The Hemodynamics of Calcific Aortic Valve Disease
1. Aortic Valve Physiology

1.1 Function and structure

1.1.1 Function

The aortic valve (AV) is located at the junction between the left ventricle and the aortic root. Coordinated and full opening of the AV is essential to ensure unobstructed and unidirectional blood flow from the left ventricle to the aorta and to minimize ventricular workload during systole (contraction of the heart) [1]. Conversely, complete closure of the valve during diastole (relaxation of the heart) prevents blood from flowing back to the left ventricle. The valve opens and closes more than 100,000 times daily to regulate blood flow in response to changes in transvalvular pressure [2].

1.1.2 Structure

The AV consists of three semi-lunar leaflets attached to an annulus, which is a ring of fibrous tissue at the base of the leaflets (Figure 1) that provides support and maintains the shape of the valve.

Figure 1: AV anatomic position: a) cross sectional view; b) top view (https://www.adam.com)
AV leaflets consist of three distinct layers: 1) the fibrosa, which has a corrugated surface and faces the aorta; 2) the ventricularis which is smooth and faces the left ventricle of the heart; and 3) the spongiosa, which is located between the fibrosa and the ventricularis (Figure 2).

The fibrosa consists mainly of circumferentially aligned collagen fibers, which are composed mainly of proline, hydroxyproline and glycine in a triple helical structure [3]. The particular architecture of the fibrosa makes the valve stiffer in the circumferential direction than in the radial direction [4]. The primary collagen subtypes present in the fibrosa are collagen I, III, and V [5].

The ventricularis mainly consists of elastin fibers, with some interspersed collagen, which makes the ventricularis more extensile than the fibrosa [6,7]. Because elastin is a coiled hydrophobic structure consisting mainly of the amino acids alanine, valine, leucine and glycine, it is considerably less stiff than collagen [3]. Besides, the fibers in the ventricularis have much less directionality than those in the fibrosa.
Located between the ventricularis and the fibrosa, the spongiosa consists of loose, watery connective tissue containing mainly glycosaminoglycans. These long, multi-chain proteins bind water readily and give the spongiosa a gelatinous, watery consistency [8]. The semi-fluid nature of this layer permits the fibrosa and the ventricularis to easily slide over each other as the leaflet deforms during the cardiac cycle.

1.2 AV cellular biology

The cells contained within the AV play an important role in valvular function [9]. The cells populating the leaflet are broadly categorized as: 1) aortic valve endothelial cells (AVECs) on the surfaces of the leaflets, and 2) aortic valve interstitial cells (AVICs) that populate the body of the leaflets and form an integral network along with the extracellular matrix (ECM) (Figure 2).

1.2.1 AVECs

AVECs are located on both surfaces of the leaflets and are responsible for maintaining a non-thrombogenic blood contact surface and transmitting biochemical and mechanical signals to the interstitial cells [10]. AVECs express von Willebrand factor (a blood glycoprotein) and endothelin-1 and nitric oxide [11,12], that both play important roles in homeostasis.

1.2.2 AVICs

Three cellular phenotypes can be distinguished in the AVIC population: myofibroblasts, fibroblasts and smooth muscle cells [9,13-15]. The myofibroblasts account for a large percentage of AVICs and have characteristics of both fibroblasts and smooth muscle cells. The myofibroblast phenotype is characterized by prominent stress fibers associated with α-smooth muscle actin (α-SMA), and is also involved in cell proliferation, migration and ECM remodeling [15]. The fibroblast phenotype is characterized by prominent synthetic and secretory organelles
and is involved in matrix regulation and production. Fibroblasts are responsible for synthesizing ECM components that are critical for the structural support of the tissue [15]. Lastly, smooth muscle cells promote the contractile properties of the valve.

### 1.3 Hemodynamic environment

![Diagram](a)

**Figure 3** Overview of AV hemodynamics: (a) during diastole, the transvalvular pressure of 80 mmHg imposes bending and tensile stretch on the leaflets; and (b) during systole, the blood forms a central jet and recirculation occurs behind the leaflets.

