

# Mechanisms of vascular damage in SSc—implications for vascular treatment strategies

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Vascular abnormalities are a major component of SSc, but little is known about the events or mechanisms that initiate vascular injury and prevent its repair. In SSc, angiogenesis is incomplete or lacking despite the increased expression of a large array of pro-angiogenic factors such as VEGF. Conflicting results have recently been published concerning the presence and role of vasculogenesis and circulating endothelial progenitor cells in SSc. It remains to be established if these endothelial progenitor cells are a marker of endothelial disease or a cause of insufficient vascular repair. Human mesenchymal stem cells (MSCs) may be an alternative source for endothelial progenitor cells, and it has been observed that the angiogenic potential of endothelial-like MSCs is reduced. Other mechanisms of vascular damage include oxidative stress and factors released from activated platelets. In addition, growth factors such as ET-1 and PDGF induce proliferation of vascular smooth muscle cells resulting in intimal thickening. For the development of new therapeutic strategies, it is important to realize that the different vascular pathologies—uncompensated loss of capillaries on one hand and vascular remodelling with a proliferative vasculopathy on the other—might require different treatment approaches.

**KEY WORDS:** Systemic sclerosis, Vascular damage, Vascular treatment, Endothelial progenitor cell, Mesenchymal stem cell, Endothelial cell, Angiogenesis, Vasculogenesis, Oxidative stress, Platelet activation.

## Introduction

SSc is characterized by a complex array of clinical features involving internal organs and the skin. A common pathogenetic leitmotif to all these features is not only fibrosis due to excessive collagen production, but is also the overall microvascular involvement, in the early phases of the disease [1]. This dictates the pace of the disease that progresses silently until the major damage to the vessel wall is done. The modifications of the microvasculature may be observed by nail-fold capillaroscopy [1] and the progression of capillary deterioration, from giant capillaries to architectural modification and desertification, can be easily followed. Therefore, a major problem for the clinician is modulation of the process leading to vessel modifications and the capacity we may have to foster 'regeneration' of the damaged and disappeared vessels. In this review, we will present the state-of-the-art mechanisms of vascular damage in SSc and outline strategies to develop novel vascular therapies for this disease.

## Angiogenesis and vasculogenesis

In most adult organisms, endothelial cells (ECs) are quiescent with turnover rates estimated to be in the order of years, with the exception of the reproductive cycle in fertile females and in wound healing or tissue regeneration [2]. Angiogenesis, the creation of new blood vessels from pre-existing ones, mainly depends on the activation, proliferation and migration of ECs, and is driven by angiogenic stimuli that also induce proteolytic enzymes cleaving extracellular matrix (ECM). The balance between pro-angiogenic and anti-angiogenic factors tightly regulates angiogenesis [3]. This event is highly complex and requires a dynamic, temporally and spatially regulated interaction between ECs, soluble angiogenic growth factors and ECM molecules. On one hand, EC

proliferation and new vessel formation is characteristic of several diseases such as cancer and macular degeneration [2], while on the other hand EC death is also a typical feature of diseases such as atherosclerosis, allograft vasculopathy, heart failure, diabetic retinopathy and SSc.

Vasculogenesis is defined as the formation of new vessels from progenitor cells. Bone marrow-derived cells contribute to physiological and pathological vascular remodelling throughout ontogeny and adult life. Following tissue ischaemia, progenitor cells are mobilized from their bone marrow or peripheral niches into the circulation, adhere at sites of vascular lesion and differentiate into a variety of mature cell types according to their origin and the local environment [4]. Impairment in this pathophysiological process due to either low numbers of circulating progenitor cells or dysfunctional progenitor cells, leads to inadequate vascular repair. Vascular repair is a complex process that includes mobilization, chemotaxis, adhesion, proliferation and differentiation of progenitor cells. Although homing of progenitor cells into bone marrow has been extensively studied [5], migration of precursor cells into peripheral tissues and differentiation into mature cells is poorly understood so far. The regenerative ability of progenitor cells following organ injury is well established. Progenitor cells promote structural and functional repair in several organs such as the heart, liver, kidney or brain. For instance, CD34+ cells have been described to be recruited to the ischaemic myocardium, differentiating into cardiac and vascular cells, and restoring cardiac function. Progenitor cells migrate to sites of vascular injury and differentiate not only into an endothelial phenotype (vascular repair), but also into vascular smooth muscle cells or foam cells contributing, therefore, to neointimal formation and eventually to vascular disease [6]. Moreover, Tanaka and colleagues [7] showed that bone marrow cells contribute to neointimal hyperplasia after mechanical vascular injuries. However, it is poorly understood as to which factors influence the fate of progenitor cells in damaged tissues.

