Intelligent antithrombotic nanomedicines: Progress, opportunities, and challenges

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Abstract
Antithrombotic therapy has long been a trade-off between antithrombotic effects and hemorrhagic risk. With the aid of nanotechnology, antithrombotic drugs have realized and will further realize highly controllable targeting, disease microenvironment responsive release, imaging-guided treatment, and reversal agents’ development in parallel with novel antithrombetics. Preclinical studies have indicated that intelligent antithrombotic nanomedicines show potential in preventing hemorrhagic risk, evading the balancing act of current antithrombotic treatments. However, none of these nanomedicines have come into clinical trials. In this review, we present a strategic summary of the field of nanoantithrombetics, which is needed to guide the design and application of the next generation of antithrombotic nanomedicines. Herein, we present the recent progress in the preclinical studies of this field, which can be divided into three categories: fibrinolytic, anticoagulant, and antiplatelet therapies. In addition, we provide an outlook on how future antithrombotic nanomedicine design can better overcome the current translational challenges for clinical use.

KEYWORDS
anticoagulant therapy, antiplatelet therapy, antithrombotic nanomedicines, bleeding risk, fibrinolytic therapy

1 | INTRODUCTION

Thrombotic events, the causes of death in the majority of cardio-cerebrovascular diseases, directly result from pathological thrombogenesis or thrombus exuviation-induced vascular embolism.¹,² Excessive activation of multiple factors in the coagulation cascade triggers downstream thrombin and platelet activation, alongside the formation of a fibrin network that eventually converts into a thrombus.¹ The newly formed thrombus can partly
or even completely block local blood supply within almost any type of blood vessel. Without immediate medical intervention, thrombosis can progress quickly into ischemia and necrosis of the affected tissues, including in myocardial and cerebral infarction. Based on the embolism location of thrombosis, thrombotic diseases can be generally categorized into arterial thrombosis and venous thromboembolism. Arterial thrombosis, including acute myocardial infarction and ischemic stroke, causes the greatest mortality worldwide. Most arterial thrombosis is caused by a local occlusion of cardiac-derived blood clots. Arterial thrombosis often arises from cardiac diseases including atherosclerosis and atrial fibrillation. Local blood flow occlusion activates the sympathetic nervous system upon the stimulation of the endothelial wall, accelerating the release of thrombosis-inducing components, including 5-hydroxytryptamine and histamine. These thrombosis-inducing components activate platelets and the coagulation cascade to further induce local tissue necrosis. Venous thromboembolism mainly includes deep venous thrombosis and pulmonary embolism. The morbidity rate of venous thromboembolism is much higher than arterial thrombosis, significantly affecting many patients' quality of life. The pathogenesis of venous thromboembolism is more diverse and complicated. To simplify, venous thromboembolism could be induced by abnormal changes in the fibrinolytic and/or pro/antiocoagulant system. In detail, abnormally inefficient fibrinolytic system including the nonfunctional plasminogen and plasminogen activators and the increased plasminogen activator inhibitors; abnormal procoagulant system including the increased prothrombin and fibrinogen, the abnormal fibrinogenemia, and the increased activity of Factor VIII; and abnormal antiocoagulant system including the defecction or decrease in antithrombin, heparin cofactor-II, protein C, and protein S have potential to induce the formation of venous thromboembolism. In addition, endothelial injury can also lead to venous thromboembolism.

In clinical practice, fibrinolytic, anticoagulant, and antiplatelet drugs are the three main options to combat thrombotic diseases, at present. As implied by their descriptions, these three strategies function through the respective elimination of fibrin network, inhibition of coagulation cascade and platelet activation. Several evidence-based clinical guidelines provide instructions for the use of these three classes of antithrombotic treatments (Table 1). Antiplatelet therapy is one of the main choices for secondary prevention of acute thrombotic diseases, such as coronary heart disease and ischemic stroke, and is also an important treatment for ST-segment elevation heart attacks and ischemic strokes. Anticoagulation therapy is mainly used for various types of venous thromboembolism and perioperative administration of various surgical procedures (such as percutaneous coronary intervention) and is also an important option for thromboprophylaxis. Thrombolysis is mainly used for early treatment of acute thrombotic diseases (such as heart attack, ischemic stroke, and pulmonary thromboembolism).

| Diseases/indications | Treatment options |
|----------------------|-------------------|
| Early stage of ischemic stroke (onset time \( t < 4.5 \) h) | rt-PA thrombolysis (preferred), angioplasty, or antiplatelet |
| Early stage of ischemic stroke (6 h < \( t < 24 \) h) | Angioplasty, rt-PA thrombolysis (preferred), or antiplatelet |
| Early ST-segment elevation myocardial infarction | Pre-heparinized PCI (preferred), thrombolysis, or antiplatelets |
| Early non-ST-segment elevation myocardial infarction | Antiplatelets (important means), PCI, CABG, or long-term anticoagulation |
| Pulmonary thromboembolism | rt-PA thrombolysis (preferred), parenteral or long-term oral anticoagulation |
| Secondary prevention of coronary heart disease | Antiplatelets (preferred), antihypertensives and/or statins |
| Secondary prevention of ischemic stroke | Antiplatelets (preferred), statins and/or antihypertensives |
| Venous thromboembolism | Parenteral or long-term oral anticoagulation (preferred) |

Abbreviations: rt-PA, recombinant tissue plasminogen activator; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting.

