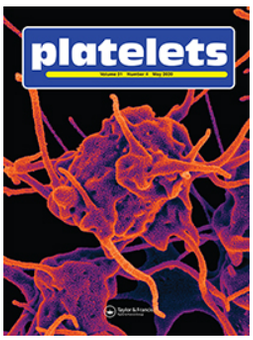

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Atherosclerotic plaque injury-mediated murine thrombosis models: advantages and limitations

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Abstract

In spite of current treatment strategies, myocardial infarction and stroke are still major causes of death worldwide. These events are triggered by damage of an atherosclerotic plaque, resulting in occlusive thrombus formation. Mouse studies have significantly contributed to our understanding of the mechanisms of atherogenesis and of thrombosis following plaque injury, but the extent to which the mouse serves as an accurate model of human disease is open to discussion. In this review, we provide a detailed overview and comparison of the described mouse models for atherothrombosis including their (dis)advantages. Herein guidance is provided on how to select a suitable atherothrombosis model for research questions primarily relevant to the field of thrombosis.

Keywords

Atherosclerosis, coagulation, murine atherothrombosis, plaque erosion, plaque rupture, platelets

History

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Introduction

In spite of current treatment strategies, myocardial infarction (MI) and stroke are still major causes of death [1]. The emergence of MI or stroke is preceded by damage of an atherosclerotic plaque, resulting in thrombus formation, which can either grow and thereby occlude a vessel or cause vessel occlusion by embolization. Classically, the rupture of a so-called vulnerable plaque, exposing blood to thrombogenic material residing within the plaque, is regarded as the major cause of acute coronary syndrome (ACS). However, plaque erosion of the overlying endothelium is gaining considerable attention as a cause of ACS. Eroded plaques have distinct morphological characteristics compared to a plaque that is rupture prone [2]. As a consequence, the mechanisms that trigger coronary thrombosis due to superficial erosion versus fibrous cap rupture might not be the same. Indeed, thrombi associated with superficial erosion appear more platelet-rich than the fibrinous clots triggered by plaque rupture [2].

A major preclinical approach for understanding the biology of plaque erosion or plaque rupture and subsequent thrombotic responses is the use of murine models. However, the atherosclerotic lesions observed in mouse models rarely progress to advanced stages leading to spontaneous plaque rupture with atherothrombotic vascular occlusion that are observed in humans. Therefore, various mouse models of atherosclerotic plaque injury leading to acute arterial thrombosis have been developed over the last 20 years. The extent to which these models accurately represent human disease is open to discussion. In this review, we provide a detailed overview and comparison of the described mouse models for atherothrombosis including their (dis)advantages. Hereby guidance is provided on how to select a suitable atherothrombosis model for research questions primarily relevant to the field of thrombosis and hemostasis. Inclusion criteria are the histological characterization of the extent of plaque injury and demonstration of occlusive or non-occlusive thrombotic response at the site of plaque injury.

Mouse Models for Atherosclerosis and Spontaneous Plaque Rupture

In humans, high plasma levels of low-density lipoprotein (LDL) are regarded as a major risk factor for atherosclerosis. In contrast, mice contain only low levels of LDL and are hence relatively resistant to the development of atherosclerosis. Genetic manipulations of the lipid metabolism by deletion of Apolipoprotein E (ApoE) or the LDL receptor (LDLR), often combined by feeding a high-fat, high-cholesterol diet, are the most common strategies to induce atherogenesis in mice. A main advantage of $Ldlr^{-/-}$ over $Apoe^{-/-}$ is that the lipid profile in $Ldlr^{-/-}$ is human-like whereas this is not the case for $Apoe^{-/-}$. In $Ldlr^{-/-}$ a high-fat diet is a prerequisite for atherogenesis, whereas in $Apoe^{-/-}$ it acts as an accelerator of plaque development. Indeed, at 3 months of high-cholesterol diet $Apoe^{-/-}$ mice have more advanced atherosclerotic lesions than $Ldlr^{-/-}$ mice [3]. A relatively new and upcoming approach, which enables for rapid atherogenesis in

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wild type mice, is by single adeno-associated virus (AAV)-mediated gene transfer of mutant pro-protein convertase subtilisin/kexin type 9 (PCSK9). This approach leads to increased total plasma cholesterol as well as VLDL- and LDL-cholesterol, and the development of atherosclerosis upon Western diet feeding [4]. More detailed information on the lipid metabolism and plaque composition of these mouse models for atherosclerosis is provided in comprehensive reviews by Getz et al. and Veseli et al. [3,5].

