normal metabolic and biological processes. Upregulated expressions of specific enzymes have been observed in the microenvironments of CVDs, such as atherosclerosis, MI, thrombus, and AAA, which can be exploited as stimuli for targeted delivery and triggered release of payloads at sites of action. In this aspect, different enzyme-responsive nanoplatfroms have been constructed, which can be degraded by specific enzymes to activate or expose bioactive components for subsequent diagnosis or treatment. The main mechanisms involve the degradation of short peptide sequences or esters by proteases or esterases. The major predominance of utilizing enzymes to trigger degradation of nanocarriers lies in the biological selectivity and unique specificity of enzymes to their substrates, thereby facilitating targeted and biomimetic payload release.

Thus far, different types of enzyme-responsive nanoplatfroms have been examined for diagnostic imaging and therapy of diverse CVDs (Table 4). Pathologically overexpressed enzymes involved in these systems mainly include matrix metalloproteinases (MMPs), thrombin, hyaluronidase, myeloperoxidase (MPO), β-galactosidase, phospholipase A2, cathepsin K, and plasmin. For instance, MMPs are the most common proteases used as biological stimuli to construct enzyme-sensitive nanosystems. They are a class of proteolytic enzymes that participate in proteolysis of the extracellular matrix (ECM). The overexpression of MMPs is a typical characteristic of many CVDs. In a recent study, Carlini et al. engineered an enzyme-responsive cyclic peptide nanoplatform, which flowed freely during injection and promptly formed hydrogels at the site of MI overexpressing MMP-2/9 and elastase in rats. In this case, cyclic self-assembling peptides were incorporated with the a peptide of Proline-Leucine-Glycine-Leucine-Alanine-Glycine (PLG-LAG) sequence that can be cleaved by MI-related proteases. The non-Newtonian fluid of peptide solution solidified into viscoelastic hydrogel upon triggering by overexpressed proteases in the infarcted heart, due to self-assembly of peptide β-sheets into fibrils. In other studies, by decorating with HA, hyaluronidase-sensitive nanosystems derived from magnetic iron oxide nanoparticles or reconstituted high-density lipoprotein (rHDL) core-shell nanoparticles were constructed for imaging activated macrophages by magnetic resonance imaging (MRI) and targeted therapy of atherosclerosis.

On the other hand, thrombin-sensitive nanosystems were prepared for imaging and treatment of thrombosis or stroke, by incorporating thrombin-cleavable peptide sequences (such as LVPRGS, GGLVPR|GSGGC, and NH₂-norleucine-TPRSFL-CSH) into polymers as crosslinkers. For different enzyme-sensitive nanoplatfroms, the introduced crosslinkers should be stable in the bloodstream and normal tissues, while degrade and release the payload in a well-controlled and highly selective manner after reaching the diseased sites with upregulated levels of specific enzymes. Compared with other bioresponsive nanosystems, enzyme-responsive nanoplatfroms exhibit high selectivity, while their preparation generally involves complicated procedures.

2.5 | Other bioresponsive nanoplatfroms

Besides the aforementioned nanoplatfroms, some other bioresponsive nanosystems have been investigated for imaging and therapy of CVDs. Jiang et al. constructed ATP-responsive rHDL-mimicking nanoplatforms. Ternary polyplexes composed of polyethyleneimine (PEI₃k, i.e., PEI with molecular weight of 1.8 kD), SR-A siRNA, and catalase (CAT) served as the ATP-responsive core, while the outer shell was based on phosphatidylserine-modified rHDL for targeting CD36 and SR-BI, in which pitavastatin was encapsulated. Thus formed nanoparticles
| Enzymes  | Responsive moieties                      | Nanocarriers                      | Applications       | Diseases               | Evaluation models                           | References |
|----------|------------------------------------------|-----------------------------------|--------------------|------------------------|--------------------------------------------|------------|
| MMPs     | PLGLAG                                   | Peptide-polymer amphiphile nanoparticles | Therapy           | Myocardial infarction | In vivo in rats                            | 87         |
|          | SGKPRQITA                                | Cy5.5-SGKPRQITA nanoprobe         | Imaging            | Myocardial infarction | In vivo in mice                            | 163        |
| Thrombin | LVPRGS                                   | Mesoporous silica nanoparticles   | Therapy            | Thrombosis             | /                                           | 92         |
| ACPP     | PFP-ACPP-FTL liposome                    |                                   | Imaging and therapy | Deep venous thrombosis | In vivo in rats                            | 91         |
|          | NH2-LGRMGLPGK-C-SH                       | PEG-thrombin responsive peptide-PCL nanoparticles | Therapy           | Ischemic stroke        | In vivo in mice                            | 104        |
|          | LTPRGWRLGGC                              | tP-NP-rPA/ZL006e nanoparticles    | Therapy            | Ischemic stroke        | In vitro in RAW264.7 cells, PC12 cells, and BCEC cells; in vivo in rats | 146        |
| TAP      | TAP-SiO2@AuNPs                           | Imaging                           | Thrombosis         |                        | In vivo in mice                            | 174        |
| Hyaluronidase | Hyaluronic acid                              | Iron oxide magnetic nanoparticles | Imaging            | Activated macrophages  | In vitro in THP-1, EA.hy926, and LNCaP cells | 96         |
|          | Hyaluronic acid                          | rHDL mimetic nanoparticles        | Therapy            | Atherosclerosis        | In vitro in HUVECs and RAW264.7 cells; in vivo in rabbits | 97         |
|          | Hyaluronic acid                          | HA-LT-rHDL nanoparticles          | Therapy            | Atherosclerosis        | In vitro in rabbits                        | 94         |
|          | Hyaluronic acid                          | Hyaluronic Acid/CPP/siRNA nanoparticles | Therapy           | Atherosclerosis        | In vitro in HUVECs and THP-1 cells; in vivo in mice | 95         |
| MPO      | Luminol                                  | LCD nanoparticles                 | Therapy            | Atherosclerosis        | In vitro in mouse peritoneal macrophages, RAW264.7 cells, and mouse peritoneal neutrophils; in vivo in mice | 98         |
| PhospholipaseA2 | DSPC                                         | PMINs liposomes                   | Therapy            | Thrombosis             | In vivo in mice                            | 100        |
| Cathepsin K | Cathepsin K polyclonal antibody                    | PLGA submicron particles          | Therapy            | Abdominal aortic aneurysms | In vitro in EaRASMCs and RASMCs          | 101        |

