

# Role of Endothelial Cells in Acute and Chronic Thrombosis

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## Abstract

Haemostasis encompasses a set of strictly regulated actions, such as vasoconstriction, platelet activation and blood coagulation. Endothelial cells play a crucial role in all of these processes and are an integral part of the vascular response to injury resulting in thrombus formation. Healthy endothelium expresses mediators to prevent platelet activation, including prostacyclin and nitric oxide, and to inhibit coagulation, such as thrombomodulin or RNase1. Upon activation, endothelial cells expose von Willebrand factor, integrins and other receptors to interact with activated platelets, erythrocytes and coagulation factors, respectively, resulting in blood clot formation. The endothelial cell response to cytokines and growth factors released from activated platelets and immune cells abundantly present in arterial and venous thrombi also plays an important role for thrombus resolution, whereas failure to completely resolve thrombi may initiate fibrotic remodelling and chronic vascular occlusion both in the arterial and venous tree. Therefore, endothelial cells are increasingly recognized as potential target to prevent thrombotic events and to accelerate thrombus resolution. Here, we discuss recent publications from our group in the context of other studies on the role of the endothelium during acute and chronic thrombotic events.

## Keywords

- ▶ arterial
- ▶ endothelial dysfunction
- ▶ thrombosis
- ▶ thrombus resolution
- ▶ venous

## Zusammenfassung

Die Hämostase umfasst eine Reihe von streng regulierten Abläufen wie Vasokonstriktion, Thrombozytenaktivierung und Blutgerinnung. Endothelzellen spielen eine entscheidende Rolle in all diesen Prozessen und sind ein integraler Bestandteil der vaskulären Antwort auf Verletzungen, die zur Thrombusbildung führen. Gesundes Endothel exprimiert Mediatoren zur Verhinderung der Thrombozytenaktivierung einschließlich Prostacyclin und Stickoxid und zur Hemmung der Gerinnung, wie Thrombomodulin oder RNase1. Nach der Aktivierung exponieren Endothelzellen den von Willebrand-Faktor, Integrine und andere Rezeptoren, um mit aktivierten Thrombozyten, Erythrozyten bzw. Gerinnungsfaktoren zu interagieren, was zur Bildung von Blutgerinnseln führt. Die Endothelzellenantwort auf Cytokine und Wachstumsfaktoren, die von aktivierten Blutplättchen und Immunzellen in arteriellen und venösen

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**Schlüsselwörter**

- ▶ arteriell
- ▶ endotheliale Dysfunktion
- ▶ Thrombose
- ▶ Thrombusauflösung
- ▶ venös

Thromben freigesetzt werden, spielt ebenfalls eine wichtige Rolle bei der Thrombusauflösung, während eine unvollständige Auflösung von Thromben fibrotische Umbauprozesse und einen chronischen Gefäßverschluss sowohl im arteriellen als auch im venösen Bereich auslösen kann. Daher werden Endothelzellen zunehmend als potenzielles Ziel zur Vorbeugung thrombotischer Ereignisse und zur Beschleunigung der Thrombusauflösung erkannt. Hier diskutieren wir aktuelle Publikationen aus unserer Gruppe im Zusammenhang mit anderen Studien zur Rolle des Endothels bei akuten und chronischen thrombotischen Ereignissen.

**Introduction**

Endothelial cells cover the surface of all blood vessels. They provide an important barrier between the cellular and noncellular components of the circulating blood and the interstitium; regulate tissue perfusion and supply with oxygen and nutrients; help in the recruitment of inflammatory cells and control blood pressure in conjunction with underlying smooth muscle cells and pericytes endothelial cells. The fundamental role of endothelial dysfunction for cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure and peripheral artery disease, has been established in numerous clinical and experimental studies. Despite the large body of existing knowledge, new facets and functions of the endothelium, one of the largest 'organs' of our body, continue to emerge. Moreover, changes in risk factor exposure (such as increasing age, noise or air pollution) and novel therapeutic options (such as direct thrombin or factor Xa inhibitors) have yielded additional insights into the regulation and response of endothelial cells. In this short review article, we will briefly summarize the existing knowledge on the role of endothelial cells in acute and chronic thrombosis (or thrombus formation and thrombus resolution) and also highlight recent findings obtained, among others, through interactive and interdisciplinary translational research efforts at the Center for Thrombosis and Hemostasis (CTH) at the University Medical Center in Mainz, Germany.

**Endothelial Control of Platelet Activation and Coagulation**

Healthy endothelial cells express several molecules that counteract platelet activation and prevent coagulation and thrombus formation to maintain unobstructed blood flow and tissue perfusion. The control of platelet adhesion and activation is achieved by the expression of negatively charged heparan sulfate proteoglycans on the endothelial cell surface<sup>1</sup> as well as by ectonucleotidases (such as CD39) catalysing the conversion of the platelet agonist adenosine diphosphate (ADP) released from activated platelets and red blood cells into adenosine.<sup>2</sup> Interaction of endothelial cells with platelets or stimulation with thrombin liberates prostacyclin I<sub>2</sub> (PGI<sub>2</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), two potent platelet antagonists.<sup>3</sup> The release of nitric oxide (NO) produced by endothelial nitric

oxide synthase (eNOS) represents another means by which endothelial cells contribute to the prevention of platelet activation and adhesion.<sup>4,5</sup> The parallel relaxation of vascular smooth muscle cells and vasodilation in response to NO may reduce the degree of thrombotic vessel obstruction and limit the extent of ischaemic tissue damage.<sup>6</sup> Ribonuclease 1 (RNase1)—released from specialized intracellular storage granules, the so-called Weibel–Palade (WP) bodies, upon stimulation of endothelial cells with thrombin, tumour necrosis factor (TNF)- $\alpha$  or vascular endothelial growth factor (VEGF),<sup>7–9</sup> degrades extracellular procoagulant RNA, and administration of RNase1 has been shown to delay arterial thrombus formation and blood vessel occlusion in mice.<sup>10</sup>