Whereas in humans, atherosclerotic lesions in the coronary and cerebral arteries, carotid bifurcations and abdominal aorta give rise to most clinical symptoms, in *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$ and PCSK9-AAV mice, atherosclerotic plaques are mostly observed in the aortic root, aortic arch, branch points of the brachiocephalic artery and the carotid bifurcations without significant coronary artery lesions [3,6]. However, the development of plaques can be induced in the coronary arteries of mice deficient in ApoE combined with either deficiency in the HDL scavenger receptor B1 (SR-BI) [7] or its downstream mediators Akt1 [8], endothelial nitric oxide synthase [9] or PDZK1 [10].

Since atherosclerotic lesions observed in these mouse models rarely progress to advanced stages leading to plaque vulnerability and spontaneous plaque rupture, as observed in humans, different strategies can be used to increase plaque vulnerability and spontaneous rupture with subsequent thrombosis, which can be categorized by: i) alteration of circulating lipoprotein and cholesterol levels by dietary and/or genetic manipulations, e.g. feeding *Apoe*$^{-/-}$ or *Apoe*$^{-/-}$/*Ldlr*$^{-/-}$ mice a high-fat diet for approximately 3 months or feeding *Apoe*$^{-/-}$/*Srbi*$^{-/-}$ mice a standard chow diet for 6 weeks [3,7], ii) applying a tandem stenosis around the carotid artery of *Apoe*$^{-/-}$ mice [11], iii) continuous infusion of angiotensin II in *Apoe*$^{-/-}$ mice fed a high-fat diet for 4 weeks [12], iv) crossbreeding *Apoe*$^{-/-}$ mice with mice presenting a mutant fibrillin-1 allele (Fbn1C1039G), producing an impaired elastin structure of the vessel wall leading to arterial stiffening and large vulnerable atherosclerotic plaques [13], v) inducing blood hypercoagulability in *Apoe*$^{-/-}$ by overexpression of tissue factor (TF) or prothrombin, by silencing activated protein C or by inhibiting its thrombomodulin-dependent generation [14,15]. We refer to recent reviews by Ouweneel et al. [16] and Oppi et al. [17] for a more in-depth description of these atherothrombosis models, including the site and frequency of thrombosis and a link to the original research papers. The incidence of luminal thrombosis ranges widely (~5–75%) between the models, which in theory can allow for monitoring of the effect of antithrombotic medication on the presence of thrombi by using histology. However, since the incidence and vascular location of spontaneous plaque rupture remains unpredictable, various methods have been developed to provoke plaque injury and acute thrombus formation, which are detailed below.

Mouse Models of Thrombus Formation after Acute Plaque Injury

The literature (Pubmed 1980-May 2019) was screened for original research articles using the following keywords: atherothrombosis mouse, plaque rupture mouse, murine atherothrombosis, murine plaque rupture, thrombus plaque mouse. The search was expanded by using the 'similar article' function in PubMed. Damage of the carotid artery of *Apoe*$^{-/-}$ mice appeared to be the model of choice to elicit an acute thrombotic response in atherosclerotic mouse vessels. Of all plaque-containing murine vessels the carotid artery is most easily accessible to apply plaque damage and monitor subsequent thrombus formation in real time. Below we provide a brief description of these models including their (dis)advantages (Table I). We excluded the inside wire model [39], which is suitable to study vascular remodeling after the adhesion of platelets to the atherosclerotic vessel wall, as no actual thrombus appeared to be formed.

