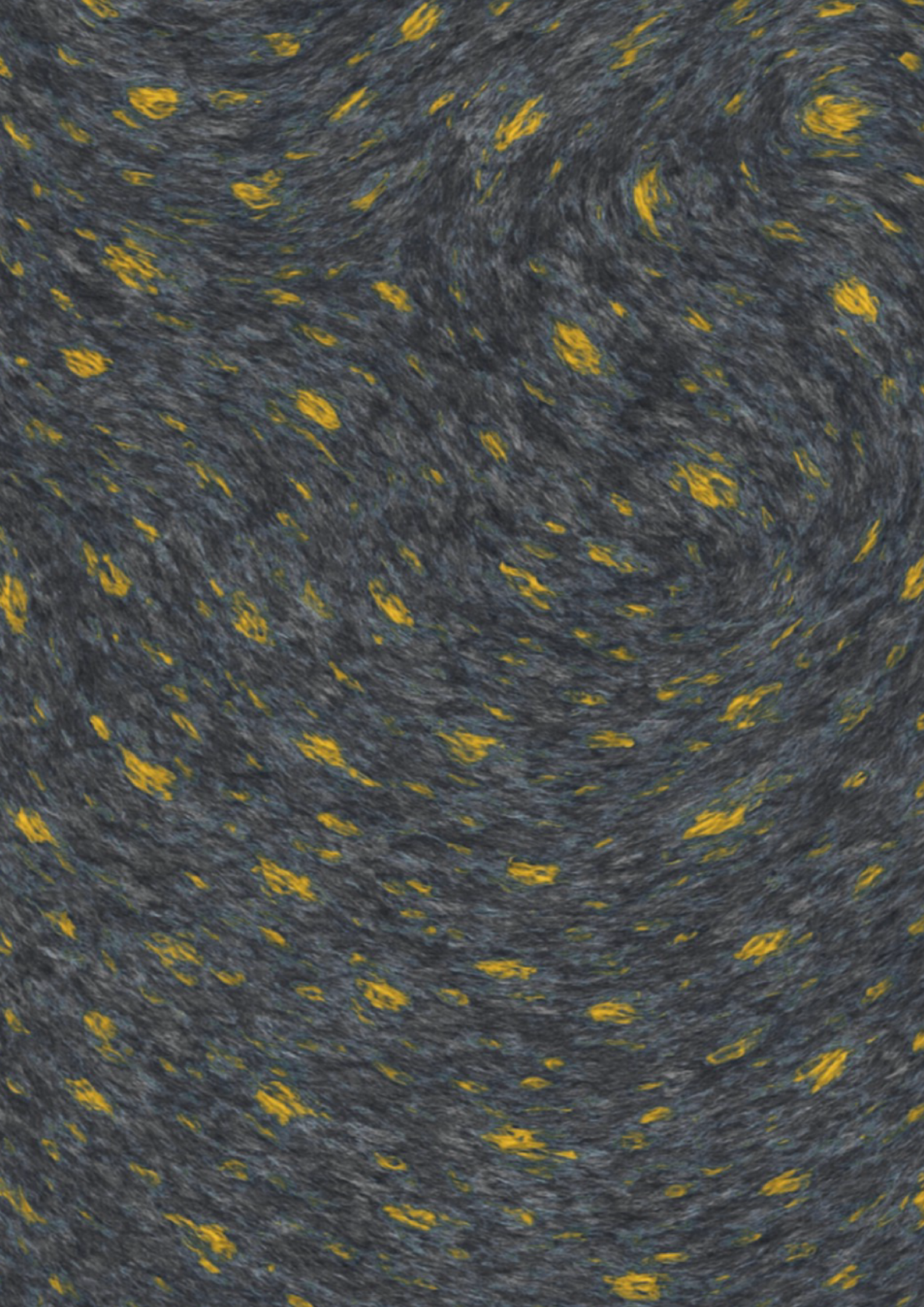
Shedding a light on vascular remodeling

Sier, V.Q.