Under physiological conditions, a healthy endothelium, platelets, coagulation factors, and the fibrinolytic system work in concert to maintain the balance between hemorrhage and hemostasis. During hemostasis, the coagulation components promote the formation of hemostatic plugs; in some pathological cases, when hemostasis is excessively present, a thrombus forms. In other cases, overreaction of the fibrinolytic system or the loss of hemostasis system function may introduce bleeding risk.

Although many thrombotic disease treatments, including antiplatelet, anticoagulation, and fibrinolytic therapies, have been clinically established, these options share the same paramount concern, namely, bleeding risk. Based on recent developments in pharmacogenomic research, genotyping is greatly advocated to improve the efficacy and safety of antithrombotic treatment. Evidences indicate that mutant CYP2C9 and VKORC1...
genotypes significantly prolong the circulation time of warfarin, thus bringing extra bleeding risk. The transformation of clopidogrel into its active form largely depends on the gene polymorphism of CYP2C19. Therefore, genetic tests should be widely used before the application of these antithrombotic drugs.\[15\] However, additional factors beyond genetics contribute to bleeding risk. Most clinically approved drugs targeting the clotting cascade have the potential to cause bleeding, due to their indiscriminate actions on both occluded and normal vessels.\[16\] For decades, large-scale clinical trials have focused on fine tuning the balance between antithrombotic effects and bleeding risk; however, the search for improved strategies to mitigate bleeding complications is still underway.

Recently, the combination of nanotechnology and antithrombotic drugs has provided hope for moving the balance toward the antithrombotic side.\[17\] With the aid of nanotechnology,\[18\] antithrombotic drugs can be made to act only at local, obstructed vessel sites by thrombus-targeting properties\[19\] and disease-specific signal-triggered drug release.\[20\] With controllable pharmacokinetic behavior and high local drug accumulation, both thromboprophylaxis and antithrombotic effects can be significantly improved.\[21\] Moreover, the precise structure design of nanoparticles makes it feasible to monitor their accumulation at thrombotic sites via the integration of various tracing agents.\[22\] In addition, highly specific, corresponding reversal agents can be developed to cease undesirable antithrombotic effects.\[23\] Thus, such a multifaceted strategy can notably lower bleeding risk, while preserving antithrombotic effects. In the current review, we highlight the recent progress of antithrombotic nanomedicines in three categories, namely, fibrinolytic therapy, anticoagulant therapy, and antiplatelet therapy (Figure 1). The design principles, current challenges, and opportunities of antithrombotic drugs are discussed. Finally, future perspectives are provided to stimulate multidisciplinary approaches to tackle the unmet medical needs.

2 | FIBRINOLYTIC THERAPY

For acute vascular occlusion diseases, such as acute myocardial infarction and ischemic stroke, surgical intervention and thrombolysis are recommended as the first-line treatment methods in clinical practice.\[8,31,24,25\] Surgical thrombectomy is highly dependent on the hospital setting and the proficiency of medical teams. This procedure has not yet been popularized in most hospitals, especially those in rural areas.\[26\] Intravenous thrombolysis, by contrast, is the most effective approach to achieve rapid thrombus removal. Fibrinolytic therapy is also a preferential treatment for patients with acute venous thromboembolism, especially pulmonary embolism.\[27\]