The AV functions in a complex mechanical environment (Figure 3) that includes pulsatile pressure, bending and tensile stress, and fluid shear stress [16]. Under physiologic conditions, the flow through the valve is pulsatile and unidirectional with a cardiac output of 5 L/min. During diastole, the AV experiences a transvalvular pressure of about 80mmHg acting perpendicular to the leaflet. The pressure imposed on the leaflets causes large bending and tensile stretch, which elongate the leaflets by 11% in the circumferential direction and 31% in the radial direction from systole to diastole [5] (Figure 3a). During systole, the valve opens when the ventricular pressure becomes larger than the aortic pressure (transvalvular pressure ~ 0mmHg). The blood forms a central jet and recirculation occurs behind the leaflets (Figure 3b).

The fluid shear stress experienced by the AVECs is an important component of the valve hemodynamic environment. It results from the relative motion between the leaflet and the blood.
flow. Computational and experimental methods have been used to quantify the native valvular shear stress environment. Two-component laser-Doppler velocimetry (LDV) was utilized to measure the shear stress on the surface of a polymeric valve [17]. The maximum wall shear stress on the leaflet surface was found to be 79 dyn/cm\(^2\), while the wall shear stress measured 83 mm downstream of the valve was 10-17 dyn/cm\(^2\) at a flow rate of 7.5 L/min and 52-104 dyn/cm\(^2\) at a flow rate of 22.5 L/min. Due to the complex leaflet dynamics, it is difficult to accurately quantify the native leaflet wall shear stress. Ge et al. developed a computational fluid dynamics model of a trileaflet valve that offers fine spatial and temporal resolutions while maintaining near-physiologic valve opening/closing characteristics [18]. Although the leaflet deformation was prescribed, based on a separate structural deformation calculation [19], the model was able to capture the side-specificity of the leaflet wall-shear stress previously described by in vivo magnetic resonance phase-velocity mapping measurements [20], as well as the peak-systolic wall-shear stress (79 dyn/cm\(^2\)) captured by LDV measurements [17]. Based on the model predictions, the physiologic surface-averaged shear stress experienced by the ventricularis consists of a pulsatile waveform varying between 0 and 79 dyn/cm\(^2\) over a cardiac period of 860 ms, while that experienced by the fibrosa consists of a bidirectional oscillatory waveform ranging from -8 to +10 dyn/cm\(^2\) (Figure 4). However, due to the sub-physiologic flow rates and the prescribed motion of the leaflets employed in the simulations, the computed surface-averaged shear stress was only an approximation of the native shear stress.
2. Calcific Aortic Valve Disease

Calcific aortic valve disease (CAVD) causes a significant human and economic burden in both developed and developing countries and is now recognized as an important worldwide public-health problem. The prevalence of CAVD in the western world correlates with age and affects 26% of the population above 65 years of age [21], while in developing countries, it is secondary to rheumatic fever which accounts for 25-40% of all cardiovascular diseases and is responsible for 33-50% of all hospital admissions [22].

CAVD is characterized by structural changes such as increased thickness, stiffness and calcification of the leaflets, which cause the heart to weaken and prevent efficient blood flow. The initial phase of the disease, known as aortic valve sclerosis (AVSc), consists of the mild thickening of the valve tissue. The advanced stages of the disease, known as calcific aortic stenosis (CAS), consist of the formation of calcific lesions on the leaflets, which causes serious impairment of leaflet motion and, in turn, a limited blood flow through the valve (Figure 5).

2.1 AVSc and CAS

AVSc is due to the thickening of the roots of the aortic leaflets but produces no obstruction and no hemodynamic instability. Although a systolic outflow murmur may be
auscultated during physical examination, there are no clinical symptoms reliably associated with AVSc.