The endothelium also plays a primary role in the prevention of thrombin generation. Endogenous heparan sulphates in the endothelial glycocalyx bind the potent thrombin inhibitor antithrombin (AT).<sup>11</sup> Endothelial cells also express specific receptors that control coagulation by binding thrombin and converting its coagulant into anticoagulant properties. Thrombomodulin, constitutively expressed on endothelial cells, in conjunction with endothelial cell protein C receptor (EPCR) accelerates the thrombin-catalysed activation of protein C to generate activated protein C (APC), a circulating serine protease with potent anticoagulant activity via irreversible inactivation of factors Va and VIIIa.<sup>12,13</sup> Loss or inactivation of endothelial thrombomodulin, for example, in response to TNF $\alpha$ ,<sup>14</sup> predisposes to coagulation activation and thrombosis. In this regard, plasma levels of soluble thrombomodulin were found to be elevated in patients with ST segment elevation myocardial infarction (STEMI) developing cardiogenic shock.<sup>15</sup>

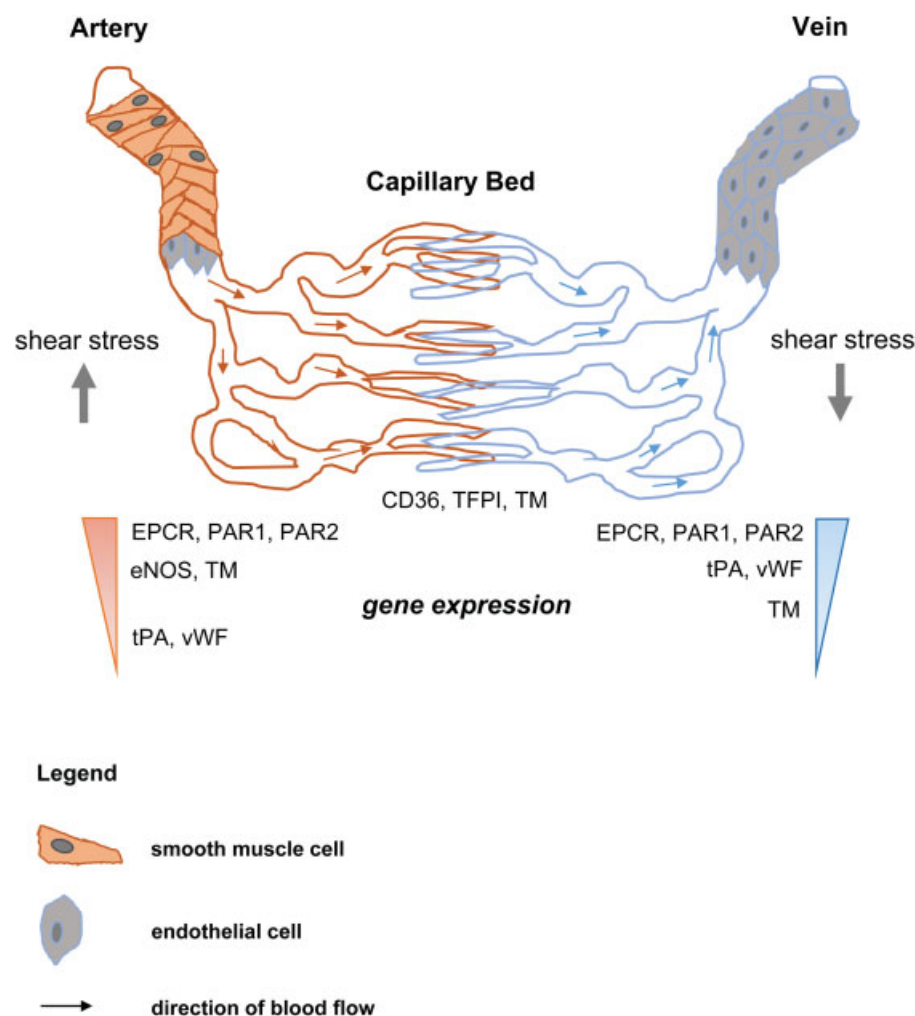
Endothelial cells also express tissue factor pathway inhibitor (TFPI), which binds and inhibits the factor VIIa/tissue factor (TF) complex, thus preventing initiation of the extrinsic coagulation pathway.<sup>16</sup> Mice with endothelial-specific deletion of TFPI exhibit accelerated thrombus formation in response to ferric chloride-induced arterial injury,<sup>17</sup> and lower plasma TFPI levels have been reported in patients with STEMI,<sup>18</sup> ischaemic stroke<sup>19</sup> or deep vein thrombosis.<sup>20</sup>

The aforementioned properties of endothelial cells describe functions of healthy endothelium and are typically lost or shifted to a prothrombotic phenotype under the influence of cardiovascular risk factors, inflammatory or procoagulant stimuli, a phenomenon described as 'endothelial dysfunction' (see later).

