crystals

MDPI

Review

Nanocarrier-Based Management of Venous and Arterial Thrombosis

Mihai Ștefan Cristian Haba [1,†], Dragomir N. Șerban [2], Ionela Lăcrămioara Șerban [2], Ivona Maria Tudorancea [3], Raluca Maria Haba [4], Minela Aida Mărănducă [2,†], Daniela Maria Tănase [1,†], Radu Iliescu [5] and Ionuț Tudorancea [2,*]

1 Department of Internal Medicine, Grigore T. Popa University of Medicine and Pharmacy, 700115 Iași, Romania; mihai.haba@umfiasi.ro (M.Ș.C.H.); tanasedm@gmail.com (D.M.T.)
2 Department of Physiology, Grigore T. Popa University of Medicine and Pharmacy, 700115 Iași, Romania; dragomir.serban@umfiasi.ro (D.N.Ș.); ionela.serban@umfiasi.ro (I.L.Ș.); minela.maranduca@umfiasi.ro (M.A.M.)
3 Faculty of Pharmacy, Grigore T. Popa University of Medicine and Pharmacy, 700115 Iași, Romania; tudorancea.ivona.maria@email.umfiasi.ro
4 Faculty of General Medicine, Grigore T. Popa University of Medicine and Pharmacy, 700115 Iași, Romania; raluca-maria-e-vreme@d.umfiasi.ro
5 Department of Pharmacology, Grigore T. Popa University of Medicine and Pharmacy, 700115 Iași, Romania; radu.iliescu@umfiasi.ro
* Correspondence: ionut.tudorancea@umfiasi.ro
† These authors contributed equally to this work.

Abstract: Cardiovascular diseases represent the leading cause of mortality worldwide, with recent epidemiological studies revealing an increasing trend of prevalence and incidence globally. Among cardiovascular disorders, both arterial and venous thrombosis and particularly their acute life-threating complications such as ischemic stroke, acute myocardial infarction, deep venous thrombosis and pulmonary embolism are responsible for more than 25% of all deaths worldwide. The modern approach following progresses in anticoagulant, thrombolytic and antiaggregant therapies has significantly improved the prognoses of these conditions in the last past decades. However, several challenges still remain such as achieving the optimal drug concentration at the injured site, reducing the shortcomings of drug resistance and the incidence of life-threatening hemorrhages. Nanomedicine is a well-known field of medicine in which atomic and molecular structures ranging between 0.1–100 nm are used in various domains due to their specific mechanical, electrical, thermal and magnetic properties. Recent experimental and clinical evidence have shown that nanotechnology could be a safe, effective and an appealing approach for various non-cardiovascular and cardiovascular diseases such as thromboembolic conditions. In this review, we have described the most promising nanotechnology-based approaches not only for the diagnosis, but also for the treatment of vascular thrombotic diseases.

Keywords: nanocarriers; venous thrombosis; arterial thrombosis

Citation: Haba, M.Ș.C.; Șerban, D.N.; Șerban, I.L.; Tudorancea, I.M.; Haba, R.M.; Mărănducă, M.A.; Tănase, D.M.; Iliescu, R.; Tudorancea, I. Nanocarrier-Based Management of Venous and Arterial Thrombosis. *Crystals* **2022**, *12*, 450. https://doi.org/10.3390/cryst12040450

Academic Editor: Dritan Siliqi

Received: 24 February 2022
Accepted: 22 March 2022
Published: 23 March 2022

1. Introduction

Cardiovascular diseases represent the leading cause of mortality worldwide, recent epidemiological studies revealing an increasing trend of prevalence and incidence globally [1,2]. Among cardiovascular disorders, both arterial and venous thrombosis and particularly their acute life-threating complications such as ischemic stroke, acute myocardial infarction, deep venous thrombosis and pulmonary embolism are responsible for more than 25% of all deaths worldwide [3]. For venous thromboembolism (VTE), the Virchow's triad described over a century ago still provides an unique model to understand the physiopathology of clot formation which requires the interrelation between three major factors: a hypercoagulability state of blood, venous endothelial injury and venous stasis [4,5]. On the other hand, occlusive arterial thrombosis usually requires three different key factors

such as high-shear stress flow, the exposure of sub-endothelial derived prothrombotic factors and pathological activation of platelets [6]. Understanding the pathophysiology involved in the alteration of the balance between pro- and anticoagulant factors leading to blood coagulation is important not only for disease control, but also for appropriate therapeutic management. Since the physiopathology of clot formation is complex, the proper treatment usually requires antiplatelet drugs in the case of arterial thrombosis while for venous thrombotic events agents targeting specific factors in the cascade of coagulation are mainly indicated [7,8].

Nanomedicine is a well-known field of medicine in which atomic and molecular structures ranging between 0.1–100 nm are used in various domains due to their specific mechanical, electrical, thermal and magnetic properties [9]. Although nanoparticles were initially used in the field of oncology, their attractive and innovative properties made possible their translation in other various domains such as cardiovascular system. Recent experimental evidences have shown that nanotechnology could be a safe, effective and an appealing approach for different thromboembolic diseases such as venous and arterial thrombosis [10]. In this review, we have described promising nanotechnology-based approaches for diagnosis and management of vascular thrombosis.

2. Nanocarriers for the Management of Venous Thromboembolism

2.1. Pathophysiology of VTE

Venous thromboembolism (VTE) represents an important global health issue, with more than 10 million cases occurring every year worldwide, which results in a significant economic burden of USD $7 billion in the USA alone [11]. The main pathophysiological manifestation of venous thrombosis are deep vein thrombosis (DVT) and pulmonary thromboembolism (PE). DVT represents the formation of thrombi at the level of the deep veins, particularly at the inferior limbs and at the pelvis venous system. For patients with DVT, there is a 50% chance that the thrombus dislodges from the venous wall, provoking the embolization of the pulmonary arteries [12]. Through pulmonary embolism, VTE represents the third-most important cause of vascular death, following acute myocardial infarction and stroke [13]. Incidence of venous thrombosis ranges from 100 to 200 events per 100,000 patients/year [14]. However, these numbers are rising continuously due to the increased prevalence of comorbidities associated with this condition such as heart failure, obesity and cancer. Furthermore, this rise is accentuated by the constant ageing of the global population, as VTE is encountered in 1/100 people older than 80 years [15].

The short term mortality of pulmonary embolism of approximately 20% is constantly decreasing, due to improvements in diagnosis and treatment. However, VTE is considered a chronic condition with a 30% rate of recurrence at 10 years and worsening overall prognosis with each occurrence. The main complications of VTE consist in the development of post-thrombotic syndrome (PTS), which develops in 20–50% of patients with DVT and the development of chronic thromboembolic pulmonary hypertension which is encountered in up to 4% of PE [16].

The pathophysiology of thrombosis was proposed by Virchow et al. in 1856 and it consists of changes in blood flow (low flow or stasis), changes in the structure of the vessel wall (wall damage) and a pathological activation of the coagulation cascade (hypercoagulability) [5]. Understanding these three components help to identify and prevent the risk factors associated with the development of VTE. Venous stasis usually appears as a result of many interconnected factors such as prolonged immobility (long distance travel, perioperative or orthopedic immobilization, severely ill patients confined to bed), partial or complete venous obstruction (pregnancy, intraabdominal or pelvic tumors) and increased blood viscosity (polycythemia, hypergammaglobulinemia, dysproteinemias). Venous endothelium damage results after direct trauma or subsequent to injury caused by thrombin, low oxygen tension, exposure to endotoxins or inflammatory cytokines (interleukin −1 and tumor necrosis factor). These tissue mediators can be overly expressed in the context of chronic systemic pathologies such as heart failure, malignancy and chemotherapy and

obesity. Hypercoagulability is a consequence of an altered balance between the activation of the coagulation cascade and the decreased activity of fibrinolytic system [17].