## Vascular damage in SSc

In SSc pathogenesis, chronic inflammation plays a role in EC ageing and damage. Vascular abnormalities are a major component of SSc, but little is known about the events or mechanisms that initiate vascular injury, prevent its repair and lead to loss of angiogenesis [8]. Early stages of SSc are characterized by an exaggerated angiogenic response, later followed by fibrosis.

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The earliest clinical symptoms of SSc relate to disturbances in the peripheral vascular system [1] that may precede other manifestations by years. Fibrotic stages of SSc with the full-blown clinical symptoms, often fail to exhibit prominent perivascular skin infiltration, which can be found in the early inflammatory stage of SSc. The initial events leading to vascular alterations are poorly understood but are thought to involve EC injury. EC damage impairs vascular supply, leading to tissue hypoxia, and promotes inflammation and fibrosis. In response to damage, there is enhanced expression of EC adhesion ligands that promote inflammatory cell attachment, transmigration into the interstitial space and tissue infiltration. The loss of viable ECs also leads to loss of protective and vasodilating cytokines. In addition, vascular smooth muscle cells proliferate in response to growth factors resulting in intimal proliferation, matrix deposition in the vessel wall and eventually complete occlusion of the vascular lumen during the course of the disease. Thus, vascular disease is both functional and structural: with reversible vasospasm as well as a reduction in the capillary density followed by obliterative vasculopathy. It is likely that prolonged EC perturbation and activation may lead to dysfunction and irreversible loss of integrity, with cell detachment and persistent tissue injury. EC damage with apoptosis resulting in the loss of capillaries, is considered as one of the earliest changes in the pathogenesis of SSc [9].

### SSc: a model for loss of angiogenesis

Despite the reduced capillary density, there is paradoxically no sufficient angiogenic response in SSc. Tissue ischaemia leads usually to the expression of angiogenic growth factors (e.g. VEGF), which then initiate angiogenic sprouting by inducing vasodilatation, proliferation and migration of ECs, and stabilization of the lumina to form new vessels [10]. VEGF is the major regulator of neovascularization. Plasma levels of VEGF are elevated in SSc and this could stimulate angiogenesis [10]. Microvascular ECs (MVECs) from SSc patients showed urokinase-type plasminogen activator-receptor (u-PAR) truncation between domains 1 and 2, a cleavage that is known to impair u-PAR functions [11]. The u-PAR cleavage occurring in SSc MVECs was associated with overexpression of MMP-12. Overproduction of MMP-12 by SSc MVECs accounts for the cleavage of u-PAR and the impairment of angiogenesis *in vitro* and may contribute to reduced angiogenesis in SSc patients.

The role of vasculogenesis in SSc is less clear. Endothelial progenitor cells might be recruited for vascular repair during active disease [12]. However, conflicting results have been published concerning the presence and role of circulating endothelial progenitor cells in SSc [12]. Avouac *et al.* [13] demonstrated increased circulating endothelial progenitor cell levels in SSc, supporting their mobilization from bone marrow. Furthermore, the subset of patients with digital vascular lesions and high severity score displayed lower endothelial progenitor cell counts, suggesting increased homing at this stage [13]. On the other hand, there is data suggesting that vasculogenesis might be impaired in SSc. This was demonstrated by the presence of a substantially reduced number of bone marrow-derived circulating endothelial precursors as compared with either healthy subjects or patients affected by RA [14]. The lowest values of these immature cells were observed in SSc patients with active fingertip ulcers, suggesting that the inadequate recruitment of bone marrow-derived circulating endothelial precursors might be related to an impaired vascular repair mechanism [14]. It remains to be established if these endothelial progenitor cells are a marker of endothelial disease or a cause of insufficient vascular reparation. Endothelial progenitor cells are important in vasculogenesis, and may also be involved in other systemic features of inflammatory rheumatic disorders [15]. Mesenchymal stem cells (MSCs) might be another source of endothelial progenitor cells. MSCs show a

normal pattern of biological markers and functional properties in SSc, but the angiogenic potential of endothelial-like MSCs is reduced [16]. These cells, when seeded on Matrigel, have lower ability to form capillary-like structures, giving rise to endothelial networks that appeared incomplete and forming thinner vessels, even after VEGF and stromal-derived factor (SDF-1) stimulation, suggesting that endothelial repair may be affected in SSc starting from the bone marrow.