Ultrasound-induced Plaque Injury

In the model of ultrasound-induced plaque injury, the tip of an ultrasound probe is pressed against the carotid plaque shoulder and ultrasound is applied for 10 s, as established by two research groups (Table I) [18,19]. In both cases, the application of ultrasound results in loss of the endothelial cell layer and presence of adherent platelets in contact with plaque material (Figure 2b), collagen exposure, and luminal formation of a platelet-rich thrombus, accompanied by intra-plaque intrusion of erythrocytes and fibrin formation. Formed thrombi are non-occlusive and display a biphasic kinetic, with a maximum in thrombus size reached after 30–60 s, after which the thrombus size progressively declines within 10–15 min. Thus, the application of ultrasound induces only mild injury of the plaque resulting in the formation of relatively small non-occlusive thrombi.

Inside Needle-induced Plaque Injury

Two groups have reported successful injury on an atherosclerotic plaque using a suture needle. The needle is either introduced through a collateral of internal carotid in a non-plaque area [25,26] or directly into the plaque [19] (Figure 1). The duration of the scratching varies according to the model, with the needle being moved forward and backward only twice [25] or the scratching being performed for 3 to 4 min until rupture of the plaque is observed under the optical microscope [19]. Scratching leads to the formation of either a small thrombus on the surface of the plaque [25] or a larger fibrin-rich thrombus in contact with collagen-rich plaque material, which reaches maximum size just after the needle is removed and gradually decreases over the next 15 min (Figure 2a) [19]. In both cases, plaque damage is more pronounced than upon ultrasound application, with rupture of the atherosclerotic plaque and exposure of the plaque material to the blood (Figure 2a,b) [19,25], leading to the formation of a thrombus that is larger in size [19].

Ligation-induced Plaque Injury

In the ligation-induced model of plaque injury, the common carotid artery is ligated near the carotid bifurcation for 5 min to induce plaque rupture, after which the wire is removed [28]. Although this injury technique is fairly straightforward to perform and induces rapid platelet adhesion to the damaged vessel wall, it does not always appear to promote thrombus formation. In 16-week-old *Apoe*$^{-/-}$ mice fed a high-fat diet, thrombus formation was minimal as evaluated by intravital fluorescence microscopy [28]. In contrast, an abstract reported reproducible formation of non-occlusive platelet-rich thrombi that remained present for a prolonged time (intravital microscopy and histology) [29]. Other investigations using the ligation-induced model studied vessel-wall remodeling rather than atherothrombosis and report no information on initial plaque injury. In summary, it has not been clearly described yet to which extent the atherosclerotic plaque is injured by ligation.

Ferric Chloride-induced Plaque Injury

To our knowledge, only three research groups have used ferric chloride ($FeCl_3$) to induce thrombosis in plaque-containing vessels [30–32] (Table I). The size of the thrombus is larger and occlusive as compared to the non-occlusive thrombi formed in the case of ultrasound or needle injury, indicating that the severity of the vascular damage is likely to be greater upon $FeCl_3$ application. Of the three groups, two applied $FeCl_3$ to the normal-appearing segment of the carotid artery [31,32], questioning the extent of actual plaque injury. Unfortunately, none of these studies

Table I. Overview of mouse models of thrombotic response after acute plaque injury.