Thrombolytic drugs take effect by promoting the translation of plasminogen into fibrinolytic plasmin to directly dissolve the fibrin network that supports the rigid structure of the thrombus, which comprises platelets, red blood cells (RBCs), and leukocytes.\[12\] However, considering the indiscriminate action of fibrinolytics in the circulation, these agents unpredictively degrade existing emboli that maintain the integrity of blood vessels elsewhere in the patient’s body, thus introducing a high risk of hemorrhage in greater than 1% of patients.\[28,29\] The most fatal event, intracranial hemorrhage,\[30\] can occur when fibrinolytics affect the blood brain barrier,\[31\] directly causing intracranial hemorrhage.\[32\] With a delay of onset time of treatment,\[33\] the benefit of surgery or fibrinolytic therapy decreases, whereas the risk of hemorrhage increases. Limited by the relationship between treatment time and risk-benefit ratio, the clinical guidelines\[8,34\] have established strict treatment time windows for both surgical treatment and fibrinolytic therapy.\[24\] In addition, the extremely short half-life of fibrinolytic drugs requires the inconvenience of continuous intravenous drip for several hours.\[29\] On account of the disadvantages of fibrinolytic drugs, such as bleeding risk and short half-life, tenecteplase, a tissue plasmin activator (rt-PA) candidate with a prolonged half-life, was once considered a viable option to avoid bleeding and improve the curative effect.\[35\] Local injection of fibrinolytic drugs was also considered to generate a response specifically at thrombotic sites.\[36\] However, in two independent clinical trials of tenecteplase\[36\] and catheter-directed thrombolysis,\[37\] only noninferiority results were achieved in comparison with normal systemic fibrinolytic drugs. Importantly, fibrinolytic therapy has a high risk of hemorrhage and a shallow applicable treatment site, which leads to a series of contraindications for fibrinolytic therapy to be excluded before the treatment can be applied.\[25\] To broaden the therapeutic window in both time and dose ranges, progress has been made to introduce advanced drug delivery system into fibrinolytic therapy. Considering the complex intravascular environment of thrombi, the combination of targeted delivery and specific biomarker-triggered drug release at the site of thrombus is a potential approach to overcome the obstacles to safe and effective fibrinolytic therapy.

An ideal thrombolysis delivery system should exhibit good biocompatibility in the circulation, bypass the physiological hemostatic clots, and selectively dissolve newly formed thrombi. To these ends, Muzykantov’s group covalently conjugated the fibrinolytic protein, rt-PA, onto the surface of RBCs. As RBCs naturally exist in the blood with a long circulation half-life, this conjugate was
Specific components and/or physicochemical characteristics at the thrombus site can be selected as targeting ligands or responsive triggers for thrombosis-related disease treatment. Typical functional mechanisms of antithrombotic nanomedicines are illustrated below in panels (A-C).

(A) Fibrinolytic therapy mainly applies to acute arterial thrombus consisting of an abundant fibrin network. In this situation, abnormal hemodynamics, such as high shear stress, can serve as responsive signals. Highly activated biomarkers on platelets, such as CD62p and GPIIb/IIIa (CD41/CD61 complex), can also be applied as targeting ligands.

(B) Anticoagulants aimed at inhibiting key intermediates in the coagulation cascade, including Factor Xa and thrombin (Factor IIa), to stop the gradual formation of venous thromboembolism. Due to the slow blood flow, the thrombus is rich in thrombin and its hemodynamics is different from acute thrombus. Therefore, thrombin can serve as both a target and a responsive stimulus for controlled drug release. The negative feedback loop of thrombin can enhance the efficiency and safety of this anticoagulant nanomedicine.

(C) Antiplatelet drugs inhibit platelet activation via binding onto/inside resting platelets. As platelets take a central role in almost all types of thrombus, antiplatelet therapy can be applied in numerous thrombosis-related indications, in contrast to fibrinolytic and anticoagulant therapies. In addition to fibrin networks and abnormal shear stress, antiplatelet nanomedicines can also utilize inflammation or oxidative stress signals, such as peroxides, as responsive signals.

expected to significantly prolong the plasma half-life of rt-PA from <5 to >60 min. Indeed, the rt-PA-carrying RBCs significantly inhibited stable cerebral thrombosis, restored cerebral blood supply, reduced side effects, such as postembolism edema, and reduced bleeding risk, compared with the free rt-PA treatment group. Although RBCs or RBC membrane-coated nanoparticles can serve as a fibrinolytics carriers with high biocompatibility, the formulations still lack active targeting to increase local drug concentration and decrease bleeding risk. Inspired by the pathological behavior of platelets during thrombus formation, researchers have developed platelet membrane biomimetic nanoparticles to target delivery of fibrinolytic drugs to thrombus sites. The use of platelet
Platelet membrane-cloaked nanoparticles have a broad-spectrum thrombolytic effect. (A) Platelet membrane-cloaked nanoparticles efficiently target the thrombus site and exert a thrombolytic effect. (B) Nanoparticles achieve blood flow recirculation for acute pulmonary embolism. (C) Nanoparticles reduce ischemic area. (D) A significant thrombolytic effect was elicited by nanoparticles in a mouse model of mesenteric thrombosis, with prolonged treatment time, as observed by stereomicroscopy. (E) Coagulation indices and bleeding time assays demonstrate a greater safety profile of nanoparticles compared to common thrombolytic drugs. Reproduced from the study by Xu et al. with permission. Copyright 2020, Wiley-VCH

