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Role of smooth muscle cells in coronary artery bypass grafting failure

Kerry Wadey[1], Joshua Lopes[2], Michelle Bendeck[2], Sarah George[1]

[1] Bristol Medical School, Research Floor Level 7, Bristol Royal Infirmary, Bristol, BS2 8HW

[2] TBEP, University of Toronto, 661 University Ave., Rm 1432, Toronto, Ontario, M5G 1M1

Corresponding Author:

Professor Sarah Jane George

School of Clinical Sciences,

Research Floor Level 7,

Bristol Royal Infirmary,

Bristol,

BS2 8HW

UK

Email: s.j.george@bristol.ac.uk

Telephone: 00 44 117 3423154

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ABSTRACT

Atherosclerosis is the underlying pathology of many cardiovascular diseases. The formation and rupture of atherosclerotic plaques in the coronary arteries results in angina and myocardial infarction. Venous coronary artery bypass grafts (CABG) are designed to reduce the symptoms of atherosclerosis in the coronary arteries by diverting blood flow around the atherosclerotic plaques. However, vein grafts suffer a high failure rate due to intimal thickening that occurs as a result of vascular cell injury and activation and can act as 'a soil' for subsequent atherosclerotic plaque formation. A clinically-proven method for the reduction of vein graft intimal thickening and subsequent major adverse clinical events is currently not available. Consequently, a greater understanding of the underlying mechanisms of intimal thickening may be beneficial for the design of future therapies for vein graft failure. Vein grafting induces inflammation and endothelial cell damage and dysfunction, that promotes vascular smooth muscle cell (VSMC) migration and proliferation. Injury to the wall of the vein as a result of grafting leads to the production of chemoattractants, remodelling of the extracellular matrix and cell-cell contacts; which all contribute to the induction of VSMC migration and proliferation. This review focuses on the role of altered behaviour of VSMCs in the vein graft and some of the factors which critically lead to intimal thickening that predisposes the vein graft to further atherosclerosis and reoccurrence of symptoms in the patient.

Keywords:

Vascular smooth muscle, Vein graft, Cadherin, Extracellular matrix.

SAPHENOUS VEIN GRAFTING

Atherosclerosis is the underlying pathology in many cardiovascular diseases. Coronary artery bypass graft (CABG) surgery is the most frequently performed surgical intervention for relieving symptoms associated with ruptured or occlusive coronary artery atherosclerotic plaques [1, 2]. Surgery entails usage of non-native, un-diseased vessel (most often the saphenous vein, internal mammary artery or radial artery) for diversion of blood from the aorta to the coronary artery, distal to the stenosed region, thereby re-vascularising cardiac tissue. Despite improvements in surgical techniques and medical management, long-term efficacy of bypass grafts, especially saphenous vein grafts, remains restricted due to complete or significant occlusion (restenosis) of the conduits or continued progression of atherosclerosis in native coronary arteries [3]. Though arterial grafts exhibit superior patency rates to saphenous vein grafts [4-10], the latter is most commonly used as it is easier to harvest and manipulate, and its length allows for reaching of any coronary artery and making of multiple grafts [11]. However, the prognosis is such that 10-15% of saphenous vein grafts are predisposed to early failure within the first year of surgery, and up to 50% suffer late vein graft failure within 10 years [1, 2, 6]. Numerous studies have attempted to identify treatments for effectively ameliorating late vein graft stenosis though no validated interventional strategies currently exist. Advances in our understanding of the epidemiology and aetiology of saphenous vein graft failure are thus essential for the design of preventative therapies.

PATHOGENESIS OF SAPHENOUS VEIN GRAFT FAILURE

Thrombosis, intimal hyperplasia and accelerated atherosclerosis are the primary pathophysiological events underlying saphenous vein graft failure (Figure 1) [12]. Thrombosis typically occurs within one month of surgery in 3-15% of patients [13, 14] and is a direct consequence of endothelial dysfunction [12]. Disruption to the endothelium is in turn a product of high pressure distension used to overcome vasospasm during surgery and to disrupt the venous valves [15], and tissue handling during harvesting and implantation of the vein conduit [16]. Focal endothelial loss is most prominent at the sites of anastomosis [3]. After implantation the vein graft is subjected to considerably altered blood flow and wall stress than from its original source of the venous system. This is also thought to contribute to endothelial cell damage and loss from the luminal surface [17]. Loss of the endothelial monolayer and endothelial dysfunction induce adherence and accumulation of fibrin, platelets and

neutrophils to the luminal surface of the vessel wall [18, 19] and causes an attenuation in production of thrombolytic agents, including tissue plasminogen activator (tPA) [20], and thrombomodulin – a protein cofactor normally expressed on the surface of endothelial cells that complexes with thrombin to activate protein C, a potent anticoagulant [21]. Furthermore, the inherent antithrombotic properties of veins are comparatively weak with respect to arteries; for example, expression of anticoagulant heparin sulphate, and production of platelet activation inhibitors including nitric oxide (NO) and prostacyclin, is notably reduced in veins with respect to arteries [12, 17]. The propensity for thrombus formation is also amplified by such factors as intact venous valves, vein distension, reduced flow and anastomotic error [3]. Thus, even when saphenous vein graft surgeries are performed optimally, there remains an inherent predisposition for generation of thrombi. Further endothelial damage and dysfunction is caused after Implantation into the arterial circulation, this is discussed in more detail later in this review.

