Review Article

The vessel wall: A forgotten player in post thrombotic syndrome

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Summary
Much research in venous thrombotic diseases focuses on the acute thrombotic process such as anticoagulation and risk factors. Deep-vein thrombosis directly leads to post thrombotic syndrome, and this can cause significant patient disability. Little research has focused on the vein wall injury response to the thrombotic inflammatory insult. Herein, we review what is currently known about this process with emphasis on the matrix, mediators, and vascular medial smooth muscle response after thrombotic injury. Translational therapies and potential future agents are reviewed.

Keywords
Cell-matrix interactions, deep-vein thrombosis, inflammation, matrix metalloproteinases

Introduction
Deep-vein thrombosis (DVT) is an important clinical problem in the United States, affecting up to 250,000 patients each year (1). Aside from pulmonary embolism, many patients will experience a post-thrombotic syndrome (PTS), manifested by valvular dysfunction, skin changes, and in the most severe cases, stasis ulcers resulting in significant morbidity.

PTS epidemiology and current treatment
PTS affects approximately 20–30% of patients with DVT and is classified under the umbrella of chronic venous insufficiency (CVI) (2, 3). The syndrome is defined clinically, and comprised by a number of symptoms and signs, including leg aching, swelling, pigmentation and ulceration. The primary pathophysiology is venous hypertension, related to both venous obstruction, and valvular incompetence. Obstructive CVI is often more debilitating than primary valvular insufficiency (4). Although not all patients affected will have all of these symptoms, PTS causes significant morbidity and afflicts a group of patients that would otherwise be healthy and active. It produces a significant reduction in measures of quality of life (QOL) and in some studies, produces a greater impairment of QOL than chronic obstructive pulmonary disease or angina. In severe cases, amputation may be necessary (5). In spite of the scope of this problem, the available treatments are limited. Therapy consists mostly of compression and ulcer care, and no specific pharmacologic treatments directed at decreasing vein wall fibrosis or improving damaged vein function exists.

Aggressive therapies with pharmacomechanical thrombus (PMT) clearance are now being actively studied with the idea that thrombus removal will decrease the long-term development of PTS. While empirically this is appealing, no level I evidence is yet available. Ongoing randomised controlled trials (RCTs) will answer this question from solid data within the next several years. However, not all patients will or should undergo this more aggressive and costly treatment (6). Indeed, not all patients with DVT at risk for PTS will be eligible for PMT (7, 8). Thus, pharmacological and other novel treatments will still be needed.

Multiple studies have demonstrated the role of the inflammatory response in promoting DVT resolution and organisation, as well subsequent vein wall injury (9–14). However, the effect of the thrombus on the cells comprising the native vein wall is less well understood, but seems to result in an increase in vein wall stiffness, increased vein wall thickness, decreased vein wall compliance, and aberrations in the typical venous matrix architecture (Fig. 1) (15).

Thrombus resolution involves fibro-cellular organisation of the thrombus, peripheral thrombus constriction and fragmentation, re-canalisation, and intimal thickening (16). The initial inflammatory response is marked by PMN influx, followed by monocyte influx, peaking at post-thrombotic day 8 (17). The specific order in which inflammatory cells are recruited into the thrombus and the growth factors and cytokines elaborated by these cells have been shown to influence both matrix remodelling and vein wall cellular proliferation (12, 14, 18–20).

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Vascular smooth muscle cells (VSMCs) are highly specialised cells that are responsible for blood vessel contraction and relaxation (21). In normal venous circulation, VSMCs function largely to maintain and regulate venous tone, controlling the return of blood from the periphery back to the heart. Vascular injury is associated with increased VSMC proliferation and migration, and a switch to a cellular synthetic state (22, 23). The VSMCs are also responsible for synthesis of extracellular matrix (ECM) components, production of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), which have demonstrated importance in models of arterial vascular injury (24, 25).

Much of our current insight into VSMC response to injury and intimal proliferation has been gained through studies of the arterial circulation, and the response of “arterialised” vein grafts to injury. Although many of the same regulators function to control VSMC proliferation following DVT, several factors limit the reciprocal applicability of these observations; namely, the different local oxygenation level, lower shear stress, and the fact a thrombus is in juxtaposition with the vessel wall. It is becoming increasingly apparent that even within the arterial circulation, populations of VSMC respond differently to injury (21).

