Summary

Coronary artery disease (CAD) is one of the main causes of morbidity and mortality worldwide.[1, 2] Animal models have increased our understanding of the development and treatment of CAD, but there is an ongoing need to further increase our understanding of the disease, especially in more complex situations, such as in a background of diabetes mellitus (DM).[3] Diabetic patients have a 2 to 6 fold higher risk to encounter adverse events associated with CAD than non-diabetic patients.[4] With an expected DM epidemic, a deeper understanding of the development and treatment of CAD is becoming increasingly important.[3] For this purpose, a swine model seems most representative. Moreover, swine can be rendered diabetic mimicking the human situation of multiple co-morbidities, develop atherosclerotic lesions at anatomical locations similar to humans [5-7] and allow for coronary stent-implantation as well as invasive assessment of the coronary vasculature.[8]

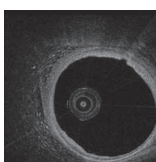
Compared to the traditionally used coronary angiography that solely provides a representation of the lumen, invasive imaging techniques such as optical coherence tomography (OCT) and near-infrared spectroscopy (NIRS) enable highly detailed imaging of the coronary morphology and the vascular response to stent-implantation.[9-11] This is important, as understanding the vascular response to stent-implantation can improve CAD treatment. Thorough evaluation of the vascular response to drug-eluting stent (DES) implantation increased our understanding of not only the advantages, but also the disadvantages of DES, such as permanent caging with or without malapposition, jailing of side branches and late stent thrombosis.[12, 13] Consequently, bioresorbable vascular scaffolds (BVS) have been developed in an attempt to overcome these limitations.[12]

This thesis evaluates the development of CAD in a background of diabetes mellitus and the treatment of CAD by BVS, using intracoronary imaging techniques.

Intracoronary imaging of coronary artery disease development

Part I describes the intravascular imaging techniques that can be used to assess coronary atherosclerosis and discusses the safety and methodological considerations of OCT.

A number of catheter-based intravascular imaging techniques have been developed to overcome one of the major limitations of coronary angiography, namely the direct assessment of specific morphological components in the coronary vessel wall.[9-11] In **Chapter 2** the abilities of these intravascular imaging techniques, including intravas-



cular ultrasound (IVUS), IVUS-VH, iMAP IVUS, integrated-backscatter-IVUS, OCT, NIRS and angioscopy, to diagnose coronary atherosclerosis and their potential to guide clinical decision-making, are discussed.

Chapter 3 reports on a large single-center registry investigating the safety of intracoronary imaging by OCT in an unselected patient population of 1142 patients with varying indications for imaging. OCT was successfully performed with a low incidence of imaging-related complications (0.6%). This incidence was similar to the incidence of imaging-related complications observed in 2476 patients undergoing IVUS during the same study period in the same center. Importantly, the observed complications were self-limiting after retrieval of the imaging catheter or easily treatable in the catheterization laboratory. These findings indicate that OCT is safe to use in an unselected and heterogeneous group of patients with a very low event rate.

Quantitative OCT analysis can however be complicated by catheter displacement. The impact of longitudinal catheter displacement during image acquisition on the quantitative assessment of BVS is examined in an experimental setting in **Chapter 4**. Variation in the assessment of the imaged scaffold length was high. Although this did not affect global measurements such as mean lumen, mean scaffold and mean coverage area, measurements in specific scaffold regions were affected, hampering serial evaluation of matched cross-sections. Importantly, longitudinal catheter displacement due to coronary artery motion has also been described in humans, suggesting that such quantitative measurements are also vulnerable to errors in human studies.[14] Dedicated analysis methods with per frame analysis or using software allowing for synchronized evaluation of matched segments, could be more suitable for serial analysis in specific scaffold regions in cases with apparent longitudinal catheter displacement.

Preclinical evaluation of coronary artery disease

In **Part II**, animal models used to assess coronary atherosclerosis are discussed and micro- and macrovascular coronary dysfunction and atherosclerosis development is investigated in swine with and without diabetes mellitus.

Several animal models have been used for the evaluation of experimental atherosclerosis. However, most of the studies are performed in transgenic and knockout mice, rats or rabbits, not taking into account the multifactorial nature of the disease. Non-transgenic animal models that more closely mimic human CAD seem more appropriate. In **Chapter 5** several animal models used for the assessment of experimental atherosclerosis are discussed and the preliminary results of a study evaluating CAD development in swine using OCT, IVUS and histology are presented. The presence

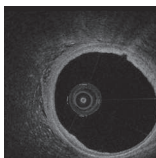
of metabolic alterations resulted in coronary lesion development in swine within 9 to 12 months. OCT and IVUS enabled plaque characterization in-vivo in a diseased swine model and proved to be complementary to each other for the evaluation of CAD development, which may be ideal for studying in-vivo CAD development as well as new coronary diagnostic and therapeutic interventions.

