Post-interventional vascular remodeling: novel insights and therapeutic strategies

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CHAPTER 1

General Introduction

Cardiovascular Disease: a brief introduction

Cardiovascular diseases (CVD) rank as the leading cause of death worldwide, taking an estimated 18 million lives each year. As a result of – amongst others – the global obesity pandemic, aging population and physical inactivity, the prevalence and incidence of CVD are still increasing.[1]

The most common underlying cause of most CVD is atherosclerosis, which is characterized by accumulation of cells and lipids in the vessel wall leading to (partial) occlusion of the large-to mid-sized arteries. This can lead to insufficient blood supply to the tissue downstream of the occlusive atherosclerotic lesion, resulting in angina pectoris (chest pain) or claudicatio intermittens (leg pain). Classically, atherosclerosis has been viewed as a disease that is primarily driven by hypercholesterolemia, hypertension and tobacco use. In recent years, however, this paradigm has shifted and non-classical factors such as inflammation and blood hemodynamics have gained more attention. Indeed, atherosclerosis recently has been acknowledged as a chronic inflammatory disease, in which both the innate and the adaptive immune system play a central role.[2, 3]

Activation of the endothelium, a monolayer of specialized cells that separate the lumen from the vessel wall, is the first step in development of an atherosclerotic plaque.[4, 5] Under physiological conditions, the endothelium functions a semi-permeable barrier that tightly regulates extravasation of fluids, ions and circulating cells into the vessel wall.[5] Through the expression of different adhesion molecules, the endothelium can allow immune cell infiltration into the vessel wall. Crossing of the endothelial monolayer by leukocytes without causing vascular leakage requires concerted action with the endothelial cells (ECs).[6] After transendothelial migration of leukocytes, for example to clear invading pathogens, the endothelium should return to a quiescent state to prevent uncontrolled and excessive extravasation of immune cells into the vessel wall.[7]

In the context of atherosclerosis, the endothelium is constantly activated which leads to endothelial dysfunction and retention of oxidized low-density lipoproteins (oxLDL) in the subendothelial layer.[5] Consequently, monocytes migrate into the vessel wall where they differentiate into macrophages to clear oxLDL.[8] Excessive oxLDL in the vessel wall triggers formation of lipid-laden foam cells, which mainly originate from monocytes/macrophages, but can also be derived from other cell types.[9] Regardless of their origin, foam cells contribute to pathological thickening of the intimal layer of the vessel wall, which is termed intimal hyperplasia (IH), by eliciting an inflammatory response that is followed by proliferation of predominantly VSMCs and fibroblasts.[10] These cells usually reside in the medial and adventitial layer of the vessel wall, but can migrate to the intima upon vascular damage. Both the inflammatory as well as the proliferative response can lead to hypoxia in the vessel wall itself as a result of increased metabolic demand.[11] This provokes intraplaque angiogenesis, which is the ingrowth of neovessels into the hypoxic vessel wall, to facilitate the increased oxygen demand. Unfortunately, these neovessels are

immature and therefore highly susceptible to vascular leakage, which in turn fuels inflammation and atherosclerotic plaque development.[12]

Growth of the atherosclerotic plaque often limits blood flow, which results in hypoxia downstream of these occlusive arterial lesions. When atherosclerotic cardiovascular disease (such as coronary and peripheral artery disease – CAD / PAD) are diagnosed, the primary focus of treatment is on preventing future lesion growth and improving plaque stability to impede disease progression. Consequently, clinicians aim to address behavioral risk factors – tabacco use, unhealthy diet – as well as biological risk factors – hypercholesterolemia, hypertension – through a combination of lifestyle-advice and medical therapy.[13, 14] This, unfortunately, only has a modest effect in preventing (progression of) CVD. Additionally, patients with existing comorbidities, such as auto-immune diseases[15], chronic kidney disease[16] and diabetes[17] are at increased risk of developing CVD.

To alleviate atherosclerotic disease-associated symptoms, these patients require (endo)vascular interventions, such as balloon angioplasty with(out) stent placement or bypass surgery, which aim to restore blood flow downstream of these occlusive lesions.[18] Another example of a vascular intervention is the creation of an arteriovenous fistula in patients with end-stage kidney disease. During this procedure, a vein is directly connected to an artery to allow sufficient blood flow for hemodialysis access.[19]

The increase in CVD prevalence has resulted in a significant rise in the number of vascular interventions performed annually.[20] The clinical success of these vascular interventions depends on adaptation of the vessel wall to the altered hemodynamic situation. This process of post-interventional vascular remodeling, however, is often accompanied by maladaptation of the blood vessel which is mainly characterized by IH and accelerated atherosclerosis. Overall, this causes (re)stenosis after the intervention leading to a large number of patients requiring re-interventions.[21] This is associated with a decrease in quality of life for patients and high economic burden for society.

The **aim** of this thesis is to enhance our understanding of the pathophysiological process that underly post-interventional vascular remodeling and to assess the efficacy of novel therapeutic strategies to hamper adverse vascular remodeling, which can ultimately be used to improve patient care.

Post-interventional vascular remodeling

Adaptation of blood vessels to altered hemodynamic forces after vascular interventions, as discussed in this thesis, can be divided into three different categories:

1) **arterial** remodeling, which is seen after transluminal balloon angioplasty with(out) stent placement or bypass surgery using an arterial graft;
2) **venous** remodeling, as induced after bypass surgery using a venous graft;
3) **arteriovenous** remodeling, as observed after creation of an arteriovenous fistula (AVF) for hemodialysis access.

The improvements in clinical success of (endo)vascular interventions have been mainly achieved through evolution of existing treatments, such as the development of drug-eluting stents, rather than invention of new therapies or technological advances that ameliorate post-interventional vascular remodeling. As a result, we are currently lacking options to abrogate the maladaptive response that is observed after an intervention in a large number of patients. Although negative arterial, venous and arteriovenous remodeling each yield different clinical problems, the pathophysiological mechanisms that drive these phenomena are largely shared between them. Thrombosis and technical failure are major drivers of early failure following a vascular intervention, which is typically observed within in the first months after the procedure. Intimal hyperplasia and (accelerated) atherosclerosis cause mid- as well as long-term failure of vascular interventions, which occurs in the months or years following the intervention. The process of vascular remodeling is already induced during the vascular intervention: physical damage during surgery induces cellular damage, especially to the endothelium. Additionally, when vessels are harvested, the deprivation of blood flow induces ischemia. Together, the physical damage and ischemia serve as the first trigger for inflammation, before the vascular intervention is even finished.[22, 23] In this thesis, we focus on the biological processes that are observed as soon as the vascular intervention is completed and how these can be therapeutically targeted to improve surgical outcomes, which in the end will contribute to improved patient care.

Hemodynamics

Upon completion of the vascular intervention, the vessel wall is exposed to significantly higher blood pressure which causes distension of the vessel wall. The altered morphology of the vessel wall results in perturbed blood flow. The changes in blood flow are sensed by endothelial cells that in turn also respond to these alterations. Depending on the type of flow, the cellular response varies. Laminar blood flow, as observed in physiological situations and often termed ‘stable flow’, induces elongation of endothelial cells and alignment of the cell body along the direction of the flow.[24] Moreover, a sequence of biochemical and genetic signaling events is triggered, including production of cytokines and small molecules that have anti-

inflammatory and vasodilatory effects, such as nitric oxide.[25] The alterations in cell shape are in part mediated by activation of small RhoGTPases, which regulate remodeling of the actin cytoskeleton, intercellular junctions and focal adhesions.[26] The activation of these RhoGTPases, such as Rac1, is in turn regulated by guanine nucleotide exchange factors (GEFs). The importance of cell morphology is illustrated by RhoGEF Trio, which controls endothelial barrier functions by controlling the anatomical shape and junctional integrity of endothelial cells through modulation of RhoGTPase activity.[27] Overall, laminar blood flow yields vaso-protective effects, through a variety of mechanisms, that ultimately limit atherogenesis.

Atherosclerotic plaques arise in curved or branched vascular regions, such as the carotid bifurcation, due to the multidirectional oscillatory flow, which is also termed 'disturbed flow'.[28] In contrast to laminar flow, disturbed flow promotes endothelial dysfunction by augmenting oxidative stress through increased production of reactive oxygen species.[29] Upon disturbed flow, nuclear factor-κβ (NF-κβ) signaling is activated with initiates endothelial inflammation by increased expression of adhesion molecules such as (Intercellular / Vascular Cell Adhesion Molecule – ICAM / VCAM – or E-selectin) as well as production of pro-inflammatory cytokines such as IL1, IL6 and CCL2.[30] Moreover, endothelial cell proliferation as well as apoptosis is increased. [31] Altogether, disturbed flow generates a pro-inflammatory and proliferative response that drives atherogenesis.[30]

Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) play a major role in regulating cellular responses to hemodynamic alterations.[32] Laminar flow promotes phosphorylation of YAP/TAZ, which signals cytosolic retention and inactivation of YAP/TAZ signaling. Upon disturbed flow, YAP/TAZ translocates into the nucleus to promote transcriptional activation of its targets via interactions with e.g. transcription enhancer activator domain transcription factors (TEADs).[33] Activation of YAP/TAZ signaling increases endothelial inflammation, resulting in increased adherence of monocytes to the endothelium. In regions of disturbed flow, YAP/TAZ signaling mediates atherogenesis. Moreover, therapeutic inhibition as well as genetic deletion of YAP resulted in a reduction in plaque development.[32, 34] This underscores the importance of the endothelium for vascular homeostasis and exemplifies how loss of the endothelial barrier function can prompt uncontrolled infiltration of e.g. leukocytes into the vessel, which amplifies the initial inflammatory response to surgery and signals the onset of chronic inflammation in the vessel wall.

In addition to activation of the endothelium after exposure to disturbed flow after an intervention, the arterial blood pressure together with the surgical damage, also results in (partial) de-endothelialization of the vessel wall.[35] Consequently, the extracellular matrix of the subendothelial layer is exposed which can lead to thrombosis. Together with technical failure, thrombosis is the main cause for short-term failure.[36]

Specifically in arteriovenous remodeling, the sharp angle between the artery and the vein inevitably induces disturbed flow. This leads to a pro-thrombotic and pro-inflammatory as well as pro-atherogenic microenvironment around the site of the arteriovenous anastomosis. Consequently, the disturbed flow represent a major problem that is associated with thrombosis and adverse vascular remodeling that limits clinical success of arteriovenous fistulas. Several modifications of the initial surgical technique, including changes in fistula angles[37], artery-to-vein (A-V) configuration rather than vein-to-artery[38] and external support devices (such as VasQ[39]), have been proposed to improve hemodynamics by optimizing fistula geometry (partly based on computational fluid dynamics). The results from these studies are quite promising demonstrating a decrease in both short- as well as long-term failure, underscoring the importance of hemodynamics in post-interventional vascular remodeling.