Within this framework, mediators of VSMC proliferation, migration, and differentiation fall into the broad categories of inflammatory and growth-derived factors, ECM-derived factors, and blood-borne and endothelial factors. Molecules of each group are present in the resolving thrombus as well as following vascular injury, and have the potential to direct cellular changes in the post-thrombotic environment. The purpose of this review is to provide an update about the role of the VSMC in vascular injury with special focus on the venous system, and the potential targets for translational therapies. Specifically, this review will focus on the role of inflammatory and growth factors, the ECM, proteinases, and the endothelium in the pathophysiology and potential avenues to mitigate the development of PTS.

**Inflammatory/growth factor mediators**

An overview of inflammatory/growth factor mediators is given in Figure 2.

**MCP-1**

The secreted factor monocyte chemotactic protein (MCP-1) is a potent and specific chemotaxant and activator of monocytes, basophils, and blood-borne mesenchymal stem-cells (MSC) (26). MCP-1 exerts its effects primarily through chemokine receptor CCR2, commonly found on most leukocytes and endothelial cells (27). Vascular injury is able to rapidly induce secretion of MCP-1 from VSMCs, endothelial cells, fibroblasts, and monocytes themselves (28). In resolving experimental venous thrombi, levels of MCP-1 are elevated, and are believed to be largely responsible for the observed monocyte influx peaking at post-thrombotic day 8 (10). Interestingly, exogenous MCP-1 has been shown to accelerate experimental venous thrombus resolution (29), but the specific effect on the venous wall after thrombosis is unknown. Several studies have also indicated a role for MCP-1, via the CCR2 receptor, in VSMC proliferation and VSMC progenitor recruitment. Most studies have utilised VSMC derived from saphenous
veins and not native arterial segments (26, 30–33). CCR2 is undetectable by RT-PCR in uninjured arterial VSMC. However, following polyethylene cuff arterial injury, CCR2 gene expression is detectable by day 3, and remains elevated through day 21 post-injury (34). Although this finding provides evidence that CCR2 is present in injured vascular tissue, CCR2 expression may be limited to infiltrating monocytes in response to MCP-1. Several studies have demonstrated increased levels of MCP-1 post arterial injury, and immunohistochemical staining shows co-localisation of MCP-1 and α-smooth muscle actin (αSMA), a marker of smooth muscle cells (35, 36). CCR2 -/- mice demonstrated significantly reduced neointimal proliferation post arterial injury as compared to their wild-type counterparts, as well as reduced VSMC incorporation of 5-BrdU, a marker of cellular proliferation (35).

MCP-1 also appears to modulate cultured VSMC proliferation (30–32). Several studies on both rat and human VSMCs showed VSMC proliferation after treatment with MCP-1 (30, 31). However, another study suggested that MCP-1 was in fact inhibitory towards VSMC growth (32). Although the role of MCP-1 and CCR2 in vascular remodelling remains incompletely characterised, we direct the reader to Schober et al. for a more comprehensive review of cytokines in vascular remodelling (37).

In addition to its ability to modulate VSMC proliferation, MCP-1 plays a role in the recruitment of MSC to areas of vascular injury. There is ongoing debate as to the role of recruited MSCs in neointima formation and medial vessel thickening. Current evidence suggests that MSC derived cells are capable of differentiation into VSMC-like cells and can occupy up to 10% of the area of a vascular lesion (26, 38, 39). Zhang et al. demonstrated that exogenous administration of MCP-1 was capable of recruiting MSCs and inducing their differentiation into VSMC-like cells (26).

**Transforming growth factor-β (TGF)-β**

TGF-β is capable of regulating VSMC growth, proliferation, and ECM secretion. TGF-β represents one member of a large superfamily of multifunctional signalling peptides including activins, inhibins, and bone morphogenetic proteins (BMPs) (40). VSMCs, along with myofibroblasts, macrophages, and other hematopoietic cells, have been shown to be potent secretors of TGF-β. After secretion, TGF-β must be activated through a process that can involve plasmin, thrombospondin-1, and MMPs (41), all found within the thrombus microenvironment and injured vascular tissue (15, 42). In part, the effects of TGF-β are dependent on the effector cell receptor subtypes. For example, with vascular injury, a switch in TGF-β receptor subtype predominance from type II to type I on VSMCs has been observed (43). Type II dominant receptor activation has been shown to promote expression of contractile proteins, whereas type I dominant receptor activation promotes ECM production, expression of plasminogen activator inhibitor (PAI-1) and the TIMPs, favouring matrix accumulation and slow DVT resolution (43, 44).