Abbreviations: ACPP, activatable cell-penetrating peptide; DSPC, distearylphosphatidylcholine; HA, hyaluronic acid; LCD, luminol-conjugated β-cyclodextrin; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; PCL, polycaprolactone; PEG, poly(ethylene glycol); PFP, perfluoropentane; PLGA, poly(lactide-co-glycolide); PMINs, platelet microparticle-inspired nanovesicles; RASMCs, rat aortic smooth muscle cells; rHDL, reconstituted high-density lipoprotein; TAP, thrombin-activatable fluorescent peptide.
promptly released siRNA upon intracellular triggering by ATP, since the PEI_{1.8k}-derived supramolecule and the oxygen-boosting agent CAT can accelerate intracellular ATP production. Compared with the control, DiR-labeled nanoparticles showed 3.3-fold higher retention in atherosclerotic lesions after i.v. administration. Moreover, 3-month treatment with the ATP-rHDL nanotherapy induced a 65.8% reduction in the atherosclerotic plaque area in apolipoprotein E-deficient (ApoE −/−) mice. To improve the performance of nanocarriers and afford much more specificity and sensitivity to diseased sites of the cardiovascular system, a series of multi-responsive nanoplatforms simultaneously responsive to two or several stimuli have been exploited. As a typical example, our group designed pH/ROS dual-sensitive nanoparticles by incorporating a pH-responsive material a pH-responsive material of acetalated α-cyclodextrin (ACD) and an ROS-responsive material a ROS-responsive material synthesized by functionalizing β-cyclodextrin with an oxidation-labile compound 4-(hydroxymethyl)phenylboronic acid pinacol ester (PBAP) (OCD),\textsuperscript{106} in view of the acidic and oxidative microenvironment in vascular inflammation. In this case, ACD was synthesized by acetalation of β-CD, while OCD was obtained by conjugating PBAP onto β-CD. The hydrolysis behaviors of ACD/OCD nanoparticles (i.e., AOCNPs) were dependent on both pH and H\textsubscript{2}O\textsubscript{2}. By changing the weight ratio of ACD/OCD, the responsiveness of corresponding nanoparticles can be readily regulated. Optimized AOCNPs were packaged with RAP to obtain a dual-sensitive nanomedicine RAP/AOCD NP, which showed notable anti-restenosis effects in rats after i.v. administration. In another work by Murry et al., they synthesized a pH- and temperature-sensitive material poly(N-isopropylacrylamide-co-propylacrylic acid-co-butyl acrylate). The aqueous solution of this copolymer was liquid at room temperature at pH 7.4, while it assembled into hydrogel at 37°C at pH 6.8.\textsuperscript{107} This responsive system provided spatiotemporally controlled delivery of basic fibroblast growth factor (bFGF) to the infarcted myocardium in rats after injection, thereby effectively promoting angiogenesis, enhancing blood flow, and ameliorating cardiac function.

### 3.1 Heart diseases

MI, resulting from occluded coronary arteries, is currently the primary cause of cardiovascular mortality and disability worldwide,\textsuperscript{111} leading to over 2.4 million deaths in America and over 4 million deaths in Northern Asia and Europe.\textsuperscript{112} MI occurs when the nutrient and oxygen sources of the myocardium are reduced by coronary artery blocking. MI can result in thinning of the heart wall, slippage of myocardial cells, and ventricular dilatation, progressively causing massive apoptosis of cardiomyocytes and left ventricular remodeling.\textsuperscript{112}

Extracellular pH values in the region of myocardial ischemia are approximately 6.0–6.5.\textsuperscript{113} In view of the mildly acidic microenvironment in an acute infarct, Davis et al. developed p38-inhibitor SB239063-loaded microparticles for direct cardiac injection, using an acid-sensitive polymer PCADK.\textsuperscript{48} The PCADK-derived pH-responsive microparticles could retain in the myocardium, slowly release loaded drug molecules over weeks, inhibit p38 activation in macrophages, and eventually degrade into 1,4-cyclohexanediol (an indirect food additive approved by FDA) and acetone. In comparison to drug-containing microparticles based on PLGA, a non-responsive and biodegradable polymer, a single cardiac injection of SB239063/PCADK microparticles more remarkably decreased fibrosis and improved cardiac function post-MI, without triggering inflammatory responses. Later, the same group prepared acid-labile polyketal PK3 nanoparticles for site-specifically delivering Nox2 siRNA to the infarcted heart.\textsuperscript{49} Such pH-responsive nanoparticles significantly decreased Nox2 expression and enhanced recovery of cardiac function after intramyocardial injection in MI mice. To regulate the release rate of growth factors in the post-MI heart, Christman et al. fabricated pH-responsive microparticles with three different hydrolysis profiles by utilizing acetalated dextran (AcDex) with tunable sensitivity.\textsuperscript{114} Similarly, dual-peptide functionalized AcDex-based nanosystems were constructed, which showed high endocytosis by M2 macrophages and desirable targeting ability to the infarcted heart tissue.\textsuperscript{115}

On the other hand, the abnormally high level of nicotinamide adenine dinucleotide phosphate oxidase has been detected in the MI region, which is a primary source of ROS in the infarcted heart. The overexpressed ROS play an important role in the initiation of MI and subsequent reperfusion treatment, mainly by affecting cellular homeostatic imbalance, death of cardiomyocytes, local inflammation, and fibrosis.\textsuperscript{116,117} A large number of ROS-responsive nanoplatforms in the treatment of typical CVDs.
of ROS-responsive nanomedicines have been fabricated for site-specific payload delivery and targeted treatment of MI. Wang et al. prepared an injectable hydrogel system containing ROS-sensitive self-aggregated nanodrugs. The hydrogel was formed by an ROS-responsive polymer HB-poly(β-amino esters) (PBAE) and thiolate-modified HA (HA-SH), which was strengthened by tanshinone IIA nanoparticles decorated with a polydopamine layer (TIIA@PDA NPs), owing to chemical cross-linking of PDA and thiolate. Thus established injectable hydrogel showed H$_2$O$_2$-responsive degradation in the injured heart and significantly enhanced cardiac functions by improving ejection fraction and reducing infarction area, along with suppressing the level of inflammatory factors, including TNF-α, interleukin (IL)-1β, and IL-6. Yao et al. constructed oxidation-degradable and methylprednisolone-loaded polyurethane fibrous cardiac patches to treat MI, which showed good intrinsic antioxidant activity in vitro. Implantation of patches in MI rats greatly protected the myocardium from oxidative injury and significantly decreased cardiomyocyte apoptosis. After 28 days of treatment, the ROS-responsive fibrous patches effectively enhanced angiogenesis and cardiac functions as well as significantly inhibited cardiac remodeling, in comparison to the non-responsive fibrous patch.

