Aortic valve disease

Exploring methods, models, and mechanisms

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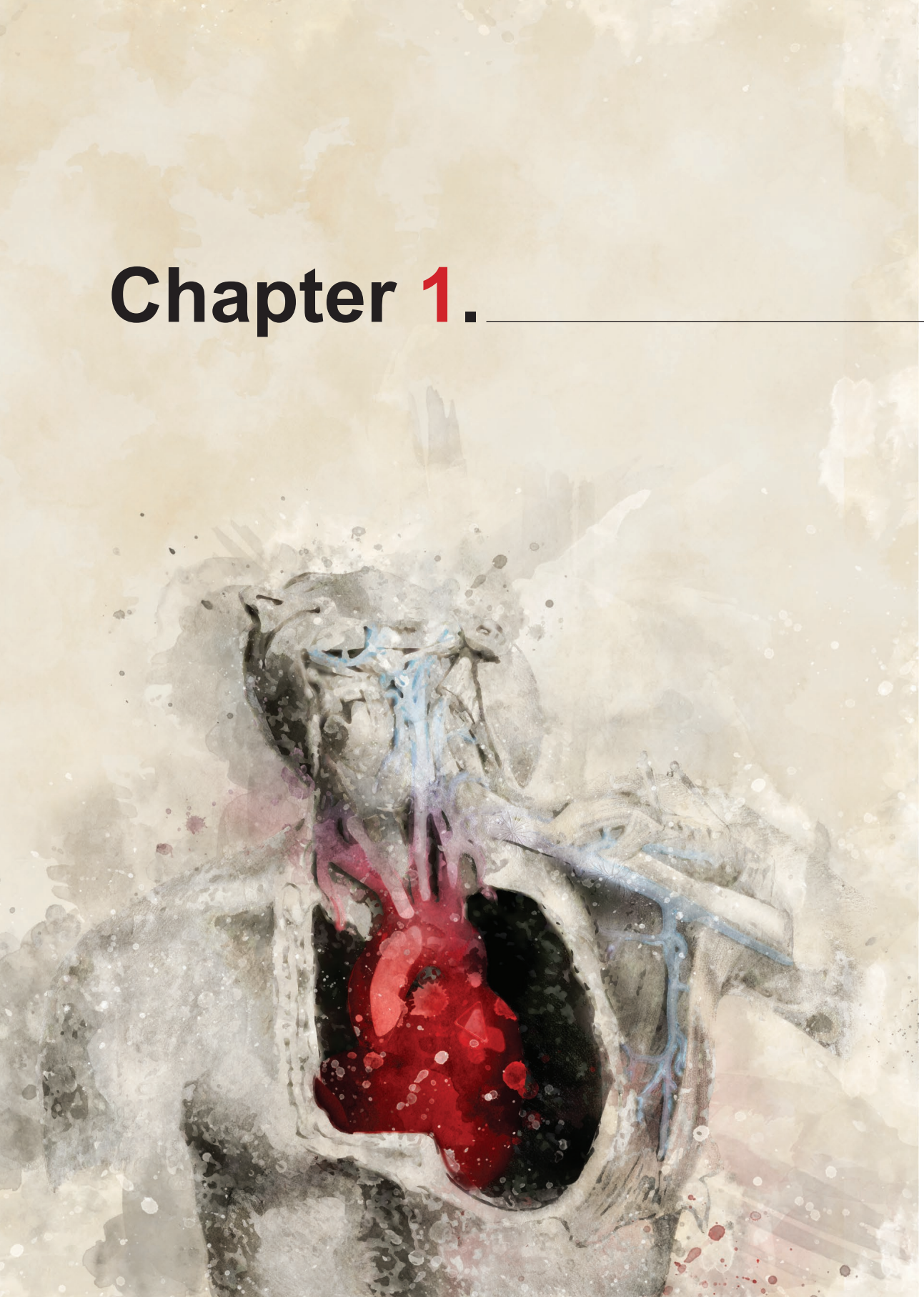
Chapter 1.