Nie’s group described a platelet membrane-coated nanosystem (PNP-PA) that carries the fibrinolytic drug, rt-PA, to overcome the bleeding risk, narrow treatment window, and short half-life associated with rt-PA administration (Figure 2A). They found that PNP-PA can improve the pharmacokinetic behavior of rt-PA, target drug delivery to the thrombus site via multiple receptors involved in thrombosis on the surface of platelet membrane, and optimize the drug’s pharmacokinetics. This easily prepared nanomedicine elicited an overall improvement of fibrinolytic effects in different mouse models (Figure 2B-D). On account of its targeting ability, PNP-PA exhibited a lower bleeding risk than rt-PA, as evidenced by activated partial thromboplastin time (aPTT), fibrinogen concentration, and bleeding time (Figure 2E). In addition to the need for specific targeting to the thrombotic site, the rapid release and action of fibrinolytic drugs are also required for effective thrombolysis. Some groups have established
different thrombin-sensitive nanomaterials, including albumin nanoparticles and layer-by-layer-assembled polymer complexes, that can specifically release plasminogen activators at the site of thrombus. Shear stress has also been applied as a trigger for the controlled release of fibrinolytics encapsulated in nano/microparticles that can disaggregate under high shear stress at pathological thrombotic sites. External stimuli can likewise elicit a responsive release of fibrinolytic drugs. For example, an Au@Si nanoparticle with a thermal-sensitive myristic alcohol group can responsively release its payload, urokinase (uPA), under near-infrared irradiation, eliciting enhanced thrombolysis in a mouse tail thrombosis model compared to the uPA-treated group.

3 | ANTICOAGULANT THERAPY

The risk factors associated with venous thromboembolism include mainly age, genotyping, surgery, decreased exercise, a history of thrombosis, and malignant tumors. These factors are related to changes in blood composition, causing an imbalance of the blood coagulation/anticoagulation systems. In the early stages of venous thromboembolism, a “rouleau,” a typical RBC aggregation, is gradually formed under relatively slow blood flow velocity and turbulence. If a thrombosis formed in veins breaks into smaller emboli under certain conditions, fatal pulmonary embolism can occur by blockage of the downstream pulmonary artery and capillaries. Anticoagulant drugs are the first choice for the treatment of venous thromboembolism. However, if an acute pulmonary embolism forms, surgical thrombectomy and thrombolysis are also important treatment choices. Anticoagulant therapy mainly includes heparin-based anticoagulants, thrombin inhibitors (e.g., dabigatran), factor Xa inhibitors (e.g., rivaroxaban, apixaban, and edoxaban), and vitamin K antagonists (e.g., warfarin). Among these drugs, heparin is the most general type of parenteral anticoagulant. Heparin is also the first choice for recurrent venous thromboembolism and tumor patients with thrombosis. However, the differences in individual response lead to a differentiated pharmacokinetic behavior of heparin, which can introduce a high risk of bleeding and thrombocytopenia. In addition, heparin is mainly derived from animals and its impurity can further lead to a limited curative effect and undesirable side effects. Decreasing the rates of both hemorrhage risk and thrombocytopenia is an important consideration in the development of heparin-based and other venous thromboembolism treatments. Similar to arterial thromboses, venous thromboembolism therapy also carries potential bleeding side effects. When long-term clinical administration is required, hemostasis parameters, such as aPTT, D-dimer, international normalized ratio, and other blood tests, need to be regularly monitored to dynamically regulate the administration regimen and dosage.

Nanomedicine-based anticoagulants that alleviate bleeding risk have undergone preclinical studies. As most nanomedicines can only be intravenously injected, research on anticoagulants has mainly focused on injectable, heparin-based anticoagulants, polypeptides, and RNA/DNA-based pharmacophores. Almost all anticoagulants ultimately lead to the inactivation of thrombin. Therefore, thrombin activity can serve as an indicator to evaluate the efficiency of anticoagulants. Thrombin-responsive peptides have been widely applied in nanomedicine design to realize controlled release of anticoagulants at thrombotic sites. The released anticoagulants can inhibit the activity of thrombin, which will in turn lower the drug release rate. Such a “negative feedback regulation” of heparin release leads to a significant decrease in bleeding risk for both nanoparticles and transdermal microneedles. Several other synthesized polymers and nanomaterials have been designed as alternative control methods, such as photoreversible gold nanoparticles that have been shown to govern the effects of both heparin and warfarin with comparable efficiency.