Considering overproduced enzymes after the initial period of MI, a series of enzyme-sensitive nanoplatforms have been employed to treat MI. A prominent work by Purcell et al. presented MMP-degradable injectable hydrogels based on HA which can be injected into the MI area and release rTIMP-3 (a recombinant tissue inhibitor of MMPs), since the used cross-linker of MMP-cleavable peptide (GGRMSPMPV) can be degraded by active MMPs. Hydrogel formation was verified by MRI when aldehyde and hydrazide functionalized HA blended instantly via a dual-barrel syringe. Intramyocardial injection of MMP-cleavable hydrogels containing rTIMP-3 remarkably decreased MMP expression in the injured area in a porcine MI model, while MMP activity was not changed in the distal myocardium. Further studies demonstrated that localized delivery of the MMP-sensitive hydrogel capable of releasing a recombinant TIMP is a promising strategy to interrupt adverse post-MI remodeling. Nguyen et al. designed enzyme-sensitive micelles assembled by peptide-polymer amphiphiles that can be selectively recognized and degraded by MMP-2 and MMP-9 for specific accumulation and sustained retention in the MI heart tissue. After i.v. injection, the developed nanoparticles underwent a morphological switch from globular materials to a network-like scaffold, in response to overexpressed MMPs in the heart tissue post-MI. The in situ formed scaffold can be maintained at the diseased site for 28 days. Nevertheless, therapeutic advantages of this type of MMP-responsive and transformable nanoparticles remain to be demonstrated. To construct MMP-responsive hydrogels for on-demand release of growth factors in the MI area, Zhao et al. prepared a recombinant protein glutathione-S-transferase (GST)-TIMP-bFGF by combining bFGF, an MMP-2/9-degradable peptide PLGLAG (TIMP), and GST, which was further encapsulated in a GSH-modified collagen hydrogel. The substrate peptide TIMP was degraded by MMP-2/9 after local injection of the hydrogel to the injured site of MI rats, followed by bFGF release, thereby inhibiting MMP activity, enhancing angiogenesis and promoting MI recovery by increasing vascularization and alleviating myocardium remodeling.

HF, caused by left or global ventricular dysfunction, is the most common cardiovascular reason for hospital admission of patients over 60 years old. HF is highly correlated with systemic inflammation and the overproduction of intracellular ROS. In a doxorubicin-induced murine HF model, the levels of ROS, TNF-α, MCP-1, inducible nitric oxide synthase (iNOS), and eNOS were significantly reduced by H$_2$O$_2$-responsive PVAX nanoparticles, in which a bioactive agent vanillyl alcohol could be triggerably released to scavenge intracellular free radicals. In a recent study, captopril-loaded mesoporous silica nanoparticles (MSNPs-Cap) attached with a boronic ester-based H$_2$O$_2$-sensitive probe were studied to ameliorate KillerRed-induced HF in zebrafish. For the heart tissue experiencing contractile failure, increased myocardial H$_2$O$_2$ can react with the probe to dissociate α-CD molecules on the surface of nanoparticles, resulting in captopril release accompanied by “turn-on” of fluorescence. Treatment of HF-bearing zebrafish with MSNPs-Cap notably improved cardiac output and heart rate.

### 3.2 | Atherosclerosis

Atherosclerosis, characterized by arterial wall remodeling, is the major cause of a wide range of fatal CVDs, such as MI, heart attacks, and ischemic stroke. As a chronic inflammatory disease with oxidative stress, atherosclerosis is initiated by endothelial dysfunction as well as retention and accumulation of different lipoproteins in the subendothelial layer. Increasing evidence has demonstrated that massive generation of ROS notably accounts for the pathogenesis of atherosclerosis. Overexpressed ROS promote low-density lipoprotein (LDL) oxidation in the first stage of atherosclerosis, disrupt redox-dependent signaling in the blood vessel wall, and regulate genes correlated with cardiovascular functions. Also, inflamed atherosclerotic lesions are characterized by mildly acidic microenvironments, and pH was found to be around 5.5–7.5
and 6.5–8.5 in rabbit and human atherosclerotic plaques, respectively.31

Taking advantage of the elevated levels of ROS and local acidification in the atheromatous plaques, our group constructed ROS- or pH-responsive nanoplatforms for the treatment of atherosclerosis, by packaging RAP into nanoparticles derived from PBAP-conjugated β-CD or acetalated β-CD.44 Either ROS or pH-responsive RAP release was demonstrated by in vitro tests. Cellular stud-

ies indicated that both nanotherapies could effectively inhibit macrophage migration and suppress foam cell formation. Compared with the non-responsive PLGA nanotherapy, both pH- and ROS-sensitive nanotherapies more notably inhibited the development of atherosclerosis and enhanced stability of atherosclerotic plaques in ApoE−/− mice. Of note, responsive nanomedicines were safe after long-term i.v. administration. Recently, Wang et al. con-

structed a biomimetic and bioresponsive nanoplatform based on macrophage membrane-modified ROS-sensitive nanoparticles containing atorvastatin for atherosclerosis treatment.23 In addition to improved targeting efficiency of nanoparticles to the lesion site, the macrophage membrane can decrease the expression of proinflammation cytokines. This multifunctional nanotherapy demonstrated superior therapeutic effects in atherosclerosis. Also, ROS-responsive micelles loaded with celastrol and andrographolide were engineered to effectively treat atherosclerosis by scavenging ROS and decreasing inflammatory responses.79–80

Since overproduced ROS are involved in the etiology of numerous inflammatory diseases, while existing antioxidants exhibit multiple limitations, our group designed an ROS-sensitive and broad-spectrum ROS-scavenging material by sequentially conjugating Tempol and PBAP onto β-CD.127 The developed bioactive material, defined as a material based on β-cyclodextrin simultaneously conjugated with Tempol and phenylboronic acid pinacol ester; (TPCD), could efficaciously eliminate H2O2, hypochlorite, superoxide anion, and free radical. Correspondingly, TPCD nanoparticles (TPCD NP) displayed potent anti-
inflammatory and antioxidative activity in different animal models. We also found that TPCD NP remarkably mitigated H2O2-induced cell apoptosis and effectively suppressed foam cell formation from either VSMCs or macrophages.128 After i.v. injection, TPCD NP consider-
ablely retained in atherosclerotic plaques by passive targeting and remarkably delayed progression of atheroscle-

rosis in ApoE−/− mice via eliminating excessive ROS, reducing inflammatory responses, and decreasing infil-

tration of inflammatory cells in atherosclerotic lesions. Consequently, ROS-responsive and intrinsically bioactive nanoparticles are promising candidate nanotherapies for atherosclerosis treatment.