Intimal hyperplasia is a consequence of VSMC accumulation and their deposition of extracellular matrix (ECM) (Figure 1). Most veins display fibrosis of the intimal or medial compartments prior to grafting [22], though further postoperative intimal thickening can impinge on the lumen by up to 25% within the first 4-6 weeks following surgery [12]. Though rarely enough to induce significant stenosis alone [23], intimal hyperplasia serves as a foundation for accelerated, superimposed atherosclerosis [24]. Intimal fibrosis, in part, precipitates from transient ischemia within the graft. Hypoxia has been detected in the vein graft and it has been proposed that chronic exposure in contrast to transient hypoxia promotes intimal thickening in grafts. In response to hypoxia there is a marked induction of superoxide radical formation in the vessel wall and an attenuation in endothelial cell production of anti-mitotic agents including prostacyclin, NO, and adenosine directly promoting intimal VSMC migration and replication [25, 26]. In addition, re-endothelialisation over non-occlusive thrombi – platelet and fibrin deposits at the luminal surface – allows for progressive organisation of the thrombi into fibrotic tissue; subsequently, platelets release various growth factors and cytokines compounding VSMC proliferation and thereby intimal thickening [19]. Such mitogens are also secreted by endothelial cells, macrophages and VSMCs themselves [17, 18, 27]. Neointima formation is further exacerbated by increased wall stress and distension of the vein graft when subjected to pulsatile pressures of the arterial system [3, 12].

This is described in more detail later in this review. Elevated tangential stress promotes production of mitogenic agents including platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and endothelin 1, and inhibits synthesis of anti-proliferative factors such as transforming growth factor-β (TGF-β) and NO [17, 27, 28]. All these elements combined confer on saphenous vein grafts a high susceptibility to generation of restenotic lesions.

The focus of this review is the role of altered behaviour of VSMCs within the vein graft. This is due to both inherent differences in the composition of the vein and divergent behaviour of venous VSMCs (v-VSMCs) and arterial VSMCs (A-VSMCs), see Figures 2 and 3. Within the native vein and artery in their natural environment the VSMCs predominantly exist in a quiescent phenotype. The main function of the VSMC is contraction and thereby regulation of blood flow and pressure. VSMCs are not terminally differentiated and can switch from the quiescent contractile differentiated phenotype to a de-differentiated synthetic phenotype [33]. In veins there is a higher incidence of de-differentiated VSMCs than arteries. This switch occurs in response to a number of stimuli including hypoxia, endothelial dysfunction and inflammation [33] and is also induced prior to grafting by surgical preparation of the vein [34]. De-differentiation involves VSMCs loss if contractile proteins such as α-smooth muscle actin, myosin heavy chain and smoothelin, and enhanced extracellular matrix and inflammatory cytokines production [35]. This phenotypic transformation observed in grafts at 1 month plays a major pathophysiologic role in intimal thickening.

Though the widely accepted rationale for development of neointimal hyperplasia is that mature, quiescent medial VSMCs de-differentiate into proliferative and synthetic VSMCs [29-31], over the past decade, an array of studies have identified alternative vascular progenitors that may contribute to the overall neointimal VSMC population. Controversy still remains over the origin of the intimal vein graft cells in vein graft. It is considered by some that intimal cells are exclusively from the vein graft [37]. Others proposed that bone marrow or "graft-extrinsic" cells are the major source of intimal cells [38]. A more recent study by Liang et al showed that ~54 % of SMA-α-positive cells in the neointima of mouse vein grafts originated from the flanking recipient artery [39]. Interestingly bone marrow cells did not contribute to the SMCs of the neointima in vein grafts. This is consistent with a previous study in which all graft

cells migrated from the flanking artery without any contribution from putative progenitor cells in the blood in arterial allografts in mice [40]. Not all the host-derived cells expressed the SMC marker a-smooth muscle actin (~10% were negative), which may represent the de-differentiated VSMCs. It is also of note that some of the neointimal VSMCs within the vein graft were from other sources, possibly SMC progenitors [41, 42], endothelial cells [41, 43], or adventitial cells [44, 45]. Using nanofibrous vascular grafts interposition into the carotid artery of athymic rats Tang et al demonstrated that multipotent vascular stem cells (MVSC) differentiated into mature VSMCs [46]. However, Liang et al concluded that de-differentiation of SMCs in the anastomosed arteries are the major contributor to intimal thickening [39]. Additional strategies are required to further examine the contribution of MVSCs to intimal thickening, such as a tamoxifen-inducible SMC-Cre reporter and/or SOX10-Cre reporter mice. Whether alternative vascular progenitors play a salient role in the generation of proliferative and synthetic VSMCs in human grafts remains unclear and subject to debate and is most likely dependent on the degree of injury to the graft either during or following the procedure as this influences the release of factors that induce homing of stem cells to the site of injury. To date fate tracing experiments have not been performed in a suitable large animal model of vein grafting to determine directly whether neointimal cells are derived from mature VSMCs or from progenitor cells. Notwithstanding the involvement of progenitor cells, it is still presumed that mature, quiescent medial VSMCs, at least to an extent, participate in intimal thickening and formation of occlusive lesions.