Model Brief description	Thrombus size; measurement	Components involved in thrombus formation[*1]	Diet and mouse age	Advantages	Disadvantages	Ref
Ultrasound						
Ultrasound treatment from outside the vessel at the plaque shoulder. Mild injury of the plaque with loss of the endothelial cell layer, collagen exposure and luminal formation of a platelet-rich thrombus, accompanied by intra-plaque intrusion of erythrocytes and fibrin formation [18,19].	Small, non-occlusive; microscopy with labeled platelets and histology	ADP, CD40L, collagen, fibrin, FXI, FXII, thrombin	Western-type, 16% fat and 0.15% chol for 18–20 wks, start at wk 4	Local damage, real-time kinetics	Rapid increase and decrease thrombus size	[18,20–22]
		ADP, collagen, fibrinogen, PGE2, thrombin	Chow with 3% fat for 45 wks			[19]
Inside needle						
Plaque injury is induced by scratching the plaque with a suture needle, leading to plaque rupture and exposure of collagen-rich plaque material.	Large fibrin-rich thrombus in contact with collagen-rich plaque material, non-occlusive; microscopy with labeled platelets or mMRI	ADP, collagen, fibrinogen, FX, PGE2, thrombin	Chow with 3% fat for 45 wks	Local damage, real-time kinetics. Scratching of the plaque in combination with real-time monitoring fosters eliciting a consistent thrombogenic response	Requires skilled experimenter	[19,23,24]
		Fibrin, PGE2	Chow for > 55 wks ± 5 wks high fat diet		Requires highly skilled experimenter since the needle is introduced through small collateral of external carotid artery	[25–27]
Ligation						
Suture around vessel of interest is tightened for 5 minutes. Vascular damages triggers platelet adhesion only, without clear thrombus formation.	Single platelets, non-occlusive; microscopy or PET imaging with labeled platelets	ADP, GPVI, TxA_2	Diet with 0.25% chol for 8–12 wks, start at wk 4	Straightforward to perform	No evidence provided for plaque injury and/or the presence of thrombi	[28,29]
$FeCl_3$						
Filter paper with 5-20% $FeCl_3$ applied on adventitial surface of vessel at the site of a plaque [30] or adjacent to a plaque [31,32].	Large, occlusive fibrin-rich; doppler flow probe (TTO)	Platelet dense granules	Diet with 42% calories from fat for 16–18 wks, start at wk 4	Straightforward to perform, duration and concentration of $FeCl_3$ treatment can be adjusted to modulate extent of damage	$FeCl_3$ causes severe damage in all layers of the vessel wall (adventitia, media, intima). No histological analysis of plaque injury	[32]
		FVII and TF, no role for FXI and FXII	High fat chow for >90 days, start at wk 12			[30]
		PAI-1	Diet with 21% fat and 0.15% chol for 14 weeks, start at wk 6-8			[31]
Photochemical						
Free radical formation upon illumination of a photosensitive dye, injected intravenously, by laser light resulting in plaque damage.	Large, occlusive fibrin-rich; doppler flow probe (TTO)	FX, PAI-1, thrombin, TF, Type I IFNs, no role for FXI and FXII	Chow for 30 wks or western diet for 10 wks, start at wk 8–10	Local damage, straightforward to perform	No detailed histological analysis of plaque injury	[30,33–38]

All studies were performed in the carotid artery of *Apoe*$^{-/-}$ mice.

Abbreviations: adenosine diphosphate (ADP), CD40 ligand (CD40L), cholesterol (chol), glycoprotein VI (GPVI), factor VII (FVII), factor X (FX), factor XI (FXI), factor XII (FXII), ferric chloride ($FeCl_3$), interferons (IFNs), molecular magnetic resonance imaging (mMRI), plasminogen activator inhibitor-1 (PAI-1), positron emission tomography (PET), prostaglandin E2 (PGE2), thromboxane A2 (TxA_2), time to occlusion (TTO), tissue factor (TF), week(s) (wk(s)).

AD1: the involvement of platelet and plasma components in thrombus formation was tested using pharmacological inhibitors or knock-out mice.