In **Chapter 6** the development of CAD was investigated in swine with and without DM using in-vivo OCT, NIRS and coronary computed tomography angiography (CCTA) and ex-vivo vascular function testing and histology. OCT and NIRS enabled the assessment of the gradual development of early atherosclerosis, whereas CCTA was not able to detect the mainly small, non-calcified lesions. Throughout the study, OCT, NIRS, CCTA, vascular function testing and histology demonstrated no differences in early atherosclerosis development between diabetic and non-diabetic swine, suggesting that macroscopic atherosclerosis development was not influenced by hyperglycemia in these swine.

The effect of hyperglycemia and hypercholesterolemia on vascular function of small coronary arteries, e.g. side branches of the main conduit coronary arteries, was investigated in swine in **Chapter 7**. Ex-vivo vascular function testing demonstrated that the balance of the different contributors to vascular tone of small coronary arteries changes during the progression of the atherosclerotic disease in both diabetic and non-diabetic swine. Early in the disease, at 2.5 months follow-up, impaired endothelium-mediated vasodilation in response to bradykinin was compensated by a reduced endothelin-1 (ET-1) dependent vasoconstriction in DM swine, while no alterations in either bradykinin or ET-1 responses were observed in non-DM swine. In contrast, at 15 months follow-up the balance was dominated by increased vasoconstriction to ET-1 in both DM and non-DM swine, while the endothelium-dependent vasodilator mechanisms seemed paradoxically restored in DM swine and were no longer different from non-DM swine. Hence, findings obtained early on in the disease are not a predictor for the future evolution of the dysfunction, emphasizing the importance of longitudinal studies of vascular function in DM and CAD.

Bioresorbable vascular scaffolds for the treatment of coronary artery disease

Part III elaborates on BVS for the treatment of CAD and the vascular response to BVS-implantation is investigated at long-term follow-up in patients, and shorter-term follow-up in diabetic and non-diabetic swine.



Various types of bioresorbable scaffolds are discussed in **Chapter 8**. Some of them are used in an experimental setting, whereas others are already used in clinical practice. Before BVS can be widely used in clinical practice, it is important to understand the vascular healing response to BVS-implantation. Intravascular imaging by OCT can improve our understanding of the vascular healing response. Therefore, in **Chapter 9,** the vascular healing response 5 years after first-in-man BVS-implantation was investigated by OCT. Implantation of the bioresorbable scaffold led to a restoration of the vascular phenotype at long term by configuration of a non-obstructive plaque, which was created by the consolidation of the underlying plaque, neointima, and resorbed struts. Tissue characterization of the formed 'neo-plaque' was performed both by a human analyst and automated software assessing tissue attenuation. Both assessments were consistent, showing in the majority of cases the formation of a stable plaque with low amount of necrotic core that was covered by a signal-rich tissue layer separating the underlying thrombogenic plaque components from the lumen, potentially shielding the plaque. However, this favorable response was not universal and the observation of a patient with recurrent asymptomatic plaque rupture suggests a need for optimum lesion coverage and continued secondary prevention strategies to optimize vascular healing after BVS-implantation.

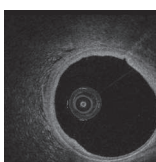
Hence, the need to understand the vascular response to BVS-implantation remains, especially in more complex situations. In **Chapter 10**, the vascular response to BVS-implantation was investigated in swine with and without DM. Swine with and without DM, fed a fast-food diet, received single BVS in two or more coronary arteries and were investigated using in-vivo intracoronary OCT, polarization-sensitive (PS)-OCT and NIRS. After sacrifice at either 3 or 6 months after BVS-implantation, ex-vivo histology and polymer degradation analysis were performed. Late lumen loss was relatively high in all swine within 3 months after BVS-implantation (~60%), stabilized from 3 to 6 months follow-up, and demonstrated a highly heterogeneous neointima, suggestive of neoatherosclerosis formation. Between diabetic and non-diabetic swine, no difference was observed, suggesting that neoatherosclerosis developed in these swine, independent of the presence of DM. Although it is not completely clear to what extent these observations can be extrapolated to the clinical arena, the considerable neoatherosclerosis development under diet-induced dyslipidemia may point at neoatherosclerosis as an important contributor to BVS failure at long-term.

Discussion and future outlook

Considerations for the assessment of coronary artery disease

Compared to the traditionally used coronary angiography, intracoronary imaging offers new levels of anatomical detail and new dimensions of information for the diagnosis and treatment of CAD (**Chapter 2**), paving the way to an improved understanding and therapeutic targeting of atherosclerosis.[9-11, 15] However, a routine use of intracoronary imaging in guidance of CAD treatment is currently not supported, although intracoronary imaging is safe (**Chapter 3**) and indications for clinical use were proposed.[16] Further establishment of the indications for clinical application seems warranted and may be accommodated by randomized studies assessing intracoronary imaging versus angiography guidance.