Mechanical stress

In addition to the disturbed flow that can activate and damage the endothelium, the increased hydrostatic pressure and cyclic strain after surgery amplify the mechanical stress that is exerted on the vessel wall. Cells residing in the vascular wall can 'sense' mechanical forces and respond to these mechanical cues by altering gene expression and epigenetic programming, which leads to a myofibroproliferative response.[40] Especially in (arterio)venous remodeling, the shift from low and constant blood flow – as seen in the venous circulation – to high and pulsatile arterial flow results in an enormous increase in cyclic strain.[41] Exposure of human saphenous veins, which are commonly used for venous bypass surgery, to coronary mechanics *ex vivo* induced phenotypic switching of vascular smooth muscle cells (VSMCs) from a contractile to a synthetic phenotype.[41] Both in naïve and accelerated atherosclerosis, this switch has been described to actively contribute to lesion development.[42, 43] In human saphenous veins, this transition to synthetic VSMC phenotype was associated with a consistent release of thrombospondin-1, a pro-fibrotic matricellular protein that has chemoattractant effects fibroblasts,[41] and saphenous vein progenitor cells, another cell type that has recently been described to to actively contribute to intimal hyperplasia. Additionally, thrombospondin-1 also mediates differentiation of fibroblasts into myofibroblasts upon injury.[44]

In addition to phenotypic switching of VSMCs, mechanical strain also stimulates VSMC proliferation, release of pro-fibrotic factors such as collagen and transforming growth factor-β (TGF-β) and activity of metalloproteinases (MMPs), which overall results in extracellular matrix remodeling and a fibrotic response.[45, 46] Interestingly, VSMCs derived from the internal mammary artery (IMA) do not exhibit increased proliferation by pulsatile stretch[47], thus underlining the importance of mechanical stress in specifically (arterio)venous remodeling.

Preventing maximal distension in e.g. venous grafts, could reduce mechanical stress and diminish the myofibroproliferative response that leads to intimal

hyperplasia. Small clinical trials (VEST I-IV, US, Venous External Support) have demonstrated a decrease in neointima formation in externally supported venous grafts compared to non-supported vein grafts, although this increase did not translate into a decrease in mid- and long-term patency.[48-51] In fact, the patency exhibits a non-significant trend towards decreased patency upon external support, highlighting the need to identify the molecular mechanisms that underly the reduction in intimal hyperplasia in order to target these pathways without the potential side-effects of a synthetic, external graft. Interestingly, preclinical evidence has suggested that external stenting of venous grafts prevents intimal hyperplasia by decreasing YAP-signaling as well as VSMC proliferation and dedifferentiation.[52] The reduction in neointima formation by external stenting was further amplified by coating these external stents with rapamycin[52], a drug with potent anti-inflammatory and anti-proliferative effects that has already long been used for drug-eluting stents for PCI, demonstrating that not only internal but also external coating of stents could provide clinical benefits.[53]

Reendothelialization

Introduction of the drug-eluting stent, in which an antiproliferative drug coats the scaffold, has led to a significant reduction of restenosis.[54] Long-term trials, however, have revealed that the antiproliferative drug also inhibits reendothelialization after the intervention.[55] Rapid reendothelialization requires proliferation of endothelial cells that are located proximally as well as distally of the denudated region and should prevent thrombosis. Additionally, venous endothelial cells can also contribute to the reendothelialization process.[56]

The decreased endothelial cell proliferation in drug-eluting stents has led to an increase of late (>30 days) and very late (>12 months) thrombosis.[57, 58] To promote reendothelialization, stents coated with CD34-antibodies have been developed to capture circulating endothelial progenitor cells that have been demonstrated to orchestrate repair processes in damaged tissues.[59] Use of these stents has been shown to decrease the risk of thrombosis although this was also associated with increased target vessel revascularization, indicating higher failure, whilst underscoring the important role of the endothelium in post-interventional vascular remodeling.[60]

Preserving the integrity of the endothelium during vein graft surgery has been proposed as one of the potential mechanisms to explain the increased patency of vein grafts harvested via the ‘no touch’ technique compared to conventional harvesting, which includes stripping of all tissue surrounding the vein.[61] The surgical damage inflicted by conventional harvesting, however, extends beyond the luminal endothelium, it also impacts the medial and adventitial architecture and the perivascular adipose tissue (PVAT).[62]

Intimal hyperplasia

The hemodynamic changes, mechanical stress and surgical damage together incite a myofibroproliferative response through a variety of mechanisms. Excessive proliferation of predominantly vascular smooth muscle cells as well as fibroblast(like) cells, extracellular matrix remodeling and inflammation are key factors that can drive intimal hyperplasia.

Immediately after the intervention, numerous growth factors, cytokines and chemokines are locally excreted in the vessel wall, which altogether trigger VSMC and fibroblast proliferation. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and TGF-β are well characterized growth factors known to trigger proliferation of vascular cells. These cells that reside in the medial and adventitial layer of the vessel wall do not only proliferate, but also migrate to the intima. To facilitate their migration to the intima, these cells excrete matrixmetalloproteases (MMPs), which induces extracellular matrix remodeling through degradation of collagen and various other components in the vessel wall. After surgery, particularly MMP2 and MMP9 are extensively expressed in restenotic,[63] arteriovenous fistula[64] as well as vein graft lesions.[65] Preventing degradation of the extracellular matrix could potentially prevent vascular cell migration into the intima and reduce neointima formation. Indeed, therapeutic inhibition of MMPs resulted in significant less intimal hyperplasia.[64, 66, 67] Moreover, gene therapy aimed at local overexpression of tissue inhibitor of MMPs (TIMP) 1, 2 or 3 has been demonstrated to inhibit intimal hyperplasia formation in various animal models,[68-70] whilst the efficacy of gene therapy for TIMP3 is currently being investigated in a clinical trial (G1001147/1).

Preventing vascular cell proliferation is also an attractive therapeutic approach to limit intimal hyperplasia. Expression of genes that regulate proliferation is controlled by E2F, a group of transcription factors that are key regulators of cell cycle progression.[71, 72] Using a gene therapy approach in which a decoy specifically binds E2F to block proliferation, resulted in decreased arterial neointima formation after vascular injury in rats[73] and vein graft intimal hyperplasia in rabbits.[74] Brief incubation of human saphenous veins with the therapeutic agent *ex vivo* resulted in excellent drug delivery[75], which was also safe and feasible in both peripheral[75] and coronary[76] bypass graft settings. Assessment of clinical efficacy in randomized trials in patients undergoing peripheral[77] and coronary[78] bypass surgery, however, indicated no protection from vein graft failure, demonstrating the multi-faceted nature of post-interventional vascular remodeling.

The proliferation of vascular smooth muscle cells is also accompanied by a phenotypic switch from a contractile to a mesenchymal or even fibroblast-like state, which has been suggested to accelerate intimal hyperplasia formation.[79] Not only smooth muscle cells and fibroblasts, however, contribute to intimal hyperplasia, also perivascular adipose tissue-derived stem cells[80], dedifferentiated endothelial cells and leukocytes are found in the intima.[56] In (arterio)venous remodeling,

there is also the additional question whether the cells residing in the vein graft, and specifically the intimal layer, are of venous or arterial origin. Experimental evidence in mice suggests that around the anastomotic regions both arterial and venous smooth muscle cells are the main cell type found in the neointima, whilst at the middle segment mainly synthetic smooth muscle cells of venous origin are found. Interestingly, only endothelial cells that have undergone endothelial-to-mesenchymal transition (EndoMT) of venous origin were found in the intima, whilst arterial endothelial cells are the major cellular source of reendothelialization.[56] Despite relatively small number of dedifferentiated endothelial cells found in the intima, conditioned medium of these EndoMT endothelial cells had a strong inhibitory effect on vascular smooth muscle cell dedifferentiation in vitro,[56] which could be suggestive of a regulatory role of venous endothelial cells in the development of intimal hyperplasia.

It is important to note cellular proliferation to a certain extent is required for beneficial post-interventional vascular remodeling. Not only luminal endothelial cells are required to proliferate to initiate reendothelialization, also VSMCs and fibroblasts residing in the intimal and medial need to proliferate to withstand the arterial blood flow. Beneficial, or outward, remodeling is characterized by moderate intimal hyperplasia with medial thickening whilst the lumen area is preserved – or even increased – to facilitate adequate blood flow. In contrast, negative, or inward, remodeling is characterized by excessive intimal hyperplasia and narrowing of the lumen, which leads to decreased blood flow. Proliferation in post-interventional vascular remodeling, as a result, is a delicate process with a very tight balance, that requires proliferation during early stages of vascular remodeling, but is often followed by prolonged and excessive proliferation at later stages leading to loss of patency.

Inflammation

In the last decades, inflammation has emerged as a critical driver of atherosclerotic cardiovascular disease (**Fig. 1**). Extensive pre-clinical evidence has suggested a key role of inflammatory cytokines and various immune cells in atherosclerotic plaque development.[3] The importance of inflammation to the progression of atherosclerotic cardiovascular disease was highlighted in the CANTOS trial, in which a monoclonal antibody capturing IL-1β (canakinumab) reduced major adverse cardiovascular in patients with a history of myocardial infarction and elevated baseline levels of C-reactive protein compared to control.[81] Importantly, canakinumab did not affect lipid levels, but significantly decreased high-sensitive CRP, which indicates reduced inflammation. Furthermore, the strong inflammatory component of atherosclerosis was corroborated by a decreased risk of cardiovascular patients with chronic coronary disease upon treatment with low dose colchicine.[82] This is an anti-inflammatory drug that also has broad cellular effects including alteration of leukocyte responsiveness.[83] Together, these data establish a clear and causal relationship between inflammation and atherosclerotic cardiovascular disease

and underscore the therapeutic potential of anti-inflammatory therapy to reduce disease burden. Nevertheless, the side-effects of anti-inflammatory medication in general include, amongst others, increased rates of infection and leukocytopenia. Interestingly, these side-effects were observed for anti-IL-1β treatment, but not for low-dose colchicine. Moreover, the effect sizes were relatively small for both canakinumab and colchicine, highlighting the multi-faceted nature of atherosclerosis and the great diversity in disease development. This also warrants further research to optimize anti-inflammatory treatment to specifically target local, rather than systemic inflammation to prevent potential side effects and utilize its therapeutic potential to its fullest.