Further support for TGF-β’s capability to induce matrix production comes from several studies of atherosclerosis. Injection of

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**Figure 2: Overview of selected factors involved with thrombosis, inflammation, and vessel wall response.**

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a soluble TGF-β inhibitor into ApoE-/- mice was associated with decreased plaque area and reduced fibrosis, increasing lipid content, inflammatory cells, and tendency to rupture (45). These changes suggest that TGF-β mediates fibrosis while concomitantly decreasing the surrounding inflammatory response within a vascular lesion. Deletion of Smad3, a downstream mediator of TGF-β signalling, results in increased proliferation and decreased synthesis of type-1 collagen and TIMP-1, relative to controls (46).

In addition to its profibrotic function, TGF-β also directs VSMC differentiation in vitro as marked through the up regulation of SM markers such as αSMA and smooth muscle myosin heavy chain (SM-MHC) (47, 48). Although the ability of TGF-β to promote both fibrosis and VSMC differentiation may appear contradictory, it is clear that the in vivo activity and downstream effects of TGF-β are dependent on the combination of primary receptor type present, concentration, and cellular microenvironment. For example, low levels of TGF-β promote growth, whereas high levels inhibit growth in cultured uninjured VSMCs. In explanation of this phenomenon, it is hypothesised that high levels of TGF-β results in the down regulation of platelet-derived growth factor receptor (PDGFR), thus reducing the ability of VSMCs to respond to powerful growth promoting signals (49).

The ability of TGF-β to modulate fibrosis, inflammation, and the cellular state provides evidence for a key role for TGF-β in the vein wall response, and indeed, current research suggests that the fibroproliferative response in DVT is associated with TGF-β (50).

**Fibroblast growth factor (FGF)**

Numerous studies have demonstrated that FGF, when released from sites of endothelial injury, is a key inducer of VSMC proliferation and migration (51, 52). Initial work investigating the role of FGF signalling in VSMC proliferation demonstrated that in addition to thrombin and factor Xa, FGF2 release and activation of FGFR1 was necessary to achieve maximal VSMC proliferation in response to injury (53). Additional research has demonstrated considerable pathway overlap between FGF and PDGF-BB in regards to their ability to induce VSMC proliferation and migration, and it has been suggested that the interplay between these two factors may be key to vessel restenosis (54). This may be of particular relevance in DVT, where endothelial injury and a localised inflammatory response promote signalling via both FGF and PDGF-BB.

In addition to regulating VSMC migration and proliferation, recent work has shown that FGF1 activation is capable of down-regulating αSMA and promoting a synthetic phenotype (55). In accordance with previous experiments, there appears to be considerable overlap between the FGF and PDGF-BB signalling pathways, and both factors together are synergistic in promoting a switch to the synthetic phenotype (55).

**PDGF**

PDGF has also been identified as a key modulator of VSMC phenotype and proliferation (56). Expression of PDGF has been identified in fibroblasts (57), VSMCs (58), macrophages (39), and platelets/megakaryocytes (60). PDGF has multiple effects on VSMCs, inducing a down-regulation of smooth muscle markers, promoting the switch from a contractile to a synthetic phenotype, and inducing migration and proliferation (20, 61–65). Resolving thrombi are rich in PDGF due to the accumulation and lysis of aggregated platelets, and thus the effects of PDGF on the VSMCs in the vein wall post-DVT are of significance.

PDGF reduces the amount of smooth muscle α-actin present in cultured VSMCs, an early marker for phenotypic dedifferentiation (66). In a cultured rat aorta VSMC model this down-regulation was accomplished through the destabilisation of αSMA mRNA, suggesting a post-transcriptional mechanism of VSMC phenotype modulation (61). Other models of vascular injury have demonstrated PDGF induced transcriptional down-regulation of several markers of VSMC differentiation (62, 67, 68). Importantly, expression of all known VSMC marker genes is inhibited through repressor binding to G/C rich repressor elements known CArG boxes (62, 67, 68).

Recent research has shown that PDGF induced VSMC de-differentiation is mediated by the expression of transcription factor Krüpple-like factor 4 (KLF4) (20, 47), as siRNA-mediated knockdown of KLF4 in cultured VSMCs attenuated PDGF-BB repression of the VSMC marker genes αSMA, SM-MHC, and SM22α (69).