With precisely controlled size and shape at the atomic scale, RNA and DNA nanostructures have also demonstrated potent anticoagulant capability in vitro. Engineered DNA/RNA thrombin aptamers proved advantageous both in affinity and specificity in blocking thrombin activity. Moreover, the complementary nucleotides of DNA/RNA origami allow for the generation of corresponding reversal agents, which is important to the safety of anticoagulant therapy. Nucleic acid aptamers screened with high antithrombin activity were self-folded into RNA origami nanostructure. The most exciting thing lies in that once the single-stranded DNA antidotes of the RNA origami were added, significant recovery of both clotting activity (~80% recovery) and clotting time (85 s) was observed, indicating the convenience in reversing its anticoagulant activity by virtue of the precise complementary base pairing between the DNA reversal antidotes and RNA origami nanostructure. The basic principles of reversal agents could be transferred into other platforms such as micelles. When DNA antidotes were added into plasma with anticoagulant self-assembled micelles, dramatic inhibitory effect of anticoagulation activity was observed from 5 min to 5 h. Oral nanomedicines may also be developed for anticoagulant delivery with higher bioavailability compared with present oral anticoagulants. A pH-sensitive chitosan nanoparticle that was stable in a gastric acidic environ-
ment was efficiently absorbed by the intestine to realize controllable release of low-molecular-weight heparin into the blood. Fluorescent sections of the mouse intestinal epithelium showed that the adhesion of the chitosan granules to the intestinal epithelium was significantly enhanced compared to heparin alone; the peak aPTT reached 75.3 s after oral treatment at a dose of 50 mg/kg, and both the peak area under the curve and bioavailability were significantly increased.\textsuperscript{[59]}

4 | ANTIPLATELET THERAPY

Activated platelets play key roles in the pathogenesis of thrombosis-related diseases, including atherosclerosis.\textsuperscript{[60]} Therefore, antiplatelet therapy has become an essential method of prevention or treatment of various thrombosis-related diseases.\textsuperscript{[61]} Meanwhile, the platelet is also one of the most important participants in physiological hemostasis.\textsuperscript{[1]} Therefore, it is particularly important to maximally reduce the influence of antiplatelet drugs on hemostasis while maintaining the inhibition of pathological thrombosis. Antiplatelet drugs mainly include cyclooxygenase (COX-1) inhibitors, phosphodiesterase (PDE) inhibitors, adenosine diphosphate (ADP) inhibitors, and GPIIb/IIIa glycoprotein inhibitors.\textsuperscript{[62]}

Except for COX-1 and PDE inhibitors, most antiplatelet drugs function by binding to the activation-related receptors on platelet membranes (e.g., P2Y\textsubscript{12}) to inhibit the activation pathway. Antiplatelet drugs have been widely used for the treatment of acute myocardial infarction, acute ischemic stroke, unstable angina pectoris, peripheral arterial diseases, and atrial fibrillation; as well as the secondary and tertiary prevention of cardiovascular and cerebrovascular events, such as atherosclerosis; and for the treatment of patients with a history of thrombotic diseases and perioperative patients undergoing major surgical operations, for example, percutaneous coronary intervention (PCI).\textsuperscript{[3,13,61]} The clinical demand for antiplatelet drugs is extremely high and is still growing. In the past 20 years, large-scale clinical trials have proved the effectiveness of antiplatelet drugs. Blockbuster drugs such as aspirin,\textsuperscript{[63,64]} clopidogrel,\textsuperscript{[63,65]} and ticagrelor\textsuperscript{[64,66]} have achieved great success in the antiplatelet market. Many potential antiplatelet drugs targeting various new platelet receptors are also on the way to the market.\textsuperscript{[13]} Nevertheless, the multi-target characteristic of antiplatelets has also revealed that these drugs present a wide range of bleeding risks, including gastrointestinal bleeding, intracranial hemorrhage, symptomatic hemorrhage of major organs, and hemorrhage at surgical sites.\textsuperscript{[67]} The incidence of hemorrhage varies with different drugs, dosages, and different drug combination strategies. Therefore, the risk-benefit ratio of antiplatelet drugs is the most important factor affecting their utilization. Future exploitation of antiplatelet drugs is expected to evolve in three major directions: (1) the development of new antiplatelet drugs (focusing on P2Y\textsubscript{12} and GPIIb/IIIa as drug targets) and the identification of new targets (PAR-1/4, GPVI, PDE, etc.) with the comprehensive assessment of curative effect and bleeding risk;\textsuperscript{[68]} (2) a continuous adjustment of the dosage and regimen of existing drugs (e.g., aspirin, clopidogrel, ticagrelor, tirofiban, and other classic drugs) mainly considering the risk of bleeding; and (3) expanding the indications of antiplatelet drugs, from short-term treatment to long-term treatment for patients with thrombotic risk, in perioperative coverage of PCI, coronary artery bypass grafting, and even noncardiovascular disease-related surgery. In addition, a combination strategy with anticoagulant drugs may also aid in optimizing the benefit-risk ratio.

