Review: Mechanobiology of the Aortic Heart Valve

Jonathan T. Butcher[1], Craig A. Simmons[2], James N. Warnock[3]

[1]Department of Biomedical Engineering, Cornell University, Ithaca, NY, USA, [2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada, [3]Department of Agricultural and Biological Engineering, Mississippi State University, Mississippi State, MS, USA

The aortic heart valve is a complex and sophisticated structure that functions in a mechanically challenging environment. With each cardiac cycle, blood flow exerts shear stresses, bending stress and tensile and compressive forces on the valve tissue. These forces determine a plethora of biological responses, including gene expression, protein activation and cell phenotype. Consequently, mechanical forces may influence valve remodeling or pathological changes. Understanding the mechanobiology of heart valves is a vast task. Herein, some of the recent studies that have increased current knowledge of endothelial and interstitial cell interactions with physical forces are examined. Additionally, experimental co-culture models are described that are being developed to further improve the understanding of endothelial-interstitial cell interactions. Finally, the means by which organ culture systems are being utilized to study heart valve biology, thereby providing a complementary approach to in vivo experimentation, are described.

The Journal of Heart Valve Disease 2007;17:62-73

The aortic valve, positioned between the left ventricle and aortic root, prevents retrograde flow of blood into the ventricle during diastole. Opening and closing of the valve occurs passively in response to inertial forces exerted on the valve leaflets by the surrounding blood. The forces exerted by the blood on the aortic valve leaflets are sizeable and result in shear stresses, pressure loads, and flexural deformations that are unlike those experienced by any other tissue in the body.

The mechanical behavior of the aortic valve has been well studied, particularly as it relates to the design and failure analysis of mechanical and bioprosthetic valves (1,2). Diseases of native aortic valves have also been linked to mechanical factors, largely through observations that lesions occur at sites that correlate spatially with distinct local mechanical environments. For example, the sclerotic lesions that occur in calcific aortic valve disease occur preferentially in the subendothelial space on the aortic side of the leaflet, a region that is exposed to low shear stress and disturbed patterns of blood flow (3,4). Further, calcified lesions tend to occur in regions that experience the highest bending stresses - that is, along the line of leaflet coaptation or radially from the area of leaflet attachment (Fig. 1) (5). Traditionally, it has been presumed that the forces applied in these regions disrupt the endothelium that lines the surface of the valve leaflets (6) or damage the interstitial matrix (7), leading to lesion formation. This 'wear and tear' mechanism has also been used to explain the early occurrence of sclerosis in patients with bicuspid valves, which experience hemodynamic and biomechanical stresses that differ significantly from tricuspid valves (8).

This traditional view of aortic sclerosis - that mechanical 'wear and tear' contributes to passive valve degeneration secondary to aging - is challenged by recent studies which demonstrate convincingly that aortic sclerosis is not a passive, unregulated process, but rather is an active cell-mediated process involving chronic inflammation (3,9) and active calcification (6,10-12). Consistent with this new understanding of the pathogenesis of valve disease is increasing evidence that mechanical forces may contribute to valve disease by actively regulating valve cell biology. Valvular endothelial cells and valvular interstitial cells are exquisitely sensitive to mechanical forces, often responding with unique behaviors that distinguish them from seemingly similar cells. In this review, new insights into these mechanobiological responses are

Address for correspondence:
Dr. James N. Warnock, Department of Agricultural and Biological Engineering, Mississippi State University, Box 9632, Mississippi State, MS 39762-9632, USA
e-mail: jwarnock@abe.msstate.edu

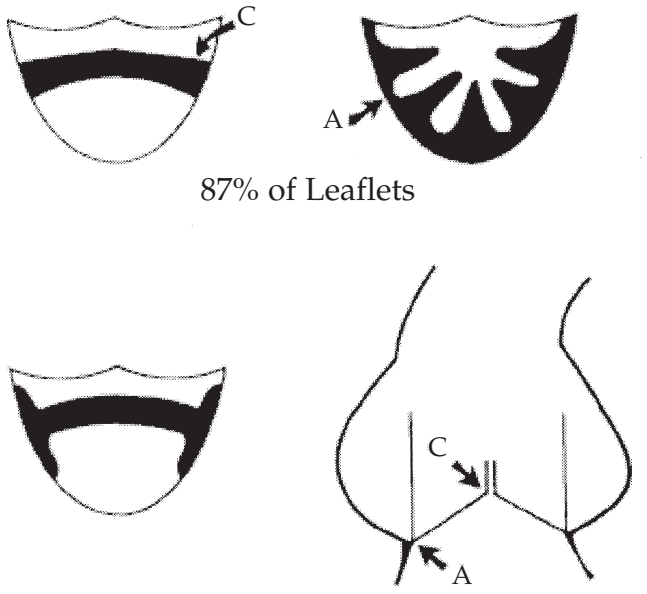

Figure 1: Patterns of calcific deposit and the areas of cusp flexion. Upper left: Coaptation pattern, where calcification occurs primarily along the line (C) of leaflet coaptation. Upper right: Radial pattern, where calcium spreads as radially oriented spokes starting at the cusp attachment area (A) and moving toward the center of the cusp. Lower left: Combination of coaptation pattern and calcium along the cusp attachment, seen quite frequently. Lower right: As the aortic valve opens and closes, the cusps of the valve are under a great deal of flexion along the area of cusp attachment (A). The cusps also undergo flexion along the line of coaptation (C). (Reprinted from: Thubrikar MJ, Aouad J, Nolan SP. Patterns of calcific deposits in operatively excised stenotic or purely regurgitant aortic valves and their relation to mechanical stress. Am J Cardiol 1986;58:304-308 (5), with permission from Elsevier.)