## Endothelial Heterogeneity Affecting Factors Controlling Haemostasis and Thrombosis

Genetic and phenotypic differences known to exist between endothelial cells from different vascular beds and organs<sup>21,22</sup> include surface receptors involved in haemostasis and coagulation control. For example, both thrombomodulin and EPCR are poorly expressed on brain microvascular endothelial cells,<sup>23,24</sup> although the implications of this observation are not clear. Tissue plasminogen activator (tPA) is strongly expressed on vein endothelium, which may contribute to the higher propensity of venous thrombi to embolize.<sup>6</sup> CD36 or platelet glycoprotein IV, one of several receptors for collagen,<sup>25</sup> is found primarily on microvascular endothelial cells.<sup>26</sup> A short schematic overview of factors controlling thrombosis and haemostasis differentially expressed in endothelial cells lining arteries, veins and capillaries is given in **Fig. 1**. The location-specific heterogeneity of endothelial cells may also contribute to the known differences in arterial, venous and microvascular thrombus composition, besides differences in hemodynamic forces between vascular beds.

Both arterial and venous endothelial cells express receptors cleaved and activated by the serine protein thrombin, protease activated receptor (PAR), which exist as four members, PAR-1 to PAR-4.<sup>27</sup> The procoagulant response of the endothelium to thrombin is largely mediated by PAR-1.<sup>28</sup> PAR-2 is expressed to a lesser extent on endothelial cells and, like PAR-1, responds to thrombin and activated coagulation factors,<sup>29</sup> and also to trypsin and tryptase.<sup>30</sup> PAR-3 and PAR-4 are not expressed on the endothelium in significant amounts. Activation of PAR-1 on endothelial cells by thrombin is responsible for the production of NO and PGI<sub>2</sub> and induces the release of von Willebrand factor (vWF) and tPA from WP bodies.<sup>31</sup> Thrombin-induced activation of PAR-1 and PAR-2 mediates the expression of TF in cultivated endothelial cells.<sup>32</sup> Of note, activation by the EPCR/APC complex switches endothelial PAR-1 signalling toward the transduction of anticoagulant and cytoprotective effects, including antiapoptotic, anti-inflammatory and proangiogenic activities.<sup>33</sup> Therefore, the usefulness of the so-called 'parmodulins' to safely activate APC-like cytoprotective signalling in endothelial cells is currently examined in several studies.<sup>34</sup>



**Fig. 1** Heterogeneity of endothelial cells with regard to factors involved in thrombosis and haemostasis. Schematic drawing showing endothelial expression of factors involved in preventing platelet activation and blood coagulation according to the presence of shear stress (artery vs. vein) and the endothelial bed (adapted from references 21–27).

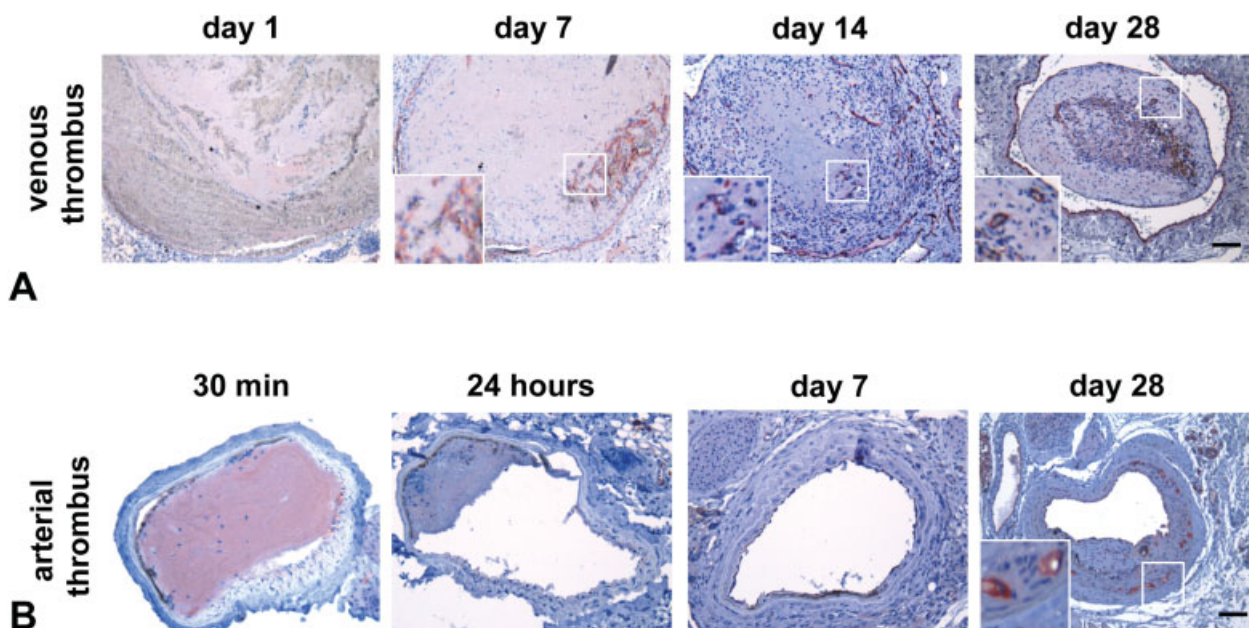
## Endothelial Integrity: An Indirect Means to Prevent Thrombosis