Beyond the first year following CABG surgery, atherosclerosis is the dominant pathophysiological process responsible for restenosis and saphenous vein graft degeneration (Figure 1) [12, 47, 48]. Atheroma development in vein grafts is similar to that in native arteries, though there are a few temporal, histological and topographical discrepancies [12]. Firstly, the progression of atherosclerosis is more rapid in saphenous vein grafts compared to native coronary arteries as chronic endothelial dysfunction [19, 49] and pronounced intimal fibrosis [24] observed in the former generate a highly atherogenic environment. Secondly, diseased vein grafts accommodate more inflammatory cells, including foam cell macrophages and multinucleate giant cells [50], and display higher rates of lipid-uptake and lipid synthesis, than native coronary arteries [51]. Lastly, morphologically, coronary artery vein graft atheromas are diffuse, concentric (Figure 1) and show poorly developed or absent fibrous caps and little

evidence of calcification, whereas native coronary atheromas are focal, eccentric and exhibit well-developed fibrous caps and calcification [50, 52-54]. However, as with native atheromas, restenotic and atherosclerotic lesions within grafts are prone to rupture, resulting in the reoccurrence of ischemic myocardial injury [55]; thus, study of the molecular dynamics underlying this pathology is essential for the design of new and improved treatments.

FACTORS INVOLVED IN ACTIVATION OF V-VSMCS

In this review we have focussed on the role of the VSMC in the formation of intimal thickening which predisposes the graft to atherosclerosis. There are a number of inherent differences in the composition and cells of the vein and the host artery which may affect the behaviour of the graft cells and thereby contribute to intimal thickening (summarised in Figures 2 and 3).

COMPOSITION OF VEIN GRAFT

It has been clearly demonstrated that the composition of saphenous vein and internal mammary artery differs considerably and this may also contribute to the divergent graft patency rates [56]. For example, in comparison to internal mammary artery, saphenous vein has high levels of dermatan sulphate, collagen and salusin-α, while low levels of heparin sulphate, esterified and free cholesterol, phospholipids, DNA, protein, total glycosaminoglycans, hyaluronic acid, chondroitin sulphate, nitric oxide (NO), salusin-β and apelin-36. Approximately 65% of the saphenous vein is collagen whilst lower amounts are present in the internal mammary artery [57]. Furthermore, the internal mammary has higher medial elastic lamina than the saphenous vein, providing greater elasticity, and reducing deformation and compliance. Intimal thickening is caused by VSMCs migrating through the internal elastica lamina and invading the intima. Therefore, the internal elastic lamina as a barrier to smooth muscle cells [58]. In addition, this difference in ECM composition and cellularity results in a compliance mis-match between the stiffer vein graft and the artery, this is further exaggerated at the anastomosis [59]. The saphenous vein contains less heparan sulphate than the internal mammary artery due to reduced cellularity [57]. Contrastingly, there is augmented dermatan sulphate in glycosaminoglycans in the saphenous vein than the internal mammary artery, which provides enhanced affinity for LDL and VLDL than other glycosaminoglycans components. This may explain in part why the saphenous vein graft has an enhanced propensity to atherosclerosis.

Divergent secretion of soluble factors may also favour intimal thickening in the vein graft. Saphenous vein secretes less NO [60], a vasodilator which inhibits thrombosis and is critical for lumen patency [61], and therefore this is thought to contribute to enhanced intimal thickening and reduced graft patency. Reduced sensitivity of the saphenous vein to vasoactive materials may also contribute to a low patency rate [57]. Moreover, A-ECs secret higher levels of endothelium-dependent relaxation factor (EDRF) than veins which also protects against intravascular thrombus formation. Moreover, GSV secretes less NO [60]. Veins also secrete different types and amounts of vasodilatory substances [56]. Lower amounts of the peptides apelin and salusin in the saphenous vein than arteries and could influence the vasodilatory properties of the graft and thereby patency.