Chapter I | General introduction

Preface

Whether facing a prolonged seated position at the office, a rise in circulating glucose and lipids after a fast-food meal, or a spike in pressure during intense exercise, blood vessels are designed to provide consistent and adequate tissue perfusion.[1-3] This is achieved through a vast and hierarchically organized vascular network, where arteries and arterioles transport oxygen-rich blood to tissues, capillaries mediate the exchange of gases and solutes, and veins return deoxygenated blood to the heart and lungs. A key feature of hemodynamic regulation is the vessel wall's fascinating ability to adapt to its environmental demands. Over time, however, this adaptive capacity declines under the cumulative burden of mechanical, metabolic, and inflammatory stressors, which may lead to maladaptive remodeling. As a consequence, the arteries of individuals with cardiovascular risk factors are particularly vulnerable to progressive luminal narrowing, a process that can ultimately lead to ischemia of the heart, brain, or limbs, and affects up to 1.5 million people in the Netherlands alone.[4-6]

Before delving into the complexities of vascular remodeling explored throughout this thesis, it is important to introduce three core elements: (i) the historical and biological foundations of the process, (ii) relevant and representative disease models, and (iii) novel analytical tools with translational potential and clinical relevance. These elements will be examined in the following sections as a prelude to the individual chapters.

i.) Long before the formal discovery of vascular remodeling and disease, the value of studying the heart and blood vessels had already been recognized by ancient humans. Often rooted in spiritual and symbolic interpretations, early understandings of the cardiovascular system gradually gave way to more anatomical and physiological perspectives. This shift laid the groundwork for a growing interest in the mechanisms underlying cardiovascular disease and, ultimately, the development of early surgical interventions. For instance, bypass surgeries using vein grafts proved life- and limb-saving, yet it appeared that their long-term patency remained suboptimal. Over the years, the biological processes underlying vascular remodeling and the progression toward advanced disease appeared increasingly complex, inspiring clinicians to work together with basic scientists to further their understanding and ultimately improve patient outcomes.

Biologically, vascular remodeling is a fundamental response of blood vessels to alterations in their environment. This process consists of structural and cellular adaptations of the vessel wall following fluctuations in blood flow and mechanical stress to accommodate new functional demands.[7] However, dysregulated vascular remodeling can lead to excessive wall thickening and luminal narrowing that ultimately compromise downstream tissue perfusion. Central to these pathological alterations of the vessel wall architecture are the disruption of coordinated cell responses, triggered by a combination of injury, inflammation, and hypoxia.[8,9]

Adverse vascular remodeling plays a critical role in atherosclerosis, where native arteries or surgically constructed bypasses occlude due to plaque formation.[8,10] Saphenous vein grafts, which are commonly used to bypass stenotic coronary and peripheral arteries, are particularly vulnerable to accelerated forms of adverse remodeling and atherosclerosis, resulting in poor long-term patency.[11-13] Mechanistically, upon exposure to the high-pressure arterial environment, vein grafts are subjected to abrupt hemodynamic shifts and mechanical strain, triggering a cascade of vascular injury.[14] In this early phase, endothelial denudation promotes platelet adhesion, thrombus formation, and infiltration of leukocytes.[15] As the graft arterializes to accommodate its new environment, vascular smooth muscle cells (VSMCs) undergo phenotypic switching, proliferate, and migrate into the intimal layer, forming a neointima that initially provides structural support.[8] However, this response frequently becomes excessive, resulting in adverse inward remodeling and graft disease, characterized by persistent intimal thickening, extracellular matrix deposition, and the establishment of a pro-atherogenic milieu.[16] Within this environment, increased lipid uptake by macrophages leads to foam cell formation, followed by macrophage apoptosis and the development of a necrotic core. Furthermore, hypoxia resulting from excessive wall thickening and inflammation promotes the ingrowth of immature neovessels and subsequent intraplaque hemorrhage, which contributes to further long-term vein graft failure.[17,18] Ironically, plaque development in these vein grafts closely mirrors advanced arterial atherosclerosis; the very disease they were originally intended to circumvent.

ii.) Accelerated vein graft atherosclerosis can be studied using a mouse interposition model, where a segment of donor vena cava is grafted into the right common carotid artery of a recipient mouse.[19,20] Traditional histological analyses have shown that this model reliably develops advanced, human-like atherosclerotic lesions under hypercholesterolemic

conditions, with key features such as the ingrowth of immature plaque neovessels.[21] Furthermore, when vein graft surgery is paired with hypercholesterolemia induced by knock-in of the defective human ApoE3 gene (ApoE3*Leiden),[22,23] the model offers an additional advantage: mice retain functional cholesterol metabolism, enabling pharmacological modulation of low-density lipoprotein (LDL) levels with therapies such as statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.[22] Given these features, this model is well suited not only for studying vein graft failure but also for investigating advanced mechanisms of atherosclerosis relevant to human disease.

iii.) Advancements in analytical technologies have enabled researchers and clinicians to monitor disease processes at an increasingly granular level, offering insights that complement or may even replace traditional methods. In the context of atherosclerosis, imaging has long played a central role in patient evaluation, particularly for assessing luminal narrowing and plaque burden. However, current imaging modalities often lack the spatial or temporal resolution required to capture dynamic disease mechanisms at or near the cellular level. Beyond technical capability, the utility of advanced imaging relies on clinical or scientific relevance: it should serve the purpose of visualizing biologically meaningful targets, related to disease progression or stabilization. To this end, large-scale human population datasets are increasingly used to identify drivers of disease, a process accelerated by advances in molecular profiling, computing power, and data integration.