CAS is usually defined by a restricted systolic opening of the valve leaflets due to the formation of calcific nodules on the fibrosa [23] (Figure 5b). CAS leads to an increased pressure gradient between the left ventricle and the aorta. To compensate for the elevated pressure gradient, the left ventricle generates an increased pressure to drive blood into the aorta, which results in left ventricle hypertrophy and a progressive decline in systolic function. Rapid progression of CAS is defined as an increase of maximal transvalvular pressure across the valve of at least 10 mmHg per year [24].

2.2 Diagnostic evaluation

The standard diagnostic evaluation of CAVD includes assessment of leaflet anatomy and extent of valvular calcification by echocardiography. The severity of CAVD can be estimated on the basis of jet velocity and valvular effective orifice area (EOA) [8], a metric defined as the ratio of the cardio-output flow through the AV to the flow velocity:

\[ EOA = \frac{(CSA)(VTI)_{LV}}{(VTI)_{AV}}. \]

Where CSA means the cross-sectional area and VTI represents flow velocity-time integral.
The degrees of severity in all adult cases have been proposed (Table 1).

Table 1. Guidelines for grading severity of CAVD [8]

<table>
<thead>
<tr>
<th></th>
<th>Jet Velocity, m/s</th>
<th>EOA, cm²</th>
</tr>
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<tbody>
<tr>
<td>AVSc</td>
<td>&lt;2.5</td>
<td>Normal</td>
</tr>
<tr>
<td>Mild CAS</td>
<td>2.5-3.0</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Moderate CAS</td>
<td>3.0-4.0</td>
<td>1.0-1.5</td>
</tr>
<tr>
<td>Severe CAS</td>
<td>&gt;4.0</td>
<td>&lt;1.0</td>
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2.2 CAVD pathobiology

For years, CAVD was considered a passive degenerative and irreversible process [25]. However, recent studies have evidenced that CAVD is not merely a degenerative disease related to age-associated “wear and tear” with calcium deposition, but rather an active process that involves chronic inflammation [26,27], ECM remodeling and ossification [28-32], as shown in figure 6.

Figure 6 Potential pathways depicting of CAVD pathophysiology [33].
2.2.1 Inflammation

The initiation of valvular inflammation is associated with the disruption of the leaflet endothelium. This process is followed by the oxidation of low density lipoprotein (LDL) and their uptake by macrophages that become, in turn, foam cells [34]. The accumulation of oxidized LDL and the release of inflammatory cytokines in the subendothelium, such as transforming growth factor-β1 (TGF-β1), interleukin-1β (IL-1β) [35], bone morphogenic proteins (BMPs) and tumor necrosis factor-α (TNF-α) [36] induce ECM remodeling, and local calcification. These cytokines may contribute to upregulate expression of and matrix metalloproteinases (MMPs) and calcium.

BMPs are pro-inflammatory cytokines, members of the TGF-β super family. BMP4 is involved in shear-induced inflammation and capable of activating endothelial nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase, and reactive oxygen species (ROS) production [37]. NADPH oxidase is a membrane-bound enzyme complex, which can produce ROSs. ROS production is known to activate the smad- and NFκB-pathways, which are responsible for increasing expression of inter-cellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1), which promotes monocyte adhesion to endothelial cells, a critical step in inflammation [37].

TGF-β1 is a polypeptide member of the TGF-β super family and is a secreted protein that controls many cellular functions, including ECM synthesis and controls cell growth, proliferation, differentiation and apoptosis [38]. TGF-β1 has been shown to promote ECM remodeling in porcine valve and AVIC differentiation into active myofibroblasts in a dose-dependent manner, as determined by a significant increase in α-SMA [39].
2.2.2 ECM Remodeling