DIFFERENCES IN VASCULAR CELLS

In addition, to differences in composition inherent differences in the grafted vascular cells with the host vascular cells may contribute to intimal thickening. A number of studies have demonstrated contrasting properties of arterial VSMCs (A-VSMCs) and venous VSMCs (V-VSMCs). The distinct properties of V-VSMCs may contribute to the intimal thickening and late failure of venous grafts. As hypoxia occurs at the anastomosis of synthetic grafts [62] and arteriovenous fistulas [63], it is of relevant to determine the effect of hypoxia on VSMC behaviour. VSMC migration and proliferation was enhanced in venous VSMCs compared to arterial VSMCs [64] [65]. Interestingly the mechanism of induction also differed, with V-VSMCs utilising vascular endothelial growth factor (VEGF)-A via VEGF-receptor-1 whilst arterial hypoxia-induced migration and proliferation was predominantly mediated by PDGF-BB [64, 65]. It is therefore likely that differential regulation of VSMC migration and proliferation under hypoxia contributes to the differences in intimal thickening observed in vein and arterial grafts despite hypoxia occurring in both types of graft. It should be noted however that a limitation of these studies is the use of umbilical cord cells and future studies utilising saphenous vein to compare with arterial graft VSMCs and native coronary artery VSMCs would be beneficial. It has also been proposed that the augmented proliferative responses V-VSMCs to hypoxia compared to A-VSMCs are due to the differential responses of SMC proliferation to hypoxic EC-derived growth factors such as PDGF-AB [66]. Moreover V-VSMCs express higher levels of PDGF-Rβ expression and exhibit transactivation of epidermal growth

factor (EGF)-receptor by PDGF-BB. Contrastingly, A-VSMCs, but not V-VSMCs, induce proliferation in response to PDGF-AA, presumably via activation of the PDGF-Rα/JNK1/$p27^{kip1}$ pathway [67].

V-VSMCs exhibit a more de-differentiated phenotype to A-VSMCs, exhibiting heightened proliferative and synthetic capacity [68]. In culture it is apparent that V-VSMCs are smaller, more spindle-shaped, and demonstrate higher proliferative and migratory rates than A-VSMCs. Moreover, increased SMemb expression and decreased α-actin is indicative of a more dedifferentiated phenotype in V-VSMCs, as well as enhanced expression of MMP-2 and MMP-9. Additionally, adhesion to both collagen and fibronectin was significantly lower for V-VSMCs than A-VSMCs. Notably, the expression of decorin was significantly higher in A-VSMCs than V-VSMCs and this may contribute to their altered behaviour [68]. Consequently, strategies to enhance decorin expression may be beneficial for retarding vein graft intimal thickening. A limitation of this study is the use of cultured cells and isolation of carotid arteries and jugular veins from rabbits. Future studies are required using human VSMCs from saphenous vein and coronary artery to demonstrate the generality of these findings.

Observed differences in the behaviour of VSMC derived from saphenous vein to A-VSMCs could facilitate development of new pharmacotherapy, directed specifically at venous intimal hyperplasia. Such therapeutic strategies could be formulated to the type of vessel graft and could prevent restenosis in saphenous vein grafts [67].

Differences in arterial and venous cells may not be restricted to VSMCs, and differences in arterial endothelial cells (A-ECs) and venous endothelial cells (V-ECs) may have important ramifications on vein graft failure. Short-term exposure of V-ECs to high shear stress such as that encountered in the venous graft after implantation into arterial circulation the leads to proinflammatory activation of the ECs by the induction of MAP kinase phosphatase-1 [69]. Interestingly, this does not occur in A-ECs. Dexamethasone pre-treatment of the V-ECs, 'arterializes' them by inducing MAP kinase phosphatase-1 and it is therefore possible that this approach may protect veins from inflammation. The observed differences in response of V-ECs and A-ECs may reflect epigenetic memory of the markedly different hemodynamic environments of their origin. The shear stresses in arteries are markedly higher (10 to 20

dynes/cm^2) than veins (<5 dynes/cm^2). Cultured A-ECs may be pre-adapted to the arterial environment and retain this resistance in culture, whereas v-ECs are primed by the venous environment. There are also non-hemodynamic factors which contribute to the arterial-venous heterogeneity, including epigenetic modifications and cellular signalling pathways that control A-EC and V-EC differentiation in embryonic vessels before circulation of blood [70]. The altered response to shear stress by the V-ECs may lead to the release of inflammatory and pro-proliferative factors that cause activation of the underlying V-VSMCs.

INJURY TO GRAFT

The endothelium and VSMCs of the vein graft are damaged during the preparation and the implantation of the vein graft. The graft is injured in various ways: physical damage by the use of instruments used to harvest the vein from the patient's leg, application of high intra-luminal pressure to check for leakage and disruption of the valves, deployment of clips or sutures to seal side branches. Additionally, damage is caused by implantation into the arterial circulation leading to exposure to high arterial blood pressure and flow causing further damage to the endothelium [17]. The damaged endothelium disrupts the physiological balance of the vein and this is further disturbed by release of factors from damaged VSMCs [71]. The imbalance leads to accelerated VSMC proliferation and is proportional to the degree of injury [72]. This response is considered as a wound healing response that leads to intimal and media thickening in an attempt to become arterialised. However, the thickening of the vein graft ultimately becomes problematic as the required adaptation to the arterial circulation leads to an environment that is susceptible to superimposed atherosclerosis which leads to re-occurrence of symptoms. It would be highly beneficial if grafts could be monitored for the occurrence of arterialisation to determine the point at which detrimental superimposed atherosclerosis was initiated and suitable treatments could be applied at this timepoint. However, currently this has not been established in a suitable large animal model or in humans.