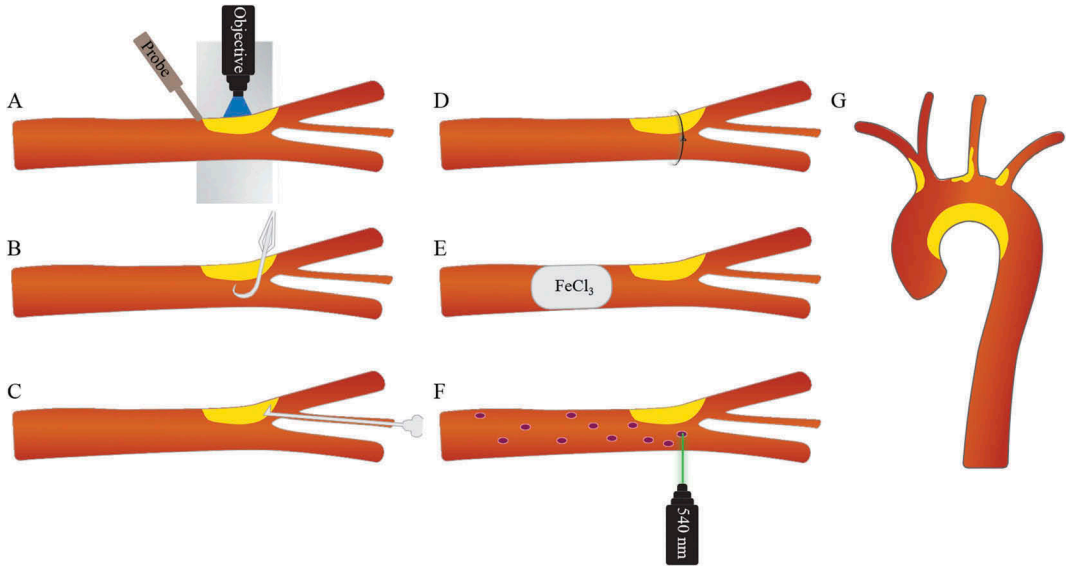

Figure 1. Schematic representation of the different models of atherosclerotic plaque injury in the mouse carotid artery.
(a) Ultrasound-induced plaque injury, (b) Inside needle-induced plaque injury according to Hechler et al. [19] (c) Inside needle-induced plaque injury according to Gross et al. [25] (d) Ligation-induced plaque injury, (e) Ferric chloride-induced plaque injury, (f) Photochemical-induced plaque injury, (g) Spontaneous plaque rupture. See Table I for original research articles on which these schematic representations are based.

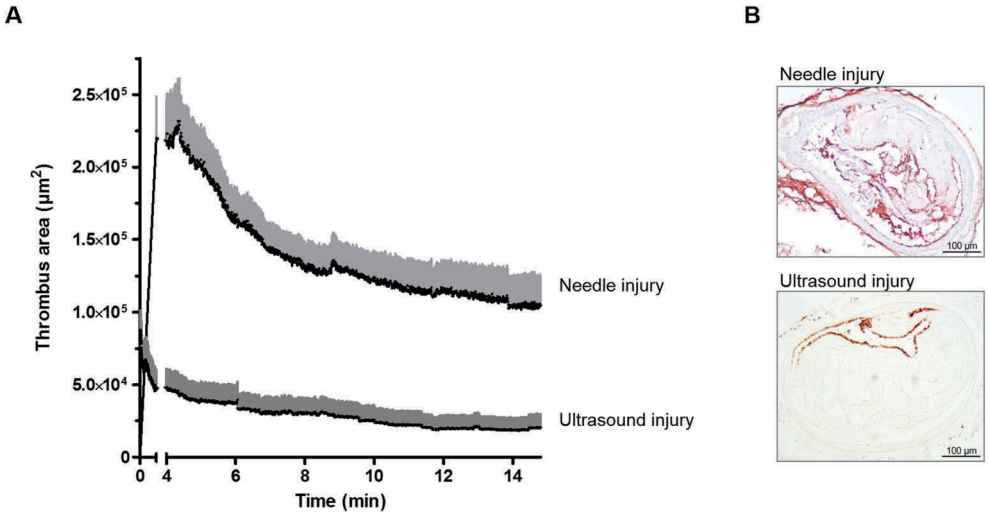

Figure 2. Thrombosis of *Apoe*$^{-/-}$ carotid artery triggered by injury with ultrasound or with a suture needle.
(a) Time-course of thrombosis in response to ultrasound injury or needle injury of carotid artery of an *Apoe*$^{-/-}$ mouse. (b) Transversal cryosection of the carotid artery at the site of injury with immunostaining for platelets (GPIbβ brown staining) with hematoxylin counterstaining only in the case of needle injury.