Physiologically, low amounts of activated procoagulation factors are present in healthy individuals but they are diluted by blood flow, inactivated by circulating antiproteinases or already-formed fibrin is hydrolyzed by plasminogen. However, the activation of coagulation at a local or a distant site promotes the initiation of VTE. Conditions that promote hypercoagulability can be local (trauma, surgery, burns) or systemic (malignancy, myocardial infarction, antiphospholipid syndrome). Furthermore, local hypoxia secondary to venous stasis has itself been incriminated as a trigger for the coagulation cascade. In pregnant women, there is an increased production of coagulation factors such as factor VII, VIII, X and von Willebrand, therefore an increased risk of VTE is constantly present during pregnancy and postpartum. Increased in vivo activation can be present also in hereditary deficiencies of anticoagulant factors such as antithrombin, protein C deficiency or resistance and protein S deficiency [13]. Age is another important risk factor, as blood coagulation increases over 45 years while the fibrinolytic process is reduced. Moreover, this reduction of physiological fibrinolysis is more significant in the lower limbs veins of the elderly, thus increasing the risk of developing DVT [17].

2.2. Nanotechnology-Based Approaches for Diagnosis and Treatment of VTE

Diagnosis of VTE is based on the detection and description of the thrombus at the level of the systemic veins or in the pulmonary artery and it is a crucial step to establish proper therapeutic management. Contemporary diagnostic approach is based on evaluation of clinical characteristics, serological markers and imaging techniques. Many plasma molecules (D-Dimers, P-selectin, factor VIII) have been proposed as biomarkers for VTE, however most of them are found predominantly at the level of the clot and less in the circulating blood. Probably the most used biomarker in clinical practice are D-Dimers, a by-product of fibrin degradation, due to their lower cost and overall availability. Unfortunately, these molecules have a very poor specificity for thrombosis and are used more in conjunction with clinical and imagistic parameters in order to rule out VTE.

Newer molecules, such as circulating micro-RNA have been shown to have significant specificity for the detection of VTE and pulmonary embolism, but the underlying pathological mechanisms that lead to their expression are not yet fully understood [18]. However, due to the fact that coagulation is a complex systemic process, trying to identify a molecule with high specificity and sensibility for thrombosis could be futile. A new approach proposes introducing "synthetic biomarkers", which are attracted by clot formation and offer thus not only the location, but also the intensity of the coagulation process. As a result, Bhatia et al. used thrombin-activatable peptide (TAP), which only reacts and is cleaved by thrombin. They coupled this peptide with a ligand and loaded the complex on iron oxide nanoworms and tested them on an experimental thromboplastin-induced thrombosis mice model. The increased level of thrombin resulted in the cleavage of the TAP and the ligand, which was released into the bloodstream. The intensity of the process was quantified by the level of expression of the ligand in urine [19]. In a further study, the same investigator team, conjugated the TAP-ligand complex with a polyethylene glycol scaffold (PEG-T1E) with a diameter of ~8 nm, which allowed subcutaneous administration [20]. With further development, these nanostructures may be used for point-of-care screening and diagnosis tools for thrombosis, especially in all clinical conditions, which are associated with a prothrombotic status.

The most commonly used methods for thrombus detection in current medical practice is the use of imaging techniques, which allow accurate detection of size and location of the pathological process. Ultrasonography (US), computer tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography showed good applicability in the detection of aged clots, but are inefficient in the early stages of thrombus formation. Furthermore, in the context of post-thrombotic syndrome or in the context of residual thrombosis they cannot identify accurately newly formed thrombi.

To further enhance imaging of clot formation, nanoparticles (NPs) can be designed to target specific components of thrombosis such as fibrin, activated platelets or factor XIII. While accumulating at the thrombosis site these nanoparticles can act as contrast agents for conventional imaging techniques.

Magnetic nanoparticles are, at the moment, the most studied contrast agent due to their excellent detection by MRI devices. Suzuki et al. attached fucoidan, a polysaccharide which binds to P-selectin in the intraluminal thrombus, to ultrasmall supermagnetic iron oxide nanoparticles. After image acquisition, these complexes presented an adequate selectivity for the thrombi, which was detected during high T2 sequence relaxation [21]. Another target for thrombus detection is the GPIIb/IIIa receptor which is overexpressed on activated platelets. Molecules that specifically bind to this complex such as cyclic Arg-Gly-Asp (cRGD) peptide and single chain antibodies (scFv) were linked to magnetic nanoparticles and their accumulation at the thrombus level was detected through MR imaging. Furthermore, since scFv bound nanoparticles presented enhanced T1 signal and decreased T2 signal, they may be used to enhance the accuracy of the diagnosis, by comparing the differences between the two acquisitions [22]. Alpha 2 antiplasmin is a molecule synthetized by the liver and acts as a modulator of fibrinolysis, through its rapid inhibition of plasmin. Furthermore, alpha 2 antiplasmin is cross-linked with fibrin by factor XIIIa in the early stages of thrombus formation. This expression of alpha 2 antiplasmin both in early and later stages of clot development makes this molecule an excellent target for nanomaterial imagistic and therapeutical delivery systems [23,24]. Temme et al. described such a technique using α2-antiplasmin peptide (α2AP)-targeted perfluorocarbon nanoemulsions (PFCs) as contrast agent. Through 1F/19F magnetic MRI, they succeeded in identifying developing clots, smaller than 0.8 mm in diameter, in murine inferior vena cava [25]. Khurshid et al. used iron oxide magnetic nanoparticles which were functionalized with ATP15 and ATP29 to bind to two distinct epitopes of thrombin. Using magnetic spectroscopy, they evaluated the Brownian rotation of the magnetic nanoparticles to characterize blood clots. Through this technique, they successfully estimated the thrombus age, differentiating between new and old thrombosis. Furthermore they were able to establish the organization level of the thrombi, as the nanoparticle relaxation time was more reduced in organized clots [26].

Nanoparticles have also been designed to improve other imagistic techniques such as CT and US. Glycol chitosan gold nanoparticles (GC-AuNPs) conjugated with fibrin specific peptide (EP-2104R) were used to adequately detect thrombosis through microCT (mCT) within 5 min after intravenous administration [27]. Kwon et al. designed a fluorescent switch system using the previously mentioned TAP molecule which was loaded on silica-coated AuNPs (SiO2-AuNPs). When reaching the thrombin areas of the clots, the TAP molecule was cleaved and florescence was emitted, while SiO2-AuNPs accumulation was detected by mCT. This dual-mode thrombus imaging system was used in vivo to identify clots in an experimental thrombotic model [28]. Due to their oscillation properties after interaction with ultrasound waves, microbubbles have been continuously studied as contrast agents for US. After coupling microbubbles with a phospholipid shell with Abciximab, a monoclonal antibody against glycoprotein IIb/IIIa, they were further used to target thrombosis site. These immunobubbles showed great potential for detecting platelet aggregates in thrombi both in in vitro and in vivo preclinical studies [29,30]. Photoacoustic imaging (PAI) is a novel imaging modality that quantifies the ultrasonic emission resulted after non-ionizing laser pulses are delivered into biological tissues. In conjunction with NP-based technologies, PAI has been used to detect clot formation [31]. Amphiphilic perylene-3,4,9,10-tetracarboxylic diimide derivatives assembled into organic semiconducting nanoparticles (cRGD-PDI) were used by Cui et al. to distinguish early thrombus from healthy vessels. Furthermore, compared with US and MRI, PAI evaluation of cRGD-PDI showed an improved information profile, including thrombus size, conformation, as well as allowing the differentiation between early and old thrombi [32].

The main purposes of VTE treatment are the inhibition of thrombus formation and readily and effective thrombus resolution. Prompt and precise treatment is vital, considering the risk of embolization of DVT and the high mortality rates of pulmonary embolism. Furthermore, incomplete thrombus resolution is associated with an increased risk of VTE recurrence, developing post-thrombotic syndrome and developing thromboembolic pulmonary arterial hypertension. Anticoagulation strategies remain the main staple for VTE treatment and prophylaxis, and it consists in interruption of the pro-thrombotic processes while relying on the physiological fibrinolytic processes to break down the clot. The most commonly used anticoagulants in clinical practice are unfractionated heparin, low molecular weight heparin (LMWH), vitamin K antagonists (VKAs) and direct oral anticoagulants (DOAC). All of these drugs have important benefits and their specific group indications, but anticoagulation therapy alone is not able to address massive clots, resulting in a residual thrombotic burden and in the development of PTS. Although the benefits of anticoagulants are clearly demonstrated, initiation of this therapy may associate also severe hemorrhagic complications. Thus, assessing the hemorrhagic risk before administration of anticoagulants is a mandatory procedure. In case of massive pulmonary embolism associating hemodynamic instability, urgent clot removal is mandatory, commonly achieved by intravenous thrombolytic agents. Thrombolysis may also be required in selected patients with iliofemoral deep vein thrombosis associating a high risk for thrombus embolization. The limitation of thrombolytic therapy is a result of the significant risk of hemorrhagic events, as shown by the Pulmonary Embolism THrOmbolysis (PEITHO) Trial, where thrombolysis was associated with a 9% absolute increase in major bleeding and a 2% higher absolute risk of hemorrhagic stroke [33].