In addition, a number of methodological shortcomings of current analysis techniques should be overcome in order to enhance the prognostic potential of the currently available intracoronary imaging techniques. Our observation in **Chapter 4** that significant errors in the accurate analysis of lumen measurements by intracoronary OCT can occur due to a relatively slow pullback speed and frame rate acquisition of the catheter, suggests that the development of OCT imaging with faster pullback speeds and higher frame rates could help improve such analysis.[17] Additional developments should be aimed at more detailed and user-independent quantitative tissue characterization. OCT, for example, offers possibilities for quantitative tissue characterization by exploiting the optical attenuation coefficient for tissue identification or measure tissue birefringence using polarization sensitive OCT (PS-OCT).[18-20] Dual-modality intracoronary imaging tools, such as a combination of OCT and NIRS or NIRS and IVUS imaging for simultaneous microstructural and molecular imaging, may also improve intracoronary atherosclerosis assessment.[21] Furthermore interesting is the development of photoacoustic imaging. Photoacoustic tomography uses a short-pulsed laser beam to diffusively penetrate into tissue. Upon absorption of the light by the target, photoacoustic waves are generated and used to reconstruct, at ultrasound resolution, the optical absorption distribution that reveals optical contrast.[22, 23] Ongoing development of these new intracoronary imaging techniques that allow for the accurate and longitudinal assessment of structural information of plaques, intraplaque biochemical activity or plaque features at cellular level, may improve our understanding of atherosclerosis development and its therapeutic targets.



The need for a sophisticated animal model mimicking human-like coronary artery disease

Several animal models for the assessment of atherosclerosis have been discussed in the past decades.[8, 24-28] However, only a few allow evaluation of coronary atherosclerosis that closely resembles coronary atherosclerosis in humans.[29] Besides the porcine diabetes/ hypercholesterolemia model[30] studied by our group, the low-density lipoprotein (LDL) receptor knockout swine, mimicking familial hypercholesterolemia,[31-33] and the more recently developed D374Y-PCSK9 transgenic swine,[28] that exhibit increased plasma LDL levels, demonstrated promising results regarding the assessment of 'human-like' coronary atherosclerosis.[8, 27, 34] However, although swine enable in-vivo longitudinal intracoronary imaging as well as ex-vivo vascular function testing and histological examination of coronary atherosclerosis as demonstrated in **Chapter 6**, conflicting results have been reported regarding the severity of the atherosclerotic disease and the effect of hyperglycemia on the development of coronary atherosclerosis.[5, 28, 30-32, 35] Several factors, including strain difference, age of the swine, duration of the disease, the toxins used to induce diabetes and the type and amount of diet given to the swine may be responsible for the differences observed between the different studies. Moreover, the LDL-receptor knockout swine fed a normal, low calorie, diet demonstrate atheromas by 18 months of age and fully developed coronary atherosclerotic lesions by 24 months of age,[31] whereas the PCSK9 transgenic swine fed a high fat/ high cholesterol diet develop complex progressive human-like coronary atherosclerotic lesions at approximately 12 months of age.[28] Furthermore, in **Chapter 7** we demonstrated a different vascular dysfunction at 2.5 months follow-up than at 15 months follow-up in hypercholesterolemic swine fed a fast-food diet with and without diabetes mellitus, suggesting that the type and amount of vascular dysfunction depends on the time of evaluation. The need to understand what constitutes the most optimal swine model to assess coronary atherosclerosis development remains.[27, 36] A study evaluating several swine strains under different 'atherosclerotic' conditions may improve our understanding of atherosclerosis development in swine, and may help to construct the ideal model to study experimental 'human like' coronary atherosclerosis. Additionally, future studies should consider longitudinal assessment of coronary atherosclerosis development in aging swine with or without diabetes mellitus and include risk factors such as hypertension.

The future of bioresorbable vascular scaffolds

BVS were introduced in an attempt to overcome the limitations of the current gold standard treatment of CAD, namely DES-implantation, which is hampered by permanent caging with or without malapposition, jailing of side branches and late stent thrombosis.[12, 37, 38] In **Chapter 9** we demonstrated that BVS are associated with a favorable healing response at long-term follow-up in a selected group of patients with relatively non-complex lesions. However, clinical studies demonstrating that the vascular healing response following BVS-implantation in an unselected patient population translates into a better clinical outcome at long-term are needed. Although non-inferiority of BVS compared to metallic stents in relatively selected non-complex situations was demonstrated in a number of studies, [39-45] a recent comparison between the BVS and everolimus-eluting DES demonstrated a similar vasomotor reactivity and a larger late luminal loss in the BVS group than the DES group 3 years after implantation.[46] Additionally, conflicting results from registries with several studies demonstrating increased rates of scaffold thrombosis following BVS-implantation in more complex lesions have been reported.[47-50] In the first clinical study assessing the pathomechanisms of BVS thrombosis in-vivo, suboptimal implantation appeared the main substrate for BVS thrombosis, highlighting the need for optimal lesion preparation and possibly for the use of intracoronary imaging techniques to optimize stent implantation.[51] Additionally, we documented neoatherosclerosis development within 3 months after BVS-implantation in swine with and without DM fed a fast-food diet (**Chapter 10**), which may point at neoatherosclerosis as an important contributor to BVS failure at long-term. The distinct healing patterns observed in our preclinical and clinical studies (**Chapter 9 and 10**) need to be better understood. Future studies should consider investigating the use of intracoronary imaging to optimize lesion preparation, sizing of the device and accurate scaffold implantation. Furthermore, larger randomized studies focused on outcome in more complex patient populations are needed to support the use of BVS in complex lesions.