summarized, their implications for the normal valve function and disease are discussed, and some of the systems with which aortic valve mechanobiology is studied are described.

Valvular endothelial cells are a distinct subphenotype

Although the hemodynamic stimulation of endothelial-mediated vascular response has been studied for several decades, virtually nothing is known about the response of valvular endothelial cells to shear stress. Vascular endothelial cells characteristically align parallel to the direction of fluid flow (13,14), and secrete vasoactive agents such as endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) to stimulate underlying smooth muscle cells to contract or relax in order to maintain wall shear stress in a defined range (15). Valvular endothelial cells also express vasoactive agents, and studies have shown that isolated valve leaflet strips will contract in the presence of these factors only when a confluent endothelial layer is present (16). Contraction by aortic valve strips is only about 10-20% that of arterial strips with forces less than 1 mN (17), which is much less than the passive in vivo tissue stresses (75 g/mm^2) (18). Although interstitial cell contraction in response to agonists does result in changes in flexural stiffness (19), a functional consequence of these agents for valves in vivo seems secondary, at best, based on the available data (20,21). Arterial shear stresses vary between 10-25 dynes/cm^2 depending on location within the vascular tree (22). Shear stresses on aortic valve leaflet surfaces are much more difficult to quantify because of the constant and rapid motion of the leaflets. Results from several studies suggest that peak wall shear stresses range from 30 to 1,500 dynes/cm^2 (23,24). Fluid flow on the inflow and outflow surfaces are very different. Blood flow on the inflow (ventricularis) side is strongly unidirectional and pulsatile, whereas flow on the arterial (fibrosa) side is much lower and more oscillatory (25). Until recently, it was believed that valvular and vascular endothelial cells were phenotypically similar. Valvular endothelial cells in vivo align circumferentially on the leaflet surface, which is perpendicular to the direction of fluid flow, and in contrast to arterial endothelial cells (26). Pathological endothelial activation in vivo is somewhat similar in valvular and arterial endothelial cells. Both cell types will present inflammatory mediators such as vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin on their surface when activated in vivo (27), but only the endothelial cells on the outflow side of the valve appear to do this.

The present authors have performed studies to further characterize the valvular endothelium. Butcher et al. first investigated the hemodynamic regulation of valvular endothelium by exposing valvular and arterial endothelial cells to identical shear stress conditions (steady unidirectional shear stress). As shown in Figure 2, it was found that valvular endothelial cells align perpendicular to the direction of fluid flow, in contrast to arterial endothelial cells (28). It was further demonstrated that this alignment difference was regulated by changes in: (i) integrin clustering (α1 integrin); (ii) plaque formation (vinculin); (iii) integrin-mediated signaling (focal adhesion kinase; FAK); and (iv) cytoskeletal (f-actin) arrangement. Furthermore, this process could be disrupted in both cells in vitro by blocking Rho kinase (with Y-27632) and calpain (calpain inhibitor-1). PI 3-kinase, on the other hand, regulated arterial but not valvular endothelial cell alignment. These results demonstrated that valvular endothelial cells respond uniquely to

J Heart Valve Dis
Vol. 17. No. 1
January 2008

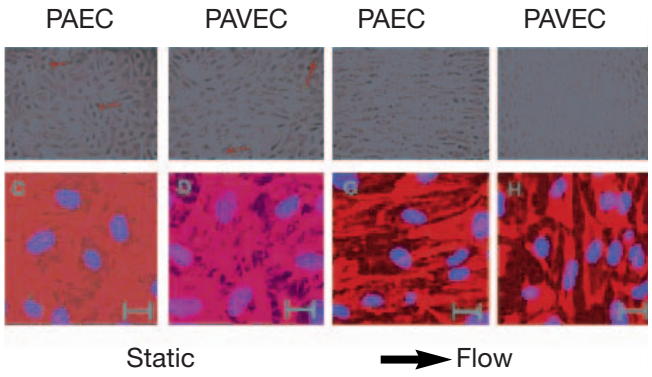

Figure 2: Images of pulmonary artery endothelial cells (PAECs) (A, C, E, G) and pulmonary artery vascular endothelial cells (PAVECs) (B, D, F, H) in static and steady fluid flow environments. A-D) Static culture. E-H) After 48 h of 20 dynes/cm^2 steady laminar shear stress. The top panels are phase-microscopy images; the bottom panels are laser confocal microscopy images. Cells were stained for f-actin (red) and cell nuclei (blue). The flow direction is horizontal left to right. Scale bars = 50 mm. (Figure reprinted from Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. Arterioscler Thromb Vasc Biol 2004;24:1429-1434 (28), with permission from Lippincott, Williams & Wilkins.)

shear stress, which may underlie more important phenotypic differences.

