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Shedding a light on vascular remodeling

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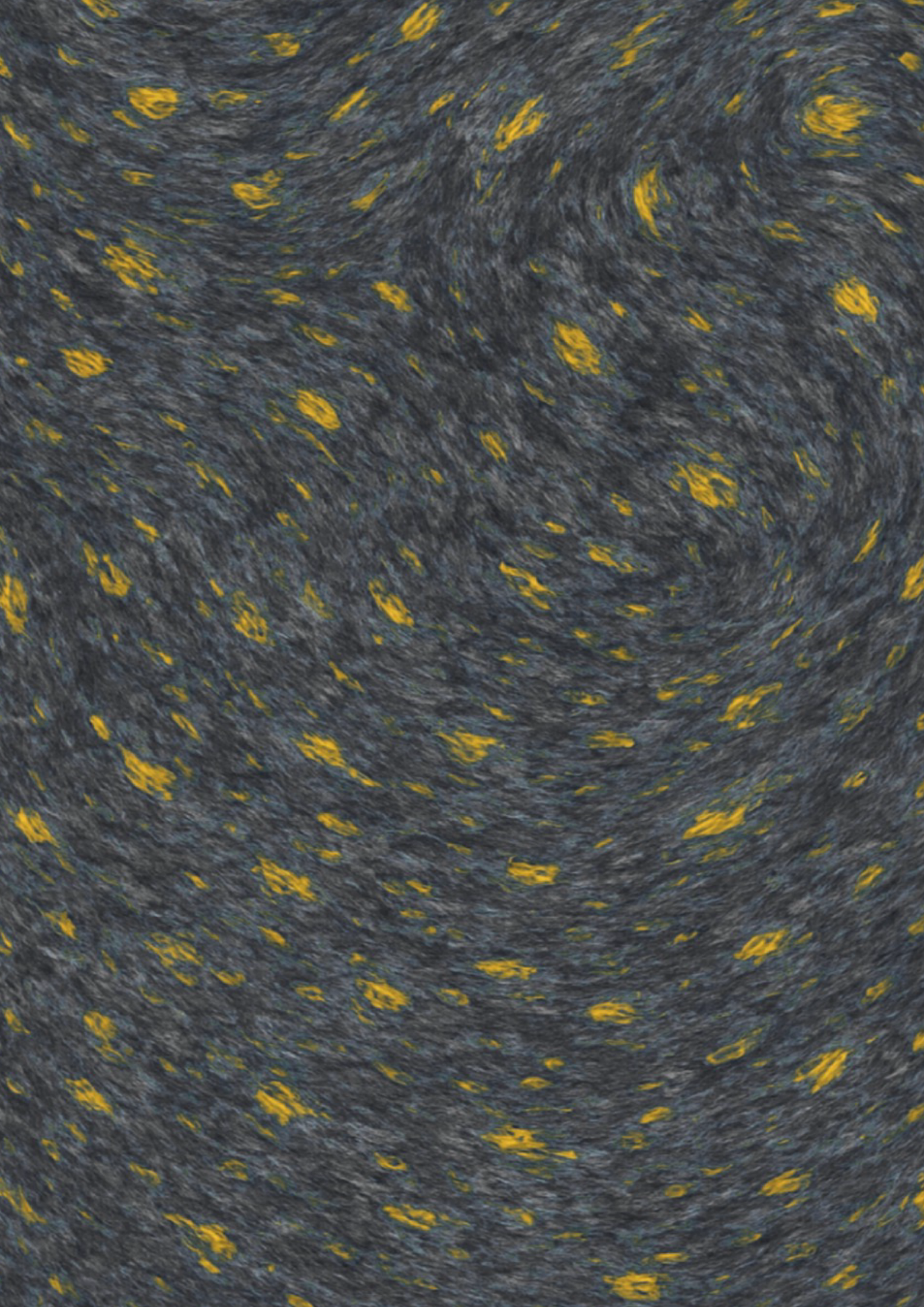

Chapter X | General summary & Future perspectives

General summary

Vascular remodeling refers to the structural and functional adaptation of blood vessels in response to physiological or pathological stimuli. Although this process is essential in maintaining homeostasis, maladaptive remodeling contributes directly to the initiation and progression of atherosclerosis. This chronic, occlusive disease compromises arterial patency and leads to reduced tissue perfusion, ultimately resulting in ischemic events such as myocardial infarction or critical limb ischemia. In clinical practice, atherosclerosis affects not only native coronary and peripheral arteries but also undermines the success of surgical revascularization procedures intended to restore blood flow to affected tissues. Particularly in patients undergoing coronary or peripheral bypass surgery by means of autologous vein grafts, long-term patency is often compromised by an accelerated form of atherosclerosis. Vein graft disease (VGD), mirroring advanced coronary atherosclerosis, is characterized by the development of a necrotic core, a persistent pro-inflammatory environment, and the ingrowth of immature neovessels, which collectively promote plaque destabilization and luminal occlusion. Despite major advancements in medical therapy and surgical revascularization techniques, a significant unmet clinical need persists in identifying, monitoring, and therapeutically targeting the biological mechanisms that drive advanced atherosclerosis, particularly in patients with complex occlusive disease requiring bypass surgery.