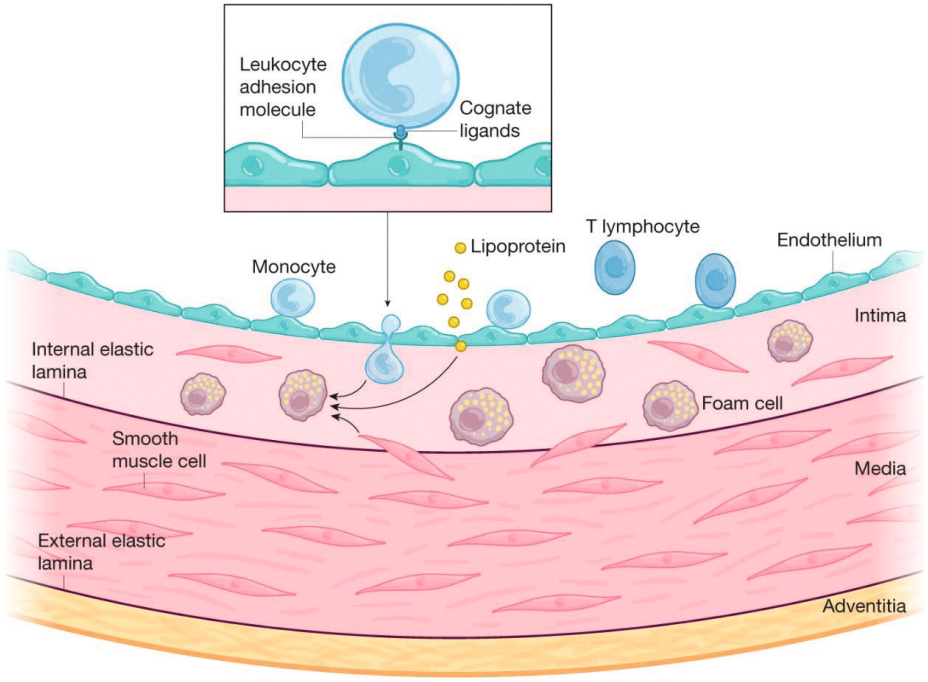

Figure 1. Initiation of atherosclerosis.

Pathologic activation of the endothelium results in loss of endothelial barrier function as well as upregulation of leukocyte adhesion molecules, altogether leading to subendothelial retention of lipoproteins and subsequent migration of leukocytes into the vessel wall. The inflammatory response, amongst others, will drive vascular smooth muscle cell migration and proliferation, thus ultimately initiating atherosclerotic plaque formation. [Adapted from Libby P. The changing landscape of atherosclerosis. Nature, 2021]

Vascular intervention itself induces vascular damage which – together altered hemodynamics after the procedure – triggers local production and secretion of numerous growth factors, chemokines and pro-inflammatory cytokines, such as IL and CCL, in the vessel wall. Additionally, VSMCs and fibroblast proliferate and migrate

to the intima, during which these cells induce extracellular matrix remodeling. The products that are released during the degradation process, e.g. cell and extracellular matrix fragments, are damage-associated molecular patterns (DAMPs), which can act as endogenous ligands for toll-like receptors (TLRs).[84] These membrane-bound receptors are key drivers of the innate immune response and are expressed on a variety of vascular cells, including endothelial cells, vascular smooth muscle cells and macrophages. After surgery, these DAMPs and other TLR-ligands (such as Tenascin-c) are locally expressed in the vessel wall[84] and elicit activation of nuclear factor-κB (NF-κB).[85] The surgical damage itself serves as the initial trigger for the (local) inflammatory response, which is further amplified by the matrix turnover as a result of hemodynamics, mechanical strain and vascular cell migration. All in all, this results in the excessive release of pro-inflammatory cytokines, such as tissue necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and c-c motif chemokine ligand 2 (CCL2), which prompts recruitment of leukocytes into the vessel wall and yields local inflammation in the vessel wall after surgery.[86]

To a certain extent, acute inflammation early after vascular interventions is required to a certain degree to 'kickstart' post-interventional vascular remodeling and induce outward remodeling. The issue is that this short period of acute inflammation after the vascular intervention is often not resolved and transitions into long-term, chronic inflammation, which is associated with inward remodeling. The two phases of inflammation are best exemplified by the expression of TLR4 in murine vein grafts. TLR4 expression peaks one day after surgery (acute), followed by a period of hardly no TLR4 expression in the vein graft, until it surges again at later timepoints (chronic).[84] The hyperinflammatory response to surgery distinguishes post-interventional vascular remodeling from, for example, native atherosclerosis. Moreover, it is a critical determent of long-term success: aggravating the local inflammatory in the vessel wall in mice by periadventitial delivery of lipopolysaccharide during surgery increased development of arterial restenosis.[84] In contrast, inhibiting both acute and chronic inflammation by systemic administration of dexamethasone (long-term) decreased neointima decreased neointima formation in mice in both arterial and venous remodeling after surgery.[87, 88] Strikingly, short-term dexamethasone (7days) yielded similar protection as long-term (28 days), underscoring the crucial role of early inflammation as a critical regulator of post-interventional vascular remodeling.[88] Furthermore, the potential of dexamethasone to inhibit arterial intimal hyperplasia was amplified when administered locally, which also accentuates the important distinction between systemic and local inflammation.[87]

The release of DAMPs and pro-inflammatory cytokines induces generation of reactive oxygen species (ROS). The excessive production of ROS can lead to oxidative stress in all layers of the vessel wall and lead to cell damage, necrosis and apoptosis due to their direct effects on protein, lipids and DNA.[89] The damage, specifically to the DNA, causes cellular stress. In response to DNA damage, poly ADP-ribose polymerase (PARP) is activated to facilitate DNA repair by recruiting DNA repair

machinery to damaged DNA sites.[90] PARP-1 is the most studied nuclear enzyme.[91] Although the role of PARP-1 is initially beneficial and allows cells to respond to DNA damage, excessive PARP-1 activation is associated with a wide variety of disease, including CVD.[92] Extensive activation of PARP induces inflammation, partly through activation of NF-κB and production of pro-inflammatory cytokines.[93] PARP activation is therefore regarded as a common mediator of the inflammatory process in multiple CVD.[94]

In mice, genetic deletion of PARP1 diminished atherosclerotic lesion development by upregulating tissue inhibitor of metalloproteinase-2, which prevented vascular smooth muscle cell migration. Moreover, the infiltration of macrophages into the plaque was reduced.[95, 96] Additionally, when PARP-1 was therapeutically inhibited, neointima formation after carotid wire injury was restricted in rats, whilst also the infiltration of leukocytes into the vessel wall was impeded.[97] Mechanistically, the reduced recruitment of leukocytes after surgical injury could in part be explained by decreased NF-κB activation and reduction in CCL2 expression in PARP1$^{-/-}$ macrophages, which indicates an attenuated response on a cellular level to injury.[96]

The production and secretion of CCL2 is a key driver of inflammation. CCL2, formerly known as monocyte chemoattractant protein-1 (MCP-1), is a chemokine that can mobilize monocytes from the bone marrow and attracts them to sites of inflammation, including inflamed blood vessels, thus amplifying the inflammatory response.[98] In mice that lack CCL2, or its receptor CCR2, the formation of atherosclerotic plaques is reduced, signaling its importance in native atherogenesis.[99, 100] Additionally, the CCL2-CCR2 axis has been demonstrated to critically regulate post-interventional vascular remodeling. Pharmacological inhibition of CCR2 signaling reduced neointima formation after arterial wire injury in mice.[101] Furthermore, also adverse venous remodeling was attenuated when expression of CCL2 or CCR2 was inhibited through gene therapy approach.[102, 103] In patients, expression of CCL2 in carotid plaques corresponds with macrophage infiltration, MMP activity and other markers of inflammation.[98] All this evidence suggest a key role for CCL2 in the development and an atherosclerotic plaque and therefore, the therapeutic potential of CCL2 inhibition to prevent plaque progression was assessed in a clinical trial. Although selective CCL2 inhibition increased stent patency after percutaneous coronary interventions, there were no significant differences in incidence of major adverse cardiovascular events.[104] Overall, these studies highlight the crucial role of inflammation in atherogenesis and post-interventional vascular remodeling, whilst highlighting the need for individualized and more targeted therapy, rather than general immune-inhibition, to increase chances of clinical success.

Macrophages

The inflammatory response after the intervention is orchestrated by different leukocyte subsets at different timepoints. During surgery, systemic CCL2 levels increase which mobilizes monocytes from the bone marrow.[105] After the procedure,

circulating monocytes will adhere to the activated or even denudated endothelium in response to the excretion of pro-inflammatory cytokines and upregulation of adhesion molecules. After extravasation, monocyte differentiate into pro-inflammatory or anti-inflammatory macrophages depending on the micro-environment in the vessel wall.[106] In the vessel wall, especially during early stages of vascular remodeling, pro-inflammatory macrophages represent the vast majority of leukocytes that are found in the vessel wall, due to the cytokine storm after surgery.[86, 107] After the initial inflammatory response, macrophages should become more repair associated and undergo phenotypic switching from a pro- towards anti-inflammatory profile. Pro-inflammatory macrophages, formerly known as M1 macrophages, typically express CD80-CD86 (costimulatory marker), CCR2 and inducible nitric oxide (iNOS), whilst anti-inflammatory macrophages, formerly known as M2 macrophages, are characterized by expression of CD206 and Arginase1.[108]

The arrival of single cell omic techniques have revealed that the classical distinction between pro- (M1) and anti-inflammatory macrophages (M2) has been too simplistic. Different single cell transcriptomic studies have identified four main macrophage subsets in atherosclerotic plaques.[109-112] There are two inflammatory macrophage populations: one that includes macrophages expressing IL-1β, whilst the other population predominantly expresses TNF-α, which indicates a certain degree of heterogeneity in response to inflammation in the atherosclerotic plaque. Additionally, a resident-like macrophage population was identified that appears to play a role in maintaining vascular homeostasis and could therefore be regarded as more anti-inflammatory.[113] Interestingly, another population mainly expressed genes involved in lipid metabolism and cholesterol transport / efflux. These TREM2^{hi} macrophages were therefore identified as candidate foam cells. Interestingly, the absence of pro-inflammatory gene expression challenges the concept of directly lipid-driven inflammation in macrophages, and is suggestive of indirect lipid-driven inflammation through necrosis of foam cells for example.[106]

Above all, the single cell transcriptomic studies of the atherosclerotic plaque have affirmed the plasticity of this cell type. These data can be used to identify potential therapeutic targets to guide the transition of macrophages from a pro-inflammatory phenotype that promotes plaque instability and progression towards an anti-inflammatory and repair-associated phenotype. Additionally, this transition could also stimulate outward remodeling of blood vessels by facilitating inflammation regression after surgery. Interestingly, statin-therapy is known to exert anti-inflammatory effects, in addition to their cholesterol-lowering effect.[114, 115] Mechanistically, statins have been demonstrated to inhibit YAP as well as NF-κβ signaling in macrophages, but also in other cell types, which blocked excretion of pro-inflammatory cytokines such as IL-6.[114-116] These pleiotropic effects could provide a rational for aggressive statin therapy after vascular interventions to restrain plaque inflammation.