Because of the many possible differences that could exist between valvular and arterial endothelial cells, cDNA microarrays were used to compare the transcriptional profiles of these cells in vitro. Total RNA was isolated from porcine aortic and aortic valve endothelial cells that were cultured in either static or shear stress conditions, and hybridized together in one of four comparison groups. Approximately 10% of all genes profiled were significantly different in each comparison group at a significance level of $p < 0.005$ (29). These changed transcripts were then compared globally using ontological hierarchies according to prominently expressed biological themes. Significantly expressed biological themes included cell proliferation, oxidoreductase activity, migration, cytoskeletal reorganization, and differentiation. Deeper investigation of genes involved in the pathogenesis of valvular diseases revealed several important observations. First, valvular and arterial endothelial cells expressed genes involved in oxidative response similarly, but valvular endothelium was inherently less inflammatory than arterial endothelial cells. Steady shear stress was protective against pro-oxidation and pro-inflammation in both endothelial cell types. Second, valvular and arterial endothelial cells were both prone to calcification, but by different means. Valvular endothelial cells expressed genes involved in chondrogenic differentiation to a greater degree than arterial endothelial cells, but arterial endothelial cells expressed osteogenic genes to a greater degree. Shear stress was inhibitory of chondro/osteogenic differentiation in either cell type. These results correlate well with in vivo observations of valvular degeneration (30-32). Both inflammatory and calcific degeneration initiate on the fibrosa side of the valve, which is known to experience a lower, more oscillatory shear stress.

A separate microarray comparison by Simmons et al. profiled the transcriptional expression of valvular endothelium isolated directly from both sides of normal adult pig aortic valve leaflets (33). Over 584 transcripts were found to be differentially expressed in situ by the endothelium on the fibrosa versus ventricularis side of the valve. These transcriptional profiles identified for the first time globally distinct endothelial phenotypes on opposite sides of the aortic valve. Several over-represented biological classifications were identified among the differentially expressed genes. Of note, multiple inhibitors of cardiovascular calcification were significantly less expressed by the fibrosa-side endothelium. These data suggest that the fibrosa side of the valve, which is prone to lesion formation and exposed to recirculating flow, is permissive to calcification. However, this apparent vulnerability was balanced by an enhanced antioxidative gene expression profile on the fibrosa side, suggesting that the fibrosa of the normal valve may be protected against inflammation and lesion initiation.

While the side-dependent endothelial phenotypes correlated spatially with differences in the local hemodynamics, a comparison of the results of Simmons et al. and Butcher and colleagues showed the story to be more complex. Only 15% of the genes significantly changed in the Simmons study were also differentially expressed in the Butcher study. Of these genes, 64% of those expressed to a greater degree by ventricularis endothelial cells were also up-regulated by steady shear stress. Only 44% of the fibrosa side specific gene expression, however, was also down-regulated by shear stress in valvular endothelial cells. These results suggest that the side-specific differences in valvular endothelial gene expression may be due to differences in hemodynamic profiles on each side, but that there may also be unique aspects of valvular endothelial phenotypes from each side. It is suspected that the former case is true, but additional controlled experiments using shear profiles from both sides of the valve must be conducted in order to confirm this. It is also important to note that both of these studies were conducted by cross-hybridizing porcine RNA to human microarrays. This was done for two reasons: (i) porcine valves are easier to obtain while selecting for variables such

as age, gender, and disease state than human valve leaflets; and (ii) porcine microarrays were not available at the time of these studies. Because cDNA microarrays contain 200-400 bases in each probe, they are less sensitive than oligonucleotide arrays to species mismatch. In fact, Simmons et al. validated their microarray data by quantitative reverse transcription-polymerase chain reaction (RT-PCR), and found that validation rates for porcine RNA on human cDNA microarrays (83%) were similar to those obtained from human mRNA hybridized to human cDNA microarrays (33). Nonetheless, it is likely that some genes may have been missed. More importantly, however, is that newer microarray technologies can print entire genomes (~30,000 genes) on a single chip, which is almost three times more than the 12,000 genes available at the time of these studies. Therefore, future studies using whole-genome, species-specific arrays may yield even more insights into valvular endothelial function.