ECM remodeling in CAVD involves not only extensive fiber accumulation, but also the breakdown of matrix components through degradation and disorganization of collagen and elastin fibers. A perturbation of the equilibrium between fiber accumulation and degradation can lead to pathological ECM remodeling and compromised valvular function. The disorganization of collagen bundles and the fragmentation of elastin fibers contribute to valvular dysfunction and loss of leaflet elasticity, which contributes to the development of CAVD via the upregulation of MMPs, their tissue inhibitors (TIMPs) and cathepsins [35,36,40,41]. MMPs are a family of zinc- and calcium-dependent enzymes that are mainly produced by inflammatory cells and can degrade collagen, elastin fibers and proteoglycans [42]. MMP activity is mediated mainly by binding with endogenous TIMPs. The local balance between MMP and TIMP expression and activity regulates the extent of tissue remodeling. MMP1, 2, 3, 9 and TIMP1, TIMP2 have been shown to be involved in the pathogenesis of CAVD [40,41,43]. In comparison to fresh valves, the expression levels of MMP1, 2, 3 and 9 are increased in CAS valve [40]. It has been demonstrated that cytokines such as TNF-α can contribute to cell proliferation and increased expression of MMPs and cathepsins in diseased valves [36].

Cathepsins K, L, and S are potent elastolytic proteases that have been associated with atherosclerotic plaque progression [44] and dysfunctional heart valves [45]. They can efficiently degrade elastin and collagen fibers [46]. A recent study by Helske et al revealed that cathepsin S, and K are the sub-types that are upregulated in stenotic AVs [47].

2.2.3 Ossification
The areas of calcification colocalize with areas of oxidized LDL accumulation and inflammatory cell expression. As shown in in vitro study, oxidized LDL and cytokines produced by T-lymphocytes stimulate calcified nodule formation by valvular fibroblasts through pathways activated by BMPs [48]. BMPs are present in human AV lesions [49], which promote osteogenesis through the Wnt/Lrp5/β-catenin pathway [50] or Runx2/Cbfa1 pathway [51]. In the Wnt/Lrp5/β-catenin pathway, secretion of Wnt3a leads to the formation of the Lrp5/Wnt3a/Frizzled receptor complex, which increases β-catenin expression. When β-catenin enters into the nucleus, it activates transcription factors associated with bone formation.

In addition, mutations in NOTCH1, a transcription factor that normally represses Runx2/Cbfa1, have been shown to lead to valvular calcification [52]. Thus, the osteogenic Runx2/Cbfa1 pathway may be activated not only by BMP2, but also by specific genetic abnormalities. Following their activation by Runx2, fibroblasts differentiate into valvular myofibroblasts with an osteoblast-like phenotype and express osteopontin, a protein involved in bone formation. Osteopontin mRNA expression correlates with the degree of valvular calcification. Besides, Alkaline phosphatase (Alk phos) activity is also involved in the development of calcific nodules and bone formation [28].

2.3 CAVD management

The current treatment for CAVD includes surgical valve replacement and pharmacological control of risk factors.

2.3.1 Valve Replacement

Since the first successful heart valve replacement in 1952 by Dr. Hufnagel, there have been over 4 million (300,000 annually) prosthetic replacement implants worldwide of over 50
developed artificial heart valve designs [53,54]. These designs are categorized into two types, mechanical and bioprosthetic valves.

2.3.1.1 Mechanical Valves

Currently, the most prevalent mechanical valve is the bileaflet valve (Figure 7a). Mechanical valves have good EOA and durability. However, mechanical valves are prone to thromboembolic complications[55], which may cause valve obstruction and/or regurgitation. Therefore, patients with mechanical valve require lifelong anticoagulation therapy [56].