To improve the efficiency of VTE management, local delivery of beforementioned therapies can bypass important disadvantages such as their inhibition in systemic circulation or the risk of life-threatening hemorrhagic events. This can be achieved by using a targeted nanoparticle approach which may act as carriers for anticoagulant or thrombolytic molecules with increased therapeutic efficiency and reduced adverse effects. Furthermore, NPs can be used to carry molecules that can potentially reduce venous remodeling and decrease the risk of PTS.

One of the most investigated structures designed for thrombolytic agent encapsulation are liposomal NPs. They are organic lipid-bilayer composites, synthetized from natural amphipathic molecules, therefore decreasing the risk of toxicity. Bader et al. developed an echogenic liposome by using a lipid monolayer shell encapsulated with octofluropropane and loaded with tissue plasminogen activator (tPA). This NPs had a high affinity to blood clots due to the surface-modified targeting molecule. Moreover, locally ultrasound application was used to adequately deliver the tPA [34]. Aside from liposomes, polymeric NPs and dendrimers have been constantly investigated as a potential carrier for therapeutics. Wadajkar et al. synthesized poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with collagenase which significantly reduced the weight of the clots after 80 min of incubation [35]. In another experiment, Colasuonno et al. developed an erythrocyte-inspired PLGA NP, conjugated with tPA. In a murine model of VTE, intravenous administration of 2.5 mg/kg of this NP led to 90% recanalization of blood clots, while free tPA recanalized only 40% [36]. Poly ethylene glycols (PEG) and PLGA can be also used to encapsulate LMWH in order to achieve a more sustained release with better antithrombotic activity and more stable aPTT levels [37]. Dendrimers are complex nanomolecules that are organized in a branching pattern with a central core, offering an excellent site for therapeutics incorporation. Several studies that incorporated streptokinase and tPA into dendrimeric structures showed both enhanced lysis rates and stability when compared to administration of free thrombolytics [38–40]. Nanoparticles used for the management of VTE are summarized in Table 1.

Table 1. Nanocarriers for management of VTE.

Nanocarriers	Mechanism	Application
[1] TAP-ligand complex with a polyethylene glycol scaffold (PEG-T1E) [20]	• TAP mediated release of ligand after interaction with thrombin rich clots	• point of care diagnosis
TAP-ligand complex with a polyethylene glycol scaffold (PEG-T1E) [25]	• cross-linked to fibrin by active factor XIII	• contrast agent for [1] MRI imaging
Iron oxide magnetic nanoparticles which were functionalized with ATP15 and ATP29 [20]	• binding to thrombin epitopes	• magnetic spectroscopy used to evaluate Brownian rotation of the magnetic nanoparticles • differentiation between early and old thrombus
Amphiphilic perylene-3,4,9,10-tetracarboxylic diimide derivatives assembled into organic semiconducting nanoparticles (cRGD-PDI) [32]	• [1] PAI evaluation of cRGD-PDI expression at thrombosis site	• evaluating thrombus size, conformation • differentiation between early and old thrombus through PAI
Octofluropropane [34], **poly(lactic-co-glycolic acid) (PLGA) nanoparticles** [35] **and dendrimers** [39] **loaded with [1] tPA**	• NPs encapsulating tPA	• delivery of optimal doses of thrombolysis without drug degradation
Magnetic nanoparticles wheels loaded with tPA [41]	• magnetic field guided delivery of nanoparticles at thrombus site	• magnetic field guided chemical and mechanical lysis
Acrylamide (AAm), N-(3-aminopropyl) methacrylamide hydrochloride (APM) and TAP nanocapsules loaded with tPA [42]	• TAP mediated release of tPA after interaction with thrombin rich clots	• disease microenvironment drug release
Elongated tobacco mosaic virus (TMV) loaded with streptokinase [43]	• special geometry of viral structures leads to accumulation at thrombus site	• specific drug release out of a biological-based nanostructure

[1] Abbreviations: TAP—thrombin-activatable peptide; tPA—Tissue plasminogen activator; MRI—magnetic resonance imaging; PAI—photoacoustic imaging.

Besides preserving the bioavailability of anticoagulant and fibrinolytic molecules, nanotechnology-based therapy ideally should be able to provide a good specificity for thrombosis site, to increase the efficacy and also reduce drug release in non-thrombotic areas. This can be achieved by external or internal delivery systems. External delivery systems consist mainly in ultrasound or magnetic guided release and lysis. As described above, US guided NPs thrombolysis may be an exciting approach, but it has some drawbacks due to attenuation of US waves while passing through tissues, making deep vascular structures inaccessible. Magnetic fields can bypass these limitations by concentrating tPA loaded magnetic nanoparticles (MNP) at the level of the clot. These MNPs are developed by using superparamagnetic iron oxide crystals of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). In an in vitro thrombotic model, Hu et al. used magnetite nanorods coupled with 6% tPA and guided them with a magnetic field to increase the lysis rate [44]. In another study, Tasci et al. achieved enhanced fibrinolysis through an innovative approach which combined chemical and mechanical mechanisms. They designed magnetically microwheels, which were coupled with biotinylated tPA. The microwheels were guided using a magnetic field to the plasma clot interface where the tPA molecules were released. In addition, through a ~10 mT rotating magnetic field, the microwheels were induced a corkscrew-like motion, which increased the lysis rate six-fold faster than tPA alone [41]. Internal delivery systems are based on targeting the components of the disease microenvironment, which can be further used as a trigger for local drug release. Therefore, thrombin, which plays a key role in the coagulation cascade, can be used as a triggering molecule. Li et al. used acrylamide (AAm), N-(3-aminopropyl) methacrylamide hydrochloride (APM) and TAP nanocapsules

to protect packaged tPA from the physiological degradation in the blood flow. At the level of the thrombus, as a result of the thrombin-TAP interaction, tPA was released with remarkable fibrinolytic activity [42]. Other microenvironment-based released systems have been designed, such as H_2O_2 increased concentration from thrombus site [45], which will be discussed in the next paragraphs.

Besides classical inorganic and organic NPs, efforts were made to design biologically based nanostructures to improve biocompatibility. Due to the fact that platelets play a crucial role in thrombus formation, attempts were made to mimic platelet membrane structures resulting in platelet membrane-nanoparticles (PMN). Hu et al. loaded the surface of PMN with tPA, showing great specificity and thrombolysis effect on a lung thrombosis model [46]. An interesting approach for biomimetic delivery system was proposed by Park et al., which loaded streptokinase on the elongated tobacco mosaic virus (TMV). The special geometry of this viral structure resulted in increased accumulation at the level of the thrombi and good thrombolytic effect in a murine model of DVT [43].

Taken together, all of these preclinical experimental approaches for both the diagnosis and treatment of VTE by using nanotechnology offer encouraging perspectives for their translation to clinical practice.