BIOMECHANICAL FACTORS

Biomechanical factors are a major contributor to the incidence and distribution of bypass graft disease [59]. Disturbed local haemodynamics are known to be involved in the formation of intimal thickening. It has been illustrated that end-to-side graft –artery implantation is

responsible for disturbed flow patterns which make the vein susceptible to remodelling in the occurrence of intimal thickening [73]. Low-wall shear stress and high-wall mechanical stress or strain are two major factors [74]. Additionally, a range of secondary factors are involved including vessel geometry; vessel movement; vessel wall characteristics and the presence of reflection waves [75]. However, to date no surgical differences have been made to significantly reduce intimal thickening by alteration of biomechanical factors. Increasing the awareness of the effects of the biomechanical factors on the vein graft may facilitate new approaches to reduce the failure rate of coronary artery vein grafts.

It is apparent that flow pattern regulates cell proliferation and migration. Using a venous polymer implant in rats, vortex blood flow induced VSMC migration and neointimal hyperplasia, whereas the reduced vortex blood flow in the modified vein graft strongly suppressed these effects via the phosphorylation of ERK1/2 and myosin light chain kinase [76]. Elevated cyclic stretch is thought to be one of the major contributors to intimal thickening due to the differences in the venous and arterial environment [12]. Moreover, mechanical stretch *in vitro* causes a differential effect on VSMCs from different sources. Enhanced proliferation is not observed in A-VSMCs after stretching at 0.05 and 1 Hz when compared with unstretched controls. By contrast, stretching of V-VSMCs both at 0.05 and 1 Hz result significantly augmented proliferation compared with static controls [77, 78], via IGF-1 receptor activation and serum-, glucocorticoid-regulated kinase 1 [79, 80]. Thereby it can be proposed that the accelerated V-VSMC proliferation, within vein grafts *in vivo*, is partially the response of the induction of proliferation of V-VSMCs to mechanical stimulation. It is plausible therefor that a biomechanical engineering approach with adaptive training of V-VSMCs prior to grafting could possibly improve vein graft patency.

Speculatively, movement may have an effect on graft patency. At the junction between the stationary aorta and the partially mobile vein graft there may be increased local wall stresses and strains as well as the graft being exposed to a repeated torsional or pendular movement depending on the site of the anastomosis. Experimental external stents which reduce some of the biomechanical forces reduced intimal thickening [81], however this did not translate into clinical benefit in humans due to kinking of the graft within the external stent [82]. Development

of a flexible, biodegradable external stent that can surround both the anastamosis and the mid-portion of the graft is essential.

The vein graft is proposed to be subjected to high blood pressure and flow stagnation in systole and high flow-induced high shear stress and potentially erosion in diastole. To examine this Piola et al established an ex vivo flow model. They observed that although pulsatile stimulation did not affect vessel wall integrity and size, it caused partial endothelial denudation, apoptosis in the vessel wall, and altered medial and intimal thickness [83]. These data could mimic the early vessel remodelling events in vein grafts.

As mentioned previously the composition of the ECM and the cellularity (and thereby the amount of cell-cell cadherin mediated contacts) of veins differs from that of arteries and may contribute to the altered behaviour of VSMCs in grafts by enhancing migration and proliferation. Therefore the role of some of the components of the ECM and cadherin contacts in the regulation of VSMC behaviour will be discussed in more detail in the following sections.

EXTRACELLULAR MATRIX

ECM synthesis not only serves as marker of the active VSMC phenotype, but many of the matrix molecules produced are bioactive and regulate proliferation and migration.

Elastin

Elastin is a stable matrix molecule deposited in vessels during embryonic and early neonatal life. It is a hydrophobic protein composed of cross-linked tropoelastin molecules. Intact elastin inhibits VSMC proliferation to help maintain vessel quiescence. Elastin-deficient mice die early after birth from complete arterial occlusion, which is secondary to robust proliferation and migration of VSMCs from the medial into the intimal layer [85]. With the development of vascular disease, there is significant degradation and disruption of elastin. Elastin degradation is mediated by at least three groups of elastases; serine proteases (i.e. neutrophil elastase and cathepsin G), cysteine proteases (i.e. cathepsin L, S, K and V) and MMPs (i.e. MMP-2, -7, -9, and -12) [86]. Elastin degradation results in the accumulation of elastin-derived peptides which have been shown to increase VSMC proliferation and migration [87].