The integrity of the endothelial layer per se may influence the thrombotic response. An increase in vascular permeability (such as during inflammation) may lead to a shift of fluids, albumin and molecules with a similar molecular weight, including AT and protein C, from the intravascular compartment into the extravascular space and thus reduce the amount of natural anticoagulants while at the same time increasing blood viscosity. Following thrombosis, reconstitution of endothelial integrity and coverage of prothrombotic extracellular matrix proteins present in the vessel wall and exposed after injury or atherosclerotic plaque rupture represents another important function of this cell type. Factors modulating endothelial proliferation and migration, including VEGF or transforming growth factor- $\beta$  (TGF $\beta$ ), are released from activated platelets.<sup>35</sup> Platelet-derived VEGF is bioactive, accumulates in thrombi<sup>36</sup> and may act as a local proangiogenic agent enhancing recanalization.<sup>37</sup> Conversely, neutralization of VEGF or inhibition of VEGF signalling has been shown to impair venous thrombus revascularization and, consequently, resolution.<sup>38,39</sup> Platelet granule secretion may thus accelerate reconstitution of endothelial integrity following injury, which induces endothelial and smooth muscle cell quiescence,<sup>40</sup> but also prevents further activation of the clotting cascade and thrombus propagation by creating a barrier between blood and the thrombus surface.<sup>41</sup> Enhancing the regenerative capacities of the endothelium may thus constitute an indirect antithrombotic strategy. In this regard, several studies including that of our group have examined the potential of endothelial progenitor cells to enhance revascularization after arterial injury<sup>42,43</sup> and to

promote venous thrombus resolution.<sup>44</sup> On the other hand, we could recently show that TGF $\beta$  released from activated platelets does not alter the thrombotic response to arterial injury, but impairs lesion re-endothelialization and promotes neointima formation,<sup>45</sup> in line with its role as a negative regulator of endothelial cell proliferation.<sup>46</sup> Moreover, TGF $\beta$  is a potent profibrotic factor and may convert endothelial cells into myofibroblasts.<sup>47</sup> On the other hand, 'unleashing' angiogenic growth factor signalling, for example, by inhibition of protein tyrosine phosphatase-1B (PTP1B) in endothelial cells, may result in unrestricted proliferation and premature cell senescence, as recently shown by us in mice with conditional genetic deletion of PTP1B in endothelial cells and after pharmacological inhibition of PTP1B in human endothelial cells.<sup>48</sup> Endothelial cells are present during thrombus resolution, both in the venous and the arterial system, and can be detected using CD31 immunostaining (→Fig. 2). Interestingly, CD31 (or PECAM1) was shown to actively participate in venous thrombus resolution, as shown in mice with genetic PECAM1 deficiency and humans after acute deep vein thrombosis.<sup>49</sup>

## Role of the Endothelium to Prevent Blood Loss after Vessel Injury

Endothelial cells are not only equipped to ensure continuous, undisturbed blood flow by preventing platelet and leucocyte adhesion, but are also part of the first line of defence following vascular injury. For example, stimulation of endothelial cells with thrombin, histamine or bradykinin results in the acute release of endothelin-1,<sup>50</sup> which triggers rapid vasoconstriction in smooth muscle cells to prevent blood loss after vascular injury. Within WP bodies, endothelial cells also



**Fig. 2** Endothelial cells in venous and arterial thrombus resolution. Typical immunohistochemical images showing CD31-positive endothelial cells (red signal) at different time points following experimental induction of venous (A; IVC ligation) and arterial (B; ferric chloride injury) thrombosis. Zoom-in pictures are shown in the left corner of the picture. Scale bars represent 100  $\mu$ m.



store preformed haemostatic proteins, such as vWF, a large multimeric adhesion glycoprotein which stabilizes factor VIII, and binds to GPIb and GPIIb/IIIa integrin receptors expressed on platelets or to extracellular matrix proteins such as collagen.<sup>51</sup> By linking endothelial cells with activated platelets and collagen fibrils exposed after tissue damage, vWF plays a major role in haemostasis controlled by the endothelium, and experimental studies have demonstrated the importance of vWF-mediated platelet adhesion for venous thrombus formation.<sup>52</sup> Following the lag phase of platelet-dependent adhesion and aggregation for wound healing to prevent blood loss, ADAMTS13 (which stands for disintegrin-like and metalloprotease with thrombospondin type 1 repeats-13), a vWF-specific metalloproteinase synthesized in and bound to the surface of endothelial cells,<sup>53</sup> cleaves ultra-large vWF multimers to generate less thrombogenic fragments.<sup>54</sup> Deficiency (genetic or acquired) in ADAMTS13 results in excessive platelet aggregation and disseminated deposition of vWF- and platelet-rich thrombi and has been discovered as pathomechanism underlying thrombotic thrombocytopenic purpura.<sup>55,56</sup> Moreover, reduced plasma ADAMTS13 activity and increased plasma vWF are risk factors for acute myocardial infarction<sup>57</sup> and ischaemic stroke,<sup>58</sup> among others. ADAMTS18, another endothelial cell-derived member of this family, is cleaved and activated by thrombin to disintegrate and oxidatively fragment platelet aggregates.<sup>59</sup> Thus, endothelial cells assist in primary clot formation after injury, but also are equipped with 'tools' to remove these aggregates and to restore tissue perfusion.