Exposure of monocytes and macrophages to endogenous atherogenic stimuli, such as oxLDL, can lead to epigenetic and metabolic reprogramming of these cells.[117] This can lead to a long-lasting pro-inflammatory phenotype of these cells. When re-exposed to a previously encountered stimulus, the inflammatory response of the immune system will then be amplified compared to the initial exposure.[118] This concept of trained immunity can significantly affect post-interventional vascular remodeling and induce adverse remodeling. Myocardial infarction, for example, drives trained immunity of monocytes, thus accelerating atherogenesis.[119] Moreover, this augment immune response further escalates upon re-interventions and hamper clinical success. Overall, this phenomenon further emphasizes the importance and therapeutic potential of anti-inflammatory therapy, which could guide transition of macrophages towards an inflammation-resolving phenotype to prevent adverse vascular remodeling.

T cells

Although atherosclerotic cardiovascular diseases have classically been viewed to be driven by the innate immune system, it has recently become increasingly clear that also the adaptive immune system can contribute atherogenesis. In fact, single cell technologies have revealed an abundance of T cells in advanced, human atherosclerotic plaques. Moreover, these T cells display an activated phenotype. Additionally, a strong association between the number of activated T cells and plaque progression has been identified, which indicates an active role of the adaptive immune system during atherogenesis.[109, 110, 120]

In mice, multiple T cell populations have been identified at different stages of plaque development, which implicates that T cells are involved in and actively contribute to atherosclerotic lesion development.[111] The (causal) role of T cells in atherogenesis and post-interventional vascular remodeling, however, remains to be fully understood due to the large variety of T cell subsets and opposing functions.

Three signals are required for T cell activation: i) recognition of an antigen (presented by an antigen presenting cell) by the T cell receptor, ii) activation of the T cell by its costimulatory molecule and iii) a polarizing cytokine signal.

Naïve T cells are precursors for all T cell subsets. These naïve T cells can be activated by various immune cells, for example dendritic cells, which can take up antigens and consequently function as an antigen presenting cells. Following activation of the T cells receptor and costimulation in lymphoid organs, naïve T cells proliferate and differentiate into effector and memory T cell subsets. Central memory T cells are characterized by their presence in blood and lymphoid organs, whilst effector-memory T cells are characterized by rapid effector function upon activation, production of various (inflammatory) cytokines and their rapid infiltration into inflamed tissue.

Antigen presenting cells as well as T cells are commonly found in the vessel wall under physiologic conditions. In the context of atherosclerotic lesions, the number of

antigen presenting cells is greatly increased, whilst their phenotype is also distinctly altered.[121, 122] In addition to invading pathogens, antigen presenting cells can also present damage-associated molecular patterns (DAMPS), such as oxLDL, glycated proteins, lipids and nucleic acids, which are frequently found in plaques.[123] This is accompanied by expression of costimulatory molecules such as CD40, CD80 and CD86, which are normally not expressed in healthy vessels, and leads to T cell activation.[124] After vascular interventions, the altered hemodynamics and surgical injury together induce a cytokine storm, necrosis of vascular cells and extracellular matrix remodeling (the products of this process can act as DAMPs). Altogether, this leads to a micro-environment in the vessel wall that favors T cell activation.

The importance of T cell costimulation for both native and accelerated atherosclerosis is corroborated by clinical as well as preclinical evidence. For example, $CD40^+$ dendritic cells have been observed in carotid plaques as well as stenotic atherosclerotic vein grafts,[125] whilst soluble CD40L levels closely associated with restenosis following PCI.[126, 127] CD40L-deficiency in $CD4^+$ T cells and dendritic cells reduced atherogenesis by decreasing T cell activation and infiltration into the plaque.[128] Similar effects were observed for CD137, which binds to CD137L to induce T cell activation. In human atherosclerotic lesions, CD137 was expressed mainly on $CD8^+$ T cells.[129] In mice, agonistic stimulation of CD137 aggravated atherogenesis and increased $CD8^+$ T cell infiltration into the plaque.[129] In contrast, genetic silencing reduced atherogenesis.[130] Furthermore, genetic deletion as well as therapeutic inhibition of costimulatory receptor CD28 decreased arterial neointima formation in mice by diminishing leukocyte infiltration into the vessel wall.[131] This receptor, CD28, can be activated by expression of CD80 and CD86, whilst it can also be inhibited when it binds to co-inhibitor molecule cytotoxic t-lymphocyte antigen (CTLA)-4.[132] These inhibitory molecules, such as CTLA-4 and programmed death-ligand (PD-L)1, prevent T cell activation.[133]

Cancer cells often express co-inhibitory molecules to evade the immune recognition and subsequent clearance by immune cells. Immune checkpoint inhibitors (such as anti-CTLA-4 and anti-PD-L1) are therefore frequently used to induce immune activation in patients with cancer. The increased activation of immune cells, however, also profoundly affects the cardiovascular system. Clinical evidence convincingly demonstrates that patients with cancer receiving these immune-checkpoint inhibitors are at increased risk of atherosclerotic plaque progression, as evidenced by increased incidence of myocardial infarction, coronary revascularization and ischemic stroke.[134] In mice, immune checkpoint inhibition results in increased inflammation in native atherosclerosis,[135] whilst also driving arterial neointima formation.[131] Furthermore, anti-PD-L1 treatment prevented arteriovenous fistula maturation by inhibiting VSMC proliferation and increasing endothelial dysfunction.[136] Moreover, anti-PD-L1 treatment also aggravated macrophage polarization towards an inflammatory phenotype, thus emphasizing the interplay between different immune cell subsets and other cell types in the vessel wall.[136] Overall,

these data warrant close monitoring of cardiovascular health in patients receiving immunotherapy.

Intraplaque angiogenesis

Both the inflammatory as well as the myofibroproliferative response that are observed after surgery result in increased oxygen consumption.[137] Together with the progressive thickening of the vessel, which hampers oxygen diffusion, this can lead to plaque hypoxia, which contributes to atherogenesis. From a molecular point of view, the absence of oxygen prevents degradation of hypoxia-inducible factor (HIF)-1α by prolyl hydroxylases (PHD)1, 2 and 3. As a result, HIF-1α is stabilized and can translocate into the nucleus.[138, 139] This results in the transcriptional activation of downstream targets of HIF-1α, including VEGF, which drives an angiogenic response to overcome hypoxia. In the atherosclerotic plaque, HIF-1α is predominantly expressed by macrophages, which triggers the ingrowth of neovessels into the hypoxic plaque, termed intraplaque angiogenesis [140-142] These neovessels, however, are immature and therefore susceptible to intraplaque hemorrhage. Consequently, the body's physiologic response to hypoxia becomes pathologic, since intraplaque hemorrhage fuels plaque inflammation as a result of uncontrolled extravasation of erythrocytes and leukocytes.[12] Limiting intraplaque angiogenesis and intraplaque hemorrhage could therefore prevent plaque growth.

Upon the production and secretion of growth factors, such as VEGF and bFGF, in the vessel wall, neovessels usually grow into the hypoxic plaque from the adventitia. When VEGF is produced and secreted in the hypoxic vessel wall, it can bind to VEGFR2 which enhances the dimerization of VEGFR2 and increases receptor activity to promote intracellular (angiogenic) signaling.[143] VEGF can also bind to VEGFR1 (which is mainly expressed on monocytes as well as macrophages) or VEGFR3 (which is mainly expressed on lymphatic endothelial cells), but VEGFR2 is the main receptor and is mostly expressed on vascular endothelial cells.[144] The VEGF gradient induces proliferation of endothelial cells and migration of tip cells, which are followed by stalk cells, to migrate towards the angiogenic stimulus.[143, 144] Multiple therapeutic strategies that aim to reduce VEGF-VEGFR2 signaling have been designed to decrease angiogenesis in cancer[145] or retinopathy.[146]

In the context of atherosclerosis, when VEGFR2 signaling was inhibited by an antibody, the atherosclerotic lesion was decreased, whilst plaque stability was increased. Surprisingly, the number of neovessels was not reduced, whereas the number of extravasated was significantly decreased, indicating less intraplaque hemorrhage.[141] In another study that aimed to reduce atherogenesis by blocking bFGF-signaling, it was found that blockade of bFGF-signaling did inhibit intraplaque angiogenesis as well as intraplaque hemorrhage. Lesion size, however, was unaffected, whilst the plaque inflammation and infiltration of macrophages into the plaque was diminished.[147] This shows that neovascularization actively contributes to plaque growth and lesion instability. Moreover, it demonstrates that the quality,

rather than the quantity, of these neovessels determines plaque fate. Restoring the endothelial barrier function of intraplaque neovessels, could therefore potentially prevent atherosclerotic plaque growth and improve post-interventional vascular remodeling.

Perivascular adipose tissue (PVAT)

The endothelial cells usually grow into the hypoxic plaque from the vasa vasorum of the adventitia.[148] Proliferation of these endothelial cells appears to be strongly regulated by perivascular adipose tissue (PVAT), which surrounds both arteries and veins.[149] Although PVAT has long been overlooked in vascular biology, it is now recognized as an paracrine organ that regulates vascular function. Under physiologic conditions, PVAT contributes to vascular homeostasis through excretion of anti-inflammatory cytokines and vasodilators, such as NO.[150] Dysfunctional PVAT, however, can instigate vascular disease by inducing release of pro-inflammatory and pro-angiogenic cytokines, such as CCL2.[151]

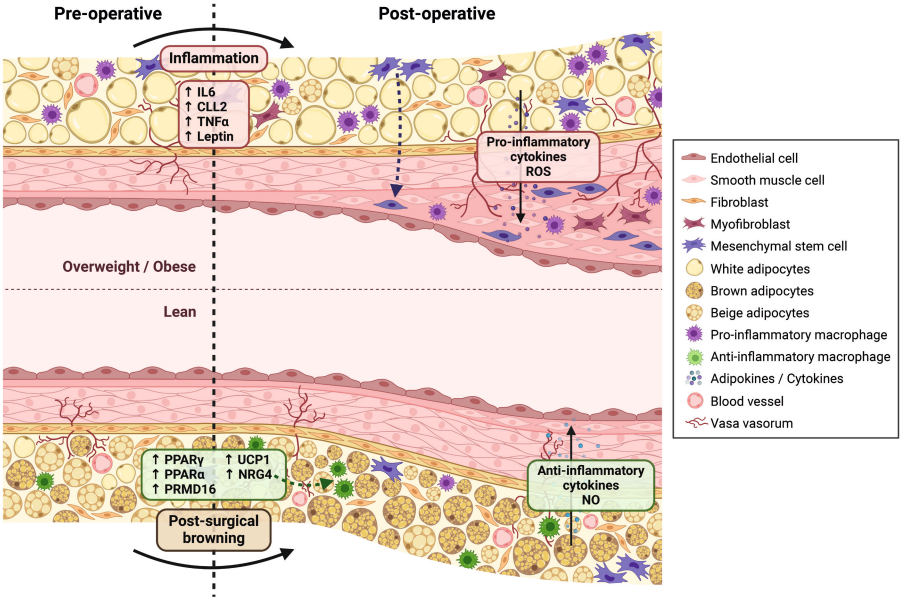

Figure 2. Preoperative and postoperative changes in perivascular adipose tissue (PVAT) in lean versus overweight/obese conditions.