3. Nanocarriers for the Management of Arterial Thrombosis

3.1. Physiopathology of Arterial Thrombosis

The pathogenesis of arterial thrombosis is complex, dynamic, and still not fully understood. In the vast majority of cases, arterial thrombosis occurs usually in the settings of the complications of an atherosclerotic plaque [9], like plaque rupture, which commonly led to the acute clinical manifestations, such as myocardial infarction, ischemic stroke or acute limb ischemia [47]. Current experimental evidence shows that the formation of arterial thrombi, as a complication of plaque rupture, further requires the pathological activation of platelets aggregation and adhesion to the arterial wall, both mechanisms being triggered by the release of pro-thrombotic messengers from injured endothelium [48]. With the exposure of the thrombogenic molecules from atherosclerotic plaque, platelets adhere and binds the subendothelial factors such as collagen and von Willebrand factor (vWF). Once activated, thrombocytes start to locally release adenosine diphosphate (ADP) and thromboxane A2 (TXA2) to further activate other platelets [49,50]. ADP play a major role in platelet activation and adherence through their P2Y1, P2Y12 and P2X1 receptors.

It is now reported that P2Y1 and P2Y12 receptors are required for phospholipase A2 activation which further induced arachidonic acid release and TXA1 synthesis [49]. The platelets activation is readily followed by conformation changes in glycoprotein (GP) IIb/IIIa, which further facilitates platelets aggregation by increasing the affinity of platelets to fibrinogen and vWF [50]. From a clinical point of view, these mechanisms of platelet aggregation are of paramount importance since P2Y12 and GP IIb/IIIa inhibitors represent essential therapies in the setting of acute or chronic arterial thrombotic events [51–53].

Concurrently with thrombocytes activation and aggregation, recent evidence has shown that the initiation of coagulation pathway is also triggered by the exposure of tissue factor (TF) from injured endothelium. Briefly, the exposed TF binds to factor VII(a) and coagulation is triggered leading to thrombin synthesis which further initiates the conversion of fibrinogen into fibrine and clot formation [54]. At once, thrombin further amplifies both platelets activation and thrombus formation trough the activation of protease-activated receptor (PARs)-1 and 4, factor V, VIII and XI and eventually, factor XIII is activated to stabilize the clot. Interestingly, the interplay between the platelets and the thrombin pathway is mediated by a factor released from platelets at the injured site—phosphatidylserine—on which the intrinsic tenase and prothrombinase complexes assemble [50].

Recent experimental and clinical evidence show that pathogenesis of arterial thrombosis is incompletely understood, and further studies are necessary to better and completely describe all the pathological mechanisms involved in this particular condition. This is paramount importance due to the continuous need for the development of new therapeutic

strategies and to improve not only the survival rate of patients suffering from these acute, life-threating conditions, but also to ameliorate their prognosis and quality of life over the long term.

As mentioned above, arterial clot formation involves a complex and dynamic pathogenesis. Current antiplatelet drugs have only partial benefits regarding the prevention and treatment of acute or chronic thrombotic events [3]. Although current antiplatelets have been developed to inhibit thrombocytes activation or biding in crucial points from thrombus formation cascade, in a significant percentage of cases, this pathological cascade is not successfully inhibited since 5–50% of patients are classified as antiplatelet resistant [6].

3.2. Nanotechnology-Based Approaches for Treatment of Arterial Thrombosis

Recent technological advance made possible the development of novel therapeutic agents to improve not only the specificity, but also the efficacy of new-generation antiplatelets drug for both prevention and treatment of arterial thrombosis [3]. In daily clinical practice, antiplatelets drugs such as adenosine diphosphate (ADP), phosphodiesterase (PDE), cyclooxygenase (COX-1) and GPIIb/IIIa inhibitors are the mostly used not only for the treatment but also for primary, secondary and tertiary prophylaxis of cardiocerebrovascular diseases such as acute and chronic coronary syndrome, peripheral artery disease or stroke [55]. Among them, antiplatelets drugs like aspirin and P2Y12 inhibitors have been shown to be the most effective in such clinical scenarios and currently they have a I class of indication in the majority of guidelines [56–61]. Despite their well-documented efficacy, current antiplatelet therapies have disadvantages, such as a modest bioavailability, a low degree of penetration of thrombi, off-target side effects and an increased risk of moderate to severe bleeding [8]. Moreover, in many patients, an adequate degree of secondary prophylaxis is challenging to be achieved due to "antiplatelet resistance" which defines the inability to protect individuals against future cardiovascular events despite the fact that they continue to use antiplatelet drugs [62,63]. Growing evidence from experimental nanotechnology have proved that a plethora of sensitive nanostructures may be considered to overcome the limitations of the present antiplatelet therapy. Although the main focus was to enhance the antiplatelet effect at the injured site, recent studies have reported that nanocarriers have several significant advantages such as an increased targeting capability, an improved bioavailability and fewer off-target side-effects.

In the next paragraphs we will discuss novel strategies in the field of nanomaterial-based antiplatelet therapies, which are targeting newly described key components in the cascade of thrombus formation such as H_2O_2 and shear-stress mechanisms.

3.2.1. H_2O_2 Targeting Strategies

Growing evidence has shown that H_2O_2 scavenging is a promising approach since high levels of this product promote the expression of pro-inflammatory cytokines and platelet-endothelium interaction, leading thus to the clot formation. Based on this pathological mechanisms, Lee et al. have developed aspirin polyconjugate particles (T-APP) not only to specifically target the arterial thrombus, but also to locally induce antithrombotic effects in a H_2O_2 dependent mechanism. To scavenge H_2O_2 during the oxidation reaction, they have conjugated the major active ingredient of aspirin-ethyl salicylate (ESA) to the side chains of random copolymer. To specifically target the thrombus, the authors have used fibrin-specific pentapeptide, Gly-Pro-Arg-Pro-Pro (GPRPP) during the development of T-APP. Moreover, to demonstrate that nanoparticles have an adequate thrombus specificity, they bound IR780 to T-APP and thus they were able to use fluorescence imaging of blood clots formed in cell culture plates. They have shown in vitro experiments that T-APP have a remarkable thrombus specificity as demonstrated by a strong fluorescence signal within the clots treated with this compound. They further tested if T-APP have anti-platelet activity based on H_2O_2 clot modulation, by using an in vivo animal model of tail transection and $FeCl_3$-induced arterial thrombosis. Interestingly, their results have shown that T-APP has a higher capability to increase both bleeding time and bleeding volume when compared to

the equivalent ESA, due to the ability of T-APP to specifically target blood clots. Moreover, in the $FeCl_3$-induced carotid arterial thrombosis, T-APP significantly accumulate within arterial thrombus in the first 60 min after administration as revealed by the fluorescence signal. Taken together, these results clearly demonstrates that T-APP are characterized by a high specificity and efficacy. Since increased level of H_2O_2 is able to induce the expression of pro-inflammatory cytokines at the level of thrombotic site further promoting platelet activation, the authors tested also if T-APP is able to decrease locally the expression of TNF-alpha and sCD40L. Remarkably, T-APP significantly suppressed both TNF-alpha and sCD40L local expression when compared to APP, indicating that this fibrin specific ligand-GPRPP- plays a key role in mediating not only anti-thrombotic activity, but also local specific anti-inflammatory effects. These encouraging results pave the way for further preclinical studies to better characterize and assess the antiplatelet, antithrombotic and anti-inflammatory effects of T-APP nanoconjugates, not only through their site-specific releasing properties of anti-platelets agents such as ESA, but also by the modulation of the mechanisms involved in H_2O_2 scavenging [64].

By targeting the same H_2O_2-induced pro-thrombotic mechanisms initiated from activated platelets and endothelial cells from thrombus site, Zhao et al. have developed H_2O_2-responsive platelet membrane-coated nanoparticles loaded with an antithrombotic agent-argatroban, which is able to induce a direct inhibition of thrombin, fibrin formation and inactivation of coagulation factors V, VIII and XIII. Once the thrombus site is targeted by platelet-coated nanoparticles, the H_2O_2 degradable polymer—poly vanillyl alcohol-co-oxalate (PVAX)—releases the encapsulated drug in this highly abundant H_2O_2 environment. Essentially, efforts were made to design and develop argatroban-loaded polymeric nanoparticle (PNPArg) coated with a platelet membrane for thrombus specificity in order to readily expend H_2O_2 and concurrently release the antithrombotic drug in a controlled manner.