2.3.1.2 Bioprosthetic Valves

Most bioprosthetic valves consist of tissue leaflets preserved in glutaraldehyde and mounted on a metal or plastic stent, and a sewing ring made of fabric. In contrast to mechanical valves, bioprosthetic valves mimic native heart valve operation by providing unobstructed central flow. Figure 7b-d shows different types of bioprosthetic valves [57]. Bioprosthetic valves have better histocompatibility than mechanical valve, but a limited durability (10-15 years) due to the progressive degradation of the biological material. For this reason, patients with bioprosthetic valves often require multiple surgeries. The first commercial porcine valve was the Hancock valve introduced in 1970. Since then, additional bioprosthetic valves have been

![Figure 7 Different types of prosthetic valves. A, Bileaflet mechanical valve (St Jude); B, stented porcine bioprosthesis (Medtronic Mosaic); C, stented pericardial bioprosthesis (Carpentier-Edwards Magna); D, stentless porcine bioprosthesis (Medtronic Freestyle);](image-url)
developed, using a variety of other biological materials, like bovine heart valve. The Carpentier-Edwards Perimount valve is the only pericardial valve widely available in North America [54].

2.3.2 Pharmacological Control of Risk Factors

To date, no medical therapy addresses the root cause of CAVD. Some medical management of concomitant conditions that correlate with CAVD would be desirable. For example, Beta-blockers or calcium blockers can be used to decrease heart rate, lessening the load on the heart and relieving angina [58]. Vasodilators can be used to lessen the work of the heart by dilating or relaxing the blood vessels. Since CAVD shares many risk factors with atherosclerosis, which may be prevented by cholesterol lowering[59], some studies investigated the effects of cholesterol-lowering drugs and CAVD progression. Although many studies did demonstrate a slowing of CAS with statins (e.g., atorvastatin or rosuvastatin [32,60]), these findings are disputed. A trial, published in the New England Journal of Medicine in 2008, failed to find any beneficial effect of intensive cholesterol lowering on the course of AV stenosis [61].

Therapeutic strategies would constitute a non-invasive and effective alternative to the current surgical valve replacement approach. These new pharmacological approaches would not only help relieve symptoms and decrease the risk of further heart damage, but also address the root cause of CAVD[58]. The development of new medications requires the knowledge of the molecular pathways/mechanisms of valvular pathogenesis, which remains limited.

3. AV Mechanobiology

The AV functions in a complex mechanical environment, including pressure, cyclic stretch, as well as shear stress that drives critical cell-ECM process. Therefore, understanding the
effect of the mechanical environment on AV biology is very important to better understand normal AV function and disease progression.

3.1 Effects of pressure on AV biology

The pressure imposed on the leaflets varies throughout the cardiac cycle, thereby changing the bending and tensile stretch and the length of the leaf. A significant increase in collagen synthesis was demonstrated under both steady and cyclic conditions at 140 mmHg and 170 mmHg, however this increase was not statistically significant at 100 mmHg [62]. In another study, the combination of high pressure magnitude (150–190 mmHg) and high frequency (2 Hz) resulted in significant increases in collagen and GAG synthesis in porcine AV leaflets [63]. These observations suggest the potential sensitivity of AV leaflets to abnormal pressure.

3.2 Effects of stretch on AV biology

Cyclic stretch is another mechanical stress experienced by AV leaflets that contributes to prevent the back flow into the left ventricle during diastole. With increasing age, the valve tissue becomes less extensible. Multiple studies have demonstrated that varying tissue stretch levels alter expression of cytokines, enzymatic activity, and protein biosynthesis in the AV [64-68]. It was reported that AVICs respond to local tissue bending and tensile stretch by altering cellular stiffness via collagen biosynthesis [16]. Balachandran et al. demonstrated that cyclic stretch was responsible for regulating the contractile phenotype of the valvular cells, with elevated stretch resulting in increased α-SMA expression and reduced sulfated glycosaminoglycan [65,68].

3.3 Effects of shear stress on AV biology

Since shear stress is an important mechanical stress, the mechanobiology of shear stress has been an active research area. The decrease in EOA experienced during CAVD leads to increased aortic jet velocity and, in turn, altered leaflet wall shear stress. Although many studies
have been carried out to characterize the biological responses of vascular endothelial cells to shear stress, studies on AVECs are few. According to previous studies, alterations in shear stress affect the biosynthetic activity of valvular cells in different aspects.