The aim of this thesis is to *shed light* on which mechanisms underlying vascular remodeling can be modulated to improve outcomes in advanced atherosclerosis, with a focus on novel strategies for disease monitoring, therapeutic modulation of disease-driving processes, and the identification of potential patient-specific targets. This objective is pursued through an integrative approach that combines mechanistic studies with translational animal models and large-scale human population data.

Chapter II examines the historical origins of our understanding of atherosclerosis, providing a chronological foundation for its discovery until the development and standardization of cardiovascular bypass grafting. Long before the term existed, calcified vascular lesions consistent with advanced atherosclerosis were already present in preindustrial populations, as demonstrated by computed tomography of mummified remains from ancient Egypt, Peru, and other early civilizations. However, a conceptual

understanding of the disease only emerged following centuries of anatomical discovery. Initially viewed through symbolic and spiritual lenses, the heart and vessels were gradually redefined through empirical investigation by Greek, Roman, and Islamic scholars. Early misconceptions, such as the separation of arterial and venous systems and the belief in invisible interventricular pores, persisted until corrected by anatomical dissections and comparative studies during the Renaissance.

As anatomy gave way to pathology, clinical observations of angina, claudication, and arterial stiffening in the 18th and 19th centuries set the stage for defining atherosclerosis as a chronic, multifactorial condition. The identification of lipid-laden plaques and inflammatory cells within the vascular wall supported a growing understanding of the mechanisms behind arterial occlusion. These insights coincided with the development of vascular surgical techniques, including end-to-end anastomosis and bypass grafting. The pioneering work of surgeons such as Kunlin, Carrel, and Goetz transformed atherosclerosis from a disease observed at autopsy to one that could be actively bypassed. In doing so, they laid the clinical and conceptual groundwork for modern revascularization strategies aimed at restoring perfusion in patients with advanced atherosclerotic disease.

In **Chapter III** the potential of endoglin-based molecular imaging is systematically explored as a strategy to monitor the development of cancer and cardiovascular disease. Endoglin, or CD105, is a transmembrane glycoprotein that serves as a coreceptor for ligands of the transforming growth factor beta (TGF-β) family and modulates signaling through ALK1 and ALK5 pathways in endothelial cells. It is highly expressed at sites of angiogenesis and inflammation, making it a relevant target for detecting active vascular remodeling. Across a variety of imaging platforms, including positron emission tomography, magnetic resonance imaging, near-infrared fluorescence, and ultrasound, preclinical studies consistently demonstrated the feasibility of detecting endoglin-positive neovessels in models of tumor growth, myocardial infarction, hindlimb ischemia, and aortic aneurysm. While most studies were performed in cancer models, where endoglin expression is prominent in tumor vasculature and sometimes in malignant or stromal cells, selected cardiovascular models confirmed its feasibility for imaging disease-associated neovascularization. The reviewed evidence shows that endoglin-targeted tracers can identify regions of endothelial activation and intralesional angiogenesis, offering a potential

means to assess disease progression and therapeutic effects in vivo. While clinical translation is still in early stages, the technical feasibility across animal models and tracer platforms supports the further development of endoglin-based imaging as a tool for studying and managing diseases characterized by abnormal vascular remodeling such as advanced atherosclerosis.

Building on the identification of endoglin as an imaging biomarker for vascular remodeling, **Chapters IV** and **V** explore the role of TGF-β signaling in this process, specifically focusing on the type I receptors known as activin receptor-like kinases (ALKs). First using both genetic and pharmacological approaches in murine vein graft models, we demonstrated that inhibition of ALK1 consistently leads to enhanced outward remodeling, but at the expense of increased vascular inflammation, characterized by augmented macrophage infiltration and elevated pro-inflammatory cytokines such as IL-6 and MCP-1. Although plaque angiogenesis was unaffected by ALK1 blockade, structural instability increased, reflected by reduced smooth muscle cell content and collagen deposition, resulting in more frequent plaque dissections. Next, the differential contributions of ALK1 and ALK5 to vein graft remodeling were characterized using murine models of vein graft implantation and carotid artery injury, alongside analyses of human saphenous vein samples obtained from coronary artery bypass graft (CABG) patients. In these studies, TGF-β signaling through ALK5 was associated with promotion of smooth muscle cell contractility and fibrotic remodeling, while ALK1 activation drove distinct transcriptional programs linked to smooth muscle cell migration and neointima formation. Single-cell RNA sequencing of human saphenous vein smooth muscle cells exposed to TGF-β revealed separate subpopulations with dominant ALK1 or ALK5 signaling profiles, suggesting divergent cellular phenotypes contribute to remodeling outcomes. Furthermore, pharmacological inhibition of ALK1/2-mediated Smad1/5 phosphorylation in vivo reduced neointimal thickening in both vein graft and arterial injury models, confirming the role of this pathway in pathological vascular remodeling. Collectively these data indicate that while ALK5 promotes a contractile and fibrotic smooth muscle cell phenotype, ALK1 signaling facilitates a migratory, synthetic phenotype associated with neointima formation and vascular inflammation. Therapeutic strategies that modulate TGF-β receptor signaling may improve vein graft durability and limit adverse remodeling, yet due to its diverse functions