On a mouse model of FeCl-induced carotid arterial thrombosis, the authors confirmed the capability of these nanoparticles to specifically target the injured site through the interaction of platelet-coated membrane with several signaling molecules such as GPIIb/IIIa, CD61, P-selectin and P2Y12. Moreover, the authors have shown also that PNPArg are able to significantly reduce the local expression of pro-inflammatory cytokine such as TNF-alpha and sCD40L, suggesting that PNPArg are able to exhibit anti-inflammatory actions. Finally, the authors have shown that PNPArg developed anti-thrombotic activity, since their administration led to a noteworthy inhibition effect on thrombus formation. Taken together, these results suggest that PNPArg have not only anti-platelets properties due to the presence of argatroban, but also anti-oxidation and anti-inflammatory activity through the H_2O_2 scavenging effects. The authors concluded that this promising approach may be used for the treatment of various thrombotic diseases due to above mentioned benefits and also due to their proper biocompatibility, as supported by the lack of toxicity in both in vitro and in vivo studies [65].

The benefits of inhibition of reactive oxygen species (ROS) at the thrombus site by nanomedicine were also confirmed by Kang et al. in a study in which they imagine for the first time a fibrin-targeted imaging and anti-thrombotic nanomedicine-FTIAN-used as a theragnostic system for vascular thrombosis. This concept combines the advantages provided by photoacoustic imaging such as high spatial resolution, deep penetration of ultrasound imaging and chemical specificity of optical imaging with contrast agents which possesses two major characteristics: the ability to specifically target thrombus and the capability to develop antithrombotic activity. Thus, to target fibrin at the thrombus site, scavenge H_2O_2 and prevent platelet activation accordingly, they developed FTIAN from near infrared (NIR) fluorescent dye-conjugated boronate antioxidant polymers (fBAP) and fibrin-targeting lipopeptides. The authors confirmed that this theragnostic approach is feasible since their results from in vivo experiments have shown that FTIAN specifically imaged thrombosed vessels and also exert anti-inflammatory effects since they were able to decrease locally the expression of TNF-alpha and sCDL40L. Moreover, the authors

assessed the potential of FTIAN to be used as thrombus-specific drug carrier by loading these nanocarriers with tirofiban—an antithrombotic agent. Their encouraging results have shown that tirofiban-loaded-FTIAN are also able to specifically target the thrombus and have potent anti-platelet and anti-thrombotic activity [66].

The same promising approach of using ROS not only as a target but also as a smart trigger for controlled drug release into the thrombus microenvironment was used by Gong et al., which imagined and developed H_2O_2-responsive thrombus-targeting red blood cell (RBC) membrane-cloaked dextran–tirofiban conjugate nanoparticles (T-RBC-DTC NPs). They hypothesized that phenylboronic ester linkage used to conjugate tirofiban to dextran will develop scavenging properties due to oxidation reaction, being thus able to oxidize and cleave H_2O_2. Once the dextran–tirofiban linkage is cleaved, the latter is specifically released at the thrombus site being thus able to inhibit platelet aggregation by interfering with glycoprotein IIb/IIIa.

Although the platelet membrane is usually indicated to specifically target the thrombus site, the authors considered that tirofiban may alter the biding receptors which can impact the targeting capability of these nanostructures. Accordingly, they have used red blood cell (RBC) membrane coated with a clot-binding peptide Cys-Arg-Glu-Lys-Ala (CREKA), which is known to have a high affinity to fibrin and is thus able to increase its thrombus-targeting capability. Using in vitro and in vivo experiments, the authors demonstrated that their nanostructures are able to specifically accumulate at the thrombus site and suppress clot development. Moreover, T-RBC-DTC NPs efficiently reduced both the expression of TNF-alpha, sCD40L and ROS generation at the injured site, suggesting thus an anti-inflammatory and anti-oxidant effect. Based on this encouraging results H_2O_2 scavenging and ROS-triggered therapeutic action at the thrombus site may be a reliable option for the treatment of thrombotic diseases [67].

3.2.2. Hemodynamic Shear-Stress Responsive Nanoparticles

Recent evidence have shown that in a medium-sized artery, platelets present a tendency to bind directly to the sub-endothelial von Willebrand factor (vWF) at shear rates greater than 630 s^{-1}. Experimental studies have shown that vWF suffer conformational changes at different shear stress rates. Thus, under low shear stress, vWF has a globular molecular shape and the platelets binding sites are actually covered and unavailable. With increasing shear stress, vWF elongates from a string to a mesh molecular conformation with 10,000 times as many platelets binding sites at shear rates > 10,000 s^{-1}. Further, a positive feedback mechanism is triggered for platelet activation and for arresting new circulating platelets leading to thrombus formation [68]. Under physiological conditions, this is a key mechanism by which a hemorrhage is stopped. In pathological conditions such as atherosclerotic disease high shear rates are locally developed at the site of arterial injury leading to platelet activation and thrombus formation [8]. The presence of an increased high shear stress in those areas affected by atherosclerotic plaques may be an attractive approach to develop shear-stress sensitive nanoparticles, which may be used for targeted therapies.

Recently, Griffin et al. [6] tested the hypothesis by which negatively charged nanoparticles (CNP) are able to inhibit thrombus formation by reducing the degree of vWF elongation at high shear stress rates and decreasing thus the number of the platelet binding sites. This approach is novel and attractive since it uses local biophysical forces instead antiplatelets drugs. In a computational model, they demonstrated that at shear-stress rates of 6500 s^{-1} the elongation of vWF is counteracted by the addition of negatively CNP and the resulting globular molecular vWF-CNP compound is stable and does not elongate anymore. From a mechanistic point of view, the development of this stable vWF-CNP compound at high shear stress rate will mitigate the ability of platelets to bind to the active sites from vWF. Tested in vitro on a microfluidic assay of thrombus formation, this approach was able to significantly delay thrombus development without using antiplatelet drugs. By developing shear stress phosphatidylcholine (PC)-nanoparticles loaded with the GPIIb-IIIa antiplatelet drug-eptifibatide, Molloy et al. [69] reported a high anti-platelet efficacy since

their nanocapsules were able to inhibit thrombosis in vitro under stenotic and increased shear rates stress of 1000 s^{-1}.

Moreover, on an animal model of carotid artery thrombosis, they have reported that the antiplatelet drug is released locally where shear stress levels are increased due to thrombus developing to near-occlusive dimensions. These results support the hypothesis that shear-sensitive nanoparticles incorporating GPIIb-IIIa antiplatelet drug may be an effective targeted therapy to prevent thrombus formation at the injured arterial site. Moreover, due to local release of the antiplatelet drug, the systemic concentration is very low leaving thus unaffected blood coagulation parameters. In another paper, Chen et al. have developed shear-sensitive charged nanoparticles (cNP) loaded with heparin and thiolated poly-l-lysine (PLL-SH) molecules and subsequently attached to the red blood cell membrane (RBC) to provide an adequate biocompatibility and biosafety. They demonstrated that RBC-cNPs have a site-specific drug delivery capacity and high therapeutic efficiency due to local release of heparin under the stimulus of shear stress changes [70]. Shear stress sensitive liposomes were also tested by Zumbuehl et al. in a cardiovascular model mimicking healthy and constricted vessel. They have shown that 1,3-diaminophospholipid lenticular vesicles are stable in static conditions but releases drugs within constricted areas which are characterized by an increased shear stress level [71]. Taken together, these encouraging results pave the way for a continuous development of various types of nanostructures loaded with antiplatelet drugs functionalized so that their content would be released preferentially at the injured site. Nanoparticles used for the management of arterial thrombosis are summarized in Table 2.

Table 2. Nanocarriers for management of arterial thrombosis.