In a most recent work, we fabricated a pH-sensitive nanotherapy a pH-responsive anti-miR33 nanotherapy based on acetalated α-cyclodextrin (AAM) NP on acetalated β-CD and PEI1.8k to realize targeted delivery and triggerable release of an antisense oligonucleotide for microRNA-33 (i.e., anti-miR33) in atherosclerotic plaques (Figure 1).45 To further enhance the accumulation of AAM NP in plaques, AAM NP was peripherally deco-

rated with an integrin-binding peptide cRGDFK to pre-

pare an active targeting nanotherapy a pH-responsive and cRGDFK-targeting anti-miR33 nanotherapy based on acetalated α-cyclodextrin (RAAM) NP. After i.v. injection, the constructed anti-miR33 nanotherapies showed notable accumulation in the advanced atherosclerotic lesions of ApoE−/− mice, with considerable endocytosis by macrophages and endothelial cells. Of note, RAAM NP afforded more desirable outcomes. Treatment with anti-

miR33 nanotherapies significantly increased cholesterol efllux by enhancing the expression levels of ABCA1 and ATP-binding cassette transporter G1 (ABCGL), effectively promoted phenotypic switching of pro-inflammatory M1 to anti-inflammatory M2 macrophages, boosted regulatory T cell differentiation, and consequently effectively delayed atherosclerosis progression.

In view of the overexpressed hyaluronidase in the ECM of plaques,129 Liu’s group developed hyaluronidase-

responsive drug delivery systems for atherosclerosis therapy. In this aspect, HA/cell-penetrating peptide (CPP)/siRNA nanoparticles were constructed.45 HA decoration improved targeting efficiency of CPP/siRNA nanoparticles at the impaired endothelium in atheroscle-

rotic lesions by binding to the CD44 receptor, which were subsequently degraded by hyaluronidase in plaques, thereby exposing bare CPP nanocomplexes to facilitate endocytosis by macrophages. LOX-1-specific siRNA-

load nanocomplexes significantly reduced plaque inmmnlation and decreased the lesion area in ApoE−/− mice. Following the similar strategy, the same group developed HA-decorated rHDL nanotherapies with mul-

tiple targeting capability, which significantly reduced atherosclerotic plaque sizes in rabbits.94, 97

3.3 | Arterial restenosis

Restenosis, generally referred as the reobstruction of blood vessels, is a leading cause of the long-term failure rate after percutaneous coronary intervention.130 Considering acidic and oxidative microenvironments in inflamed blood vessels, we previously employed the aforementioned pH- or ROS-sensitive RAP nanomedicine based on modified β-CD materials for arterial restenosis treatment.46 These responsive RAP nanotherapies remarkably inhibited the
proliferation and migration of VSMCs in vitro. After i.v. injection, both pH- and ROS-sensitive nanomedicines more remarkably accumulated at the diseased carotid artery in rats. Consistently, both responsive nanotherapies exhibited better efficacy in attenuating neointimal formation in rats with carotid balloon injury, compared with free RAP, and a PLGA nanotherapy. More recently, we integrated the pH- and ROS-responsive β-CD materials to fabricate dual pH/ROS-responsive nanoparticles (AOCNP) containing RAP (Figure 2). In vivo study demonstrated that i.v. treatment with the dual-responsive nanomedicine RAP/AOCNP showed significantly stronger anti-restenosis effects in rats, when compared with the corresponding single-responsive RAP nanotherapies. Moreover, targeting efficiency of AOCNP at the injured carotid artery can be dramatically increased by surface decoration with a type IV collagen targeting peptide. Correspondingly, thus engineered pH/ROS-sensitive and active targeting nanomedicine displayed additionally enhanced in vivo anti-restenosis efficacy in rats, as compared to RAP/AOCNP.

3.4 | Thrombosis

Thrombosis, regulated by activated platelets and coagulation factors, can occur in any section of arterial or venous circulation, frequently manifesting as deep-vein thrombosis if embolization occurs in the legs or lungs. Thrombosis is characterized by localized blood clotting, which is the proximal cause of MI and ischemic stroke. The clinically available anti-thrombotic drugs, such as heparin, aspirin, and streptokinase (SK) are widely used to treat thrombosis. Nevertheless, the main side effect of these drugs is uncontrolled bleeding.

Thromboembolism is closely related to inflammation and oxidative stress, characterized by overproduction of ROS. In this regard, an ROS-responsive nanotherapy was prepared by heparin conjugated with deoxycholic acid (Hep-DOCA) and PVAX to realize site-specific delivery to blood clots. Using a model drug indomethacin, the Hep-DOCA/PVAX nanotherapy demonstrated H2O2-dependent release profiles. In vitro study also revealed desirable anti-thrombotic
and anti-inflammatory activity of such nanotherapy. Consequently, this oxidation-degradable nanomedicine notably inhibited inflammation-associated coagulation in a mouse tail thrombosis model. Also, ROS-responsive nanoparticle-embedded PCL/gelatin nanofiber scaffold based on PEG-based poly(β-thioether ester) and heparin-MSN loaded with 2-O-α-D-glucopyranosyl-l-ascorbic acid were developed to exert anti-thrombotic and anti-inflammatory effects. In a study by Wang et al., they fabricated antithrombogenic expanded polytetrafluoroethylene blood vessel grafts encapsulated with an ROS-sensitive prodrug PCL/ethyl salicylate (ESA)-oxalyl chloride-mPEG. ESA released from the grafts displayed an ROS-responsive profile. Cellular studies revealed that the grafts could effectively inhibit the formation of thrombosis with improved biocompatibility.

As well demonstrated, insufficient oxygen supply in the peripheral blood of blood clots results in a relatively low pH value (approximately 6.5–6.9) in the microenvironment of thrombus, which provides a chance to develop pH-responsive drug delivery systems for overcoming medical barriers. Wei et al. designed prodrug nanocarriers by linking PEG to diosgenin (a plant sterol saponin) via a Schiff-base bond. As compared to free diosgenin, the prodrug micelles more effectively inhibited platelet activation and efficiently extended activated partial thromboplastin time by regulating factor FVIII activity, thereby affording much better efficacies to treat middle cerebral artery occlusion/reperfusion (MCAO)-induced cerebral thrombosis and FeCl₃-induced venous thrombosis in rats.