**Current state of translational therapy**

There have been no major trials of anti-proliferative therapies in humans to prevent PTS. In the realm of rheumatological disease, use of anti-cytokine therapies is most advanced. For example, use of anti-MCP-1 may have benefit in immune mediated arthritis. Montelukast, a selective CysLT1 receptor antagonist attenuates the development of atherosclerotic lesions and neointimal hyperplasia, an effect which appears to be mediated through an anti-MCP-1 pathway (70). Resveratrol, a statin, inhibits tumour necrosis factor (TNF)α-induced MCP-1 production, and appears to mediate some of the anti-proliferative effects of this medication. Several studies have demonstrated the ability of an angiotensin II receptor blocker, losartan, to inhibit the activation of TGF-β in humans. One challenge here may be to make this a local effect, and avoid systemic side effects.

**Ideal agent**

Venous VSMC specific agent that promotes a contractile rather than proliferative state after injury. Inhibition of the KLF-4 transcription factor represents an attractive therapeutic target to achieve this end.
ECM-derived factors

VSMCs and vascular endothelial cells that line all vessel lumens are supported by a basement membrane composed of laminins, collagens, and glycoproteins that, in addition to serving as a physical scaffold, function to maintain a differentiated cellular state and inhibit cellular proliferation (71–73). Within the vein wall, VSMCs are interspersed with elastic fibers and an ECM composed primarily of types I, III, and IV collagens. The interstitial matrix functions to provide structure and transmit external signals to the internal environment largely via integrins (74). Matrix components such as heparan sulfate have long been known to inhibit VSMC proliferation after injury (75). Recently, syndecan-1, a heparan binding proteoglycan, has been shown to be integral to experimental arterial neointimal hyperplasia, possibly by modulating PDGF-BB (76).

Experimental DVT models consistently demonstrate alterations in vein wall ECM content and composition that likely contribute to the altered venous physiology seen in PTS (9, 10, 14, 15, 29, 74, 77, 78). Biophysical changes include an increase in vein wall stiffness, peaking at seven days following experimental thrombosis (15). This more than six-fold increase over sham controls failed to return to baseline control values out to one month, and provides evidence for lasting injury within the vein wall ECM. Alterations in collagen fiber direction and polarity, also suggesting matrix remodelling, are also present. Note that varicose vein pathology shows collagen changes differing from experimental DVT, such that collagen I increases while collagen III decreases (79, 80). Although not able to be comparatively directly studied, varicosities tend to occur with veno-dilation and venous hypertension, whereas the vein wall response due to thrombosis is inflammatory and associated with thrombus factors.

Different forms of collagen either promote or repress VSMC proliferation and differentiation (Fig. 3). VSMCs associated with polymerised type I collagen (composed of a triple helix of collagen fibrils) are arrested in the G1 phase of the cell cycle, whereas binding to monomeric collagen promotes cell cycle entry and cell proliferation (73). Polymerised collagen up-regulates p27Kip1 and P21Cip/Waf1, inhibiting Cdk2, and thus preventing progression through the cell cycle. Polymerised collagen is also capable of suppressing sites of focal adhesion, suggesting a base of fibrillar collagen retards VSMC proliferation and migration (73). Disruptions to the typical collagen architecture and subtype in DVT remove several of the blocks to cellular proliferation.

In addition to collagen, the basement membrane protein laminin retains cells in a contractile phenotype, whereas the fibronectin promotes synthetic phenotype (81). The importance of VSMC-fibronectin interaction in vivo has been shown in a rat carotid injury model. A small peptide inhibitor of fibronectin reduced fibronectin interaction in vivo and adventitia, and a 60–70% reduction in cell proliferation was observed (82). As increased levels of fibronectin have been observed in clinical DVT (83), it is reasonable to assume that alterations in fibronectin signalling play a role in vein wall response to DVT resolution. As with collagen induced signalling, transduction of these ECM-derived signals proceeds at least partially through a mechanism that culminates in changes.