Nanocarriers	Mechanism	Effects
IR780-aspirin polyconjugate particles (T-APP) [64]	• local H_2O_2 scavenging • TNF-alpha and sCD40L modulation	• increased thrombus specificity • increased local anti-thrombotic effect • decrease expression of TNF-alpha and sCD40L
Argatroban-loaded poly vanillyl alcohol-co-oxalate nanoparticle (PNPArg) [65]	• H_2O_2-responsive platelet membrane-coated nanoparticles	• increased thrombus specificity through GPIIb/IIIa, CD61, P-selectin and P2Y12 receptors • significantly reduce the local expression of pro-inflammatory cytokine • inhibition of thrombus formation
Fibrin-targeted imaging and anti-thrombotic nanoparticles (FTIAN) [66]	• local fibrin targeting • local H_2O_2 scavenging	• specifical imaging of thrombosed vessels • anti-inflammatory effects • anti-thrombotic effect when loaded with tirofiban
H_2O_2-responsive thrombus-targeting red blood cell (RBC) membrane-cloaked dextran–tirofiban conjugate nanoparticles (T-RBC-DTC NPs) [67]	• H_2O_2-responsive nanoparticles	• inhibit platelet aggregation by interfering with glycoprotein IIb/ IIIa; • reduced the expression of TNF-alpha, sCD40L and ROS generation • H_2O_2 scavenging and ROS-triggered anti-thrombotic and anti-inflammatory effects • suppressed clot formation

Table 2. *Cont.*

Nanocarriers	Mechanism	Effects
Negatively charged nanoparticles (CNP) [6]	• Shear-stress-responsive nanoparticles	• reduced the elongation of vWF • significantly delay thrombus development by blocking the active sites from vWF without using anti-thrombotic drugs
Shear stress phosphatidylcholine (PC)-nanoparticles loaded with eptifibatide [69]	• Shear-stress-responsive nanoparticles	• increased thrombus specificity • prevent thrombus formation at the injured arterial site

4. Conclusions

Vascular thrombotic diseases represent a global pandemic and are associated with high mortality and morbidity rates, being one of the most important medical and economical burdens of healthcare systems in the 21st century. The modern approach following progress in anticoagulant, thrombolytic and antiaggregant therapies has significantly improved the prognoses of these conditions in recent decades. However, several challenges still remain such as achieving the optimal drug concentration at the injured site, overcoming the shortcomings of drug resistance or reducing the incidence of life-threatening hemorrhages. Current progress in the field of nanotechnology allowed for the encapsulation of previously unstable drugs, increased specificity of these agents for thrombotic sites and improved targeted delivery of anticoagulant and antiplatelet substances to act on newly described pathological mechanisms of thrombosis. Finally, these promising reported results from experimental animal studies encourage the translation of these nanotechnologies in clinical practice, which will definitely result in significant improvements of prognoses and quality of life.

Author Contributions: Conceptualization, M.Ș.C.H. and I.T.; methodology, D.N.Ș., M.A.M., D.M.T. and R.I.; resources, I.M.T., R.M.H.; data curation, D.M.T., M.A.M.; writing—original draft preparation, M.Ș.C.H., I.T.; writing—review and editing, I.T., D.M.T., M.A.M.; visualization, I.L.Ș.; supervision, D.N.Ș., I.L.Ș., R.I.; project administration M.Ș.C.H. and I.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nicholls, M. The ESC atlas of cardiology. *Eur. Heart J.* **2019**, *40*, 7–8. [CrossRef] [PubMed]
2. Leong, D.P.; Joseph, P.G.; McKee, M.; Anand, S.S.; Teo, K.K.; Schwalm, J.-D.; Yusuf, S. Reducing the global burden of cardiovascular disease, part 2: Prevention and treatment of cardiovascular disease. *Circ. Res.* **2017**, *121*, 695–710. [CrossRef] [PubMed]
3. Cicha, I. Thrombosis: Novel nanomedical concepts of diagnosis and treatment. *World J. Cardiol.* **2015**, *7*, 434–441. [CrossRef] [PubMed]
4. Stone, J.R. Diseases of Small and Medium-sized Blood Vessels. In *Cardiovascular Pathology*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 125–168.
5. Kushner, A.; West, W.P.; Pillarisetty, L.S. Virchow Triad. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2022.
6. Griffin, M.T.; Zhu, Y.; Liu, Z.; Aidun, C.K.; Ku, D.N. Inhibition of high shear arterial thrombosis by charged nanoparticles. *Biomicrofluidics* **2018**, *12*, 042210. [CrossRef]
7. Fuster, V.; Bhatt, D.L.; Califf, R.M.; Michelson, A.D.; Sabatine, M.S.; Angiolillo, D.J.; Bates, E.R.; Cohen, D.J.; Coller, B.S.; Furie, B.; et al. Guided antithrombotic therapy: Current status and future research direction: Report on a National Heart, Lung and Blood Institute working group. *Circulation* **2012**, *126*, 1645–1662. [CrossRef]
8. Shen, M.; Wang, Y.; Hu, F.; Lv, L.; Chen, K.; Xing, G. Thrombolytic agents: Nanocarriers in targeted release. *Molecules* **2021**, *26*, 6776. [CrossRef]