Type I collagen

Type I collagen is a triple helical molecule composed of heterotrimers of α1 and α2 chains. In intimal hyperplasia type I collagen is found in abundance, and in the context of the injured vessel wall it is present in varied states of assembly or degradation each of which can have distinct effects upon VSMC migration and proliferation. Like elastin, intact or polymeric type I collagen maintains VSMC quiescence, while monomeric or degraded collagen promotes VSMC proliferation and migration. Early studies revealed that plating VSMCs on polymerized collagen resulted in suppression of growth mediated by cyclin dependent kinase inhibitors [88, 89] , while treating cells with monomeric or soluble collagen stimulated proliferation [8890] . Plating on monomeric collagen also decreased the expression of VSMC differentiation markers, and led to the upregulation of the inflammatory adhesion molecule VCAM [91]. In vivo studies of the response to injury in pig coronary arteries provided support for this notion, because accumulation of collagen was correlated with the upregulation of cyclin inhibitors and a decrease in VSMC proliferation [92] . Type I collagen also acts via receptor cross-talk between integrins and growth factors to potentiate the proliferative signaling induced by platelet derived growth factor (PDGF-BB) [93]. VSMCs are able to migrate faster across a monomeric collagen matrix than a polymerized matrix [88, 94, 95] . In fact, proteolysis of the collagen matrix is required for migration in both 2D and 3D gels, not just to degrade matrix barriers, but collagen fragments help to break up focal adhesions allowing cell release and

translocation [96, 97] . There is also evidence that cell deposit new collagen on top of existing matrix to form a provisional matrix more permissive for migration [98, 99] . Treatment of cells to inhibit collagen synthesis prevents focal adhesion formation, actin stress fiber assembly, and migration irrespective of the initial matrix used to coat the plate [100]. The differences in response to different physical states of collagen are partially related to the ease with which cells can release from the ECM, however there is also clear evidence that different physical forms of collagen can trigger activation of distinct signaling pathways.

Type VIII collagen

It is well established that type VIII collagen expression is dramatically increased in vascular injury and disease [101-104]. Type VIII collagen is a short chain, network-forming collagen composed of two chains, procollagen α1(VIII) and procollagen α2(VIII), which are encoded by two separate genes, *Col8a1* and *Col8a2* [106]. Type VIII collagen is central to the control of VSMC proliferation and migration making important contributions to the development of intimal hyperplasia. *In vitro* studies show that type VIII collagen acts as a chemotactic and haptotactic factor for VSMCs [107, 108]. VSMCs adhere less strongly to type VIII collagen compared to type I collagen, and addition of type VIII collagen to a polymeric type I collagen matrix allows release and migration of cells across the matrix, suggesting that type VIII collagen can form a provisional matrix [107]. VSMCs isolated from collagen VIII deficient mice (*Col8*$^{-/-}$) displayed reduced migration, proliferation and MMP-2 production compared to cells from wild-type mice [109]. The *Col8*$^{-/-}$ cells had prominent actin stress fibers, stable microtubules, and numerous basal focal adhesions, all due to enhanced activation of RhoA [110]. Addition of exogenous type VIII collagen suppressed RhoA, restored MMP-2 activity, and enhanced proliferation and migration in these cells [110]. These studies show that type VIII collagen regulates cytoskeletal configuration and MMP-2 expression to facilitate VSMC migration. *In vivo* studies confirmed the role of type VIII collagen in promoting VSMC proliferation and migration to the neointima. *Col8*$^{-/-}$ mice subject to wire injury of the femoral artery had less VSMC accumulation and less wall thickening compared to *Col8*$^{+/+}$ mice [111], revealing an important role for type VIII collagen in regulating VSMC migration and infiltration into the vessel neointima. Type VIII collagen [112].expression is mediated by the transcription factor KLF4, which is also a critical factor mediating the VSMC phenotypic switch [112] and therefore type VIII collagen expression would be increased during VSMC de-differentiation, promoting VSMC migration and proliferation.

CELL-CELL CONTACTS - CADHERINS

The cadherins are a superfamily of transmembrane glycoproteins that mediate homophilic cell-cell adhesions [119]. Cadherin expression is cell-type specific, and the predominant classical cadherin expressed by VSMCs is N-cadherin [120]. Extracellular domain 1 (EC1) of the classical cadherins contains a HAV binding motif, which interacts with cadherins on adjacent cells in a zipper-like fashi. Fibroblast growth factor receptor (FGF-R) also contains an HAV motif, which permits the formation of a heterodimer with EC4 within the N-cadherin molecule [121]. Whilst the extracellular domain of classical cadherins regulates homophilic cell-cell adhesion the intracellular domain modulates cell signalling. The most-well characterised signalling pathway is the β-catenin/Wnt pathway [122]. Cytoplasmic accumulation of β-catenin facilitates movement of β-catenin to the nucleus where it binds to T-cell factor (TCF), and resulting in up- or down-regulation of downstream genes [123], including those involved in the cell cycle (cyclin D1 [124] and c-myc [125]), and migration (versican [126], fibronectin [127], matrix metalloproteinase-7 (MMP-7) [128] and membrane-tethered MMP-1 (MT1-MMP) [129]). Cell signalling and thereby behaviour is also modulated by cadherins via interaction with Rho GTPases [130] and receptor tyrosine kinases, including FGF-R [131].