During thrombosis, a considerable amount of thrombin is generated to switch fibrinogen to fibrin for prompting platelet aggregation and blood clotting. Thrombin-responsive nanoplatforms have demonstrated benefits in both specificity and effectiveness for thrombosis therapy. Gu’s group developed a thrombin-sensitive microneedles for sustained heparin delivery. In this system, heparin was first conjugated to the HA chain via a thrombin-cleavable peptide GGLVR/GSGGC to obtain TR-HAHP, which was further loaded into a microneedle array. Under the normal blood environment, this microneedle patch safely penetrated the skin tissue, while it rapidly released a corresponding dose of heparin in response to an increased thrombin level. In vivo studies verified efficient long-term...
protection and thrombosis prevention by the TR-HAHP microneedle patch in a model of acute pulmonary thromboembolism. Yang et al. reported a thrombin-responsive phase-transition liposome for theranostic treatment of deep venous thrombosis. The mentioned liposome was incorporated with a liquid perfluoropentane (PFP) that can vaporize into gas by low-intensity focused ultrasound (LIFU). The PFP-loaded liposome was further peripherally modified with a fibrin-binding ligand (FTP) and a thrombin-activatable CPP (ACPP). Upon insonification with LIFU, the PFP-ACPP-FTP liposome produced gas and consequently generated high shear stress to destroy the thrombus. Compared with those of PFP-ACPP and PFP-FTP groups, thrombi in the PFP-ACPP-FTP group displayed the largest holes in thrombus tissue sections, with the deepest penetration depth of approximately 2 mm. Also, in vivo study revealed that this noninvasive nanoplatform exhibited a significant thrombolytic effect in the inferior vena cava thrombosis model of rats. In addition to thrombin, secreted phospholipase A2 (sPLA2), generated by inflammatory cells and activated platelets in the thrombus, has also been applied as a clot-relevant stimulus for the treatment of thrombosis. As a decent example, platelet microparticle (PMP)-inspired nanovesicles (PMINs) were engineered, which can bind to a thrombus by PMP-relevant molecular mechanisms and allow SK (a fibrinolytic drug) release owing to the carrier rupture caused by sPLA2. PMINs were constructed based on a PEGylated liposome platform composed of cholesterol and distearylphosphatidylethanolamine, a known substrate of sPLA2. The active clot-binding ability was achieved by surface-modification of liposomes with EWVDV- and RGD-containing peptide which can selectively bind to P-selectin and integrin GPIIb/IIIa on activated platelets, respectively. After administration of SK-loaded PMINs via the jugular vein, intravital microscopy of the carotid thrombus induced by FeCl3 revealed a significant delay in vessel occlusion, while unmodified SK-loaded vesicles and free SK showed no beneficial effects. Moreover, in vivo studies on safety profiles of SK-loaded PMINs in mice demonstrated minimal adverse effects on bleeding time, thereby posing minimal systemic thrombotic risk.

3.5 Ischemic stroke

Ischemic stroke, resulting from a thrombotic clot and ischemia, has been recognized as a lethal and disabling cerebrovascular disease. Cerebral arterial thrombosis is responsible for 80% of ischemic stroke, and it also causes ischemia and angiostenosis. Conventional therapies cannot effectively inhibit ischemic stroke for their poor capacity to traverse the blood-brain barrier (BBB) and arrive at the injured sites. As well-known, enormous toxic ROS are upregulated in the ischemic region after reperfusion, which can cause neuronal damage. In view of high ROS levels at ischemia-reperfusion sites, Lv et al. fabricated an H2O2-sensitive nanoplatform based on a boronic ester-modified dextran core, coated by a red blood cell membrane engineered with stroke homing peptide (SHp) (defined as SHp-RBC-NP). A candidate neuroprotective agent NR2B9C was packaged in SHp-RBC-NP, which was released rapidly in response to high levels of H2O2 in vitro. Compared with free NR2B9C, the nanoparticle formulation enhanced NR2B9C crossing an in vitro BBB model and protected PC-12 cells from glutamate-induced cytotoxicity. After i.v. injection in MACO ischemic rats, SHp-RBC-NP could efficiently accumulate into the brain, selectively distribute in the ischemic hemisphere rather than the nonischemic hemisphere via active targeting, and significantly ameliorate neurological deficits induced by ischemia-reperfusion. In other cases, NSCs transfected with ROS-labile nanoparticles were investigated for targeted therapy of ischemic stroke (Figure 3). To this end, a cationic polymer B-PDEA was used to condense brain-derived neurotrophic factor DNA (BDNF-DNA) into polyplex nanoparticles for transfection into NSCs. The transfection efficiency of responsive nanoparticles was significantly higher than that of commercially available agents such as PEI, Lipofectamine 2000, and ScreenFect A. In the cytosol, B-PDEA can be hydrolyzed into polyacrylic acid by intracellular ROS, leading to quick release of BDNF-DNA for efficient transcription and secretion of BDNF. After i.v. injection, NSCs transfected with ROS-responsive polyplexes of B-PDEA and BDNF-DNA migrated to the ischemic brain and significantly enhanced BDNF expression, thereby increasing the MACO mice survival rate and enabling superior functional recovery.

Proteases such as thrombin and MMP-9 are highly upregulated in the ischemic brain, which have been utilized for targeted stroke therapy. To distinguish which protease can more efficiently facilitate site-specific delivery of nanoparticles, Guo et al. developed two protease-responsive nanoparticles based on PEG-PCL materials decorated with either an MMP-9-cleavable peptide or a thrombin-cleavable peptide, which were defined as PEG-M-PCL or PEG-T-PCL, respectively. In vivo targeting studies revealed that PEG-T-PCL nanoparticles showed notably higher accumulation in the ischemic region. Further investigation indicated that PEGylated shrinkable nanoparticles exhibited higher delivery efficiency than PEG-T-PCL nanoparticles. By screening a variety of surface decoration ligands that can bind to proteins abundant in the ischemic brain, authors discovered that AMD3100-modified shrinkable nanotherapies displayed the highest targeting ability and remarkably improved...
therapeutic effects of glyburide in the treatment of ischemic stroke, mainly via potentiating binding to CXCR4 overexpressed in the ischemic region. In another work by Xin et al., they constructed a thrombin-specific bioengineered “nanoplatelet” (i.e., tP-NP-rtPA/ZL006e),146 consisted of dextran derivative nanoparticles coated with platelet membrane modified with thrombin-cleavable sequence-coupled recombinant tPA (rtPA), which can release rtPA and a neuroprotectant ZL006e sequentially at the ischemic site. Both in vitro and in vivo studies revealed that this nanoplatelet exhibited remarkably improved BBB penetration capability and potently enhanced the antischismic stroke activity of ZL006e in MACO mice.