9. Haba, M.; Ștefan, C.; Șerban, D.N.; Șerban, L.; Tudorancea, I.M.; Haba, R.M.; Mitu, O.; Iliescu, R.; Tudorancea, I. Nanomaterial-Based Drug Targeted Therapy for Cardiovascular Diseases: Ischemic Heart Failure and Atherosclerosis. *Crystals* **2021**, *11*, 1172. [CrossRef]
10. Giménez, V.M.M.; Kassuha, D.E.; Manucha, W. Nanomedicine applied to cardiovascular diseases: Latest developments. *Ther. Adv. Cardiovasc. Dis.* **2017**, *11*, 133–142. [CrossRef]
11. Grosse, S.D.; Nelson, R.E.; Nyarko, K.A.; Richardson, L.C.; Raskob, G.E. The economic burden of incident venous thromboembolism in the United States: A review of estimated attributable healthcare costs. *Thromb. Res.* **2016**, *137*, 3–10. [CrossRef]
12. Moheimani, F.; Jackson, D.E. Venous thromboembolism: Classification, risk factors, diagnosis, and management. *ISRN Hematol.* **2011**, *2011*, 124610. [CrossRef]
13. Phillippe, H.M. Overview of venous thromboembolism. *Am. J. Manag. Care* **2017**, *23*, S376–S382.
14. Heit, J.A. Epidemiology of venous thromboembolism. *Nat. Rev. Cardiol.* **2015**, *12*, 464–474. [CrossRef]
15. Martin, K.A.; Molsberry, R.; Cuttica, M.J.; Desai, K.R.; Schimmel, D.R.; Khan, S.S. Time trends in pulmonary embolism mortality rates in the united states, 1999 to 2018. *J. Am. Heart Assoc.* **2020**, *9*, e016784. [CrossRef]
16. Di Nisio, M.; van Es, N.; Büller, H.R. Deep vein thrombosis and pulmonary embolism. *Lancet* **2016**, *388*, 3060–3073. [CrossRef]
17. Line, B.R. Pathophysiology and diagnosis of deep venous thrombosis. *Semin. Nucl. Med.* **2001**, *31*, 90–101. [CrossRef]
18. Luo, M.; Du, M.; Shu, C.; Liu, S.; Li, J.; Zhang, L.; Li, X. The Function of microRNAs in Pulmonary Embolism: Review and Research Outlook. *Front. Pharmacol.* **2021**, *12*, 743945. [CrossRef]
19. Lin, K.Y.; Kwong, G.A.; Warren, A.D.; Wood, D.K.; Bhatia, S.N. Nanoparticles that sense thrombin activity as synthetic urinary biomarkers of thrombosis. *ACS Nano* **2013**, *7*, 9001–9009. [CrossRef]
20. Dudani, J.S.; Buss, C.G.; Akana, R.T.K.; Kwong, G.A.; Bhatia, S.N. Sustained-release synthetic biomarkers for monitoring thrombosis and inflammation using point-of-care compatible readouts. *Adv. Funct. Mater.* **2016**, *26*, 2919–2928. [CrossRef]
21. Suzuki, M.; Bachelet-Violette, L.; Rouzet, F.; Beilvert, A.; Autret, G.; Maire, M.; Menager, C.; Louedec, L.; Choqueux, C.; Sabourал, P.; et al. Ultrasmall superparamagnetic iron oxide nanoparticles coated with fucoidan for molecular MRI of intraluminal thrombus. *Nanomedicine* **2015**, *10*, 73–87. [CrossRef]
22. Liu, J.; Xu, J.; Zhou, J.; Zhang, Y.; Guo, D.; Wang, Z. Fe_3O_4-based PLGA nanoparticles as MR contrast agents for the detection of thrombosis. *Int. J. Nanomed.* **2017**, *12*, 1113–1126. [CrossRef]
23. Pluskota, E.; Soloviev, D.A.; Bdeir, K.; Cines, D.B.; Plow, E.F. Integrin alphaMbeta2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. *J. Biol. Chem.* **2004**, *279*, 18063–18072. [CrossRef]
24. Lu, M.; Blaine, K.P.; Cullinane, A.; Hall, C.; Dulau-Florea, A.; Sun, J.; Chenwi, H.F.; Graninger, G.M.; Harper, B.; Thompson, K.; et al. Pulmonary arterial hypertension patients display normal kinetics of clot formation using thrombelastography. *Pulm. Circ.* **2021**, *11*, 1–9. [CrossRef]
25. Temme, S.; Grapentin, C.; Quast, C.; Jacoby, C.; Grandoch, M.; Ding, Z.; Owenier, C.; Mayenfels, F.; Fischer, J.W.; Schubert, R.; et al. Noninvasive imaging of early venous thrombosis by 19F magnetic resonance imaging with targeted perfluorocarbon nanoemulsions. *Circulation* **2015**, *131*, 1405–1414. [CrossRef]
26. Khurshid, H.; Shi, Y.; Berwin, B.L.; Weaver, J.B. Evaluating blood clot progression using magnetic particle spectroscopy. *Med. Phys.* **2018**, *45*, 3258–3263. [CrossRef]
27. Kim, J.-Y.; Ryu, J.H.; Schellingerhout, D.; Sun, I.-C.; Lee, S.-K.; Jeon, S.; Kim, J.; Kwon, I.C.; Nahrendorf, M.; Ahn, C.-H.; et al. Direct Imaging of Cerebral Thromboemboli Using Computed Tomography and Fibrin-targeted Gold Nanoparticles. *Theranostics* **2015**, *5*, 1098–1114. [CrossRef]
28. Kwon, S.-P.; Jeon, S.; Lee, S.-H.; Yoon, H.Y.; Ryu, J.H.; Choi, D.; Kim, J.-Y.; Kim, J.; Park, J.H.; Kim, D.-E.; et al. Thrombin-activatable fluorescent peptide incorporated gold nanoparticles for dual optical/computed tomography thrombus imaging. *Biomaterials* **2018**, *150*, 125–136. [CrossRef]
29. Schumann, P.A.; Christiansen, J.P.; Quigley, R.M.; McCreery, T.P.; Sweitzer, R.H.; Unger, E.C.; Lindner, J.R.; Matsunaga, T.O. Targeted-microbubble binding selectively to GPIIb IIIa receptors of platelet thrombi. *Investig. Radiol.* **2002**, *37*, 587–593. [CrossRef]
30. Alonso, A.; Della Martina, A.; Stroick, M.; Fatar, M.; Griebe, M.; Pochon, S.; Schneider, M.; Hennerici, M.; Allémann, E.; Meairs, S. Molecular imaging of human thrombus with novel abciximab immunobubbles and ultrasound. *Stroke* **2007**, *38*, 1508–1514. [CrossRef]
31. Beard, P. Biomedical photoacoustic imaging. *Interface Focus* **2011**, *1*, 602–631. [CrossRef]
32. Cui, C.; Yang, Z.; Hu, X.; Wu, J.; Shou, K.; Ma, H.; Jian, C.; Zhao, Y.; Qi, B.; Hu, X.; et al. Organic semiconducting nanoparticles as efficient photoacoustic agents for lightening early thrombus and monitoring thrombolysis in living mice. *ACS Nano* **2017**, *11*, 3298–3310. [CrossRef]
33. Licha, C.R.M.; McCurdy, C.M.; Maldonado, S.M.; Lee, L.S. Current management of acute pulmonary embolism. *Ann. Thorac. Cardiovasc. Surg.* **2020**, *26*, 65–71. [CrossRef] [PubMed]
34. Bader, K.B.; Bouchoux, G.; Peng, T.; Klegerman, M.E.; McPherson, D.D.; Holland, C.K. Thrombolytic efficacy and enzymatic activity of rt-PA-loaded echogenic liposomes. *J. Thromb. Thrombolysis* **2015**, *40*, 144–155. [CrossRef] [PubMed]
35. Wadajkar, A.S.; Santimano, S.; Rahimi, M.; Yuan, B.; Banerjee, S.; Nguyen, K.T. Deep vein thrombosis: Current status and nanotechnology advances. *Biotechnol. Adv.* **2013**, *31*, 504–513. [CrossRef] [PubMed]