Matrix metalloproteinases (MMPs)

VSMCs are capable of secreting MMPs, a family of proteinases responsible for the degradation and remodelling of various ECM components (25). In vascular injury, MMPs are believed to be chiefly responsible for metabolising existing ECM to allow for VSMC migration, proliferation, and vessel remodelling (84). In addition to direct interactions with the ECM, MMPs catalyse the release of compounds capable of modifying VSMC phenotype and promoting inflammation. Such mediators include PDGF (85), TGF-β [86], nitric oxide (87), and reactive oxygen species (88). MMP2 and MMP9 have been definitively identified as active in the process of DVT resolution (9). In a mouse stasis model of DVT, MMP9 was found to be increased at day 4 and at day 8, with a return to baseline by day 12. MMP2 expression peaked later than...
MMP9, with a significant increase observed at day 12. Importantly, the levels of the natural inhibitors of the MMPs, the tissue inhibitors of matrix metalloproteinases (TIMPs) did not differ significantly from controls at any time point out to 12 days (9).

Although MMP2 and MMP9 are both active in the process of DVT resolution, and may play compensatory roles in some environments, they have unique profiles. MMP2 is expressed by VSMCs and fibroblasts, and its relatively late expression after DVT suggests a role in remodelling of the ECM rather than degradation of the normal ECM or thrombus lysis (9). MT1-MMP, a proximal activator of MMP2, is upregulated at day 8 following experimental DVT creation, and thrombin can induce endothelial cell expression of MT1-MMP, providing a mechanism for thrombus-directed MMP-mediated ECM turnover (9).

MMP-9 is expressed by invading neutrophils and cytokine stimulated VSMCs, and has been shown to facilitate cell invasion and migration through normal ECM (89, 90). The early increase in MMP9 activity observed in thrombus resolution is largely in agreement with a neutrophilic source, but persistently elevated levels of MMP9 after the neutrophil response suggests that VSMCs or monocytes may contribute a physiologically significant amount of MMP9 following vascular injury (9, 90). In support of the importance of the neutrophil action, neutropenia in a rat model of thrombus resolution was associated with a three-fold increase in intrathrombus collagen, suggesting an impaired MMP9 response (14).

In addition to being active in the remodelling vein wall following DVT, MMPs have a demonstrated role in VSMC proliferation and migration. For example, transfection of cultured saphenous vein VSMCs with siRNA directed against MMP2 or MMP9 reduced migration in a modified Boyden chamber assay (91). In vivo arteriovenous grafting studies have also tested the ability of broad-spectrum MMP inhibitors to prevent graft hyperplasia. In a carotid-jugular porcine anastomosis model, MMP inhibition resulted in a 53% reduction of intima formation at 28 days as well as a decrease in elastinolysis (92). However, past studies have had difficulty utilising broad spectrum MMP inhibitors to prevent SMC proliferation (93). Part of the reason for this may lie in the complex role of the many MMPs in promoting vascular remodelling. Evidence supporting this tenet is that MMP9 and MMP12 but not MMP2 and MMP14 can cause N-cadherin shedding and resultant activation of β-catenin signalling and VSMC proliferation (94).

Current state of translational therapy

Many anti-proteinases are in early trials for their anti-neoplastic efficacy and anti-angiogenic properties (95, 96), and none outside of doxycycline have been tested in humans in the vascular system. Extrapolated data from small doxycycline trials suggest efficacy in the arterial wall with few side effects (97); however, this agent remains untested in venous injury. Recent experimental data suggests that doxycycline decreases vein wall thickening in a rodent model of DVT (98); however, this agent has not been tested in patients at risk for PTS. Once these agents are more clinically available, selected patients at high risk for PTS may constitute a worthwhile study cohort.

Ideal agent

VSMC-matrix stabilising agent, possibly MMP2/9 inhibitor in conjunction with anticoagulation.

Blood-borne and endothelial factors

An overview of blood-borne and endothelial factors is given in

Table 1.

Plasmin activators and inhibitors

Numerous factors in the clotting system play a role in modulating VSMC proliferation, migration, and differentiation. Chief among these is the plasmin system, the primary protease responsible for the degradation of fibrin. In this role, plasmin has been shown to affect vein wall fibrosis and cellularity post venous injury (10, 15, 99, 100). Plasmin is secreted in its pro-form as plasminogen, and must undergo an enzymatic cleavage reaction before local activation (101).

Urokinase plasminogen activator (uPA), as opposed to tissue plasminogen activator (tPA), is most important to DVT resolution, and uPA gene deletion results in impaired thrombus resolution in a mouse model (99). In addition to promoting thrombus resolution, uPA activity promotes VSMC migration and proliferation (102–104). In a study of uPA -/- or plasminogen -/- mice subject to perivascular electric arterial injury, the intima-to-media ratio was reduced three- and eight-fold, respectively, when compared to injured wild-type arteries (104). Interestingly, levels of both pro and active MMP2 and MMP9 were elevated relative to wild-type controls when assayed one week post injury, suggesting that MMP activity alone is not sufficient for VSMC migration.