### 3.6 | AAAs and other vascular diseases

AAAs, occurring primarily among elderly patients, involve slow and localized dilation of the abdominal aorta.147 Pathologically, AAAs are related to inflammation, matrix degradation, and smooth muscle cell apoptosis.148 Considering notable oxidative stress in AAAs, our group developed ROS-responsive RAP nanotherapies for AAA therapy (Figure 4).68 At the cellular level, the responsive nanotherapies OR NP notably suppressed calcification and decreased ROS-induced oxidative stress and apoptosis of VSMCs. In comparison to PLGA nanoparticles, ROS-responsive OxbCD nanoparticles showed remarkably higher accumulation in aneurysmal aortas after i.v. injection in AAA rats. Moreover, OR NP exhibited better in vivo efficacy by effectively preventing aneurysmal enlargement, attenuating calcification, and suppressing oxidative stress and inflammation. Furthermore, the aneurysmal targeting capability of OxbCD nanoparticles could be remarkably enhanced by functionalization with an integrin binding peptide cRGDK and macrophage cell membrane. Consistently, this biomimetic and bioresponsive RAP nanomedicine more efficiently prevented the progression of AAA. In view of overexpressed cathepsin K in the AAA tissue, enzyme-responsive, doxycycline-loaded submicron particles modified with a cationic amphiphile were engineered by Ramamurthi et al. for AAA therapy.101 Preliminary in vitro and ex vivo studies showed some promising findings for thus established enzyme-responsive delivery system. In other studies, ROS-responsive nanoplatorms were also investigated for peripheral arterial disease therapy,73,78,149,150 demonstrating encouraging results in different animal models.
FIGURE 4 An active targeting and reactive oxygen species (ROS)-responsive nanotherapy for the treatment of abdominal aortic aneurysms. (A) A sketch showing in vivo therapeutic mechanisms of an ROS-responsive rapamycin (RAP) nanotherapy. (B and C) Schematic illustration (B) and a typical transmission electron microscopy image (C) of an ROS-responsive and cRGDK targeting RAP nanotherapy (ROR NP). (D and E) Ex vivo images (D) and quantitative results (E) indicate accumulation of Cy7.5-labeled ROS-responsive targeting nanoparticles (ROCy7.5 NP) or Cy7.5-labeled ROS-responsive non-targeting nanoparticles (OCy7.5 NP) in aneurysmal aortas. (F) H&E, Alizarin Red, and Verhoeff-Van Gieson (VVG)-stained histological sections of aneurysmal aortas. (G and H) Quantitative analysis of the maximal diameter (G) and calcium contents (H) of abdominal aortas. Data are mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001. Reproduced with permission.© Copyright 2018, Elsevier

4 BIORESPONSIVE NANOPROBES FOR IMAGING OF CVDs

Thus far, different imaging techniques have been employed for cardiovascular imaging in the clinic, mainly including fluoroscopy/cineangiography, computed tomography (CT), single photon emission CT (SPECT), ultrasound imaging, PET, radionuclide blood pool imaging, and MRI. Commercially available imaging agents originated from small molecules typically exhibit insufficient circulation time, systemic distribution, and relatively low sensitivity. Therefore, there is an urgent need to explore novel imaging agents with high sensitivity to distinguish pathological tissues from normal ones. The overproduction of ROS and/or proteinases as well as acidic microenvironments provides a basis for discriminating inflamed tissues. In recent years, nanoplatorms or nanoprobes capable of responding to biochemical stimuli in tumor microenvironments have been widely investigated for molecular imaging and accurate diagnosis. Also, different bioresponsive luminescent nanoprobes have been examined for imaging of acute and chronic inflammatory diseases. Nevertheless, currently relative few studies have been focusing on CVDs. Although this field is still in its infancy, some available studies have revealed promising prospects of bioresponsive nanoprobes for real-time, noninvasive detection, and/or diagnosis of CVDs.

4.1 MI

Time-dependent activation of MMPs has been implicated in the cardiac response to ischemia and infarction. Consequently, the myocardial remodeling process can be monitored by imaging MMP activity. Chen et al. reported an MMP-2/9-responsive NIR nanoprobe to examine MMPs activity in the post-MI heart. In this case, a fluorochrome Cy5.5 and PEGylated poly-L-lysine were conjugated via an MMP substrate. Upon proteolytic hydrolysis, release of Cy5.5 led to the NIR fluorescence signal “ON” in the MI area. After i.v. injection of this MMP-responsive nanoprobe in mice with MI, NIR fluorescence imaging revealed the increased MMP expression at the diseased site, with the maximal activity at weeks 1–2 and persisting for 4 weeks. Similarly, Sinusas et al. developed a 99mTc-labeled MMP-targeting radiotracer (i.e., 99mTc-RP805) for noninvasive micro-SPECT imaging of MMP expression and tracing of
MMP-regulated infarct expansion.\textsuperscript{164} \textsuperscript{165} \textsuperscript{99m}Tc-RP805 showed beneficial biodistribution and elimination profiles for cardiac imaging, with 1–3 weeks of retention in the injured myocardium. Moreover, this radiotracer showed three-fold higher accumulation in the infarct heart, as compared to that in the normal heart. In another study, the short and long-circulating MMP-2/9 triggerable imaging probe ACPP and Alb-ACPP (ACPP decorated with an albumin binding ligand) were synthesized. Both dual-isotope radiolabeled nanoprobe displayed notably higher internalization in the injured heart in a Swiss mouse model.\textsuperscript{165} Of note, the enhanced retention was found to be correlated with the gelatinase level.

### 4.2 Atherosclerosis

Pro-inflammatory M1 macrophages facilitate the formation of vulnerable plaques via producing high levels of ROS and secreting inflammatory factors (such as TNF-\(\alpha\), IL-1\(\beta\), and IL-6) in atherosclerotic lesions. Therefore, overexpressed ROS and M1 macrophages are potential molecular and cellular imaging targets for assessing the development of atherosclerotic plaques and evaluating vascular inflammation and efficacy of drugs.\textsuperscript{166}