It has been clearly demonstrated that N-cadherin regulates VSMC proliferation and, migration, and that this is mediated at least in part by interaction with the FGF-R (Figure 2) [120, 121, 132-138]. VSMC proliferation is inhibited by N-cadherin, via β-catenin signalling [139]. N-cadherin cell-cell contacts are modulated by proteolytic shedding by MMPs including MMP-9 and -12, but not MMP-2 and -14 [136], which frees β-catenin permitting it's movement to the nucleus [139, 140]. Inhibiting β-catenin signalling reduces proliferation, due to a reduction in the expression of β-catenin responsive genes, cyclin D1 and p21, which are involved in cell cycle regulation [137]. In cells lacking MMPs 9 and 12 there was less β-catenin signalling, and attenuated cyclin D1 expression, presumably because N-cadherin is maintained and can therefore retain β-catenin at the membrane rather than releasing it for signalling [136]. Discrepant findings for the role of N-cadherin in VSMC migration have been reported. In some studies it was concluded that N-cadherin was pro-migratory [143, 144]. However, in another studies N-cadherin inhibited migration of VSMCs [146, 147].

Loss of cadherins during proliferation leads to the release of free β-catenin which in turn can induce Wnt signalling. Indeed it has been demonstrated that Wnt/β-catenin signalling regulates both VSMC migration and proliferation in various studies [137, 160-168, 170, 171]. The compelling evidence for the involvement of Wnt signalling in VSMC proliferation and thereby intimal thickening has led to the suggestion that activation of the Wnt/β-catenin pathway is a marker of culprit VSMCs that lead to intimal. Consequently, targeting Wnt activated VSMCs could reduce intimal thickening. This is supported by the following studies. Firstly, conditional smooth muscle specific deletion of β-catenin reduced intimal thickening in mice [173]. Additionally, the Wnt/β-catenin inhibitor XAV939 inhibited intimal thickening in mice subjected to carotid artery ligation [174]. Two recent studies using the mouse carotid artery ligation model demonstrated that selective suicide of cells with active Wnt signalling retards intimal thickening [175, 176].

SUMMARY

In summary, VSMC play a major role in the remodelling of the saphenous vein graft after implantation by contributing to the thickening of the intimal. Accelerated intimal thickening in the vein graft is the result of a number of inherent divergent properties of the vein and its vascular cells and the biomechanical and environment factors after implantation as outlined in this review. These result in activation of V-VSMCs and modulation of their behaviour by several factors including ECM and cadherin contacts. The thickened intima is highly susceptible to superimposed atherosclerosis which leads to reoccurrence of symptoms and the need for future treatments. To improve vein graft patency strategies are required to reduce the deleterious biomechanical and environmental factors by altering surgical procedures or by application of treatments which retard the detrimental remodelling of the artery. Identification of the timepoint when beneficial remodelling and arterialisation of the vein transfers to superimposed atherosclerosis would be highly advantageous but required improved imaging techniques or identification of suitable biomarkers.

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Figure 1. Early and late vein graft failure.

Normal vein structure showing the three layers within the vessel wall: intima, media and adventitia. Processes involved in early and late vein graft failure. Early vein graft failure is driven by thrombosis. Late vein graft failure is driven by intimal thickening and superimposed atherosclerosis. Images are from Servier art.