One of the earliest recognized effects of shear stress is the elongation and realignment of endothelial cells. Butcher et al. demonstrated that valve endothelial cells aligned parallel to steady, laminar flow in contrast with vascular endothelial cells[69], and porcine AVECs exposed to 20 dyn/cm² of steady laminar shear stress exhibited a cytoskeletal alignment different than that in static endothelial cell culture, suggesting that AVECs exhibited altered responses to mechanical forces [69].

Weston et al. studied the effects of the flow environment and shear stress on valvular biology. Porcine leaflets were exposed to one of several conditions for 48 h, including steady or pulsatile flow in a tubular flow system at 10 or 20 L/min, and steady shear stress in a parallel plate flow system at 1, 6, or 22 dyne/cm². Protein, glycosaminoglycan, and DNA synthesis increased during static incubation but remained at basal levels after exposure to flow. The modulation of synthetic activity was attributed to the presence of a shear stress on the leaflet surface, which may be transmitted to interstitial cells within the leaflet matrix through tensile forces [64].

One of the important effects of shear stress is ECM synthesis and remodeling of AVECs. MMPs and cathepsins are known to play an important role in the ECM remodeling and are often expressed early in the AV diseases. Platt et al. demonstrated that steady shear stress of 25 dyn/cm² on AVECs was able to induce ECM remodeling by increasing MMP-2 and MMP-9 activity, and decreasing cathepsin L expression [70]. A subsequent study demonstrated when endothelial cells were exposed to static flow, laminar shear stress (15 dyn/cm² for 1 day) and
oscillatory shear stress (+/- 5 dyn/cm² for 1 day), the results showed that cathepsin L is a shear-sensitive matrix protease and that it may play an important role in flow-mediated vascular remodeling and atherogenic responses [71].

Another important effect of shear stress on valvular biology is the regulation of inflammation and calcification. Sucosky et al. exposed AV surface to abnormal shear stress (aortic surface was exposed to shear stress experienced by the ventricularis, ventricular surface was exposed to shear stress experienced by the fibrosa), and they demonstrated that the alteration (magnitude or/and pulsatility) could increase expression of the inflammatory markers on the aortic leaflet surface. In contrast, neither pulsatile nor oscillatory shear stress affected expression of the inflammatory markers on the ventricularis surface. The shear stress-dependent expression of VCAM1, ICAM1, and BMP4, but not TGF-β1, was significantly reduced by the BMP inhibitor noggin, whereas the TGF-β1 inhibitor SB431542 blocked BMP4 expression on the aortic surface exposed to pulsatile shear stress. These results demonstrate that the potential of pathologic valvular shear stress to activate the fibrosa in a BMP4- and TGF-β1-dependent manner, providing some potential directions for future drug-based therapies [72].

4. Proposed Work

This study is motivated by the possible pathophysiological relevance of shear stress abnormalities to disease onset and progression. For example, the accelerated progression of CAVD is accompanied by a reduction in EOA which leads in turn to an increased peak aortic velocity [74]. Similarly, hypertension (i.e., a risk factor for calcific aortic stenosis [75]) is associated with changes in transvalvular flow rate, which also translates in variations in peak aortic velocity [76]. Given the strong interactions between the blood flow and the leaflet motion,
an alteration in shear stress magnitude is presumably associated with an alteration in shear stress frequency or pulsatility. The central hypothesis of this study is that side-specific shear stress alterations contribute to AV pathogenesis by promoting tissue inflammation and altering ECM remodeling. This hypothesis will be tested by following specific aims:

- **Specific Aim 1**: Production of native side-specific shear stress on AV leaflets.
- **Specific Aim 2**: Elucidate the effects of isolated and combined alterations in shear stress magnitude and pulsatility on AV inflammation and remodeling.
- **Specific Aim 3**: Formulate a mathematical model of shear stress-induced AV inflammation and ECM remodeling progression.
References


[58]


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