Novel imaging techniques such as combined ultrasound-optoacoustics offer the potential for live and high-resolution assessment of vascular remodeling over time in both the pre-clinical and clinical setting. Combining the strengths of sound and light, this hybrid approach integrates high-frequency ultrasound for three-dimensional structural imaging with optoacoustics for compositional analysis.[24] More specifically, ultrasound employs piezoelectric elements to generate and receive acoustic waves, enabling real-time, high-resolution imaging of anatomical features of the vessel wall and lumen, with spatial resolution in the micrometer range. Optoacoustic imaging expands upon these capabilities by providing molecular contrast based on the principle of thermoelastic expansion: nanosecond laser pulses at defined wavelengths are absorbed by endogenous tissue components such as hemoglobin, lipids, or collagen, resulting in rapid localized heating and thermoelastic expansion, which in turn generates ultrasound signals. Using spectral unmixing algorithms, user-defined optoacoustic signatures relevant to vascular remodeling

can be separated, identified, and quantified within developing atherosclerotic lesions. Molecular specificity of optoacoustics can be further enhanced through the use of selective exogenous contrast agents, such as those directed against specific targets of endothelial activation.[25]

With the advent of large-scale bioinformatics and high-throughput technologies, omics approaches have become a cornerstone in identifying novel targets in complex diseases such as atherosclerosis.[26-28] These platforms enable the systematic and unbiased interrogation of biological layers, including genomic variants that confer susceptibility (genomics), ribonucleic acid (RNA) transcripts (transcriptomics), and alterations in protein levels (proteomics). When applied in the context of large (human) cohorts, these technologies enable the discovery of candidate genes, proteins, or pathways, which may be further strengthened through integrative analysis across multiple datasets and methods. Following their identification, candidate targets or pathways of interest can be functionally validated in the preclinical setting, assessing their mechanistic role and disease-specific relevance through combined in vitro and in vivo experiments. Together, this approach forms a translational framework for the prioritization of potential clinical therapeutic targets.

Outline of this thesis

With the aim of grasping its many facets, this thesis sheds a multidisciplinary light on vascular remodeling by exploring its historical foundations, integrating state-of-the-art imaging technologies, examining mechanistic pathways and drug efficacy in preclinical models, and identifying potential therapeutic targets tailored to specific patient populations.

Chapter II introduces the historical foundations of cardiovascular functioning and disease, outlining how early symbolic interpretations gave way to scientific understanding, the recognition of atherosclerosis as a multifactorial disease, and the development of modern surgical revascularization techniques.

Chapters III, IV, and V provide mechanistic insights into the development of vein graft atherosclerosis, with particular focus on transforming growth factor-β (TGF-β) signaling. More specifically, these chapters describe how specific members of the TGF-β superfamily affect vessel wall remodeling at the structural, compositional, and cellular level, as well as reviewing how these can be used to monitor disease development.

Chapters VI and VII introduce novel imaging concepts for the visualization of vascular remodeling, respectively validating the use of three-dimensional ultra-high-frequency ultrasound for structural follow-up studies and discussing the development of cell-based theranostic approaches.

The concluding two chapters build on previously established preclinical pipelines and introduce large-scale omics to translate experimental insights into clinically relevant mechanisms and potential therapeutics. In **Chapter VIII**, an integrative translational approach is used to examine the cholesterol-independent effects of PCSK9 inhibition. Using a clinically approved inhibitor in preclinical animal and in vitro models, the study identifies a previously unrecognized role in modulating inflammation, neovascularization, and plaque stability, supported by large-scale human population data. Finally, **Chapter IX** identifies sex-specific and shared protein signatures associated with incident coronary artery disease using large-scale human population data. Through the integration of plasma proteomics, cross-cohort validation, and supporting evidence for causality, novel sex-specific candidate targets are proposed to address adverse vascular remodeling.

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