In lean individuals, PVAT acquires a brown adipose tissue (BAT)–like phenotype to mitigate the proinflammatory response to surgical injury. To facilitate vascular repair, NRG4 (neuregulin-4) expression is increased in PVAT adipocytes, which promotes transitioning of macrophages toward an anti-inflammatory phenotype. This is accompanied by release of anti-inflammatory cytokines and NO. In obese individuals, PVAT exhibits an increase in white adipocytes, elevated proinflammatory cytokines (IL-6 [interleukin-6], CCL2 [CC-chemokine ligand 2], TNFα [tumor necrosis factor-α], and leptin), and a higher presence of proinflammatory macrophages before surgery. Postoperatively, this inflammatory environment hampers browning of PVAT, which leads to the production of proinflammatory cytokines along with reactive oxygen species (ROS). This induces adverse vascular remodeling, which is mediated by enhanced vascular smooth muscle cell and mesenchymal stem cell migration and proliferation. NRG4 indicates neuregulin-4; PPAR, peroxisome proliferator–activated receptor; PRDM16, PR domain containing 16; and UCP1, uncoupling protein 1. [From Kruit et al. Role of Perivascular Adipose Tissue in Vein Remodeling. ATVB, 2025]

PVAT has a distinct phenotype that differs from other adipose depots. For example, in vitro cultured perivascular, but not perirenal or subcutaneous, adipocytes were able to induce angiogenesis via increased release of VEGF.[152] Furthermore, the PVAT phenotype also depends on the vascular bed that it surrounds. In general, arterial PVAT has a more brown adipose tissue phenotype, which is associated with energy dissipation and heat production. In contrast, venous PVAT has more white or beige adipose tissue characteristics, which is associated with energy storage.[153]

High fat feeding triggers PVAT inflammation.[154] Transplantation of inflamed, as a result of high fat diet, thoracic PVAT to the carotid artery (mice do not have a lot of carotid PVAT) exacerbated neointima formation following surgical injury, partly

through increased release of CCL2.[152] Furthermore, transplantation of inflamed PVAT destabilized atherosclerotic plaques by increasing macrophage infiltration and increasing vasa vasorum proliferation.[152, 155] This indicates a critical role for PVAT in vascular disease. Moreover, the overweight and obesity which are frequently observed in cardiovascular patients can lead to dysfunctional PVAT, which could negatively impact post-interventional vascular remodeling. (**Fig. 2**)

Dietary restriction is the most effective strategy to non-invasively lose weight. Long-term dietary restriction, which encompasses calorie, protein and/or amino-acid restriction, however, is clinically not feasible to maintain for a longer period of time in most patients. The benefits of dietary restriction include reduced inflammation, improved (cardio)metabolic health and mitigation of ROS. Interestingly, these beneficial effects arise rapidly after initiation of the dietary intervention and could therefore be used to precondition patients for planned stressors such as surgical injury. This hypothesis was first confirmed when it was demonstrated that mice which were subjected to four week 30% caloric restriction (CR) or three days water only fasting, had decreased renal as well as hepatic damage after ischemia-reperfusion injury.[156] Thereafter, it was established that a one week isocaloric, protein-free diet yielded protection from both renal and hepatic ischemia-reperfusion injury to a similar extent as caloric restriction.[157] Furthermore, the protein-free diet reduced neointima formation in the carotid artery after creation of a focal stenosis.[157] Additionally, restriction of total protein, (with equal calorie intake) also improved vein graft remodeling, partly via reducing the inflammatory response after surgery.[158] Overall, these studies provide convincing evidence that short-term pre-operative dietary restriction can alter the host's disadvantageous response to (vascular) interventions to exert protective effects.

The benefits of dietary interventions largely depend on an increase in the endogenous production of hydrogen sulfide (H_2S),[159] a gaseous molecule with vasodilatory and anti-inflammatory properties.[160] Mechanistically, dietary restriction activates the transsulfuration pathway which produces H_2S as a metabolite. When the sulfur amino acids methionine and cysteine were supplemented to mice which were subjected to caloric restriction, the protection from hepatic ischemia-reperfusion injury was lost.[159] Conversely restriction of methionine and cysteine without affecting protein or calorie intake, which is also known as Methionine Restriction (MetR), induces pleiotropic benefits on cardiometabolic health.[161] These effects are partly mediated by metabolic alterations that also result in browning of various adipose depots, whereas its effect on PVAT is unknown.[162, 163]

All in all, post-interventional vascular remodeling is complex process that requires tight regulation of cellular migration and proliferation. The myofibroproliferative and inflammatory responses that are observed after vascular interventions are necessary to allow adaptation of blood vessels to the new hemodynamic situation, but often lead to maladaptation as a result of chronic inflammation and excessive proliferation. The plethora of molecular pathways and cellular effectors as well as the vascular

bed-specific differences underscore the complexity of the process as a whole, whilst also providing ample opportunities for therapeutic targeting.

Animals models of post-interventional vascular remodeling

Murine models are the preferred option to study post-interventional vascular remodeling, due to their rapid reproduction and ease of genetic manipulation. Moreover, the response to inflammation in murine models greatly mimics the human response.[164] Although the use of animals experiments can significantly enhance our understanding of disease, data should always be interpreted with caution and be validated in human samples when possible.

1. Arterial remodeling

There are multiple models to study arterial remodeling, as observed after PCI. In this thesis, we have used two different murine models to study arterial intimal hyperplasia. In one model, we place a non-constrictive, polyethylene cuff the femoral artery to incite neointima formation. The surgical damage and placement of the cuff induce primarily vascular smooth muscle cell proliferation leading to neointima formation, whilst also triggering an inflammatory response that is mainly driven by macrophage infiltration from the lumen of the arterial wall.[165] In the other model, we place a 35-gauge mandrel needle longitudinally along the common carotid artery, which is tied with a nylon suture. The removal of the needle restores blood flow, which is severely restricted and disturbed by the suture creating a focal stenosis, which results in vascular smooth muscle cell proliferation, thus offering a simple model to study hemodynamically driven intimal hyperplasia.[166] Other models, which are not used in thesis, include femoral wire injury[167] or perivascular carotid collar placement.[168]

2. Venous remodeling

Vein graft remodeling following bypass surgery can be studied by interpositioning a caval vein from a donor mouse into the arterial circulation of a recipient at the site of the right common carotid artery.[169] The harvest and surgical damage results in inflammation, whilst the arterial blood pressure causes maximal distension triggering a myofibroproliferative response. When using mice that are prone to develop hypercholesterolemia, large, human-like atherosclerotic lesions will develop that also harbor leaky neovessels. This model, therefore, is not only suited to study vein graft remodeling, but also intraplaque angiogenesis and intraplaque hemorrhage, which are normally not observed in native atherosclerosis.[141]

3. Arteriovenous remodeling

Creation of an arteriovenous fistula by anastomosing the end of a branch of the external jugular vein to the side of the common carotid artery using interrupted sutures allows to study arteriovenous remodeling using a similar configuration (end-to-side)

that is used in humans.[170] Following the surgical intervention, progressive vessel wall thickening is observed, mimicking the remodeling that is observed in humans. Another model, which is not used in this thesis, connects the abdominal aorta to the inferior caval vein through a puncture, which also induces progressive vessel wall thickening but does not reflect the configuration as created in humans.[171]

Outline of this thesis

In this thesis, we will discuss key molecular as well as cellular signaling pathways involved in post-interventional vascular remodeling. Furthermore, we will present novel therapeutic strategies to enhance efficacy of vascular interventions by targeting the pre-, peri- as well as post-operative period.

In **chapter 2**, current knowledge on the barrier function on the endothelium and its effect on the transendothelial migration process of leukocytes in the context of atherosclerosis is reviewed. We describe the central role of luminal as well as neovessel endothelial cells and how endothelial dysfunction serves as a trigger for inflammation and (accelerated) atherosclerosis. In **chapter 3**, we describe how the actin cytoskeleton regulates endothelial cell shape and junctional integrity. Moreover, we study the role junctional F-actin on vascular leakage *in vitro* and *in vivo*. The effects of increased endothelial integrity, through overexpression of RhoGEF Trio, on immune cell transendothelial migration are presented in **chapter 4**.

The interaction between immune cells, specifically macrophages, and endothelial cells are investigated in **chapter 5**, with a particular focus on the role of hypoxic signaling in macrophages and its effect on neovascularization and endothelial integrity in venous bypass grafts.

In **chapter 6**, the therapeutic potential of short-term pre-operative amino acid restriction to improve vein graft as well as arterial remodeling is studied. Additionally, we demonstrate a crucial role for the perivascular adipose tissue in transmitting the protective effects of our dietary intervention.

The importance of inflammation and its contribution to vascular remodeling is further illustrated in **chapter 7**, in which the T cell immune landscape following vein graft surgery is examined. Furthermore, monoclonal antibodies directed at newly identified T cell targets are employed to assess the effect of immune modulation on intraplaque angiogenesis and vein graft remodeling.

In **chapter 8**, we decipher the effect of a newly developed PARP-1 inhibitor to improve post-interventional arterial, venous as well as arteriovenous remodeling. In addition, we identify the cellular mechanisms through which the compound exerts its protective effects. At last, we delineate the effect of mechanical strain on saphenous vein progenitor cells and its relation to YAP-TAZ signaling *in vitro* in **chapter 9**, whilst also evaluating the expression of YAP over time in murine vein grafts, followed by assessing the therapeutic potential of an FDA-approved YAP-TAZ inhibitor to prevent adverse vein graft remodeling.