Regulation of the plasmin system is accomplished through both plasminogen activators and inhibitors. Recent evidence has demonstrated that plasminogen activators are capable of activating growth factors. For example, plasmin can activate epidermal growth factor which may drive post-injury VSMC growth and proliferation (105–108). Incubation of cultured murine aortic VSMCs with plasmin results in increased proliferation and a concomitant activation of several conventional cell growth and proliferation pathways, all of which are prevented by pharmacological inhibition of epidermal growth factor (105, 108).

Among components of the plasmin system, PAI-1 also affects vessel wall responses to injury. PAI-1 is secreted in an active form from liver and endothelial cells, and is stabilised by binding to the ECM component vitronectin (109). Elevated levels of PAI-1 have
been detected in animal models of resolving thrombi (9), and several models of vascular injury have implicated PAI-1 as a modulator of VSMC migration, proliferation, and ultimately fibrosis (110–114). This is likely due to VSMC proliferation and migration rather than driving a proliferative phenotype change (such as decrease in smooth muscle cell markers of calponin and SM-22). This profibrotic aspect is also not dependent on plasmin-PAI-1 interactions (115).

However, studies disagree on the ability of PAI-1 to promote (110, 112, 116) or inhibit (113, 114, 117) VSMC proliferation and migration. In a mouse model of transendothelial-induced arterial vascular injury, PAI-1 knockout mice displayed reduced neointimal VSMC migration (112). Alternatively, in an arterial flow reduction model, PAI-1 was shown to prevent neointimal formation through inhibition of thrombin-mediated VSMC proliferation (113). These conflicting activities of PAI-1 may be the result of the microenvironment present in arterial vs. venous circulation, or may simply reflect differences in experimental conditions throughout the literature. Based on current research, we currently believe that in the setting of venous thrombus resolution, PAI-1 promotes matrix accumulation, VSMC migration and proliferation, and increased fibrosis after DVT.

Although the mechanism by which DVT increases PAI-1 levels is not fully known, PAI-1 has been shown to be upregulated in response to TGF-β. Otsuka et al. were able to demonstrate TGF-β1 mediated increases in PAI-1, as well as subsequent PAI-1-dependent increases in VSMC migration and matrix accumulation in a murine arterial model (111). More recently it was shown that this effect is independent of plasminogen expression, suggesting a direct signalling role for PAI-1 in this process (110).

In addition to TGF-β, recent studies have also demonstrated a role for the small monomeric G-protein Rac-1 in promoting PAI-1 expression. Rac-1 has actions that promote VSMC proliferation and neointimal formation (118), as well as leukocyte trans-endothelial migration (119). Diebold et al. demonstrated that exposure of cultured arterial VSMCs to thrombin increased levels of Rac-1 and subsequently activated a Hif-1α-dependent increase in PAI-1 levels (120). Within their model system, upregulation of PAI-1 by Rac-1 and Hif-1α was essential for thrombin-stimulated VSMC proliferation, thus suggesting a mechanism for thrombin-mediated VSMC proliferation in vivo (121). As elevated levels of PAI-1 have been demonstrated in the setting of DVT, this mechanism of VSMC proliferation is likely relevant in the DVT setting.

### Table 1: Fibrinolytic factors and VSMC.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>uPA</th>
<th>Plasmin</th>
<th>PAI-1</th>
<th>TGF-β</th>
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</thead>
<tbody>
<tr>
<td>Effects</td>
<td>Soluble mediator that promotes plasminogen → plasmin conversion</td>
<td>Chief protease responsible for degrading fibrin and clot lysis</td>
<td>Elevated levels detected in animal models of resolving thrombi</td>
<td>Must be activated through a process that can involve plasmin, thrombomodulin-1, and MMPs (9, 21)</td>
</tr>
<tr>
<td></td>
<td>Important in thrombus resolution</td>
<td>Promotes DVT resolution</td>
<td>Promotes VSMC migration, proliferation, and fibrosis</td>
<td>Post vascular injury a switch to type I TGF-β receptor predominance on VSMCs promotes ECM production, PAI-1 and TIMP expression, and favors matrix accumulation / slow DVT resolution</td>
</tr>
<tr>
<td></td>
<td>Promotes VSMC migration and proliferation</td>
<td>Recent evidence demonstrating plasmin can activate FGF, thus driving post-injury VSMC growth and proliferation</td>
<td>The small monomeric G-protein Rac1, expressed in resolving thrombi, upregulates PAI-1 expression</td>
<td>Capable of modulating both fibrosis, inflammation, and cellular state depending on level of TGF-β present as well as receptor subtype predominance</td>
</tr>
<tr>
<td></td>
<td>uPA -/- mice demonstrate reductions in intimal proliferation following peri-vascular arterial injury</td>
<td></td>
<td>PAI-1 -/- mice displayed reduced neointimal VSMC migration following FeCl3 induced venous injury</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Some discrepancy regarding ability to promote or inhibit VSMC migration and proliferation, although current venous research provides support for PAI-1 as a pro-proliferative, pro-fibrotic, and pro-ECM secretory mediator.</td>
<td></td>
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### Endothelial signals