Taken together, these results highlight the fact that valvular endothelium is a distinct sub-phenotype with unique responses to hemodynamics. This most likely results from the fact that valvular endothelium is derived from specific regions of endocardial endothelium during embryonic development through a process quite unlike vasculogenesis (see reviews by Eisenberg and Markwald (34) and Drake (35)). The relatively non-contractile sensitivity of valvular endothelial cells may protect leaflets from undergoing length changes during routine vessel tone adjustment, thus ensuring unidirectional flow under varied cardiac demand. The transcriptional differences between valvular and vascular endothelial cells suggest that valvular endothelium is maintained by a different set of gene pathways, potentially regulated by the unique mechanical environment not experienced in the vasculature. Although the functional roles of valvular endothelium are only now being revealed, it is clear that these cells are important mediators of valve hemostasis.

Valvular interstitial cells respond to changes in pressure and strain

Very little is known about how valvular interstitial cells (VICs) contribute to the pathology of heart valves. The VICs are a heterogeneous and dynamic population of specific cell types that have many unique characteristics (36). At least three different cell phenotypes have been identified in mature heart valves (37), namely fibroblasts, smooth muscle cells, and myofibroblasts. Fibroblasts are responsible for remodeling the extracellular matrix (ECM), which in turn is vital for prolonged valve function and durability. In addition to synthesizing matrix molecules, fibroblasts produce many growth factors, cytokines and chemokines (38,39). Smooth muscle cells have been identified by electron microscopy, and occur either singly or arranged in thin bundles. The myofibroblasts show characteristics of both fibroblasts and smooth muscle cells; they display a highly plastic and diverse phenotype depending on tissue origin and whether the sample is normal or pathological. They also express both muscle and non-muscle regulatory and structural proteins, have contractile properties, and secrete ECM (36). In normal valves, there are relatively few myofibroblasts (40,41); however, during growth and disease, fibroblast-like VICs can switch to activated myofibroblasts that express α-smooth muscle actin (40,42,43). The factors that cause this phenotypic switch are poorly defined, but likely include transforming growth factor-b1 (44) and cytoskeletal tension generated by cell traction (44) or possibly exogenous forces.

During each cardiac cycle, the valve is subjected to shear stresses, bending stress, and tensile and compressive forces. The aortic valve has adapted to these conditions and is able to withstand this environment through constant renewal of the ECM (45). However, it is not fully known how cells respond to a change in the mechanical environment, and it is believed that adverse conditions could be responsible for the initiation and/or acceleration of aortic stenosis and calcification (5). During the cardiac cycle, the valve leaflets undergo loading and unloading, sustaining the pressure gradient in diastole but not in systole, when the pressure gradient is essentially zero (46). Under normotensive conditions, the pressure gradient across the aortic valve is 80 mmHg. An increase in the diastolic pressure to 90-99 mmHg is classified as stage I hypertension; when the diastolic pressure exceeds 100 mmHg the patient is said to have stage II hypertension. The pressure load applied to the leaflet causes an increase in leaflet length in the circumferential and radial directions, thus increasing the strain on the tissue. As strain is a function of pressure, hypertensive conditions result in increased leaflet strains. However, as the leaflet has a high elastic modulus, the radial strain increases by only 3.9% when the diastolic pressure increases from 60 to 200 mmHg (47). Additionally, the variation in load on the leaflet between diastole and systole causes a change in the circumferential curvature of the leaflet, producing additional stresses (46).

Very few studies have been conducted to investigate the response of VICs to altered pressure or strain. It has been shown that differences exist between cells isolated from the left and right sides of the heart. Cells isolated from the pulmonary valve are less stiff than those isolated from the aortic valve. The difference in stiffness correlates with the different transvalvular pressures (48). Interestingly, stiffness correlated with