provided histological analysis, which makes it difficult to assess the extent of the plaque damage. However, in healthy non-atherosclerotic mice, the application of a filter paper soaked in a $FeCl_3$ solution on the adventitial side of the carotid artery induces severe damage to all layers of the vessel wall. Based on histology and electron microscopy analyses, the following various mechanisms for initiation of thrombosis in healthy vessels have been proposed: i) formation of ferric ions-filled spherical bodies, with tissue factor on their surface, budding of the endothelium into the lumen and leading to rapid formation of a fibrin-rich thrombus [40], ii) binding of platelets to endothelial associated red blood cell-derived material rather than to the endothelium or collagen [41], and iii) $FeCl_3$-induced aggregation of plasma proteins and blood cells, independent of endothelial cells or collagen [42]. Moreover, Eckly et al. [40] have shown that $FeCl_3$ alters the ability of adhesive proteins including collagen, fibrinogen or von Willebrand factor (vWF) to support platelet adhesion *in vitro*. Of note, the study of glycoprotein VI (GPVI)-immunodepleted or *Gp6*$^{-/-}$ mice in this model of $FeCl_3$-induced thrombosis yielded contrasting results, ranging from a prothrombotic role of GPVI to no role, while there is consensus on the major role of thrombin [43].

In sum, the $FeCl_3$ model should be further characterized for studies of atherothrombosis, among others with regard to the extent of plaque damage, before clear recommendations can be made.

Photochemical-induced Plaque Injury

Eitzman, Westrick, and colleagues [33–35] were the first to perform photochemical injury of plaques in the carotid artery of mice (Table I). In this method, the photosensitive dye Rose Bengal,

injected intravenously, is illuminated by green laser light proximal to the carotid bifurcation leading to the production of reactive oxygen species and subsequent occlusive thrombus formation that persists for at least 24 h [34,44]. Histological analysis of injured atherosclerotic segments 24 h after photochemical injury demonstrates the presence of thrombi associated with the atherosclerotic lesion [34]. Thus, photochemical-induced plaque injury leads to the formation of a thrombus that is larger in size as compared to the non-occlusive thrombi formed in the case of ultrasound or needle injury. Although histological analysis confirms plaque injury [30], detailed characterization of the extent of plaque damage has yet to be carried out, in particular, to determine whether collagen-rich plaque material becomes exposed to blood. Similarly, thrombus composition has not been studied in such an atherothrombosis setting, but studies in healthy vessels have shown that thrombi predominantly consist of platelets and a large amount of fibrin, indicating involvement of thrombin [44,45].

Molecular Mechanisms of Murine Atherothrombosis

The main pathways for thrombus formation at the site of atherosclerotic plaque injury are similar to those already identified in the process of thrombus formation on a healthy mouse artery. In the ultrasound and needle injury models, atherothrombosis involves the complementary activation of platelets by the collagen-GPVI axis and the generation of thrombin and fibrin formation triggered by plaque-tissue factor. These two models are sensitive toward both antiplatelet drugs, e.g. GPIIb-IIIa antagonist eptifibatide, $P2Y_{12}$ ADP-receptor inhibitor clopidogrel and inhibitors of the common pathway of coagulation (FXa and thrombin) [18,19,23,24,46]. Yet, they display differences with respect to the role of the platelet collagen receptor GPVI, with the needle injury model being less sensitive to its inhibition as compared to the ultrasound-induced injury, which may be related to the amount of thrombin generated. Both the extrinsic (TF/FVIIa) and intrinsic (FXII, FXI) pathways of coagulation appears to contribute to atherothrombosis following ultrasound plaque injury [18,20,21], while their respective contribution in the model of needle plaque injury remains to be investigated.

In experimental atherothrombosis following $FeCl_3$ or photochemical plaque injury, a prominent role of the extrinsic coagulation pathway (TF/FVIIa) over that of the intrinsic pathway has been reported [30]. Accordingly, *Apoe*$^{-/-}$ mice presenting heterozygous deficiency for tissue factor pathway inhibitor (TFPI), the major TF antagonist, display a profound decreased time to occlusive thrombosis after photochemical plaque injury [35]. Platelet dense granule constituents appear also essential for atherothrombosis following $FeCl_3$ plaque injury [32], while the potential role of the collagen-GPVI axis has not been evaluated. In addition, endogenous fibrinolysis, regulated by plasminogen activator inhibitor-1 (PAI-1), has an important role in occlusive vascular thrombosis after $FeCl_3$- or photochemical-induced plaque injury, since PAI-1 deficiency prolonged the time to occlusive thrombosis [31,33,36].