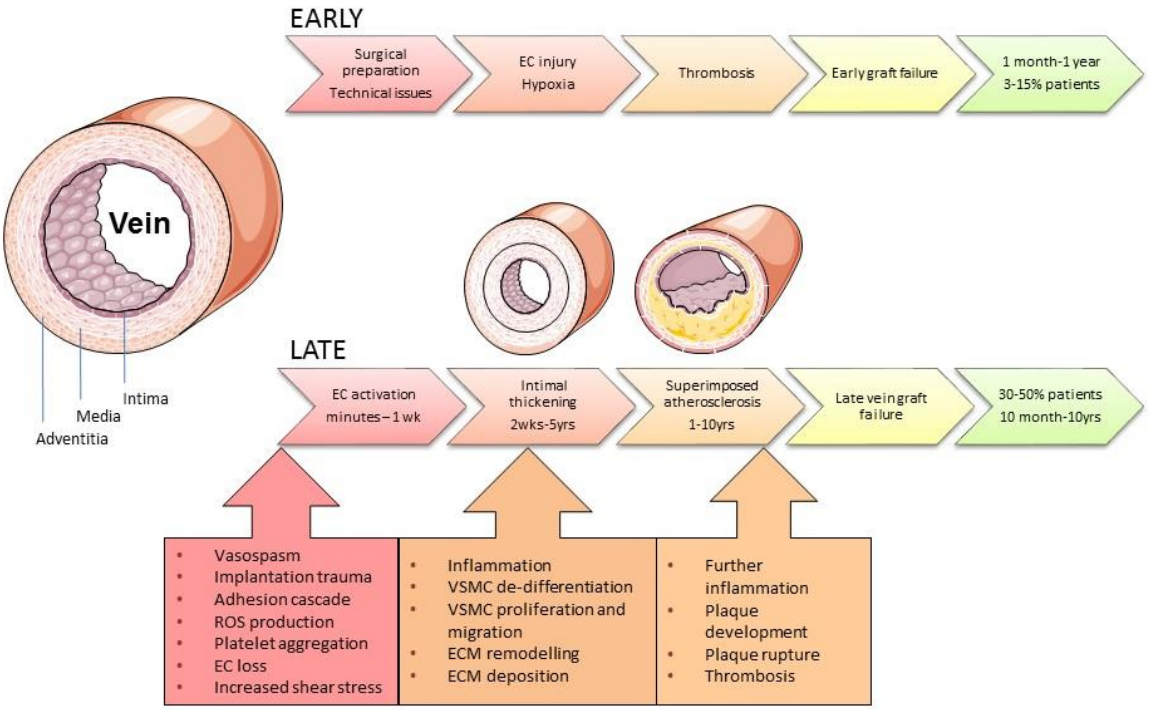
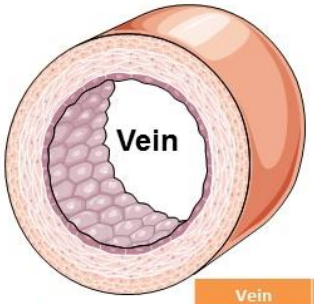
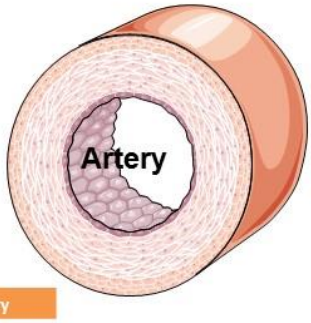


Figure 2. Comparison of venous and arterial cells.

Differences in the properties of venous and arterial cells that alter cell behaviour and may contribute to the poor patency of coronary artery vein grafts. Images are from Servier art.

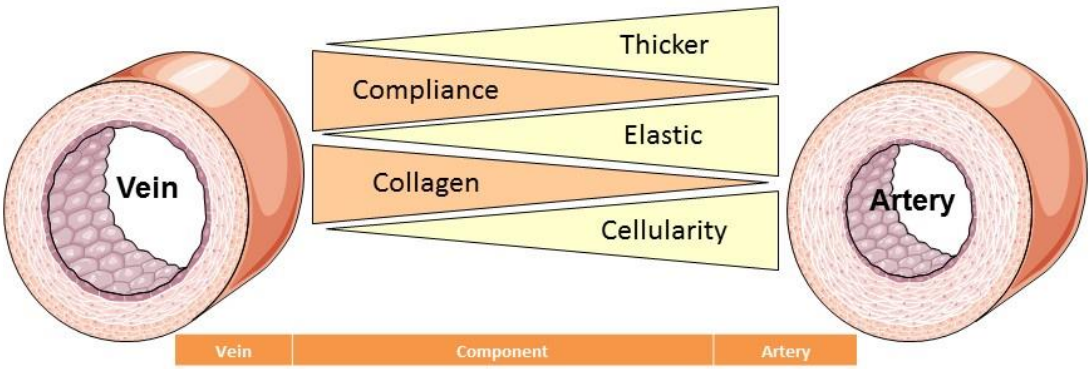




Vein	Component	Artery
Low	VSMC differentiation (contractile)	High
Low	VSMC α-actin, decorin, adhesion to collagen and fibronectin	High
Spindle	VSMC shape	Less-spindle
Small	VSMC size	Larger
High	VSMC proliferation and migration	Low
High	VSMC differentiation (synthetic)	Low
High	VSMC SMemb, PDGFR-β expression	Low
High	VSMC MMP-2 and MMP-9 expression	Low
VEGF-A	Hypoxia induced VSMC migration and proliferation	PDGFBB
High	EC: acute shear stress induced inflammation	Low

Figure 3. Comparison of veins and arteries.

Structural and compositional differences of veins and arteries that may contribute to the poor patency of coronary artery vein grafts. Images are from Servier art.



Vein	Component	Artery
Low	Heparan sulphate, Glycosaminoglycans, hyaluronic acid, chondroitin sulphate	High
Low	Esterified and free cholesterol, phospholipids	High
Low	DNA	High
Low	Protein	High
Low	NO	High
Low	Salusin–β, apelin-36	High
High	Dermatan sulphate	Low
High	Collagen	Low
High	Salusin-α	Low