36. Colasuonno, M.; Palange, A.L.; Aid, R.; Ferreira, M.; Mollica, H.; Palomba, R.; Emdin, M.; Del Sette, M.; Chauvierre, C.; Letourneur, D.; et al. Erythrocyte-Inspired Discoidal Polymeric Nanoconstructs Carrying Tissue Plasminogen Activator for the Enhanced Lysis of Blood Clots. *ACS Nano* **2018**, *12*, 12224–12237. [CrossRef]
37. Jogala, S.; Rachamalla, S.S.; Aukunuru, J. Development of PEG-PLGA based Intravenous Low Molecular Weight Heparin (LMWH) Nanoparticles Intended to Treat Venous Thrombosis. *Curr. Drug Deliv.* **2016**, *13*, 698–710. [CrossRef]
38. Fernandes, E.G.R.; de Queiroz, A.A.A.; Abraham, G.A.; San Román, J. Antithrombogenic properties of bioconjugate streptokinase-polyglycerol dendrimers. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 105–111. [CrossRef]
39. Mukhametova, L.I.; Aisina, R.B.; Zakharyan, E.M.; Karakhanov, E.A.; Gershkovich, K.B.; Varfolomeyev, S.D. Thrombolytic and fibrinogenolytic properties of bioconjugate streptokinase-polyamidoamine dendrimers in vitro. *Thromb. Res.* **2017**, *154*, 50–52. [CrossRef]
40. Wang, X.; Inapagolla, R.; Kannan, S.; Lieh-Lai, M.; Kannan, R.M. Synthesis, characterization, and in vitro activity of dendrimer-streptokinase conjugates. *Bioconjug. Chem.* **2007**, *18*, 791–799. [CrossRef]
41. Tasci, T.O.; Disharoon, D.; Schoeman, R.M.; Rana, K.; Herson, P.S.; Marr, D.W.M.; Neeves, K.B. Enhanced Fibrinolysis with Magnetically Powered Colloidal Microwheels. *Small* **2017**, *13*, 1700954. [CrossRef]
42. Li, C.; Du, H.; Yang, A.; Jiang, S.; Li, Z.; Li, D.; Brash, J.L.; Chen, H. Thrombosis-Responsive Thrombolytic Coating Based on Thrombin-Degradable Tissue Plasminogen Activator (t-PA) Nanocapsules. *Adv. Funct. Mater.* **2017**, *27*, 1703934. [CrossRef]
43. Park, J.; Wen, A.M.; Gao, H.; Shin, M.D.; Simon, D.I.; Wang, Y.; Steinmetz, N.F. Designing S100A9-Targeted Plant Virus Nanoparticles to Target Deep Vein Thrombosis. *Biomacromolecules* **2021**, *22*, 2582–2594. [CrossRef]
44. Hu, J.; Huang, W.; Huang, S.; ZhuGe, Q.; Jin, K.; Zhao, Y. Magnetically active Fe3O4 nanorods loaded with tissue plasminogen activator for enhanced thrombolysis. *Nano Res.* **2016**, *9*, 2652–2661. [CrossRef]
45. Jung, E.; Kang, C.; Lee, J.; Yoo, D.; Hwang, D.W.; Kim, D.; Park, S.-C.; Lim, S.K.; Song, C.; Lee, D. Molecularly Engineered Theranostic Nanoparticles for Thrombosed Vessels: H_2O_2-Activatable Contrast-Enhanced Photoacoustic Imaging and Antithrombotic Therapy. *ACS Nano* **2018**, *12*, 392–401. [CrossRef]
46. Hu, Q.; Qian, C.; Sun, W.; Wang, J.; Chen, Z.; Bomba, H.N.; Xin, H.; Shen, Q.; Gu, Z. Engineered nanoplatelets for enhanced treatment of multiple myeloma and thrombus. *Adv. Mater. Weinheim* **2016**, *28*, 9573–9580. [CrossRef]
47. Rumbaut, R.E.; Thiagarajan, P. *Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis*; Morgan & Claypool Life Sciences: San Rafael, CA, USA, 2010; Chapter Arterial, Venous, and Microvascular Hemostasis/Thrombosis; pp. 35–42.
48. Lippi, G.; Franchini, M.; Targher, G. Arterial thrombus formation in cardiovascular disease. *Nat. Rev. Cardiol.* **2011**, *8*, 502–512. [CrossRef]
49. Jin, J.; Quinton, T.M.; Zhang, J.; Rittenhouse, S.E.; Kunapuli, S.P. Adenosine diphosphate (ADP)-induced thromboxane A_2 generation in human platelets requires coordinated signaling through integrin $\alpha_{IIb}\beta_3$ and ADP receptors. *Blood* **2002**, *99*, 193–198. [CrossRef]
50. Olie, R.H.; van der Meijden, P.E.J.; Ten Cate, H. The coagulation system in atherothrombosis: Implications for new therapeutic strategies. *Res. Pract. Thromb. Haemost.* **2018**, *2*, 188–198. [CrossRef]
51. Chiarito, M.; Sanz-Sánchez, J.; Cannata, F.; Cao, D.; Sturla, M.; Panico, C.; Godino, C.; Regazzoli, D.; Reimers, B.; De Caterina, R.; et al. Monotherapy with a P2Y12 inhibitor or aspirin for secondary prevention in patients with established atherosclerosis: A systematic review and meta-analysis. *Lancet* **2020**, *395*, 1487–1495. [CrossRef]
52. Condello, F.; Liccardo, G.; Ferrante, G. Clinical Effects of Dual Antiplatelet Therapy or Aspirin Monotherapy after Acute Minor Ischemic Stroke or Transient Ischemic Attack, a Meta-Analysis. *Curr. Pharm. Des.* **2021**, *27*, 4140–4146. [CrossRef]
53. Valgimigli, M.; Gragnano, F.; Branca, M.; Franzone, A.; Baber, U.; Jang, Y.; Kimura, T.; Hahn, J.-Y.; Zhao, Q.; Windecker, S.; et al. P2Y12 inhibitor monotherapy or dual antiplatelet therapy after coronary revascularisation: Individual patient level meta-analysis of randomised controlled trials. *BMJ* **2021**, *373*, n1332. [CrossRef]
54. Mann, K.G.; Butenas, S.; Brummel, K. The dynamics of thrombin formation. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 17–25. [CrossRef]
55. Xu, J.; Zhang, Y.; Nie, G. Intelligent antithrombotic nanomedicines: Progress, opportunities, and challenges. *VIEW* **2021**, *2*, 20200145. [CrossRef]
56. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease—American College of Cardiology. Available online: https://www.acc.org/latest-in-cardiology/ten-points-to-remember/2019/03/07/16/00/2019-acc-aha-guideline-on-primary-prevention-gl-prevention (accessed on 21 February 2022).
57. Knuuti, J.; Wijns, W.; Saraste, A.; Capodanno, D.; Barbato, E.; Funck-Brentano, C.; Prescott, E.; Storey, R.F.; Deaton, C.; Cuisset, T.; et al. ESC Scientific Document Group 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur. Heart J.* **2020**, *41*, 407–477. [CrossRef]
58. Aboyans, V.; Ricco, J.-B.; Bartelink, M.-L.E.L.; Björck, M.; Brodmann, M.; Cohnert, T.; Collet, J.-P.; Czerny, M.; De Carlo, M.; Debus, S.; et al. ESC Scientific Document Group 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: The European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). *Eur. Heart J.* **2018**, *39*, 763–816.

59. Kleindorfer, D.O.; Towfighi, A.; Chaturvedi, S.; Cockroft, K.M.; Gutierrez, J.; Lombardi-Hill, D.; Kamel, H.; Kernan, W.N.; Kittner, S.J.; Leira, E.C.; et al. 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline from the american heart association/american stroke association. *Stroke* **2021**, *52*, e364–e467. [CrossRef]
60. Collet, J.-P.; Thiele, H.; Barbato, E.; Barthélémy, O.; Bauersachs, J.; Bhatt, D.L.; Dendale, P.; Dorobantu, M.; Edvardsen, T.; Folliguet, T.; et al. ESC Scientific Document Group 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur. Heart J.* **2021**, *42*, 1289–1367. [CrossRef]
61. ESC Guidelines on Acute Myocardial Infarction in Patients Presenting with ST-Segment Elevation (Management of). Available online: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines/Acute-Myocardial-Infarction-in-patients-presenting-with-ST-segment-elevation-Ma (accessed on 21 February 2022).
62. Capranzano, P.; Angiolillo, D.J. Tackling the gap in platelet inhibition with oral antiplatelet agents in high-risk patients undergoing percutaneous coronary intervention. *Expert Rev. Cardiovasc. Ther.* **2021**, *19*, 519–535. [CrossRef]
63. Feher, G.; Hargroves, D.; Illes, Z.; Klivenyi, P.; Liu, L.; Szapary, L. Editorial: Antiplatelet agents in stroke prevention. *Front. Neurol.* **2021**, *12*, 1674. [CrossRef]
64. Lee, J.; Jeong, L.; Jung, E.; Ko, C.; Seon, S.; Noh, J.; Lee, D. Thrombus targeting aspirin particles for near infrared imaging and on-demand therapy of thrombotic vascular diseases. *J. Control. Release* **2019**, *304*, 164–172. [CrossRef]
65. Zhao, Y.; Xie, R.; Yodsanit, N.; Ye, M.; Wang, Y.; Wang, B.; Guo, L.-W.; Kent, K.C.; Gong, S. Hydrogen peroxide-responsive platelet membrane-coated nanoparticles for thrombus therapy. *Biomater. Sci.* **2021**, *9*, 2696–2708. [CrossRef] [PubMed]
66. Kang, C.; Gwon, S.; Song, C.; Kang, P.M.; Park, S.-C.; Jeon, J.; Hwang, D.W.; Lee, D. Fibrin-Targeted and H_2O_2-Responsive Nanoparticles as a Theranostics for Thrombosed Vessels. *ACS Nano* **2017**, *11*, 6194–6203. [CrossRef] [PubMed]
67. Zhao, Y.; Xie, R.; Yodsanit, N.; Ye, M.; Wang, Y.; Gong, S. Biomimetic fibrin-targeted and H_2O_2-responsive nanocarriers for thrombus therapy. *Nano Today* **2020**, *35*, 100986. [CrossRef] [PubMed]
68. Casa, L.D.C.; Deaton, D.H.; Ku, D.N. Role of high shear rate in thrombosis. *J. Vasc. Surg.* **2015**, *61*, 1068–1080. [CrossRef] [PubMed]
69. Molloy, C.P.; Yao, Y.; Kammoun, H.; Bonnard, T.; Hoefer, T.; Alt, K.; Tovar-Lopez, F.; Rosengarten, G.; Ramsland, P.A.; van der Meer, A.D.; et al. Shear-sensitive nanocapsule drug release for site-specific inhibition of occlusive thrombus formation. *J. Thromb. Haemost.* **2017**, *15*, 972–982. [CrossRef]
70. Chen, C.; Li, S.; Liu, K.; Ma, G.; Yan, X. Co-Assembly of Heparin and Polypeptide Hybrid Nanoparticles for Biomimetic Delivery and Anti-Thrombus Therapy. *Small* **2016**, *12*, 4719–4725. [CrossRef]
71. Holme, M.N.; Fedotenko, I.A.; Abegg, D.; Althaus, J.; Babel, L.; Favarger, F.; Reiter, R.; Tanasescu, R.; Zaffalon, P.-L.; Ziegler, A.; et al. Shear-stress sensitive lenticular vesicles for targeted drug delivery. *Nat. Nanotechnol.* **2012**, *7*, 536–543. [CrossRef]