and complex interrelationships, achieving a careful balance in selectively targeting ALK receptors is essential to avoid adverse effects through unintended signaling cascades.

To allow for more accurate monitoring of vascular remodeling over time, **Chapter VI** introduces the use of ultra-high-frequency ultrasound imaging for three-dimensional follow-up of murine vein graft and arteriovenous fistula (AVF) models. We showed that this imaging approach allows for precise, non-invasive quantification of structural changes such as lumen, wall, and total vessel volumes over time, alongside site-specific hemodynamic parameters including velocity indices and flow acceleration. Using both wildtype C57BL/6J mice and atherosclerosis-prone ApoE3*Leiden mice, we were able to demonstrate the sensitivity of our imaging approach to capture distinct remodeling patterns in vivo: ApoE3*Leiden vein grafts showed pronounced wall thickening and outward remodeling, whereas C57BL/6J grafts maintained stable wall dimensions with modest lumen expansion. Hemodynamic analyses identified dynamic changes in velocity indices, primarily localized proximally in the graft, reflecting dynamic wall stiffness conditions that influence remodeling. In the AVF model, ultrasound-derived blood flow rates established a novel maturation threshold, differentiating functional from non-functional fistulas. Ultra-high-frequency ultrasound imaging was established for the structural and functional assessment of vascular remodeling in vivo, providing a powerful tool to investigate the interplay between hemodynamics and vessel wall adaptation with potential applications for preclinical therapeutic validation.

Building on the advanced imaging techniques introduced in the previous chapter, **Chapter VII** expands beyond the possibilities of current imaging technologies by exploring the use of living cells and cell-derived agents as active tracers, akin to the concept of Odysseus's *Trojan horse*. Unlike conventional tracers such as antibodies and peptides that passively target disease sites, these cell-based tracers, including monocytes and macrophages, neutrophils, T cells, natural killer cells, platelets, and mesenchymal stromal cells, offer the ability to actively home to inflammatory sites including cardiovascular disease. The chapter further explores microorganisms, as well as extracellular vesicles and synthetic particles. Nanoparticles in particular are highlighted as engineered tools with highly customizable features for imaging. Together, each class of tracers presents unique

advantages and challenges, but their continued development holds promise for advancing future (cardiovascular) disease monitoring and image-guided surgical interventions.

The final two chapters focus on vascular remodeling from a translational angle, by bridging findings from our experimental and imaging models with large-scale human population data, and providing evidence for future patient-specific therapeutic targets. **Chapter VIII** investigates the lipid-lowering-independent effects of clinically available proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors on vein graft disease. Using a unique cholesterol-modulation-sensitive murine vein graft model alongside (endoglin-based) ultrasound-optoacoustic imaging, this work demonstrates that PCSK9 inhibition promotes plaque stability by enhancing the maturation of intraplaque neovessels. This maturation is reflected in increased pericyte coverage of these vessels, reducing intraplaque hemorrhage. Additionally, PCSK9 inhibitors decrease expression of adhesion molecules such as VCAM1 on neovessels, resulting in reduced infiltration of inflammatory leukocytes, including T cells. In vitro assays further support these findings by showing that PCSK9 inhibition stimulates endothelial cell migration and neovessel sprouting. Complementary proteomic analyses from a large human population cohort confirm that PCSK9 influences pathways beyond cholesterol metabolism, including angiogenesis, inflammation, and leukocyte adhesion to the vessel wall. Together, these data position PCSK9 inhibition as a promising modulator of vascular remodeling that stabilizes vein graft atherosclerosis, supporting a potential clinical role to improve long-term patency rates in bypass surgery.