Studies described above (see also Table I) indicate that murine atherothrombosis models are suitable for testing anti-platelet and anti-thrombotic therapies. However, the atherosclerotic plaque contains a range of other factors in addition to collagen and tissue factor, which can potentially contribute to its thrombogenicity, such as adhesive proteins (vWF, fibrin/fibrinogen, thrombospondin, vitronectin, fibronectin, podoplanin), platelet-activating lipids (oxidized LDL, cholesterol and lysophosphatidic acid) or chemokines [2]. It is of importance to determine whether there are unique thrombogenic plaque components that would allow for more selective targeting of the thrombotic process at the atherosclerosis lesion site while preserving hemostasis. Another important aspect to consider is the influence of rheological conditions on thrombosis. Experimental infusion chambers with fixed stenosis, which reproduce the flow disturbance conditions found in diseased atherosclerotic arteries, have demonstrated the importance of disturbed blood circulation conditions to accelerate the deposition of platelets on thrombogenic surfaces [47]. In addition, mechanistic insight in the atherothrombosis forming process has been obtained from the perfusion of whole blood over homogenized human plaque material under arterial shear conditions in microfluidic chambers [22,46,48,49]. Recently, a high correlation has been demonstrated between flow-dependent thrombus formation determined by microfluidic devices and models of collagen-dependent murine experimental arterial thrombosis in healthy vessels [43], substantiating the suitability of using microfluidics to study aspects of the atherothrombosis forming process.

In the near future, the *in vivo* models of thrombosis on injured atherosclerotic plaques should allow assessing the potential of various targets, some of which are mentioned here: i) the GPIb-vWF axis, which is favored under conditions of high shear stress, typically encountered in stenotic arteries [50], ii) inhibition of GPVI-fibrin interaction [51,52], iii) phosphatidylinositol 3-kinase-β (PI3Kβ) which acts downstream of most platelet receptors and integrins and is critical to maintain thrombus stability at a pathological shear rate [53], or iv) inhibitors of Bruton tyrosine kinase (Btk). Btk inhibitors have been reported to selectively block GPVI-induced platelet activation upon exposure to atherosclerotic plaque material *in vitro*, while sparing shear-dependent integrin $\alpha_2\beta_1$- and VWF/GPIb-mediated platelet adhesion and aggregation on collagen, which is important for physiologic hemostasis [54].

Relevance to Human Pathophysiology and Future Outlook

Mouse models have provided us with useful mechanistic insight into plaque formation, rupture, and thrombosis. Choosing a mouse model most suitable to answer a specific research question is of vital importance. In Figure 3 guidance is provided for atherothrombosis model selection. A question that often arises is to what extent data obtained in mice are of relevance to human pathophysiology? Clearly men are distinct from mice and novel drug targets, with proven efficacy and safety in animal models, sometimes fail in clinical trials. The latter for instance occurred with the direct thrombin inhibitor melagatran that appeared to cause hepatotoxicity as a side effect in humans [55]. However, such an example does not imply a general lack of translational potential of animal models to humans. A more nuanced view and in-depth knowledge is required.

The more recent ability of linking large sets of mouse data to relevant human GWAS studies has provided the research field with new tools to assess the translational potential of murine atherosclerosis and thrombosis studies. Regarding the translation of genes involved in murine atherosclerosis to atherosclerosis in humans, two contrasting reports have been published in which human genome-wide association study (GWAS) genes associated with coronary artery disease (CAD) were linked to genes with reported involvement in murine atherosclerosis. Pasterkamp et al. [56] described for 11 out 659 mouse genes – with demonstrated involvement in murine atherosclerosis – an overlap with human genes associated with CAD. Von Scheidt et al. [57] took GWAS as starting point and showed that out of 244 human genes that are associated with CAD only 46 have been studied in mice. Strikingly, 45 out of these 46 genes significantly affect murine atherosclerosis. These conflicting results can be partly explained