### Other mechanisms of vascular damage in SSc

Oxidative stress is associated with EC ageing, due to a progressive reduction of the endogenous free radical scavengers over time. Chronic exposure of ECs to radical oxygen species induces morphological changes and impairment of cell-cell adhesion. Oxidative stress also increases vascular endothelial permeability, which is coupled with alterations in EC signal transduction. In SSc, the peroxidation product diene-conjugates and antibodies against oxidized low-density lipoproteins were found to be significantly increased [17].

The release of von Willebrand factor (vWF) into the circulation reflects the activity of the vascular disease in SSc. vWF released from the vascular endothelium of patients with SSc is also found in the perivascular and interstitial matrix. This suggests that the local microvessels of the papillary layer of the skin are damaged in SSc [18]. Most of the high molecular-weight vWF binds to the subendothelial connective tissue. The high molecular-weight vWF bound to subendothelial collagen fibres can form bridges between subendothelial matrix and platelet gpIb and gpIIb/IIIa receptors. Therefore, it contributes to platelet adhesion and aggregation, and the formation of thrombosis. Thus, the local vascular damage and the subsequent release of high molecular-weight vWF may contribute to local pathogenic processes.

Platelets adhere to activated ECs and current evidence suggests that platelets contribute to vascular remodelling. In fact, platelets are the major storage and delivery vehicles for pro- and anti-angiogenic growth factors including VEGF-A and thrombospondin (TSP), cytokines and chemokines, such as SDF-1. By site-specific deployment of these factors, platelets orchestrate the local angiogenic stimulus within a tissue and direct the recruitment and differentiation of circulating bone marrow-derived cells. These insights have profound clinical implications; activation of PDGFs or their receptors may be an effective strategy to promote capillary growth.

ET-1, predominantly synthesized by ECs and smooth muscle cells, is the strongest vasoconstrictor known, enhances mitogenesis and induces ECM formation. The capacity to regulate growth of mesenchymal cells, to induce collagen and fibronectin production and cell migration, make ET one of the main regulators of ECM synthesis and vascular and interstitial remodelling. In SSc, increased plasma ET-1 levels are involved in enhanced vasoconstriction, vascular EC proliferation, smooth muscle hypertrophy and irreversible vascular remodelling [19].

### Conclusion and consequences for therapeutic approaches

The extensive vascular damage and loss of angiogenesis makes SSc an ideal model to study novel therapeutic strategies for vascular regeneration. It has been convincingly shown that different pro-angiogenic stimuli are present in SSc that do not elicit an appropriate angiogenic response. Still, the mechanism of this lack of response and loss of angiogenesis are poorly understood. However, a number of key factors and additional mechanisms have been identified in recent years that may significantly contribute to the vascular damage in SSc.

For the development of new therapeutic strategies, it is important to realize that there are parallel vascular pathologies that might require additive therapeutic approaches. These vascular pathologies consist mainly of vasoconstriction driven

by an imbalance of vasoconstrictive/vasodilatory factors, vascular damage and loss of capillaries not compensated by new vessel formation, and a proliferative vasculopathy with prominent intimal proliferation further leading to an impaired blood flow in tissues.

### Rheumatology key messages

- Angiogenesis is impaired in SSc despite the up-regulation of a large array of pro-angiogenic factors.
- The role of vasculogenesis in SSc is controversial and needs to be addressed by further studies.
- Other mechanisms of vascular damage in SSc include oxidative stress and platelet activation.
- Loss of capillaries without compensation by angiogenesis/vasculogenesis and a proliferative vasculopathy with intimal proliferation, are the main vascular features in SSc.
- Novel therapeutic approaches should address both of these main vascular pathologies in SSc.

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