J Heart Valve Dis
Vol. 17. No. 1
January 2008

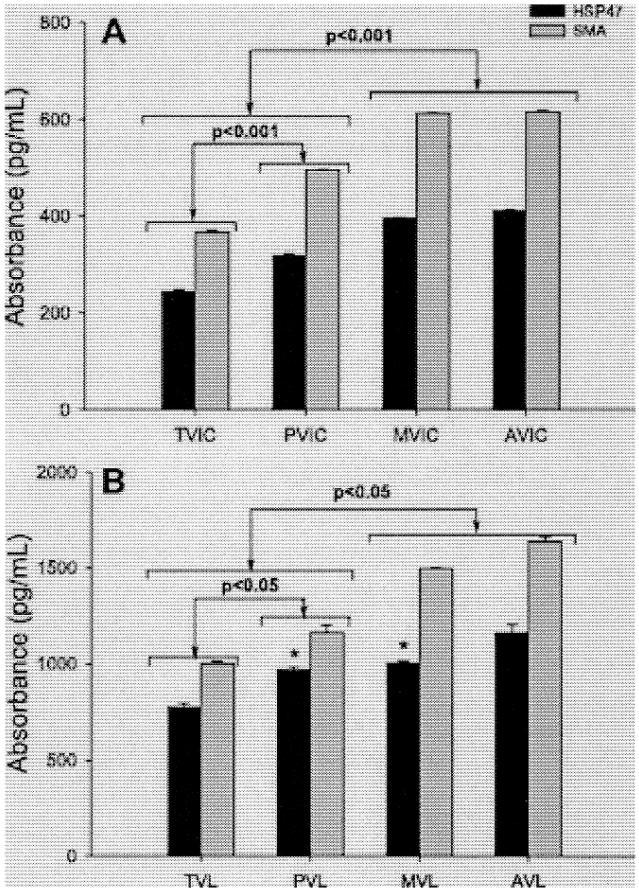

*Figure 3: ELISA results for smooth muscle a-actin (SMA) and heat shock protein 47 (HSP47). A) SMA and HSP47 absorbance from in vitro VIC populations. Significant differences were found between aortic VIC (AVIC) and mitral VIC (MVIC) populations compared with pulmonary VIC (PVIC) and tricuspid VIC (TVIC) populations ($p < 0.001$). Protein levels were significantly greater in PVICs than TVICs. B) SMA and HSP47 absorbance from in-situ VICs of explanted heart valve leaflets: tricuspid (TVL), pulmonary (PVL), mitral (MVL), and aortic (AVL). Note the difference in data range (y-axis) between in-situ and in vitro VICs. As with in vitro VICs, left-side valve protein levels were significantly greater ($p < 0.05$) than right-side valve levels, and PVL levels were greater than TVL levels. HSP47 was not statistically different between MVL and PVL. *$p = 0.786$. (Reprinted from Merryman WD, Youn I, Lukoff HD, et al. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis. Am J Physiol Heart Circ Physiol 2006;290:H224-H231 (48), with permission from the American Physiological Society.)*

α-smooth muscle cell actin content and collagen biosynthesis (see Fig. 3). Collagen synthesis has also been shown to be controlled by strain. Cells stretched at 10%, 14% and 20% show a significant ($p < 0.01$) increase in [^{3}H]-proline incorporation. Additionally, RT-PCR experiments demonstrated that 14% stretch up-regulated levels of mRNA for COL3A1 gene (type III collagen), but did not increase the expression of COL1A1 gene (type I collagen) in VICs. Hence, collagen synthesis by VICs is dependent upon the degree and duration of stretch (49). These studies confirm the earlier findings of Yoganathan and co-workers who, by using an ex-vivo culture system, showed that increased pressure caused an up-regulation in collagen synthesis (50,51). Furthermore, when pulmonary VICs were exposed to aortic pressure levels, as occurs following the Ross operation, collagen and sulfated glycosaminoglycan (sGAG) synthesis were increased significantly (52). This demonstrates that VICs are capable of remodeling the ECM in response to changes in the mechanical environment. However, this response may also indicate a pathological reaction. In atherosclerosis, activated smooth muscle cells contribute to the atheroma through the synthesis of the matrix proteins required for retention of lipoproteins, and by the production of monocyte chemoattractant protein-1 (MCP-1) and VCAM-1 (53). In the aortic valve, stenosis of a previously healthy valve is thought to be analogous to atherosclerosis, with an abnormal lipid profile and hypertension possibly playing an important role (54). Population-based studies show strong correlations between aortic stenosis/calcification and hypertension (31,55-58). This theory has been supported further by the fact that elevated cyclic pressure is a potent stimulus for the up-regulation of VCAM-1 mRNA expression and the down-regulation of osteopontin (OPN) mRNA expression. Porcine VICs were exposed to cyclic pressures representing normotensive, stage I hypertensive and stage II hypertensive conditions for 2 hours. At the conclusion of the experiment, mRNA expression was measured using semi-quantitative RT-PCR, whereupon a proportional increase in VCAM-1 mRNA expression was observed, relative to static controls (59). VCAMs are responsible for adhesion, migration and the accumulation of monocytes and T cells. OPN, which was shown to be significantly down-regulated under simulated hypertensive conditions, inhibits mineralization, suggesting that it has a protective role in the formation of ectopic calcification (60-63).Thus, OPN down-regulation may indicate that it is not involved in - or is not necessary for - inflammation, but that the tissue will become more susceptible to ectopic calcification.

in vitro co-culture models of heart valves

Several studies conducted by the Mayer group have attempted to engineer heart valves in vitro for in vivo implantation, with encouraging - but sub-optimal - results. These authors used cells isolated from a variety

of accessible blood vessels and mesenchymal stem cells to populate biodegradable polyglycolic acid (PGA) (64-67) and poly-L-lactide (PLLA) (68) polymers. Thus, it was shown that mechanical stimulation regimens in vitro can condition these cells to remodel the scaffold and secrete ECM (64). In earlier studies, valves were implanted for up to eight months in juvenile sheep animal models, and although cell marker expression and matrix structure resembled that of native valves, significant transvalvular pressure gradients persisted (42). This limitation may be due to the lack of valvular specific phenotypes used in these studies and/or a lack of understanding of how mechanical forces interact with valvular cells. Nonetheless, in vitro tissue models represent an excellent means to understand cellular interactions in well-controlled environments.