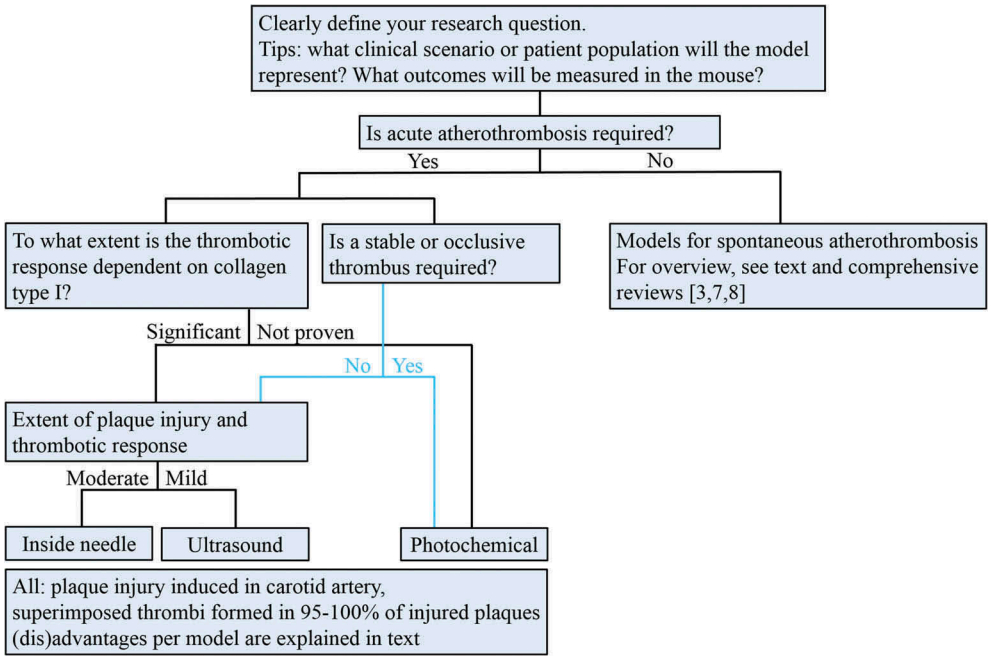

Figure 3. Guidance for atherothrombosis model selection. This flow chart may serve as a starting point for considerations in selection of an atherothrombosis model. See the text for additional explanation. Inclusion criteria are histological characterization of the extent of plaque injury and demonstration of occlusive or non-occlusive thrombotic response at the site of plaque injury.

by differences in approach, such as selection criteria for inclusion of mouse models.

A different approach was employed by Baaten et al. [43], whom developed a novel synthesis method to quantitatively compare studies on the role of mouse genes in arterial thrombosis and overcome limitations caused by sample size and differences in methodology. Of the 431 studied mouse genes 60 genes showed a consistent effect on murine arterial thrombosis. For these 60 genes, an overall high homology on the nucleotide level was present with the human orthologs. Also, a network was constructed with human protein orthologs of 267 genes with modifying effects on murine arterial thrombosis. This network covered substantial gene sets identified in GWAS of stroke, CAD, platelet count and volume, and related studies. Each approach has its strengths and limitations. Understanding the model characteristics is of vital importance. For the study by Baaten et al. [43] holds among others that genes with a role in the vascular component of thrombotic disorders are underrepresented. However, GWAS only detect variants that are common in the population and whose effects on risk (odds ratio) are large enough to become significant at the very stringent genome-wide significance level (10^{-7}–10^{-8}). Statistical power is in turn strongly dependent on population size and, importantly, an association that arises from a GWAS does not necessarily imply a causal relationship [58]. Hence, studying candidate genes that emerge from human GWAS in mouse models could either confirm the prediction made GWAS and/or provide new (contrasting) information.

In sum, in the past mouse models have provided use with useful insights in molecular mechanisms of atherothrombosis. Even with upcoming technologies, murine atherothrombosis models are of great value and indispensable for obtaining unique information with regard to all in vivo components of this process.

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Conflict of interest

The authors report no relevant conflict of interest.

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