Because of the associated bleeding risks, the therapeutic effects of the two most widely used antiplatelet drugs, aspirin\textsuperscript{[69]} and clopidogrel,\textsuperscript{[63]} are restricted by their limited dosage, which is lower than the dosage used in preclinical studies.\textsuperscript{[70]} As a result, the drugs’ effectiveness in preventing local vascular necrosis is slightly over 20%.\textsuperscript{[63,69]}

In addition to dose limitation, platelet susceptibility and the thrombogenic environment are also responsible for their limited effect.\textsuperscript{[61]} Although antiplatelet drugs inhibit the transformation of resting platelets to their activated state, they cannot actually realize the reversal of activated platelets back to the resting state. Antiplatelet drugs indiscriminately and systemically weaken the hemostatic ability of platelets, which inevitably increases the bleeding risk when blood vessels are damaged elsewhere in body.

Nanomedicine-based approaches hold great potential for the specific targeting of antiplatelet drugs to the thrombus site. When a thrombus occurs, an alteration of the local microenvironment ensues. Judiciously designed antiplatelet drugs can selectively accumulate at such sites where platelet function can then be selectively inhibited. With this approach, hemorrhage risk can be effectively reduced.\textsuperscript{[62]} Compared with antibody-drug conjugates, antiplatelet nanomedicine is more flexible and variable in the selection of drugs and functional nanomaterials. Antiplatelet drugs prevent the activation of resting platelets, thus indirectly inhibiting fibrin formation. However, antiplatelets are not able to affect the activation state of the coagulation cascade. Therefore, most existing antiplatelet nanomedicines aim at targeting the key components in active platelets and the fibrin network, where the antiplatelet process takes place. One example of this is the coupling of aspirin to RGDV to form an antiplatelet polypeptide self-assembling micelle targeting the GPIIb/IIIa receptor. In a rat carotid artery thrombus
model, the weight of thrombus in the nanoparticle-treated group remained significantly reduced (nanoparticle group, 0.1 nmol/kg, compared with aspirin group, 16.7 μmol/kg). This self-assembling micelle simply realized thrombus enrichment, preventing resistance or desensitization to aspirin.\[71,72\] Considering the complex condition of thrombus, oxidative-responsive nanomaterials may enhance the release profile of nanomedicines.\[73\] Tirofiban-loaded liposomes, modified with a fibrin-targeting peptide along with an \( \text{H}_2\text{O}_2 \)-responsive cleavable peptide, were able to significantly shorten bleeding time while fully recovering the blood flow in a carotid artery thrombosis model.\[74\] Ingber’s group found that “platelet decoys” (Figure 3A), formed from activated platelets themselves after special handling, can also exhibit potent antiplatelet properties. Unlike normal platelets, the platelet decoy is stripped of its thrombogenic properties after removal of most of its inner proteins (Figure 3B), but retains its binding interactions with peripheral cells and is therefore antithrombotic. In a microfluidic blood vessel model, few platelet decoys adhered to the collagen chamber. However, the platelet decoys inhibited platelet activation to a similar degree as the antiplatelet drug, abciximab (Figure 3C). Complete restoration of blood flow was observed in a rabbit model of carotid artery thrombosis (Figure 3D); in vivo, the ability to inhibit platelet aggregation was similar to that of aspirin and abciximab. Platelet decoys are a fast-acting antiplatelet therapy that is also feasible for intravenous infusion in patients with tumor-associated thrombosis, although the current formulations have the disadvantage of a short half-life.\[75\]