General introduction



General introduction

Aortic stenosis (AS), the narrowing of the aortic valve opening, is the most common valvular heart disease in middle to high-income countries. It is relatively uncommon at young age, but its prevalence increases exponentially after the sixth decade, with reported numbers increasing from <1% in those younger than 70 years to >10% in the elderly population (≥75 years of age) [1]. The combination of increased prevalence with age, and the increasing life expectancy and world population, make AS a growing health problem; projections indicate a doubling of the number of elderly patients in need of aortic valve replacement by 2050 and a tripling by 2060 [1, 2]. Risk factors for AS are partly similar to traditional cardiovascular risk factors including age, hypertension, male gender, high cholesterol, high lipoprotein(a), diabetes, bicuspid valve, baseline valve area, and smoking [3].

Narrowing of the aortic valve opening restricts blood flow from the left ventricle into the ascending aorta and may ultimately lead to congestive heart failure. Asymptomatic AS is currently managed by primary prevention, early diagnosis, and watchful waiting. Left untreated, progression into symptomatic AS leads to death within 2 years in over half of the patients [4]. Currently, the only treatment option for severe symptomatic AS is aortic valve replacement (AVR), and although AVR greatly extends and improves survival and quality of life, available mechanical and biological valve prostheses come with significant deficiencies.

AS is most commonly caused by progressive fibrosis and calcification, but it can also be induced by other factors including exposure to radiation and rheumatic fever. Research on fibrocalcific aortic valve disease (CAVD) has made great leaps over the last 20 years, but these efforts have yet to result in a pharmacological therapy to prevent onset, slow progression, or reverse changes associated with CAVD. For many years, AS was thought of as simply a degenerative age-related disease, but now it is clear that the initiation and progression of asymptomatic aortic sclerosis to CAVD-induced AS is an active process that involves many biological mechanisms. While *in vitro* research attempts to understand the mechanisms of CAVD by investigating the behavior of aortic valve interstitial cells (VIC) in culture, another division of research focusses its effort on developing an improved living aortic valve bioprostheses for AVR. *In situ* tissue engineered and decellularized xenografts represent attractive clinical alternatives to overcome limitations of current prostheses.

Aortic valve function and design

To appreciate the complexity involved in research regarding both CAVD and heart valve tissue engineering, we first need to understand the function and shape of the aortic valve. Because behind its deceptively simple appearance, hides a complex tissue whose homeostasis is very much linked to the interaction between the cells that inhabit its surface and interior matrix, and its extremely dynamic microenvironment.

The aortic valve is situated between the left ventricle and the aorta, and performs a number of vital functions. First, the aortic valve ensures unidirectional flow of blood with minimal resistance and regurgitation. Furthermore, the left- and right-coronary sinuses feed the coronary arteries and shape the coronary blood flow, and are therefore essential for myocardial function. The aortic valve opens during systole when pressure in the contracting left ventricle exceeds the pressure in the aorta, and then closes due to diastolic backpressure at ventricular relaxation. During these cycles, the aortic valve is exposed to a myriad of dynamic mechanical forces such as cyclic flexure, shear stress, and compressive and tensile stresses, and it does so over 100.000 times per day and about three billion times in an average lifetime (Figure 1) [5].

To cope with this harsh environment, the aortic valve uses clever architecture. The normal aortic valve consists of three semilunar leaflets (or cusps) suspended in the aortic root at the left ventricular outflow tract, with their free edges coaptating at closure. Congenital malformations of the aortic valve however, with a lower or higher number of leaflets, are relatively common and affect about 1-2% of the population; bicuspid aortic valve is the most prevalent malformation. Each leaflet is about 500 μm thick and composed of three histologically and mechanically distinct extracellular matrix (ECM) layers from aortic to ventricular side: lamina fibrosa, lamina spongiosa, and lamina ventricularis (Figure 1). The main load-bearing layer is the highly aligned collagen-rich fibrosa layer at the aortic side of the leaflet. Due to its circumferential fiber alignment, the fibrosa layer is able to withstand great tensile stresses. On the opposite side of the leaflet, the ventricularis layer is mainly composed of collagen and a network of radially aligned elastin fibers that are thought to assist in maintaining collagen fiber orientation, and to recoil collagen fibers to their original folded conformation upon valve opening. The central spongiosa layer is a highly hydrated glycosaminoglycan-rich loose connective tissue layer and is believed to act as cushioning and lubrication between the outer layers. The biomechanical properties of the valve leaflets are mainly defined by this tri-laminar structure [6].