### Endothelial Dysfunction and Aberrant Clot Formation

Endothelial dysfunction, defined as a shift of the properties of healthy endothelial cells toward a proadhesive, proinflammatory and prothrombotic phenotype, can be induced by a variety of conditions, including hyperlipidaemia, diabetes and smoking, and often is accompanied by an abnormally increased risk for thrombosis, but also has been implicated in impaired thrombus resolution. Activated, dysfunctional endothelial cells may contribute to the pathogenesis of thrombosis by altering the expression of pro- and antithrombotic factors. For example, stimulation of endothelial cells with proinflammatory cytokines, such as TNF $\alpha$  and interleukin-1, upregulates the production of TF and vWF, while attenuating the expression of thrombomodulin, NO and PGI<sub>2</sub>.<sup>60</sup> Of note, the majority of studies reporting TF expression in activated endothelial cells has been performed in cultured cells, whereas the endothelial expression of TF in vivo is controversial.<sup>61</sup>

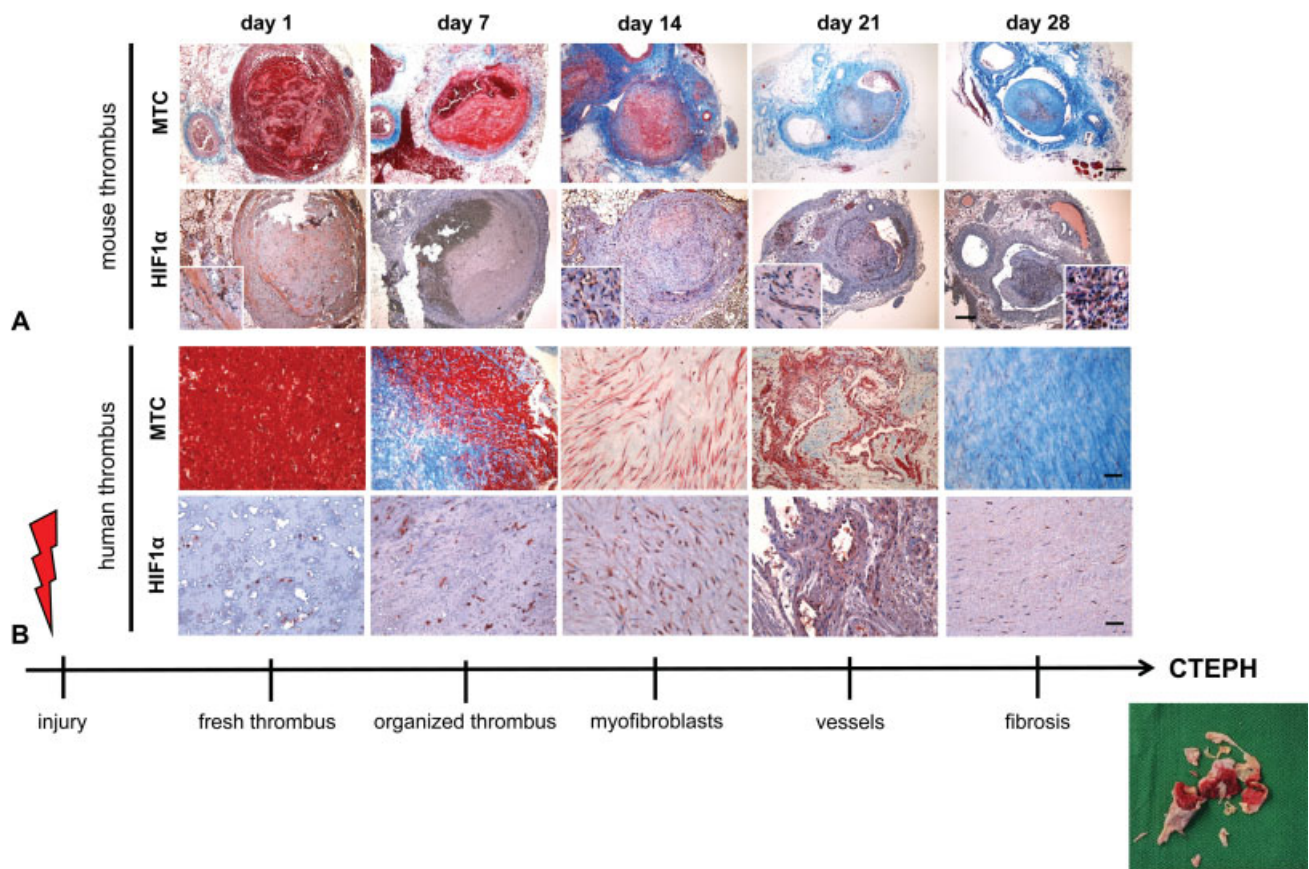
Endothelial dysfunction may also be induced by hypoxia identified as a strong prothrombotic stimulus, in particular for venous thrombosis. For example, hypoxia associated with venous stasis has been shown to activate TF expression in monocytes<sup>62</sup> or to upregulate the antifibrinolytic factor plasminogen activator inhibitor-1 (PAI-1) in cultivated endothelial cells,<sup>63</sup> which may contribute to impaired thrombus resolution. Hypoxia was also found to promote endothelial release of vWF and platelet binding.<sup>64</sup> Although per se not sufficient to

cause thrombosis, hypoxia was shown to promote the initiation and propagation of venous thrombosis in mice.<sup>65</sup>