5 | DISCUSSION

Current thrombosis treatments include thrombolytic, anticoagulant, and antiplatelet therapies, which have different advantages and indications in clinical practice. The design of intelligent nanomedicines for antithrombotic therapy should carefully reference the clinical principles correspondingly. As mentioned above, although several promising nanomedicine-based antithrombotic drugs have emerged, all of them have a long way to go before clinical use. Intelligent antithrombotic nanomedicines have shown advantages in thrombi-targeted delivery, responsive drug release, optimized pharmacokinetics, and real-time imaging ability in preclinical animal models. To achieve these purposes, different nanoplatforms are explored for drug delivery, including liposomes, self-assembled micelles, nucleic acid origami, polymers, perfluorocarbon nanoparticles, cell membrane cloaking nanoparticles, and so on. The major examples of intelligent antithrombotic nanomedicines are summarized in Table 2. Although there are various nanocarriers designed for antithrombotic drug delivery, all of them share similar basic designing principles, including targeting, drug release, and optimized pharmacokinetics. All these improved properties contribute to the increased efficacy and lower bleeding risk in preclinical studies. Therefore, intelligent antithrombotic nanomedicine brings great hope for the expansion of indications, increases in therapeutic time window, and decreases in contraindication and therapeutic dosages. In this section, we discuss the limi-
| Nanocarriers design                              | Antithrombotic entities | Types               | Intelligent features (disease targeting or drug release)                                                                                                                                                                                                 | Refs.                  |
|------------------------------------------------|-------------------------|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| Cationic peptide/PEG self-assembled micelles   | Heparin                 | Anticoagulation     | Thrombin-responsive peptide release of heparin, involved in thrombin degradation; “self-titration” negative feedback regulation                                                                                                                                  | [56]                    |
| Hyaluronic acid hydrogels in transdermal microneedles | Heparin                 | Anticoagulation     | Thrombin-responsive peptide-responsive breaks release heparin                                                                                                                                                                                                 | [57]                    |
| RNA origami                                     | Antithrombin RNA fragment designed by computer | Anticoagulation     | RNA aptamer fragment-specific binding to thrombin                                                                                                                                                                                                                                                                  | [23]                    |
| Amphiphilic polymer micelles                    | Polymeric g-quadruplex folding antithrombin fragment | Anticoagulation     | RNA aptamer fragment-specific binding to thrombin                                                                                                                                                                                                                                                                  | [54]                    |
| Gold nanoparticles                              | Antithrombin polymer structure | Anticoagulation     | Thrombin-binding DNA aptamer fragments coupled to nanogold via self-assembled block polymers on the nanogold surface                                                                                                                                   | [58]                    |
| Perfluorocarbon nanoparticles                   | PPACK peptide           | Anticoagulation     | High-affinity inhibition of thrombin by PPACK peptide                                                                                                                                                                                                     | [55]                    |
| Chitosan nanoparticles                          | Low-molecular-weight heparin | Anticoagulation     | pH-sensitive chitosan stable in a gastric acidic environment, absorbed by the intestine and adheres to the mucous membrane, achieves slow release of low-molecular-weight heparin.                                                                 | [59]                    |
| Phospholipid self-assembled micelles            | Tirofiban               | Antiplatelet        | Fibronectin-targeting peptide, hydrogen peroxide responsive borate polymer                                                                                                                                                                                   | [74]                    |
| Peptide self-assembled micelles                 | Aspirin                 | Antiplatelet        | Aspirin-coupled RGDV peptide, targeting GPIIb/IIIa receptor                                                                                                                                                                                               | [72]                    |
| Platelet decoy                                  | Platelet decoy          | Antiplatelet        | Platelet decoys lose their thrombogenic properties after removal of internal proteins but retain their binding interactions with peripheral cells and are thus antithrombotic.                                                                                       | [75]                    |
| Liposome                                        | Eptifibatide            | Antiplatelet        | The Brij76 fragment on liposomes is characterized by a shear-response release of the drug eptibatide.                                                                                                                                                      | [47]                    |
| Phospholipid polymer self-assembled predrug     | Aspirin                 | Antiplatelet        | GPRPP fibronectin-targeting peptide                                                                                                                                                                                                                       | [88]                    |
| Hydrogel                                        | rt-PA                   | Fibrinolysis        | Thrombin-responsive peptide for rt-PA release                                                                                                                                                                                                               | [89]                    |
| Albumin nanoparticles                            | rt-PA                   | Fibrinolysis        | GPIIb/IIIa targeting peptides for thrombus targeting thrombin-responsive peptide for rt-PA release                                                                                                                                                          | [44]                    |
| Polymer core-shell nanostructure                 | uPA                     | Fibrinolysis        | GPIIb/IIIa targeting peptides for thrombus targeting thrombin-responsive peptide for rt-PA release                                                                                                                                                          | [45]                    |
| Pharmacoprotective electrostatic polymer shell layer | rt-PA                   | Fibrinolysis        | Thrombin-responsive peptide                                                                                                                                                                                                                               | [46]                    |

(Continues)
TABLE 2 (Continued)

| Nanocarriers design | Antithrombotic entities | Types | Intelligent features (disease targeting or drug release) | Refs. |
|---------------------|-------------------------|-------|--------------------------------------------------------|-------|
| Gold core mesoporous silicon nanoparticles | uPA | Fibrinolysis | Myristol temperature-controlled phase change causes nanoparticles to disintegrate and release encapsulated uPA. | [49] |
| Long-circulating hybridized liposomes | SK | Fibrinolysis | P-selectin targeting peptide, RGD peptide (targeting GPIIb/IIIa), DSPC phospholipid (for leukocyte sPLA2) degradation | [50] |
| RBCs | rt-PA | Fibrinolysis (Thromboprophylaxis) | Red blood cell surface-coupled rt-PA for prolonged circulation and relative selectivity of newly formed thrombi | [39] |
| Self-assembled micelles | MTCA pharmacophore | Fibrinolysis | RGDV thrombus-targeting peptide | [91] |
| Fe₃O₄@SiO₂/SDS core | rt-PA | Fibrinolysis | Magnetic targeting and ultrasonic-activated release | [92] |

...tions and existing obstacles of the current antithrombotic nanomedicines and discuss possible directions for more intelligent designs to bring this field closer to the clinic.