Park et al. fabricated an NIR fluorescence nanosensor that can specifically detect ROS in M1 macrophages in unstable plaques. The mentioned ROS-responsive nanoprobe was prepared by chemically conjugating chlorin e6 (Ce6) to HA through an ROS-sensitive thiokeetal linker.\textsuperscript{62} Thus established HA-thioketal (TK)-Ce6 nanoprobe emitted strong NIR fluorescence signals following ROS-triggered hydrolysis, enabling effective targeting and imaging of M1 macrophages overexpressing ROS and CD44 in atherosclerotic lesions of ApoE\textsuperscript{−/−} mice. Also, an ROS-responsive theranostic nanosystem with aggregation-induced emission bioimaging capability was constructed for dimensionaldetection and precise therapy of inflammation.\textsuperscript{167} In this study, a diagnosis-therapy compound two-photon fluorophore conjugated with prednisolone (TPP) was first synthesized by bridging a two-photon fluorophore to prednisolone through an oxidation-sensitive linkage oxalate. Subsequently, TPP was packaged into micelles assembled by an amphiphatic diblock copolymer poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)-2-methylthioethanol methacrylate (PMEMA) to form multifunctional nanoparticles (defined as TPP@PMM). After i.v. administration of TPP@PMM, the location of atherosclerotic lesions was evidently visualized by two-photon imaging in ApoE\textsuperscript{−/−} mice. Furthermore, treatment with TPP@PMM afforded impressive anti-atherosclerotic activity by notably reducing inflammatory responses. In other studies, a BSA-based nanoprobe simultaneously responsive to GSH and H\(_2\)O\(_2\) was developed to examine the stability of atherosclerotic plaques by photoacoustic imaging.\textsuperscript{164} This nanoprobe enabled accurate visualization of the redox state relevant to inflammation in oxidized LDL-activated macrophages and in a murine model of atherosclerosis. Furthermore, i.v. injection of this nanoprobe may distinguish unstable plaques at different development stages in plaque-bearing ApoE\textsuperscript{−/−} mice, due to their distinct redox states.

Considering the central role of MPO in acute inflammation, Rutt et al. reported an in vivo MRI agent to image the MPO expression in atherosclerotic lesions in rabbits, in which bis-5-hydroxytryptamidediethylenetriaminepentaacetate gadolinium [MPO(Gd)] was synthesized.\textsuperscript{168} MPO(Gd)-based MRI revealed improved and sustained signal intensities in the atherosclerotic vessel wall, showing more than two-time enhancement of signals in regions with high levels of MPO. Therefore, this strategy could effectively reveal areas with increased MPO activity in rabbit atherosclerotic plaques. Nguyen et al. developed a thrombin-sensitive nanoprobe to detect atherosclerotic lesions.\textsuperscript{169} This responsive probe (DPRSFL-ACPP) was synthesized by incorporating a thrombin-cleavable linker DPRSFL derived from proteinase-activated receptor 1 (PAR-1) into ACPP. Active thrombin in serum cleaved DPRSFL-ACPP, and the product retained in atherosclerotic plaques. Moreover, the fluorescence intensities in mice and ex vivo human atheromas depended on the plaque load that was positively associated with thrombin activity. Accordingly, this approach is feasible for early diagnosis and imaging of severe atherosclerosis in surgery. In addition, the enhanced MMP expression in atherosclerotic lesions can regulate vascular remodeling and subsequent degradation of ECM in the fibrous cap. Consequently, an MMP-2/9-targeting molecular imaging probe, that is, 123I-N-arylsulfonyl iminodiacetyl monohydroxamate ([123I]10c), was fabricated to image unstable atherosclerotic plaques.\textsuperscript{170} This [123I]10c probe showed efficient accumulation in atherosclerotic plaques in ApoE\textsuperscript{−/−} mice after i.v. administration. Further histological examination indicated that [123I]10c mainly distributed in the lesion areas overexpressing MMP-2 and MMP-9. In another study, an MMP-2/9 ligand 18F-labeled (R)-2-(4-(4-fluorobenzamido)phenylsulfonamido)-3-(1H-indol-3-yl)propanoic acid (18F-1) and a specific MMP-9 inhibitor 3H-labeled methyl 4-[3-formylyhydroxyamino]-4-(4-(3-trifluoromethoxy-phenoxy) phenylsulfonyl)buty]l benzoate (3H-2) were evaluated by in vitro autoradiography. The results revealed that both inhibitors could stably bind to atherosclerotic plaques.\textsuperscript{171} Accordingly, these probes can be used as promising agents for non-invasive detection of atherosclerosis.
4.3 Thrombosis

Taking advantage of unique properties of thrombi with overproduced H$_2$O$_2$ and fibrin, Jung et al. constructed a thrombus-specific theranostic nanoagent based on IR780 (an NIR fluorescence dye)-conjugated boronated maltodextrin (FBM). This nanosystem afforded enhanced photoacoustic signals via production of H$_2$O$_2$-triggered CO$_2$ bubbles, which simultaneously exerted anti-thrombotic effects (Figure 5). Such nanotherapy demonstrated time-and H$_2$O$_2$-dependent photoacoustic responses and peaked at 15 min, while IR780-loaded PLGA particles displayed photoacoustic intensities independent of time and H$_2$O$_2$. Subsequently, FBM nanoparticles were decorated with a pentapeptide GPRPP that can target thrombi. Thus obtained T-FBM nanoparticles showed enhanced fluorescence signals at thrombosis sites at 5 min and reached peak at 30 min after injection in a FeCl$_3$-induced arterial thrombosis model. T-FBM nanoparticles revealed notably improved anti-thrombotic efficacy, as compared to FBM nanoparticles. The same group also developed another fibrin-targeting, H$_2$O$_2$-responsive imaging, and anti-thrombotic nanomedicine by conjugating an NIR fluorescence agent IR280 onto an H$_2$O$_2$-eliminating boronate polymer decorated with fibrin-specific lipopeptide, termed a fibrin-targeted imaging and antithrombotic nanomedicine prepared from near-infrared (NIR) fluorescent dye-conjugated boronate antioxidant polymers and fibrin-targeting lipopeptides (FTIAN), for diagnosis and treatment of thrombosis. On the other hand, Kwon et al. fabricated thrombin-sensitive silica-coated gold nanoparticles functionalized with a thrombin-activatable fluorescent peptide (TAP) (TAP-SiO$_2$@AuNPs) for thrombus imaging (Figure 6). In vitro study revealed that TAP-SiO$_2$@AuNPs displayed excellent fluorescence-quenching capability in a normal blood vessel, while NIR fluorescence signals were notably enhanced upon thrombin exposure, owing to the cleavage of TAP. Moreover, thrombotic lesions could be obviously visualized via fluorescence/micro-CT dual imaging in an in situ
thrombotic mouse model after i.v. treatment with TAP-SiO$_2$@AuNPs. In addition, the previously mentioned thrombin-responsive PFP-ACPP-FTP liposomes were used for real-time monitoring of the thrombolysis process by ultrasound and photoacoustic imaging. In vitro imaging tests showed that the photoacoustic intensity was linearly enhanced with the 1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide (DIR) content in DIR-labeled liposomes at 750 nm. After i.v. injection of liposomes and LIFU treatment in a rat model of inferior vena cava thrombosis, the ultrasound B-mode, contrast mode, and photoacoustic intensities were notably enhanced for the targeting liposomes. The maximal contrast was detected at 5 min, and the PFP-ACPP-FTP liposomes showed ultrasound B-mode raising by about three times, contrast-enhanced ultrasound mode by approximately 30 times, and photoacoustic signal enhancement about eight times, which were stronger than those of other liposomes. Collectively, these results demonstrated that this thrombin-responsive nanoplatform can be utilized for real-time monitoring of the thrombolysis process via photoacoustic and ultrasound imaging.