REFERENCES

1. Roth, G.A., et al., *Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study.* J Am Coll Cardiol, 2020. **76**(25): p. 2982-3021.
2. Libby, P., *The changing landscape of atherosclerosis.* Nature, 2021. **592**(7855): p. 524-533.
3. Soehnlein, O. and P. Libby, *Targeting inflammation in atherosclerosis — from experimental insights to the clinic.* Nature Reviews Drug Discovery, 2021. **20**(8): p. 589-610.
4. Libby, P., et al., *Atherosclerosis.* Nat Rev Dis Primers, 2019. **5**(1): p. 56.
5. Sluiter, T.J., et al., *Endothelial Barrier Function and Leukocyte Transmigration in Atherosclerosis.* Biomedicines, 2021. **9**(4).
6. Grönloh, M.L.B., et al., *Endothelial transmigration hotspots limit vascular leakage through heterogeneous expression of ICAM-1.* EMBO Rep, 2023. **24**(1): p. e55483.
7. Vestweber, D., *How leukocytes cross the vascular endothelium.* Nat Rev Immunol, 2015. **15**(11): p. 692-704.
8. Woollard, K.J. and F. Geissmann, *Monocytes in atherosclerosis: subsets and functions.* Nat Rev Cardiol, 2010. **7**(2): p. 77-86.
9. Gui, Y., H. Zheng, and R.Y. Cao, *Foam Cells in Atherosclerosis: Novel Insights Into Its Origins, Consequences, and Molecular Mechanisms.* Frontiers in Cardiovascular Medicine, 2022. **9**.
10. Déglise, S., C. Bechelli, and F. Allagnat, *Vascular smooth muscle cells in intimal hyperplasia, an update.* Front Physiol, 2022. **13**: p. 1081881.
11. Marsch, E., J.C. Sluimer, and M.J. Daemen, *Hypoxia in atherosclerosis and inflammation.* Curr Opin Lipidol, 2013. **24**(5): p. 393-400.
12. Parma, L., et al., *Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis.* Eur J Pharmacol, 2017. **816**: p. 107-115.
13. Borén, J., et al., *Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel.* Eur Heart J, 2020. **41**(24): p. 2313-2330.
14. Nakano, S., et al., *JCS 2022 Guideline Focused Update on Diagnosis and Treatment in Patients With Stable Coronary Artery Disease.* Circ J, 2022. **86**(5): p. 882-915.
15. Conrad, N., et al., *Autoimmune diseases and cardiovascular risk: a population-based study on 19 autoimmune diseases and 12 cardiovascular diseases in 22 million individuals in the UK.* Lancet, 2022. **400**(10354): p. 733-743.
16. Jankowski, J., et al., *Cardiovascular Disease in Chronic Kidney Disease: Pathophysiological Insights and Therapeutic Options.* Circulation, 2021. **143**(11): p. 1157-1172.
17. Malmberg, K. and L. Rydén, *Myocardial infarction in patients with diabetes mellitus.* Eur Heart J, 1988. **9**(3): p. 259-64.
18. Doenst, T., et al., *PCI and CABG for Treating Stable Coronary Artery Disease: JACC Review Topic of the Week.* J Am Coll Cardiol, 2019. **73**(8): p. 964-976.
19. Bylsma, L.C., et al., *Arteriovenous Fistulae for Haemodialysis: A Systematic Review and Meta-analysis of Efficacy and Safety Outcomes.* Eur J Vasc Endovasc Surg, 2017. **54**(4): p. 513-522.
20. Jim, J., et al., *Population-based analysis of inpatient vascular procedures and predicting future workload and implications for training.* J Vasc Surg, 2012. **55**(5): p. 1394-9; discussion 1399-400.
21. Fearon, W.F., et al., *Fractional Flow Reserve–Guided PCI as Compared with Coronary Bypass Surgery.* New England Journal of Medicine, 2021. **386**(2): p. 128-137.
22. de Vries, M.R., et al., *Vein graft failure: from pathophysiology to clinical outcomes.* Nat Rev Cardiol, 2016. **13**(8): p. 451-70.

23. Brahmbhatt, A., et al., *The molecular mechanisms of hemodialysis vascular access failure.* Kidney Int, 2016. **89**(2): p. 303-316.
24. Jr., R.S., et al., *Fluid shear, intercellular stress, and endothelial cell alignment.* American Journal of Physiology-Cell Physiology, 2015. **308**(8): p. C657-C664.
25. Noris, M., et al., *Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions.* Circ Res, 1995. **76**(4): p. 536-43.
26. Tzima, E., et al., *Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression.* Embo j, 2002. **21**(24): p. 6791-800.
27. Klems, A., et al., *The GEF Trio controls endothelial cell size and arterial remodeling downstream of Vegf signaling in both zebrafish and cell models.* Nature Communications, 2020. **11**(1): p. 5319.
28. Fernández-Friera, L., et al., *Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical Atherosclerosis in a Middle-Aged Cohort.* Circulation, 2015. **131**(24): p. 2104-2113.
29. Hwang, J., et al., *Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion.* J Biol Chem, 2003. **278**(47): p. 47291-8.
30. Tamargo, I.A., et al., *Flow-induced reprogramming of endothelial cells in atherosclerosis.* Nature Reviews Cardiology, 2023. **20**(11): p. 738-753.
31. Dimmeler, S., et al., *Fluid Shear Stress Stimulates Phosphorylation of Akt in Human Endothelial Cells.* Circulation Research, 1998. **83**(3): p. 334-341.
32. Wang, K.C., et al., *Flow-dependent YAP/TAZ activities regulate endothelial phenotypes and atherosclerosis.* Proc Natl Acad Sci U S A, 2016. **113**(41): p. 11525-11530.
33. Cunningham, R. and C.G. Hansen, *The Hippo pathway in cancer: YAP/TAZ and TEAD as therapeutic targets in cancer.* Clin Sci (Lond), 2022. **136**(3): p. 197-222.
34. Wang, L., et al., *Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow.* Nature, 2016. **540**(7634): p. 579-582.
35. Conway, C., et al., *Acute Stent-Induced Endothelial Denudation: Biomechanical Predictors of Vascular Injury.* Front Cardiovasc Med, 2021. **8**: p. 733605.
36. Modi, K., M.P. Soos, and K. Mahajan, *Stent Thrombosis*, in *StatPearls*. 2024, StatPearls Publishing Copyright © 2024, StatPearls Publishing LLC.: Treasure Island (FL).
37. Bharat, A., M. Jaenicke, and S. Shenoy, *A novel technique of vascular anastomosis to prevent juxta-anastomotic stenosis following arteriovenous fistula creation.* J Vasc Surg, 2012. **55**(1): p. 274-80.
38. Bai, H., et al., *Artery to vein configuration of arteriovenous fistula improves hemodynamics to increase maturation and patency.* Sci Transl Med, 2020. **12**(557).
39. Dillavou, E.D., et al., *VasQ U.S. pivotal study demonstrates the safety and effectiveness of an external vascular support for arteriovenous fistula creation.* J Vasc Surg, 2023. **78**(5): p. 1302-1312.e3.
40. Garoffolo, G. and M. Pesce, *Vascular dysfunction and pathology: focus on mechanical forces.* Vascular Biology, 2021. **3**(1): p. R69-R75.
41. Garoffolo, G., et al., *Coronary artery mechanics induces human saphenous vein remodelling via recruitment of adventitial myofibroblast-like cells mediated by Thrombospondin-1.* Theranostics, 2020. **10**(6): p. 2597-2611.
42. Chen, R., et al., *Phenotypic Switching of Vascular Smooth Muscle Cells in Atherosclerosis.* Journal of the American Heart Association, 2023. **12**(20): p. e031121.
43. Wadey, K., et al., *Role of smooth muscle cells in coronary artery bypass grafting failure.* Cardiovasc Res, 2018. **114**(4): p. 601-610.

44. Forbes, T., A.G. Pauza, and J.C. Adams, *In the balance: how do thrombospondins contribute to the cellular pathophysiology of cardiovascular disease?* American Journal of Physiology-Cell Physiology, 2021. **321**(5): p. C826-C845.
45. O'Callaghan, C.J. and B. Williams, *Mechanical strain-induced extracellular matrix production by human vascular smooth muscle cells: role of TGF-beta(1).* Hypertension, 2000. **36**(3): p. 319-24.
46. Stanley, A.G., et al., *Mechanical strain-induced human vascular matrix synthesis: the role of angiotensin II.* J Renin Angiotensin Aldosterone Syst, 2000. **1**(1): p. 32-5.
47. Predel, H.G., et al., *Implications of pulsatile stretch on growth of saphenous vein and mammary artery smooth muscle.* Lancet, 1992. **340**(8824): p. 878-9.
48. Goldstein, D.J., et al., *External Support for Saphenous Vein Grafts in Coronary Artery Bypass Surgery: A Randomized Clinical Trial.* JAMA Cardiol, 2022. **7**(8): p. 808-816.
49. Taggart, D.P., et al., *A prospective study of external stenting of saphenous vein grafts to the right coronary artery: the VEST II study.* Eur J Cardiothorac Surg, 2017. **51**(5): p. 952-958.
50. Taggart, D.P., et al., *Long-term performance of an external stent for saphenous vein grafts: the VEST IV trial.* J Cardiothorac Surg, 2018. **13**(1): p. 117.
51. Taggart, D.P., et al., *A Randomized Trial of External Stenting for Saphenous Vein Grafts in Coronary Artery Bypass Grafting.* Ann Thorac Surg, 2015. **99**(6): p. 2039-45.
52. Yang, Q., et al., *A novel biodegradable external stent regulates vein graft remodeling via the Hippo-YAP and mTOR signaling pathways.* Biomaterials, 2020. **258**: p. 120254.
53. Spaulding, C., et al., *Sirolimus-Eluting versus Uncoated Stents in Acute Myocardial Infarction.* New England Journal of Medicine, 2006. **355**(11): p. 1093-1104.
54. Lagerqvist, B., et al., *Long-Term Outcomes with Drug-Eluting Stents versus Bare-Metal Stents in Sweden.* New England Journal of Medicine, 2007. **356**(10): p. 1009-1019.
55. Finn, A.V., et al., *Vascular responses to drug eluting stents: importance of delayed healing.* Arterioscler Thromb Vasc Biol, 2007. **27**(7): p. 1500-10.
56. Wu, W., et al., *Mature Vascular Smooth Muscle Cells, but Not Endothelial Cells, Serve as the Major Cellular Source of Intimal Hyperplasia in Vein Grafts.* Arterioscler Thromb Vasc Biol, 2020. **40**(8): p. 1870-1890.
57. Lüscher, T.F., et al., *Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications.* Circulation, 2007. **115**(8): p. 1051-8.
58. Iakovou, I., et al., *Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents.* Jama, 2005. **293**(17): p. 2126-30.
59. Pelliccia, F., et al., *Endothelial Progenitor Cells in Coronary Artery Disease: From Bench to Bedside.* Stem Cells Transl Med, 2022. **11**(5): p. 451-460.
60. Pelliccia, F., et al., *Endothelial Progenitor Cells in Coronary Atherosclerosis and Percutaneous Coronary Intervention: A Systematic Review and Meta-Analysis.* Cardiovasc Revasc Med, 2022. **42**: p. 94-99.
61. Souza, D., *A new no-touch preparation technique. Technical notes.* Scand J Thorac Cardiovasc Surg, 1996. **30**(1): p. 41-4.
62. Tian, M., et al., *No-Touch Versus Conventional Vein Harvesting Techniques at 12 Months After Coronary Artery Bypass Grafting Surgery: Multicenter Randomized, Controlled Trial.* Circulation, 2021. **144**(14): p. 1120-1129.
63. Shen, J., et al., *Distribution and Dynamic Changes in Matrix Metalloproteinase (MMP)-2, MMP-9, and Collagen in an In Stent Restenosis Process.* Eur J Vasc Endovasc Surg, 2021. **61**(4): p. 648-655.
64. Shih, Y.C., et al., *MMP-9 Deletion Attenuates Arteriovenous Fistula Neointima through Reduced Perioperative Vascular Inflammation.* Int J Mol Sci, 2021. **22**(11).