Very few studies have been performed to investigate valvular cell interactions. Early studies by Lester and Gotlieb using mitral valve leaflet organ cultures have shown that both endothelial and interstitial cells participate in tissue repair due to small endothelial denudations (69). This repair process involves changes in polarity and migration of the endothelial cells, and activation (increased α-smooth muscle actin expression) and proliferation of the interstitial cells. This research group went on to show that interstitial cell activation and migration in wounds was mediated by inducible nitric oxide synthase (iNOS) and fibroblast growth factor-2 (FGF-2) (70).

As a first approximation, the complex trilaminar valve leaflet can be modeled as a three-dimensional (3-D) matrix filled with VICs, and the surface lined with endothelium. Recently, aortic endothelial and interstitial cells were cultured together in type I collagen scaffolds and cultured statically in vitro. The morphology of the valve cells, production of proteoglycans and organization of the ECM resembled that of normal heart valve leaflets (71). Further enhancement of this system was reported by Flanagan et al., who developed a collagen-chondroitin sulfate (CS) hydrogel (72). Supplementation with CS resulted in increased interstitial cell matrix synthesis, more confluent valvular endothelial monolayers, and more prominent endothelial basement membrane.

Recently, Butcher and Nerem used similar type I collagen hydrogels to investigate the role of hemodynamics in the valvular endothelial mediation of interstitial cell response (73). VICs inside a collagen hydrogel compacted the matrix for six days, after which the lumenal surface was seeded with valvular endothelial cells for an additional 48 hours. A schematic diagram of the experimental model is presented in Figure 4. These constructs were then exposed to 20 dynes/cm^2 steady shear stress for up to four days, with static cultures and non-endothelialized constructs serving as controls. These constructs were assessed for cell, protein, and glycosaminoglycan (GAG) content, as well as interstitial cell activation. Without endothelial cells, interstitial cells proliferated in collagen gels, and this proliferation was enhanced with shear stress. Interstitial cell constructs maintained protein content but lost GAGs over time, and shear stress enhanced this loss. An activated interstitial cell phenotype was present in the constructs for the entire period, as evidenced by the expression of α-smooth muscle actin. These results are similar to the observations reported by Lester et al. in the denuded mitral valve organ culture model (74), suggesting that these interstitial cell-only constructs represent an active remodeling valve. When these constructs were seeded with valvular endothelial cells, distinct differences were noted in interstitial cell response. Interstitial cell proliferation was stabilized, protein content increased, and GAG loss was reduced. Exposure to shear stress enhanced these effects of valvular endothelial cells. Additionally, the interstitial expression of α-smooth muscle actin was markedly reduced, suggesting that their phenotype was more quiescent. These results correlated well with published observations of VIC phenotype and matrix synthesis, suggesting that the co-culture model under shear stress is a more accurate physiological model of a valve leaflet (42). Only the effects of steady shear stress were assessed in this study, and valvular specific hemodynamic stimulation may reveal other important interactions between these two cells, which

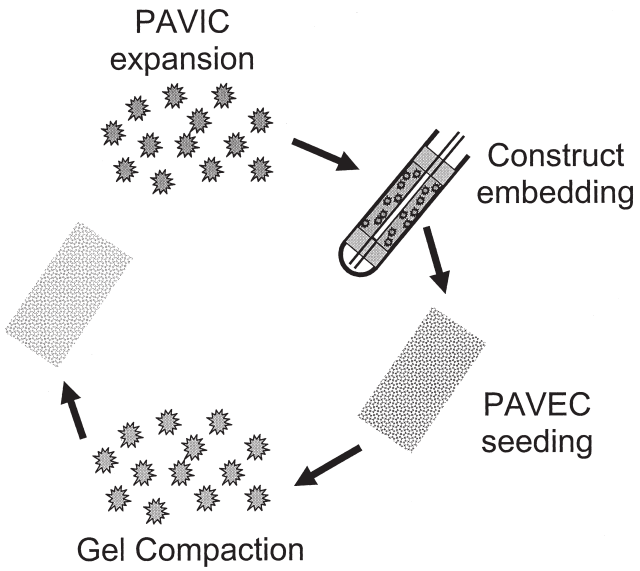

Figure 4: Schematic representation of valvular co-culture model creation. PAVEC: Pulmonary artery vascular endothelial cell; PAVIC: Pulmonary artery vascular interstitial cell.

J Heart Valve Dis
Vol. 17. No. 1
January 2008

is the focus of several ongoing studies.