Antithrombotic nanomedicines may be further refined by optimizing their modifications and particle morphology. In improving these characteristics, the nanoformulations’ affinity to normal vascular endothelium and physiological hemostasis clot may be decreased, reducing tissue uptake and keeping them in the circulation for as long as possible. In this regard, bio-inert materials as nanocarriers may be suitable choices. The rapid blood flow environment is different from that in tissues. A drug may be efficiently returned to the systemic circulation once a targeting motif is lost from a nanosystem after responsive activation. Based on the physical characteristics of particles or cells flowing within blood vessels, nanomedicines can have corresponding intravascular distributions. [76] This is largely determined by their morphological parameters, including size, [77] shape, [71] and hardness. [78] For example, particles with different sizes and hardness distribute to different regions in the blood flow. [79] First, the edge-touching behavior of particles pulls particles to the endothelium wall. When considering the collision effects of intravascular particles, soft particles similar to resting platelets [80] are more prone to deformation, whereas hard particles are more prone to centroid displacement. This displacement can promote the edge-touching behavior of micron-sized particles (e.g., collision between RBCs and white blood cells). [81] When considering the centripetal effect of blood flow itself, relatively hard particles, such as poly(lactic acid) glycolic acid copolymer particles, are more likely to be less affected than soft particles, such as soft, biomimicking membrane particles, thus leading to differential distribution in blood vessels. These physicochemical features should be carefully taken into consideration in the design of antithrombotic nanomedicines. In addition, targeting ligands and responsive triggers should be judiciously selected. The specific pathological and/or physiological characteristics in the local thrombus microenvironment can be exploited for the design of intelligent antithrombotic nanomedicines. The abnormal hemodynamics, such as the extremely high shear stress, can serve as activating signals. Highly activated biomarkers on platelets can be applied as targeting components, such as CD62p and GPIIb/IIIa (CD41/CD61 complex). The triggered release of antithrombotic agents is another advantage for nanomedicines. High levels of thrombin within a local thrombus can both serve as a target and/or a responsive stimulus for controlled drug release. The differentiation possible in intelligent nanomedicine design makes more personalized therapeutic approaches feasible, especially in patients with specific indications/contraindications.

In clinical practice, stat procedures are required on demand for the immediate reversal of any bleeding events caused by antithrombotic therapy. Protamine has been applied for neutralizing the anticoagulant or other side effects caused by heparin, [82] whereas idarucizumab can be used to neutralize the effects of dabigatran. [83] In addition, the rapid reversal agents for the hemorrhage risk of antiplatelet drugs have entered the phase II clinical trials. [84] It is expected that reversal agents for antithrombotic drugs will develop in parallel with the novel antithrombotics. With their targeting abilities and easy decoration, nanomedicines are prime candidates for the development of reversal agents. This is especially applicable to the development of effective reversal agents for intelligent antithrombotic nanomedicines. For example, the precise structure of DNA/RNA nucleic acid nanomedicines enables the facile
synthesis of complementary sequences as highly specific reversal agents.\textsuperscript{[23,54]} Similarly, the unique characteristics of antiplatelet “platelet decoys” make platelet transfusion an available choice for quick reversal.\textsuperscript{[75]} Light controllable “on-off” switch could also serve as a self-reversible antithrombotic nanomedicine design.\textsuperscript{[58]}

Due to the gradual changes in medication from intravenous administration to oral administration of both anticoagulant and antiplatelet drugs, most studies investigating antithrombotic nanomedicine mainly focus on fibrinolytic drugs. However, this does exclude the significance of the exploration of anticoagulants and antiplatelet nanomedicines. In the future, additional orally administered intelligent drug delivery systems may be developed to keep up with the trends of anticoagulant and antiplatelet therapy.

6 | CONCLUSION

In summary, current antithrombotic therapy has reached a plateau of optimizing the dosage regimens or administration dose to balance the antithrombotic effect and bleeding risk, which invariably affect the efficacy of antithrombotic drugs. Only by increasing local effective concentrations together with decreasing overall drug administration dose via nanotechnology, antithrombotics can achieve further enhanced therapeutic effects and reduced hemorrhage risk. Antithrombotic nanomedicines that exhibit both specific aggregation at thrombus sites and controllable drug release can achieve at least one goal of improving the curative effects, reducing hemorrhage risk, and rapidly reversing hemorrhage caused by antithrombotic drugs. Despite the substantial preclinical data indicating antithrombotic nanomedicines can achieve specific delivery of antithrombotic drugs, enthusiasm may be tempered by additional human clinical trials. Issues including thrombogenicity, biodegradability, and hematotoxicity should also be addressed before clinical usage.\textsuperscript{[88]}

Despite all these challenges, intelligent antithrombotic nanomedicines still hold great promise for improved net patient benefit in the near future.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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