The presence of a functional endothelium helps maintain VSMCs in a contractile and quiescent state. Endothelial cell denudation following vascular injury exposes components of the basement membrane to circulating blood, promotes activation of the clotting cascade, and deprives VSMCs of important endothelium-derived signalling factors.

Loss of endothelial cells has also been shown to promote VSMC proliferation and migration (122). Nitric oxide (NO) is a key mediator of vascular homeostasis, and is synthesised in healthy endothelium. Early studies demonstrated that addition of NO donors to cultured VSMCs reduced proliferation in a cGMP-dependent mechanism (123). Additional studies have confirmed the growth inhibitory effect of NO on cultured VSMCs, and have shown that exogenous NO administration is able to overcome the proliferative effect of both fetal calf serum and PDGF-BB (65). Indeed, reductions in endothelial nitric oxide synthase (eNOS) have been observed in experimental DVT, likely as a result of accom-
panying endothelial injury and denudation (124). More recently NO has been shown to inhibit PDGF-BB mediated activation of RhoA (125), a small G-protein that has been linked to cellular proliferation and migration through cytoskeletal rearrangements (126). RhoA also is important with VSMC contraction via phosphorylation of myosin light chain by MLCK inhibition of myosin phosphatase (127).

Acute DVT is responsible for significant mechanical and inflammatory insult to vessel walls, and endothelial loss is a well-characterised result of this process (124). In an experimental model of DVT, endothelial progenitor cells were shown to be increased in peripheral circulation, and they were observed to concentrate in areas of resolving thrombus (128). However, this work suggested that these cells reside at the thrombus-vein wall interface and it is not clear if these cells are important for repairing damaged endothelium after DVT, or if they perform some other function. No work has been done so far to evaluate exogenous progenitor cell stimulation and assessment of post-thrombotic re-endothelialisation.

Recent work has shown decreased αSMA-positive cells after experimental DVT creation with recovery to baseline levels by day 14, associated with partial endothelial recovery (124). This observation is consistent with VSMC dedifferentiation and a fibroproliferative response and partially preserved endothelial function. Pretreatment with low-molecular-weight heparin (LMWH) abrogated this negative response. After initial thrombus formation, however, treatment with LMWH failed to increase αSMA staining above the levels of untreated controls. Interestingly, LMWH treatment resulted in increased levels of vein wall collagen-III, suggesting a complex interplay of inflammatory, endothelial, and thrombus-derived factors resulting in fibrosis and ECM remodelling post-DVT.

The role of the pericyte in vascular injury is also likely important. Pericytes are small connective tissue cells that are best known for their presence in the microvasculature, oligopotent potential, and role in the regulation of blood flow (129). These have also been recognised in their role for local progenitor cells particularly of mesenchymal potential (130). Several studies have also demonstrated a key role as pericytes in maintenance of the blood brain barrier; however, their role in vascular injury remains relatively uncharacterised (131). Recent work has identified cells expressing histologic features consistent with pericytes that express high concentrations of tissue factor in both the micro- and macrovasculature (132). Although this later finding remains controversial, these macrovascular pericyte-like cells are believed to play a role in acute thrombosis and restenosis in both vein and arterial grafts. The role of such cells in the venous response to DVT has not yet been investigated, however previous work has identified these cells in intima of larger veins, thus leaving open the possibility that DVT induced vein wall injury is mediated at least partially via a pericyte response (129).
Cell adhesion molecules, particularly P-selectin, have been well studied and suggest efficacy for preventing and treating established experimental DVT (133, 134). These molecules are critical for both leukocyte trafficking as well as activation. Our laboratory has also shown that P-selectin inhibition modifies vein wall injury by down regulating profibrotic growth factors (77). It is not clear whether P-selectin inhibition directly alters the VSMC or matrix response, although further work is indicated.