Activated endothelial cells may also contribute to thrombosis via increased expression of adhesion receptors resulting in the enhanced recruitment of immune and inflammatory cells, and mice deficient in P- and/or E-selectin exhibited smaller thrombi after experimental deep vein thrombosis.<sup>66</sup> Inflammatory cells actively participate in the thrombotic response, among others by the expression of tissue factor and the release of neutrophil extracellular traps or serine proteases (such as elastase or cathepsin G) capable of activating thrombin receptors (as recently reviewed by Iba and Levy<sup>67</sup>). Previous studies have demonstrated the sequential invasion of neutrophils and monocytes to developing murine venous thrombi,<sup>68</sup> later followed by the appearance of endothelial cells and myofibroblasts.<sup>69</sup> In our own studies, we have shown that chronological stages of thrombus resolution observed in mouse venous thrombus can also be observed in PEA (pulmonary endarterectomy) samples from patients with chronic thromboembolic pulmonary hypertension (CTEPH; –Fig. 3). Furthermore, numerous hypoxic, hypoxia-inducible factor (HIF)-1 $\alpha$  and HIF2 $\alpha$ -positive cells were detected in both mouse and human thrombotic material.<sup>70</sup> Others reported increasing levels of the HIF2 $\alpha$  during venous thrombus resolution associated with nucleated cell-dense regions and areas of neovascularization within thrombi.<sup>71</sup> Regarding adaptive immunity, we could recently show in mice that CD4+ and CD8+ T cells rapidly infiltrate the thrombus and vein wall following experimental deep vein thrombosis and remain in the tissue throughout thrombus resolution.<sup>72</sup> We also found, among other, that release of interferon- $\gamma$  by activated effector-memory T cells determines neutrophil and monocyte recruitment as well as neovascularization and, ultimately, thrombus resolution. A role for interferon- $\gamma$  in delaying thrombus recanalization has also been suggested by others.<sup>73</sup>

### Novel Endothelial-Derived Mediators of Thrombosis

Experimental evidence obtained in established mouse models of arterial and venous thrombosis<sup>74,75</sup> has revealed additional endothelial-derived mediators with possible roles in thrombosis and prothrombotic disorders. For example, overexpression of tumour-suppressor protein 53 (p53), an ubiquitously expressed transcription factor involved in cell cycle control and apoptosis, was found to promote a prothrombotic endothelial cell phenotype in vitro via downregulation of Krüppel-like factor-2 and subsequent alterations in eNOS, thrombomodulin and PAI-1 expression.<sup>76</sup> Our study in mice shows the importance of p53 for the risk of thrombosis in vivo, especially in states of endothelial p53 upregulation, such as in increased age.<sup>77</sup> In this study, we could show that aging in mice was associated with p53 overexpression and apoptosis in endothelial cells lining the *inferior vena cava* (IVC). Moreover, aged mice developed more frequent and larger venous thrombi after being subjected to subtotal IVC ligation, whereas aged mice with endothelial-specific p53 deletion were protected from



**Fig. 3** Hypoxia during thrombofibrotic remodelling. Representative composite pictures of Masson Trichrome (MTC) and HIF1 $\alpha$  antibody-stained cross-sections through mouse thrombus (A) or human pulmonary endarterectomy (PEA) specimens (B) suggesting a sequence of events from thrombosis to fibrosis and the presence of hypoxia during this process. Scale bars represent 100  $\mu$ m.

venous thrombosis. Previous studies examining the effects of age on venous thrombosis also reported a larger thrombus mass in aged mice, and elevated vein wall inflammation, and increased circulating PAI-1 and procoagulant microparticle levels were suggested as prothrombotic stimuli.<sup>78</sup> Others found larger venous thrombi in aged mice to be associated with increased vein wall P-selectin expression and higher soluble P-selectin.<sup>79</sup> These and additional changes of endothelial cells with age that may underlie the prothrombotic tendency in the elderly were recently reviewed by us.<sup>80</sup>

Further analyses of primary murine endothelial cells revealed that p53 overexpression was associated with elevated expression of heparanase. The endoglycosidase heparanase is released from intracellular storage granules in response to various activation signals, including thrombin, and involved in the degradation of heparan sulphates inhibiting coagulation pathway enzymes.<sup>81</sup> The heparanase-mediated degradation of proteoglycans in the endothelial glycocalyx may also facilitate the interaction of activated platelets with the endothelium. Others found shortened times to arterial thrombosis following vascular injury and increased in-stent thrombosis in transgenic mice overexpressing human heparanase.<sup>82</sup> Importantly, we could show that inhibiting heparanase activity using TFPI-2 peptides restored the thrombotic phenotype of adult mice.<sup>77</sup> TFPI2

peptides were generated by our cooperation partners Dr. Yona Nadir and Dr. Benjamin Brenner at the Rambam Health Care Campus and Technion Israel Institute of Technology in Haifa, Israel, who had previously validated their functionality to antagonize heparanase activity and venous thrombus formation.<sup>83,84</sup> In addition to TFPI, which inhibits factor Xa and factor VIIa complexed to TF, its homologue TFPI2 antagonizes a variety of serine proteases involved in blood coagulation including factor VIIa/TF, factor Xa, factor XIa, plasmin, trypsin and kallikrein.<sup>85</sup>

### Novel Cellular Interaction Partners with Endothelial Cells during Thrombosis and Haemostasis

Important functions of endothelial cells are mediated in a paracrine manner: a classical example is NO produced and released by endothelial NO synthase, which activates soluble guanylate cyclase, cyclic GMP and protein kinase G signalling in neighbouring smooth muscle cells to control contraction and, ultimately, blood pressure.<sup>86</sup> Via NO-induced signalling activation in platelets, endothelial cells may also control platelet activation and contribute to the antithrombotic effects of healthy endothelium. Interestingly, first reports suggest that smooth muscle cells may also play a role in thrombosis.<sup>87</sup> Whether other paracrine factors released from

(dysfunctional) endothelial cells may indirectly affect thrombus formation by acting on smooth muscle cells needs to be explored in further studies.