One additional cell type that is little discussed, but may be critically important to include in engineered heart valves and/or co-culture models, is that of neurons. The presence of nerve cells in heart valve leaflets (75,76) is intriguing. Several studies performed by Chester et al. have demonstrated the capacity of heart valve leaflets to contract to a variety of neurotransmitters including serotonin (5-hydroxytryptamine) (16). During valve opening, the leaflets form a triangular orifice by decreasing leaflet length by 16% during systole, implying that they are under tension (77). Contraction in the leaflet provides the necessary tension and contributes to correct valve function. It is not known whether the contraction is caused by serotonin released from the small and regionally variant nerve supply, or from circulating vasoactive agents. Nerve branches are also often closely associated with the branching patterns of blood vessels, and may regulate the development of the vascular tree (78). Vessels are found predominantly in the basal third of valve cusps, and extend in from the commissures almost to the level of the free edge (79). This suggests that oxygen diffusion across the cusp surface is not sufficient to meet the metabolic demands of the tissue. Another study has predicted that the probable maximum oxygen diffusion distance for valve tissue would be about 0.2 mm (80). This was consistent with physical findings, implying that central tissue anoxia is avoided by the capillary bed. Hence, the presence of nerve cells may aid in the in vivo vascularization of tissue-engineered valves, thus preventing anoxic regions and a necrotic core. Future studies involving neural-interstitial cell co-cultures would be very helpful to elucidate these mechanisms and responses, and indeed this is the focus of current research.

Ex-vivo organ culture

The ECM and cellular phenotype are determinants of the response of cardiovascular cells to mechanical stimuli (81). Hence, the biological response of endothelial or interstitial cells cultured in vitro may not faithfully represent the events that occur in vivo. Animal models have the greatest physiological relevance, but they cannot distinguish between mechanical effects and other factors such as nervous stimuli and the hormonal and metabolic environment. Several research groups have turned to organ culture systems that maintain cells in their native 3-D milieu while simultaneously providing controlled mechanical conditions. The bioreactors used to culture the valvular tissue vary in complexity; the simplest systems expose samples to single mechanical forces such as shear stress or pressure, while the most sophisticated bioreactors are capable of culturing the entire valve, including the aortic root, and providing the complete physical environment.

The flow environment surrounding aortic valve leaflets has been studied using two different systems. Porcine cusps were exposed to steady and pulsatile flow for 48 hours in a tubular device, while a parallel plate system was used to assess the effect of steady shear stress (82). In the tubular system, leaflets were bisected, with half being used as a test sample and the other half as the experimental sample. The experimental group was attached to a straight tube in a staggered fashion (to minimize flow disturbances) by suturing around the attached edge. The tube was mounted in a chamber so that fluid flow would follow the in vivo direction. A peristaltic pump and a centrifugal pump were used to generate pulsatile and steady flows, respectively. The parallel plate flow chamber had six wells recessed into the bottom plate; the leaflets were placed into the wells so that they were flush with the flow surface, in order to ensure that there was no disturbance in the fluid flow. The channel height and flow rate could be varied to produce different shear stresses. Protein, GAG and DNA synthesis remained at native levels after exposure to flow, but increased during static incubation. The modulation of synthetic activity was attributed to the presence of a shear stress on the leaflet surface, which may be transmitted to cells within the leaflet matrix through tensile forces (82).

In a similar fashion, the effect of constant or pulsatile pressure on valve leaflets has been determined using a custom-designed pressure chamber (51). Valve cusps were placed in a six-well plate and placed into the chamber, which was then sealed and pressurized with 5% CO_2 plus air. Cyclic compression was made possible by adding a flexible membrane to the chamber; compressed air was fed onto the membrane, pushing it down and therefore increasing the pressure in the chamber. By controlling the compressed air with a solenoid valve, the supply could be turned on and off at a frequency of 1.167 Hz (50). These studies showed that collagen synthesis was increased under elevated pressure conditions, and that GAG synthesis was upregulated under simulated hypertensive cyclic pressure conditions (50,51).

In addition to shear stress and compression, valve leaflets undergo cyclic flexure during the cardiac cycle. A novel bioreactor was devised by Engelmayr et al. with the intention of determining if cyclic flexure had an affect on the stiffness of tissue-engineered valve leaflets. The bioreactor consisted of two identical chambers, each containing six culture wells. Situated within each well were four stainless-steel 'stationary posts' arranged orthogonally around a central channel in the floor of the culture well. Hence, a total of 12 rec-

tangular samples could be accommodated in the device, with each sample being positioned between the four stationary posts, orthogonal to the central channel (83). The system has also been used to study the way in which interstitial cell contraction can alter leaflet stiffness. Circumferential strips of porcine aortic valve leaflets were tested for flexural stiffness under normal, fully contracted and contraction-inhibited conditions. Cellular contraction resulted in a 5% increase in stiffness when flexed with the natural curvature of the leaflet, and a 48% increase in stiffness against the natural curvature. When leaflet contraction was completely inhibited, the leaflet stiffness showed a significant decrease (19).