65. Berceli, S.A., et al., *Differential expression and activity of matrix metalloproteinases during flow-modulated vein graft remodeling.* J Vasc Surg, 2004. **39**(5): p. 1084-90.
66. Lardenoye, J.H., et al., *Inhibition of intimal hyperplasia by the tetracycline derived mmp inhibitor doxycycline in vein graft disease in vitro and in vivo.* EuroIntervention, 2005. **1**(2): p. 236-43.
67. Rotmans, J.I., et al., *Matrix metalloproteinase inhibition reduces intimal hyperplasia in a porcine arteriovenous-graft model.* J Vasc Surg, 2004. **39**(2): p. 432-9.
68. George, S.J., et al., *Sustained reduction of vein graft neointima formation by ex vivo TIMP-3 gene therapy.* Circulation, 2011. **124**(11 Suppl): p. S135-42.
69. George, S.J., et al., *Inhibition of late vein graft neointima formation in human and porcine models by adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-3.* Circulation, 2000. **101**(3): p. 296-304.
70. de Vries, M.R., et al., *Plaque rupture complications in murine atherosclerotic vein grafts can be prevented by TIMP-1 overexpression.* PLoS One, 2012. **7**(10): p. e47134.
71. Zhu, Y., et al., *Vascular endothelial growth factor promotes proliferation of cortical neuron precursors by regulating E2F expression.* Faseb j, 2003. **17**(2): p. 186-93.
72. Kanai, M., et al., *Transcriptional regulation of human fibroblast growth factor receptor 1 by E2F-1.* Gene, 2009. **438**(1-2): p. 49-56.
73. Morishita, R., et al., *A gene therapy strategy using a transcription factor decoy of the E2F binding site inhibits smooth muscle proliferation in vivo.* Proc Natl Acad Sci U S A, 1995. **92**(13): p. 5855-9.
74. Ehsan, A., et al., *Long-term stabilization of vein graft wall architecture and prolonged resistance to experimental atherosclerosis after E2F decoy oligonucleotide gene therapy.* J Thorac Cardiovasc Surg, 2001. **121**(4): p. 714-22.
75. Mann, M.J., et al., *Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial.* Lancet, 1999. **354**(9189): p. 1493-8.
76. Grube, E., et al. *Phase II trial of the E2F decoy in coronary bypass grafting*. in *American Heart Association Annual Meeting (Late Breaking Clinical Trials), Anaheim, Calif*. 2001.
77. Conte, M.S., et al., *Results of PREVENT III: a multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery.* J Vasc Surg, 2006. **43**(4): p. 742-751; discussion 751.
78. Investigators*, P.I., *Efficacy and Safety of Edifoligide, an E2F Transcription Factor Decoy, for Prevention of Vein Graft Failure Following Coronary Artery Bypass Graft SurgeryPREVENT IV: A Randomized Controlled Trial.* JAMA, 2005. **294**(19): p. 2446-2454.
79. Yap, C., et al., *Six Shades of Vascular Smooth Muscle Cells Illuminated by KLF4 (Krüppel-Like Factor 4).* Arteriosclerosis, Thrombosis, and Vascular Biology, 2021. **41**(11): p. 2693-2707.
80. Gu, W., et al., *Single-Cell RNA-Sequencing and Metabolomics Analyses Reveal the Contribution of Perivascular Adipose Tissue Stem Cells to Vascular Remodeling.* Arterioscler Thromb Vasc Biol, 2019. **39**(10): p. 2049-2066.
81. Ridker, P.M., et al., *Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease.* N Engl J Med, 2017. **377**(12): p. 1119-1131.
82. Nidorf, S.M., et al., *Colchicine in Patients with Chronic Coronary Disease.* N Engl J Med, 2020. **383**(19): p. 1838-1847.
83. Cronstein, B.N., et al., *Colchicine alters the quantitative and qualitative display of selectins on endothelial cells and neutrophils.* J Clin Invest, 1995. **96**(2): p. 994-1002.
84. Karper, J.C., et al., *Toll-like receptor 4 is involved in human and mouse vein graft remodeling, and local gene silencing reduces vein graft disease in hypercholesterolemic APOE*3Leiden mice.* Arterioscler Thromb Vasc Biol, 2011. **31**(5): p. 1033-40.
85. Liu, T., et al., *NF-κB signaling in inflammation.* Signal Transduct Target Ther, 2017. **2**: p. 17023-.

86. de Vries, M.R. and P.H.A. Quax, *Inflammation in Vein Graft Disease.* Front Cardiovasc Med, 2018. **5**: p. 3.
87. Pires, N.M.M., et al., *Histopathologic alterations following local delivery of dexamethasone to inhibit restenosis in murine arteries.* Cardiovascular Research, 2005. **68**(3): p. 415-424.
88. Schepers, A., et al., *Short-term dexamethasone treatment inhibits vein graft thickening in hypercholesterolemic ApoE3Leiden transgenic mice.* J Vasc Surg, 2006. **43**(4): p. 809-15.
89. Ray, P.D., B.W. Huang, and Y. Tsuji, *Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling.* Cell Signal, 2012. **24**(5): p. 981-90.
90. Wang, Y., W. Luo, and Y. Wang, *PARP-1 and its associated nucleases in DNA damage response.* DNA Repair (Amst), 2019. **81**: p. 102651.
91. Ray Chaudhuri, A. and A. Nussenzweig, *The multifaceted roles of PARP1 in DNA repair and chromatin remodelling.* Nat Rev Mol Cell Biol, 2017. **18**(10): p. 610-621.
92. Wang, Y., et al., *A nuclease that mediates cell death induced by DNA damage and poly(ADP-ribose) polymerase-1.* Science, 2016. **354**(6308).
93. Vuong, B., et al., *NF-κB transcriptional activation by TNFα requires phospholipase C, extracellular signal-regulated kinase 2 and poly(ADP-ribose) polymerase-1.* J Neuroinflammation, 2015. **12**: p. 229.
94. Santinelli-Pestana, D.V., et al., *PARPs and ADP-Ribosylation in Chronic Inflammation: A Focus on Macrophages.* Pathogens, 2023. **12**(7).
95. von Lukowicz, T., et al., *PARP1 is required for adhesion molecule expression in atherogenesis.* Cardiovasc Res, 2008. **78**(1): p. 158-66.
96. Oumouna-Benachour, K., et al., *Poly(ADP-ribose) polymerase inhibition reduces atherosclerotic plaque size and promotes factors of plaque stability in apolipoprotein E-deficient mice: effects on macrophage recruitment, nuclear factor-kappaB nuclear translocation, and foam cell death.* Circulation, 2007. **115**(18): p. 2442-50.
97. Zhang, C., J. Yang, and L.K. Jennings, *Attenuation of neointima formation through the inhibition of DNA repair enzyme PARP-1 in balloon-injured rat carotid artery.* American Journal of Physiology-Heart and Circulatory Physiology, 2004. **287**(2): p. H659-H666.
98. Georgakis, M.K., et al., *Monocyte-Chemoattractant Protein-1 Levels in Human Atherosclerotic Lesions Associate With Plaque Vulnerability.* Arteriosclerosis, Thrombosis, and Vascular Biology, 2021. **41**(6): p. 2038-2048.
99. Boring, L., et al., *Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis.* Nature, 1998. **394**(6696): p. 894-7.
100. Gu, L., et al., *Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice.* Mol Cell, 1998. **2**(2): p. 275-81.
101. Liehn, E.A., et al., *A new monocyte chemotactic protein-1/chemokine CC motif ligand-2 competitor limiting neointima formation and myocardial ischemia/reperfusion injury in mice.* J Am Coll Cardiol, 2010. **56**(22): p. 1847-57.
102. Eefting, D., et al., *Local lentiviral short hairpin RNA silencing of CCR2 inhibits vein graft thickening in hypercholesterolemic apolipoprotein E3-Leiden mice.* J Vasc Surg, 2009. **50**(1): p. 152-60.
103. Schepers, A., et al., *Anti-MCP-1 gene therapy inhibits vascular smooth muscle cells proliferation and attenuates vein graft thickening both in vitro and in vivo.* Arterioscler Thromb Vasc Biol, 2006. **26**(9): p. 2063-9.
104. Colombo, A., et al., *A double-blind randomised study to evaluate the efficacy and safety of bindarit in preventing coronary stent restenosis.* EuroIntervention, 2016. **12**(11): p. e1385-e1394.
105. Wehlin, L., et al., *Peripheral blood monocyte activation during coronary artery bypass grafting with or without cardiopulmonary bypass.* Scand Cardiovasc J, 2005. **39**(1-2): p. 78-86.