In addition to the interaction with platelets, the main cellular mediators of haemostasis, clinical and experimental evidence also suggests that endothelial cells interact with erythrocytes, a circulating cell type involved primarily in oxygen transport, but possibly also thrombosis. Although mature erythrocytes normally do not interact with healthy endothelial cells, structurally or metabolically altered erythrocytes such as from patients with sickle cell disease<sup>88</sup> or malaria,<sup>89</sup> and also diabetes,<sup>90</sup> were shown to adhere to endothelial cells. Crystal structure modelling and cell-based adhesion assays revealed important interactions of the Landsteiner-Wiener blood group glycoprotein intercellular adhesion molecule-4 (ICAM-4) on erythrocyte membranes with  $\alpha_v$ -integrins highly expressed on endothelial cells.<sup>91</sup> ICAM-4 may also bridge the interaction of erythrocytes with the fibrinogen receptor  $\alpha_{IIb}\beta_3$  expressed on platelets<sup>92</sup> or with  $\alpha_L\beta_2$  and  $\alpha_M\beta_2$  integrins expressed on immune cells,<sup>93</sup> not only suggesting a mechanism how erythrocytes may contribute to vasoocclusive events in sickle cell disease<sup>94</sup> but possibly also other prothrombotic conditions. In this regard, it was shown that calcium-loaded erythrocytes can adhere to endothelial cells via ultra-large vWF multimer strings released from thrombin-activated endothelium.<sup>95</sup> Interestingly, splenectomy is one of the risk factors for venous thrombosis<sup>96</sup> and its chronic sequelae, such as CTEPH<sup>97</sup> and removal of the spleen (i.e., the organ filtering damaged and dysfunctional red blood cells from the circulation), was experimentally shown to be associated with larger and more persistent venous thrombi.<sup>98</sup>

## Endothelial Contribution to Thrombus Resolution

Endothelial cells express factors, including tPA, that convert plasminogen to plasmin and thus activate fibrinolysis. Endothelial cells also express urokinase plasminogen activator which is more important during pericellular proteolysis, cell migration and wound healing including the formation of a neointima after experimental arterial thrombosis.<sup>99</sup> Of note, metabolic and replicative stress are associated with increased expression of the antifibrinolytic factor PAI-1 in endothelial cells,<sup>100</sup> which may contribute to the increased risk of thromboembolic events in patients with diabetes<sup>101</sup> and older individuals.<sup>102</sup>

In addition to fibrinolysis, endothelial cells are critically involved in the restoration of vascular patency by promoting angiogenesis and the formation of new blood vessels within thrombi. Vascular obstruction and blood flow stasis result in local hypoxia and upregulation of HIF1 $\alpha$  and VEGF, as shown in mice after experimental IVC ligation and blood flow restriction.<sup>103</sup> Inhibition of HIF1 $\alpha$  degradation by administration of the prolyl hydroxylase domain inhibitor L-mimosine increased the expression of angiogenic mediators and accelerated thrombus revascularization and resolution.<sup>104</sup> The importance of VEGF for thrombus recanalization was documented in several studies,<sup>105</sup> whereas other proangiogenic

factors, including basic fibroblast growth factor, were found not to be effective with regard to acceleration of thrombus resolution, at least not in rats.<sup>106</sup> Deletion of VEGFR2, the predominant endothelial cell receptor to promote VEGF effects, also delayed murine thrombus resolution.<sup>38</sup> In addition to its role in the regulation of thrombogenesis by cleaving vWF, ADAMTS13 may modulate angiogenesis via upregulation of VEGF expression and signalling, as shown in cultivated human endothelial cells.<sup>107</sup> Mice deficient for PECAM-1 (CD31), an adhesion glycoprotein expressed on endothelial cells and platelets, exhibited larger and more persistent venous thrombi characterized by fewer vessels and less inflammatory cells.<sup>49</sup> Moreover, in patients with acute symptomatic deep vein thrombosis, serum levels of soluble PECAM-1, presumably truncated from the endothelial surface, were found to correlate with delayed thrombus resolution. Activated endothelial cells may also promote new vessel formation through release of TF-rich microparticles and paracrine stimulation of neighbouring endothelial cells.<sup>108</sup> This phenomenon has so far been observed in models of ischaemia-induced angiogenesis, but may also be of relevance during thrombus revascularization.

The abundantly present fibrin matrix provides an excellent scaffold for infiltrating immune and other cells during vascular tissue repair.<sup>109</sup> Fibrin is also a potent activator of endothelial cells that triggers the secretion of WP bodies and the release of growth factors.<sup>110</sup> In this regard, endothelial cells store, and upon stimulation with thrombin, histamine and hypoxia release angiopoietin-2 (Ang-2), the antagonist for both Ang-1 and Tie2 involved in the negative regulation of angiogenesis and promotion of vascular leakage and inflammation.<sup>111</sup> Knockdown of Ang-2 has been shown to block thrombin-induced monocyte adhesion and ICAM-1 expression.<sup>112</sup> Interestingly, a recent study involving network analysis of the proteomics identified elevated Ang-2 plasma levels as sensitive early marker and predictor of mortality in patients with disseminated intravascular coagulation, whereas endotoxemic mice with reduced Tie2 signalling exhibited excessive fibrin accumulation.<sup>113</sup>

The reciprocal interaction between activated endothelial cells and platelets may further stimulate angiogenesis, thrombus neovascularization and tissue repair by angiogenic growth factors secreted from platelets, including VEGF.<sup>114</sup> Moreover, activated platelets secrete factors that enhance the interaction of endothelial cells with immune and inflammatory cells, such as RANTES or SDF1 $\alpha$ , which may potentiate tissue repair. On the other hand, the interaction of factors released from activated platelets, such as TGF $\beta$ , with receptors expressed on endothelial cells may also result in their phenotypic conversion to mesenchymal cells (the so-called endothelial-to-mesenchymal transition;<sup>47</sup>) and contribute to the fibrotic organization of thrombus material. In this regard, we observed signs of activated TGF $\beta$  signalling in PEA specimens from patients with CTEPH (Bochenek ML (PhD) et al; 2018). Although the exact molecular mechanisms that cause the excessive pulmonary artery remodelling and development of thrombofibrosis are presently unknown, CTEPH presumably develops in response to unresolved thromboembolic material within pulmonary arteries.