Current state of translational therapy

PAI-1 inhibition would likely promote thrombus resolution and this molecule is under intense investigation as a therapeutic agent for DVT. A small peptide inhibitor is currently undergoing testing in both primates and humans (T. Wakefield, personal communication). Whether this would have clinical efficacy in preventing PTS separately from its DVT accelerating effects is unknown, but would be a reasonable secondary endpoint to assess. Of note, there is no evidence that exogenous plasmin activators increase vein wall damage, despite some vein wall injury in experimental models; indeed, rapid and complete thrombus removal is associated with less PTS (135). Exciting data from the Jupiter trial showed that those patients on a potent statin had a lower incidence of DVT as compared with placebo (136). As hyperlipidaemia is not associated with DVT, this suggests statin's benefit may be the pro-endothelial and anti-inflammatory effects. Although unlikely to be beneficial for primary prevention, for those patients at highest risk for PTS, a statin may decrease recurrence and/or mitigate PTS, and requires controlled studies.

Ideal agent

Proendothelial agent to increase re-endothelialisation and re-population after DVT vein wall injury.

Table 2: Potential therapeutic targets to decrease PTS.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Role in PTS</th>
<th>Stimulates or inhibits VSMC?</th>
<th>Example agent</th>
<th>Feasible for human therapy?</th>
</tr>
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<tbody>
<tr>
<td>Vascular SMC</td>
<td>High probable</td>
<td>Selective</td>
<td>Montelukast</td>
<td>Long term</td>
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<tr>
<td>Growth factor</td>
<td></td>
<td>+</td>
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<tr>
<td>Proteinases</td>
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<td>-</td>
<td>Doxycycline</td>
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<td>+/-</td>
<td>tPA</td>
<td>Short Medium</td>
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<td>(PAI – 1)</td>
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<tr>
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<td>Probable</td>
<td>+</td>
<td>Stem cell stimulated</td>
<td>Short and medium term</td>
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<td>(GCSF)</td>
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<td>Heparin – sulfate</td>
<td>Long term</td>
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<td>Cell adhesion</td>
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<td>P-selectin Inhibition</td>
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<td>molecules</td>
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Conclusions

Clearly, a large gap exists between understanding the basic pathophysiology of PTS and the clinical characterisation and subsequent therapy (Table 2). This is partly due to the lack of ready human specimens to study, the lack of a gravity animal model of venous hypertension, and lack of understanding of endothelial-VSMC interplay in the venous system. Of the agents for PTS, such as extract of horse chestnut seed oil or micronised purified flavonoid fraction, none have a defined mechanism that affects such as local vein wall matrix (137, 138). As compared to the fields of oncology and atherosclerosis, the investigation of agents lags for PTS. Similarly, newer anticoagulants may alter the vein wall response for the better, but oftentimes the pharmaceutical trials do not address the long-term PTS development (139).

We believe the main areas reviewed here should be focused on to decrease post-thrombotic vein wall damage. First, inflammatory and growth factors likely play a role in the VSMC response after DVT. More research is needed to determine their mechanistic roles, similar to what has been done in the arterial system. Second, the ECM, while less abundant than arteries, seems to play an important role in the injury phenotype and VSMC are the source of both the proteinases as well as the main producers of collagen. Defining mechanisms of the vein response to DVT and what may drive the VSMC contractile to synthetic state will be crucial. Third, the role of antifibrinolytics such as PAI-1, is important not only for thrombosis-thrombolysis balance, but also vein wall matrix and VSMC responses. Active experimental investigation is ongoing in this regard and interest is keen to develop a clinical PAI-1 inhibitor. Lastly, endothelial injury occurs after DVT, but the healing kinetics are incompletely understood. What therapies can accelerate this process should be the focus. Knowledge of the endothelial-VSMC interaction in the vein wall after injury is also important, based on what is known in the arterial beds.

As our understanding of the basic vascular biology underlying this process increases, we expect that it will provide a suitable framework for the development of adjunctive agents capable of decreasing PTS after the acute DVT is treated. Not only might this decrease PTS, but since a common predisposing factor for DVT is PTS (damaged vein wall), this may decrease recurrent DVT as well.
References