106. de Winther, M.P.J., et al., *Translational opportunities of single-cell biology in atherosclerosis.* European Heart Journal, 2022. **44**(14): p. 1216-1230.
107. de Jong, A., et al., *Interfering in the ALK1 Pathway Results in Macrophage-Driven Outward Remodeling of Murine Vein Grafts.* Frontiers in Cardiovascular Medicine, 2022. **8**.
108. Willemsen, L. and M.P. de Winther, *Macrophage subsets in atherosclerosis as defined by single-cell technologies.* The Journal of Pathology, 2020. **250**(5): p. 705-714.
109. Depuydt, M.A.C., et al., *Microanatomy of the Human Atherosclerotic Plaque by Single-Cell Transcriptomics.* Circulation Research, 2020. **127**(11): p. 1437-1455.
110. Fernandez, D.M., et al., *Single-cell immune landscape of human atherosclerotic plaques.* Nature Medicine, 2019. **25**(10): p. 1576-1588.
111. Zernecke, A., et al., *Meta-Analysis of Leukocyte Diversity in Atherosclerotic Mouse Aortas.* Circ Res, 2020. **127**(3): p. 402-426.
112. Cochain, C., et al., *Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis.* Circulation Research, 2018. **122**(12): p. 1661-1674.
113. Park, I., et al., *C-type lectin receptor CLEC4A2 promotes tissue adaptation of macrophages and protects against atherosclerosis.* Nature Communications, 2022. **13**(1): p. 215.
114. Fu, H., et al., *The differential statin effect on cytokine production of monocytes or macrophages is mediated by differential geranylgeranylation-dependent Rac1 activation.* Cell Death & Disease, 2019. **10**(12): p. 880.
115. Ako, S., et al., *Statins Inhibit Inflammatory Cytokine Production by Macrophages and Acinar-to-Ductal Metaplasia of Pancreatic Cells.* Gastro Hep Adv, 2022. **1**(4): p. 640-651.
116. Hilgendorff, A., et al., *Statins differ in their ability to block NF-kappaB activation in human blood monocytes.* Int J Clin Pharmacol Ther, 2003. **41**(9): p. 397-401.
117. Flores-Gomez, D., et al., *Trained Immunity in Atherosclerotic Cardiovascular Disease.* Arterioscler Thromb Vasc Biol, 2021. **41**(1): p. 62-69.
118. Riksen, N.P., et al., *Trained immunity in atherosclerotic cardiovascular disease.* Nat Rev Cardiol, 2023. **20**(12): p. 799-811.
119. Dong, Z., et al., *Myocardial infarction drives trained immunity of monocytes, accelerating atherosclerosis.* European Heart Journal, 2023. **45**(9): p. 669-684.
120. Depuydt, M.A.C., et al., *Single-cell T cell receptor sequencing of paired human atherosclerotic plaques and blood reveals autoimmune-like features of expanded effector T cells.* Nature Cardiovascular Research, 2023. **2**(2): p. 112-125.
121. Zernecke, A., *Dendritic cells in atherosclerosis: evidence in mice and humans.* Arterioscler Thromb Vasc Biol, 2015. **35**(4): p. 763-70.
122. Roy, P., M. Orecchioni, and K. Ley, *How the immune system shapes atherosclerosis: roles of innate and adaptive immunity.* Nature Reviews Immunology, 2022. **22**(4): p. 251-265.
123. Döring, Y., et al., *Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis.* Circulation, 2012. **125**(13): p. 1673-83.
124. Weber, C., et al., *CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice.* J Clin Invest, 2011. **121**(7): p. 2898-910.
125. Ozmen, J., Y.V. Bobryshev, and R.S. Lord, *CD40 co-stimulatory molecule expression by dendritic cells in primary atherosclerotic lesions in carotid arteries and in stenotic saphenous vein coronary artery grafts.* Cardiovasc Surg, 2001. **9**(4): p. 329-33.
126. Yan, J.C., et al., *Relationship between upregulation of CD40 system and restenosis in patients after percutaneous coronary intervention.* Acta Pharmacol Sin, 2007. **28**(3): p. 339-43.

127. Türker, S., et al., *Usefulness of preprocedural soluble CD40 ligand for predicting restenosis after percutaneous coronary intervention in patients with stable coronary artery disease.* Am J Cardiol, 2006. **97**(2): p. 198-202.
128. Lacy, M., et al., *Cell-specific and divergent roles of the CD40L-CD40 axis in atherosclerotic vascular disease.* Nature Communications, 2021. **12**(1): p. 3754.
129. Olofsson, P.S., et al., *CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice.* Circulation, 2008. **117**(10): p. 1292-301.
130. Jeon, H.J., et al., *CD137 (4-1BB) deficiency reduces atherosclerosis in hyperlipidemic mice.* Circulation, 2010. **121**(9): p. 1124-33.
131. Ewing, M.M., et al., *T-cell co-stimulation by CD28-CD80/86 and its negative regulator CTLA-4 strongly influence accelerated atherosclerosis development.* Int J Cardiol, 2013. **168**(3): p. 1965-74.
132. Alegre, M.-L., K.A. Frauwirth, and C.B. Thompson, *T-cell regulation by CD28 and CTLA-4.* Nature Reviews Immunology, 2001. **1**(3): p. 220-228.
133. Chen, L. and D.B. Flies, *Molecular mechanisms of T cell co-stimulation and co-inhibition.* Nature Reviews Immunology, 2013. **13**(4): p. 227-242.
134. Drobni, Z.D., et al., *Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque.* Circulation, 2020. **142**(24): p. 2299-2311.
135. Poels, K., et al., *Immune Checkpoint Inhibitor Therapy Aggravates T Cell-Driven Plaque Inflammation in Atherosclerosis.* JACC CardioOncol, 2020. **2**(4): p. 599-610.
136. Matsubara, Y., et al., *PD-L1 (Programmed Death Ligand 1) Regulates T-Cell Differentiation to Control Adaptive Venous Remodeling.* Arterioscler Thromb Vasc Biol, 2021. **41**(12): p. 2909-2922.
137. Eltzschig, H.K. and P. Carmeliet, *Hypoxia and inflammation.* N Engl J Med, 2011. **364**(7): p. 656-65.
138. Jaakkola, P., et al., *Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation.* Science, 2001. **292**(5516): p. 468-72.
139. Wang, G.L., et al., *Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension.* Proc Natl Acad Sci U S A, 1995. **92**(12): p. 5510-4.
140. van Kuijk, K., et al., *Deficiency of myeloid PHD proteins aggravates atherogenesis via macrophage apoptosis and paracrine fibrotic signalling.* Cardiovasc Res, 2022. **118**(5): p. 1232-1246.
141. de Vries, M.R., et al., *Blockade of vascular endothelial growth factor receptor 2 inhibits intraplaque haemorrhage by normalization of plaque neovessels.* J Intern Med, 2019. **285**(1): p. 59-74.
142. Sluimer, J.C., et al., *Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis.* J Am Coll Cardiol, 2008. **51**(13): p. 1258-65.
143. Sarabipour, S., K. Ballmer-Hofer, and K. Hristova, *VEGFR-2 conformational switch in response to ligand binding.* Elife, 2016. **5**: p. e13876.
144. Apte, R.S., D.S. Chen, and N. Ferrara, *VEGF in Signaling and Disease: Beyond Discovery and Development.* Cell, 2019. **176**(6): p. 1248-1264.
145. Liu, Z.-L., et al., *Angiogenic signaling pathways and anti-angiogenic therapy for cancer.* Signal Transduction and Targeted Therapy, 2023. **8**(1): p. 198.
146. Ferrara, N., *VEGF and Intraocular Neovascularization: From Discovery to Therapy.* Transl Vis Sci Technol, 2016. **5**(2): p. 10.
147. Parma, L., et al., *bFGF blockade reduces intraplaque angiogenesis and macrophage infiltration in atherosclerotic vein graft lesions in ApoE3*Leiden mice.* Sci Rep, 2020. **10**(1): p. 15968.

148. Sedding, D.G., et al., *Vasa Vasorum Angiogenesis: Key Player in the Initiation and Progression of Atherosclerosis and Potential Target for the Treatment of Cardiovascular Disease.* Front Immunol, 2018. **9**: p. 706.
149. Chang, L., M.T. Garcia-Barrio, and Y.E. Chen, *Perivascular Adipose Tissue Regulates Vascular Function by Targeting Vascular Smooth Muscle Cells.* Arteriosclerosis, Thrombosis, and Vascular Biology, 2020. **40**(5): p. 1094-1109.
150. Nosalski, R. and T.J. Guzik, *Perivascular adipose tissue inflammation in vascular disease.* British Journal of Pharmacology, 2017. **174**(20): p. 3496-3513.
151. Adachi, Y., K. Ueda, and E. Takimoto, *Perivascular adipose tissue in vascular pathologies-a novel therapeutic target for atherosclerotic disease?* Front Cardiovasc Med, 2023. **10**: p. 1151717.
152. Manka, D., et al., *Transplanted Perivascular Adipose Tissue Accelerates Injury-Induced Neointimal Hyperplasia.* Arteriosclerosis, Thrombosis, and Vascular Biology, 2014. **34**(8): p. 1723-1730.
153. Hildebrand, S., J. Stümer, and A. Pfeifer, *PVAT and Its Relation to Brown, Beige, and White Adipose Tissue in Development and Function.* Front Physiol, 2018. **9**: p. 70.
154. Chatterjee, T.K., et al., *Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding.* Circ Res, 2009. **104**(4): p. 541-9.
155. Ying, R., et al., *Endoplasmic reticulum stress in perivascular adipose tissue promotes destabilization of atherosclerotic plaque by regulating GM-CSF paracrine.* J Transl Med, 2018. **16**(1): p. 105.
156. Mitchell, J.R., et al., *Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice.* Aging Cell, 2010. **9**(1): p. 40-53.
157. Mauro, C.R., et al., *Preoperative dietary restriction reduces intimal hyperplasia and protects from ischemia-reperfusion injury.* J Vasc Surg, 2016. **63**(2): p. 500-9.e1.
158. Trocha, K.M., et al., *Short-term preoperative protein restriction attenuates vein graft disease via induction of cystathionine γ-lyase.* Cardiovasc Res, 2020. **116**(2): p. 416-428.
159. Hine, C., et al., *Endogenous hydrogen sulfide production is essential for dietary restriction benefits.* Cell, 2015. **160**(1-2): p. 132-44.
160. Hine, C. and J.R. Mitchell, *Calorie restriction and methionine restriction in control of endogenous hydrogen sulfide production by the transsulfuration pathway.* Exp Gerontol, 2015. **68**: p. 26-32.
161. Plaisance, E.P., et al., *Dietary Methionine Restriction Increases Fat Oxidation in Obese Adults with Metabolic Syndrome.* The Journal of Clinical Endocrinology & Metabolism, 2011. **96**(5): p. E836-E840.
162. Hasek, B.E., et al., *Remodeling the integration of lipid metabolism between liver and adipose tissue by dietary methionine restriction in rats.* Diabetes, 2013. **62**(10): p. 3362-72.
163. Patil, Y.N., et al., *Cellular and molecular remodeling of inguinal adipose tissue mitochondria by dietary methionine restriction.* J Nutr Biochem, 2015. **26**(11): p. 1235-47.
164. Takao, K. and T. Miyakawa, *Genomic responses in mouse models greatly mimic human inflammatory diseases.* Proc Natl Acad Sci U S A, 2015. **112**(4): p. 1167-72.
165. Lardenoye, J.H., et al., *Accelerated atherosclerosis by placement of a perivascular cuff and a cholesterol-rich diet in ApoE*3Leiden transgenic mice.* Circ Res, 2000. **87**(3): p. 248-53.
166. Tao, M., et al., *A simplified murine intimal hyperplasia model founded on a focal carotid stenosis.* Am J Pathol, 2013. **182**(1): p. 277-87.
167. Le, V., et al., *Murine model of femoral artery wire injury with implantation of a perivascular drug delivery patch.* J Vis Exp, 2015(96): p. e52403.
168. von der Thüsen, J.H., T.J. van Berkel, and E.A. Biessen, *Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice.* Circulation, 2001. **103**(8): p. 1164-70.

169. Yu, P., et al., *Rationale and practical techniques for mouse models of early vein graft adaptations.* J Vasc Surg, 2010. **52**(2): p. 444-52.
170. Wong, C.Y., et al., *Vascular remodeling and intimal hyperplasia in a novel murine model of arteriovenous fistula failure.* J Vasc Surg, 2014. **59**(1): p. 192-201.e1.
171. Yamamoto, K., et al., *The mouse aortocaval fistula recapitulates human arteriovenous fistula maturation.* American Journal of Physiology-Heart and Circulatory Physiology, 2013. **305**(12): p. H1718-H1725.