Figure 1. Schematic of mechanical forces experienced by the aortic valve. (A) laminar flow at the ventricular side during systole, and **(B)** turbulent flow at the aorta-side during diastole. Bottom figures depict the effect of these forces on VICs and VECs.

Aortic valve cells and environment

The leaflet ECM is produced, remodeled and repaired by the valvular interstitial cells (VICs) [7]. The VICs are the major cellular constituent of valves and consist of a heterogeneous population with a plastic phenotype [8]. In the mature healthy valve, at least three VIC subpopulations can be observed. (1) Most VICs have a quiescent fibroblast-like phenotype; these cells maintain valve structure and function. (2) A small physiological population (<5%) will have acquired an activated myofibroblast-like phenotype in response to altered local stress or injury; these cells express α-smooth muscle actin (αSMA) stress fibers involved in the contractile apparatus. (3) Cell of extra-cardiac origin, including macrophages, also contribute to the VIC population [9], as do valvular endothelial cells (VECs) that undergo endothelial to mesenchymal transition [10]. The VECs cover the surface of the leaflet and contribute to valve homeostasis by regulating permeability, inflammatory cell adhesion, and VIC phenotype by paracrine signaling [11].

Although the VIC may seem relatively isolated from exterior hemodynamic forces, it is highly sensitive to cues from its local environment, either indirectly through VEC paracrine signaling or secreted cytokines, or directly by mechanical deformation of the VIC and its surrounding ECM. Because VIC biosynthetic behavior affects the mechanical characteristics of the aortic valve, and at the same time the cells are stimulated by the biomechanical environment itself, there exists a delicate interplay between cells and environment to maintain valve homeostasis and proper valve functioning [12, 13]. An example to illustrate this is axial stretch during diastole, which is crucial to leaflet functioning from a mechanical point of view, as it allows the leaflet to extend and improve coaptation, while at the same time cyclic physiological stretch is essential at a cellular level to induce adequate protein expression. Because of this intricate relation, altered, pathological hemodynamics largely contribute to valve disease. The importance of hemodynamics across the valve (and valve geometry) is also underlined by the predisposition of valve disease in the bicuspid aortic valve population. It should be noted that VICs and VECs from opposite sides of the leaflet are phenotypically different, which is reflected by their potential for pathological differentiation [14, 15]. Ventricularis cells are relatively protected against calcification, while disease is primarily observed on the aortic side of the leaflet. This may be explained by the fact that the fibrosa layer is structurally different, with a relatively stiff collagen matrix as described above.

CAVD research

It is not surprising that altered hemodynamics play a large role in the initiation of valve disease. Shear stress-induced VEC dysfunction has been shown to stimulate an inflammatory response on the fibrosa surface. Increased monocyte adhesion and infiltration, increased permeability, and subendothelial lipid deposition and oxidation contribute to an inflammatory environment. Consequently, VICs can undergo activation that promotes matrix remodeling; structural disorganization, fibrosis and thickening that disrupt the layered architecture and impairs valve function. When the homeostasis is upset, a vicious cycle of reinforcing inflammation, VIC activation, remodeling, and eventually osteoblast-like differentiation of VICs and calcification can occur that, after a 'point-of-no-return', rapidly escalates from aortic valve sclerosis into symptomatic AS.