Chapter IX leverages large-scale human population data to identify novel sex-specific plasma proteomic associations with incident coronary artery disease. Analysis of over 40,000 individuals without baseline disease revealed hundreds of proteins uniquely linked to disease risk in men and women. Integrating these findings with Mendelian randomization meta-analyses involving more than one million participants provided evidence supporting potential causal roles for multiple proteins. Validation in independent cohorts further confirmed the biological relevance of top candidates. Distinct biological pathways emerged between sexes, with angiogenesis and growth factor signaling more prominent in men, and innate immunity and regulation of programmed cell death in women. Machine learning-based druggability assessment highlighted promising targets such as CTSH in men and CDKN2D in women, alongside shared candidates including TNFRSF4. These results

suggest sex-specific mechanisms in coronary artery disease and open avenues for future research, precision prevention, and treatment strategies.

Future perspectives

Understanding and overcoming maladaptive vascular remodeling remains essential to improving long-term outcomes for patients with (advanced) atherosclerotic cardiovascular disease. This thesis *sheds both a literal and figurative light* on this process by respectively introducing advanced imaging techniques and uncovering context-dependent mechanisms and patient-specific targets through integrated cellular, animal, and computational research.

Following from the approach taken in this thesis, the identification of clinically relevant mechanisms in vascular remodeling benefits from a translational research cycle that begins and ends with the patient in mind. Human population data can be used to uncover candidate mechanisms or targets (**Chapter IX**), which are subsequently validated in cellular or animal models, and ultimately re-evaluated through imaging or trial-based clinical follow-up. Fundamentally, this marks a shift from traditional hypothesis-driven research built around the familiar pathways of biology textbooks, toward unbiased data-driven discovery. Rather than starting with an idea, researchers are increasingly confronted with large tables of statistically robust associations of unknown biological relevance. Assuming the analyses have been performed correctly, the next challenge lies in interpreting these signals, deciding how they fit within existing pathophysiological frameworks, and selecting appropriate model systems for further testing. However, as datasets grow in complexity and scale, they become more open to multiple interpretations, as if researchers were each speaking their own language. Without deliberate efforts to build shared conceptual frameworks and stimulate collaboration, the field risks turning into a modern-day Tower of Babel, where the pursuit of biological meaning is fragmented and lost in translation. To safeguard against this, researchers of tomorrow must actively organize themselves in multidisciplinary teams, relying on close collaboration between basic, computational, and clinical fields.

Reflecting on the history of atherosclerosis, as outlined in **Chapter II**, it is unsurprising that clinical need and scientific discovery have shaped one another over time, together driving steady improvements in patient outcomes. A recent example is the results from the FAME 3 trial (NCT02100722), which compared fractional flow reserve-guided PCI using current-

generation drug-eluting stents to CABG in patients with three-vessel disease. When viewed alongside earlier studies like SYNTAX (NCT00114972), which enrolled patients with comparable risk profiles and anatomical complexity, both arms of FAME 3 showed numerically superior outcomes. While procedural advances and perioperative care likely contributed, the results also underscore the growing role of targeted medical therapy in supporting cardiovascular interventions. In this light, our findings in **Chapter VIII** provide experimental and population-based evidence that PCSK9 inhibition may reduce vein graft atherosclerosis, independent of its cholesterol-lowering effect. As clinically approved agents such as alirocumab and evolocumab, and newer siRNA-based compounds like inclisiran, are increasingly available, these insights raise the possibility of extending PCSK9-targeted therapy to patients undergoing surgical revascularization.

Regardless of any future developments in medical, surgical, or interventional strategies, monitoring of disease advancement will remain a cornerstone of cardiovascular medicine. As demonstrated in **Chapters III, IV, VII,** and **VIII**, increasingly refined imaging strategies enable the detection of structural, functional, and molecular features of vascular remodeling far beyond the granularity offered by current modalities. Techniques such as ultra-high-frequency ultrasound and targeted optoacoustic imaging already allow for real-time tracking of changes in vessel architecture and plaque composition in vivo, while cell-based and nanoparticle-enhanced tracers offer avenues to visualize or even modulate specific biological processes including inflammation, neovascularization, or matrix remodeling.

Advancements in imaging techniques do not only improve diagnostic precision but may also support the development of futuristic solutions to atherosclerosis. Among these are tissue-engineered vascular grafts as alternatives to autologous conduits. As outlined in **Chapter II**, early attempts at tissue-engineered vascular grafts date back to the 1980s and 1990s, with initial human applications already explored during that era. While challenges remain in matching the mechanical durability and biological performance of autologous grafts, ongoing innovations in scaffold design, biomaterials, and in vivo monitoring offer reasons for cautious optimism.

Whether or not cell-based imaging, tissue-engineered grafts, or even PCSK9-inhibitors will become standard tools in the diagnosis and treatment of vascular remodeling remains to be seen, yet their pursuit exemplifies how scientific progress often does not rely on single discoveries, but on the collective perseverance of those who dare to imagine beyond the status quo.