### 4.4 Ischemic stroke

Based on the mildly acidic microenvironment in inflammatory tissues, Lee’s group synthesized a pH-sensitive diblock copolymer containing PEG and acid-labile poly(β-amino ester) (PAE), which was further assembled into polymeric micelles containing superparamagnetic Fe$_3$O$_4$ nanoparticles. The obtained Fe$_3$O$_4$-PEG-PAE nanosystem was stable at physiological pH 7.4, while it could be completely dissolved in the medium with pH < 6.8 owing to the ionization of the tertiary amine groups. Correspondingly, the micelles showed promising MRI signals in detecting acidic pathologic areas, such as the ischemic brain area in MCAO rats and A431 subcutaneous tumors in mice. In addition, the same group developed another pH-responsive nanoplatform based on mPEG-block-poly[dopamine-2-(dibutylamino) ethylamine-L-glutamate] (mPEG-b-P(DPA-DE)LG) for MRI detection of cerebral ischemic area. Fe$_3$O$_4$ nanoparticles were also encapsulated into the micelles by interfacing with the high-affinity anchor dopamine. T$_2$-weighted MRI of the micelles showed increased negative contrast with $r_2$ relaxivity of about 106.7 mM$^{-1}$s$^{-1}$. In an MCAO rat model, MRI contrast signals in the ischemic brain area gradually increased at 1 h post-injection of Fe$_3$O$_4$-loaded pH-responsive micelles. Decrease in the maximal signal was found at 24 h owing to the accumulation of released Fe$_3$O$_4$ nanoparticles in the acidic cerebral ischemic area. Since there is dramatically decreased extracellular Ca$^{2+}$ concentration upon ischemia, Angelovski et al. fabricated a calcium-sensitive MRI nanoprobe for early diagnosis and monitoring of cerebral ischemia. The nanoprobe is based on a bismacroyclic gadolinium(III) complex (Gd$_2$L$^1$) bearing an ethylene glycol tetraacetic acid-derived calcium chelator. This smart contrast agent was able to selectively interact with Ca$^{2+}$, subsequently trigger...
in intramolecular conformational changes, and affect the longitudinal ($T_1$) relaxation time, consequently altering the longitudinal relaxation of tissues and producing Ca$^{2+}$-specific MRI signals. Molecular FMRI results demonstrated that signals gained from $\text{Gd}_2\text{L}^1$ were obviously different from those of an analogous nonresponsive chelate $\text{Gd}_2\text{L}^2$ in rats. The MRI signal intensity of ischemia in rats treated with $\text{Gd}_2\text{L}^1$ immediately reduced owing to the interaction of $\text{Gd}_2\text{L}^1$ with extracellular Ca$^{2+}$, and recovered as the brain tissue was re-perfused. By contrast, no significant changes in MRI signals were detected in rats treated with $\text{Gd}_2\text{L}^2$.

5 | CONCLUSIONS AND OUTLOOK

The clinical management of patients with CVDs has many challenges owing to unique pathoanatomic characteristics of the cardiovascular system. Main limitations of the currently used diagnostic and therapeutic agents involve the mechanistic restriction due to the size of blood vessels or the location of lesions, the indiscriminate distribution, low resolution, and relatively poor specificity and sensitivity in detection and therapy of pathophysiological changes, particularly in the case of acute cardiovascular events. Bioresponsive nanoplates show great promise to overcome above limitations, due to their ability for targeted delivery and triggerable release of drugs and/or imaging probes to diseased sites of CVDs, in response to pathologically relevant stimuli such as increased ROS, low pH, and overproduced enzymes. These advanced nanomedicines have demonstrated excellent performances in noninvasive detection, real-time assessment of therapeutic efficacy, and/or improving efficacies of different types of molecular therapeutics. Furthermore, bioresponsive theranostic nanoplates can also expand our understanding of the development processes of CVDs by facilitating precise visualization and in-depth studies on the key early molecular/cellular events under these conditions.

Despite the above-mentioned achievements, however, few bioresponsive nanotherapies and nanoprobes have been intensively examined in animal models of CVDs thus far. Especially, there are still multiple challenges for the clinical translation of existing bioresponsive nanoplates. First, the specificity of pH- or ROS-responsive imaging nanoprobes and/or nanotherapies are relatively low, since low pH or high ROS also exist in some normal organs/tissues and cells as well as in some normal physiological processes. Therefore, design strategies remain to be optimized to engineer pH- and/or ROS-responsive nanoparticles with high specificity to the diseased sites of CVDs. In particular, new sensitive moieties need to be discovered by the rational design. Whereas enzyme-sensitive nanoplates exhibit high specificity due to pathologically relevant overexpression of specific enzymes, complicated procedures and high cost are generally involved in the synthesis of frequently employed responsive peptide sequences. Currently, enzyme-responsive nanotherapies have been largely examined in the treatment of MI. In comparison to single-responsive nanoplates, multi-responsive ones show higher sensitivity. However, more sophisticated strategies and technologies are necessary for precise regulation of multiple processing variables, in order to engineer effective multi-responsive nanomedicines.

Second, acidification, redox states, enzyme overexpression, and other pathological conditions are varied in different CVDs and at different stages of the same disease, which may restrict the development of bioresponsive nanoplates with precisely controlled on-demand release profiles. In this aspect, the required optimal bio-sensitivity of nanoplates should be carefully elucidated according to the pathogenesis of specific types of CVDs. Third, some common problems such as good quality control, batch-to-batch reproducibility, and cost-efficient mass production should be taken into consideration. Last but not least, pharmacokinetic behaviors and safety profiles of bioresponsive nanoplates are drastically different from those of the corresponding small molecules. Current research primarily focuses on the development of novel bioresponsive nanomaterials, while there are almost no systemic safety evaluations on these multifunctional nanoparticles investigated for preclinical imaging and therapy. More efforts need to be devoted to this promising field to promote clinical applications of bioresponsive nanomedicines in the management of CVDs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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