CAVD research ultimately aims at developing an effective non-interventional drug therapy to stop or slow disease progression as an alternative to AVR. Although numerous cellular and molecular processes and pathways have been implicated in the above-mentioned multi-stage process that leads to eventual calcification, translation of *in vitro* results to the clinic has proven difficult. This can be explained by the complex nature of CAVD and by the lack of *in vitro* culture models that truly mimic the native aortic valve environment. In this thesis, we show that the average *in vitro* study with

isolated VICs puts little effort in recreating a biologically relevant culture model. Given the intimate relationship of VIC phenotype and their environment and the presence of VECs, results obtained in 2D models may not always translate to real patients. For example, in attempts to tackle one of the initiating steps of CAVD, lipid deposition and oxidation, *in vitro* studies showed that statins could inhibit and regress calcific nodule formation. Furthermore, statins had already shown to be effective in treatment of coronary artery disease, which together suggested a promising role for statins in AS therapy. To date, the large prospective studies however suggest no effect of statins on AS progression [16]. *In vitro* results further underline the importance of the VIC environment by showing a substrate-dependent response of VIC to statins [17]. In order to be biologically relevant, the large gap between monolayer cell culture and the human body needs to be closed. Large steps in improving the microenvironment can still be made by implementing 3D, co-culture and a dynamic environment.

In the meantime, alternative prostheses for AVR are explored. Currently used biological and mechanical valve prostheses lack viable tissue and potential for growth, which significantly affects durability, while patients with mechanical valves require additional lifelong anticoagulant treatment. *In situ* heart valve tissue engineering uses synthetic or decellularised biological scaffolds in the shape of a valve and depends on circulating and ingrowing cells to populate, absorb, and remodel the scaffold into a durable, living functional aortic valve. In theory, this approach could yield an attractive alternative to the current prostheses, but despite promising results in *in vitro* engineered heart valves two decades ago, little progress to the clinic has been made [18]. The major problem is a lack of understanding of how to induce site-relevant *in situ* tissue formation. The scaffold microstructure, topology, and geometry direct the hosts' initial immuno response and possibly the final outcome of remodeling. This leaves millions of potential combinations to explore which are, upon implantation, all in the hand of the individuals' regenerative capacity.

So far, reproducing the complex nature of the aortic valve has not been achieved both *in vitro* as *in vivo*. A collaborative effort of multidisciplinary researchers including bioengineers, biomedical scientists, clinicians, material scientists and computer scientists is needed to find an intervention for CAVD and AS from all perspectives and in all stages of disease. The aim of this thesis was to investigate several related aspects of aortic valve disease in order to provide better understanding into (1) the pathogenesis of aortic valve disease, and (2) to improve upon current models for *in vitro* research and tissue engineering alternatives.

Outline

The first part of this thesis covers histopathological studies in two often-overlooked aortic valve patient populations: In **chapters 2 and 3,** we first characterize aortic valves of patients supported by continuous-flow left ventricular assist devices (LVADs). LVADs alter hemodynamics that lead to the rapid development of pathologies in otherwise normal aortic valves. These patients are a great opportunity to study the effect of altered blood flow on aortic valve homeostasis. Second, in **chapter 4** we study radiation-associated aortic valve disease, a late sequela frequently seen after thoracic radiotherapy for breast cancer and malignant lymphoma. We focus on inflammation and remodeling to improve our understanding of the processes contributing to valve dysfunction in these patients. **Chapter 5** describes a rare congenital aortic valve malformation discovered upon necropsy.

Histopathological studies are crucial in understanding disease processes but are often endpoints, especially in human studies. To meaningfully test hypotheses and study pathological and regenerative mechanisms, *in vitro* models that represent the native aortic valve are fundamental.

In part II, we first provide an overview of the approaches and techniques used to culture aortic valve interstitial cells (VIC) in **chapter 6**. Our review suggests that most current research is low-throughput and inaccurately characterizes the three-dimensional environment in which the native aortic valve cells reside. To this end, **chapter 7** concentrates on developing a more biologically relevant VIC culture model that is compatible with high-throughput methods. Introduction of such models can accelerate research and improve translation of *in vitro* findings and drug screening to clinical trial outcomes.

Over 30 years of basic research has not yet resulted in a pharmaceutical therapy for CAVD and at present, aortic valve replacement is the only treatment option. Current biological valve replacements however lack growth and remodeling capacity, and calcify prematurely. **Chapter 8** describes our experience with a potential tissue engineering solution for valve replacement: porcine small intestine submucosa extracellular matrix. **Chapters 9 and 10** report on two rare but severe cases of complication after valve replacement.

Lastly, final thoughts are reflected in part III with a general discussion in **chapter 11** followed by a summary in **chapter 12**.

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