## Antithrombotic Therapeutic Strategies Targeting the Endothelium and Vice Versa

The aforementioned findings demonstrate that the endothelium is an essential component of the blood coagulation system and necessary to maintain normal haemostasis, whereas endothelial cell activation or injury may result in platelet activation, thrombosis and inflammation. Vice versa, factors released during platelet activation or generated during coagulation may act on endothelial cells and change their phenotype. Regarding therapeutic implications, current antithrombotic treatment regimens, including direct thrombin or factor Xa inhibitors, do not directly focus on endothelial dysfunction, but rather on the prevention of its consequences such as platelet aggregation or activation of the coagulation cascade. On the other hand, preventing the release of growth factors from activated platelets and/or the build-up of fibrin will also affect the phenotype and function of the endothelium and influence its properties during thrombus resolution and chronic wound healing processes, such as occurring in CTEPH. Pharmacological approaches to treat the prothrombotic complications of endothelial dysfunction include, but are not limited to, available or already used drugs with known endothelial-protective effects, such as angiotensin-converting enzyme inhibitors, angiotensin AT1 receptor blockers,  $\beta$ -blockers, calcium channel blockers, antioxidants, endothelial NO synthase enhancers, phosphodiesterase 5 inhibitors, or statins, which may directly or indirectly improve endothelial properties involved in the prevention of platelet aggregation and thrombus formation, and also fibrinolysis.<sup>115</sup> Although still at the experimental stage, we and others could establish the efficacy of potential novel therapeutic strategies, such as TFPI2 peptides, for their potential to limit the extent of acute venous thrombosis in mice.<sup>77,83,84</sup>

## Concluding Remarks and Outlook

Endothelial cells are an essential component of the blood coagulation system and their integrity and functionality is critical to maintain haemostasis and to prevent platelet activation and thrombosis. In addition to affecting the three components of haemostasis, as outlined in Virchow's triad of arterial and venous thrombus formation, the endothelium is crucial also for the chronic vascular response to a thrombotic event by regulation of angiogenesis, inflammation and tissue repair. Future studies will have to focus more on the reciprocal interaction of endothelial cells with coagulation factors and other vascular cell types, not only in blood but also in other haematopoietic and non-haematopoietic compartments.

### Authors' Contribution

M.L.B. and K.S. wrote the manuscript and acquired funding. Both authors have read and approved the final manuscript.

### Conflict of Interest

The authors declare no competing financial interests.

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Das Klinikum Dortmund gGmbH (in Trägerschaft der Stadt) ist als Akademisches Lehrkrankenhaus der Universität Münster ein Krankenhaus der Maximalversorgung mit über 1.400 Planbetten, ca. 4.000 Mitarbeitern und verfügt (bis auf die Psychiatrie) über sämtliche medizinische Fachrichtungen. Das Klinikum behandelt jährlich über 240.000 Patientinnen und Patienten, davon rd. 65.000 stationär.

Für das Institut für Transfusionsmedizin, Laboratoriumsmedizin und Medizinische Mikrobiologie (ITLM) (Direktor: Priv.-Doz. Dr. U. Cassens) suchen wir zum nächstmöglichen Zeitpunkt und zur Vollzeitbeschäftigung einen

## Facharzt (w/i/m) für Laboratoriumsmedizin oder Transfusionsmedizin oder einen Assistenzarzt (w/i/m) in fortgeschrittener Weiterbildung

### Wir bieten Ihnen:

- ein modernes Institut an einem großen Klinikum mit absoluter Maximalversorgung
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- einen Blutspendedienst mit mehr als 20.000 Blutspenden/Jahr (Vollblutspenden/Hämapheresen/Stammzellapheresen)
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- die komplette Weiterbildungsbefugnis in der Laboratoriumsmedizin und Transfusionsmedizin
- bei geeigneter Qualifikation wird eine Besetzung und Vergütung in der Funktion eines „Oberarztes der Laboratoriumsmedizin“ in Aussicht gestellt

### Wir erwarten von Ihnen:

- (mehrjährige) Erfahrung in der Laboratoriums- u./o. Transfusionsmedizin
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- die Durchführung und Befundung der spender- und patientenseitigen Labordiagnostik
- die Übernahme arzneimittelrechtlicher Aufgaben und Funktionen (gemäß entsprechender Erfahrung und Qualifikation)
- ein hohes Maß an Interesse und Eigeninitiative sowie Kooperationsbereitschaft und Teamfähigkeit

Die Vergütung erfolgt nach den Bestimmungen des TV-Ärzte/VKA inkl. aller im öffentlichen Dienst üblichen Sozialleistungen (einschließlich Zusatzversorgung).

Bewerbungen von Frauen sind ausdrücklich erwünscht. Bei gleicher Eignung, Befähigung und fachlicher Leistung werden Frauen nach den Bestimmungen des Landesgleichstellungsgesetzes bevorzugt berücksichtigt. Bewerbungen von schwerbehinderten Menschen sind erwünscht.

Wenn wir Ihr Interesse geweckt haben steht Ihnen für eine erste persönliche Kontaktaufnahme und weitere Auskünfte der Institutsdirektor, Herr PD Dr. Cassens, unter der Rufnummer 0231 953 19600 gerne zur Verfügung.

### Interessiert?

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