The above-mentioned systems have been useful in determining the response of valves to specific mechanical forces. However, valves experience an array of mechanical forces in vivo. Additionally, valves cultured in shear stress or pressure systems showed a decline in α-smooth muscle actin, suggesting that other physical forces are required to maintain the cell phenotype. Consequently, flow loop bioreactors have been developed to culture valves in a complete hemodynamic environment. These have evolved from left ventricle (LV) simulators (84) and bioreactors for tissue-engineered heart valves (64,85). Unlike LV simulators, flow loop bioreactors maintain cell viability and sterility. In contrast to tissue engineering bioreactors, native valves are used as opposed to cell-seeded scaffolds. Furthermore, the mechanical conditions resemble physiological and patho-physiological conditions more closely. Hildebrand et al. designed a system that utilized a computer-controlled closed-loop feedback system to manage, automatically, the mean pressure, mean flow rate, driving frequency, and shape of the pulsatile pressure waveform. The device was able to simulate normal and abnormal physiological hemodynamic conditions while maintaining heart valves in a sterile atmosphere for two weeks (86). The system demonstrated a level of hydrodynamic control exceeding that of other published pulsatile heart valve bioreactors, making it possible to perform a controlled study on the effects of dynamic pulsatile culture conditions on developing cardiovascular structures. This system therefore has great potential for tissue-engineered heart valves and, due to the versatility of the pressure control, could be used to study valve response to the Ross procedure. A subsequent report by Warnock et al. described a similar system that, in addition to controlling the mechanical conditions and providing sterility, assessed the physico-chemical environment (87). The bioreactor was fitted with silicone tubing to allow for gas transfer into the culture medium. The length of tubing and the gas flow rate were calculated to ensure that the oxygen requirements of the valve were met. A biological evaluation of the system showed that it could maintain all native characteristics of aortic valves when run under normal physiological conditions. There were no significant changes in apoptosis or cell proliferation, ECM synthesis, or leaflet morphology. In contrast to the pressure and shear stress devices, α-smooth muscle actin expression was maintained in the organ culture flow loop (88). The biological stability allows for further studies to be conducted on native heart valves subjected to altered mechanical conditions, such as those that mimic hypertension. It can also be used to investigate humoral factors implicated in valve disease, including angiotensin II or 5-hydroxytryptamine.

Future studies

Research into the mechanobiology of heart valves has only begun to scratch the surface of a very complex and intriguing issue, and consequently there are many questions that need to be addressed. First, most studies to date have only examined the effect of a single, isolated mechanical force on valve biology. However, the valve is simultaneously exposed to multiple types of mechanical forces in vivo. Specific forces such as shear stress or strain may be responsible for specific responses, but their combined effect may be either synergistic or antagonistic. The use of ex-vivo organ culture systems will be invaluable in helping to identify the combined effects of mechanical forces, without the need for animal studies. However, to truly understand valve mechanobiology, animal studies will be required to allow for the circulating humoral factors (hormones, growth factors, etc.) that undoubtedly contribute to the biological response. Second, current biological knowledge is limited. High-throughput technologies, such as DNA microarrays to examine differential gene expression, or mass spectrometry to examine protein expression, can comprehensively characterize cell phenotypic changes and regulatory pathways involved in valve function and dysfunction. The mining and dissection of these genomic and proteomic data by bioinformatics may lead to multi-scale models of regulatory processes and signaling pathways from which a more complete understanding of valve mechanobiology may emerge. Finally, the interaction between different cell types in the valve should not be overlooked.

This review has outlined initial studies that have examined the relationship between the endothelium and the interstitium. As alluded to earlier, this issue should be expanded to include nerve cells, and will have significant impact in understanding valve function and regeneration and in designing a functional tissue-engineered valve. Recommendations for future

J Heart Valve Dis
Vol. 17. No. 1
January 2008

Table I: Recommendations for future studies in valve mechanobiology.

Area of future research	Issues
Mechanical forces	• What are the combined effects of mechanical forces? • How do circulating humoral factors influence/interact with altered mechanical forces?
Biological response	• How is gene expression changed by mechanical forces? • How is protein expression changed by mechanical forces? • Which signaling pathways are regulated by mechanical forces? • Are these changes beneficial or detrimental to the valve? • Can pharmacological or molecular therapies be used to inhibit detrimental changes to valve biology?
Cell interactions	• What is the biological relationship between the endothelium and the interstitium, and how is it influenced by mechanical forces? • What role do nerve cells play in valve function and mechanobiology?

studies are summarized in Table I.

Summary

Although the mechanical environment surrounding the aortic valve has been investigated for several decades, these studies have focused predominantly on the mechanical function and potential modes of failure of mechanical and bioprosthetic valves. It is increasingly evident that mechanics are also critically important to the biological function of the native aortic valve. Valvular endothelial and interstitial cells respond to a variety of mechanical stimuli, often with unique responses that are putatively relevant to valve remodeling and pathogenesis. On the basis of the findings reviewed herein, it is apparent that altered mechanical forces play a pivotal role in the development of a pro-inflammatory state within the valve. The resulting chronic inflammation may alter cell phenotypes which, in turn, may lead to ectopic calcification, aortic sclerosis, and stenosis. An improved understanding of the role of mechanics in regulating cellular and molecular processes in the valve is likely to yield important insights into the pathophysiology of aortic valve disease. Future studies should determine how altered mechanics can stimulate cell-signaling pathways that cause endothelial cell activation or changes in interstitial cell phenotype. This goal will be best achieved through interdisciplinary efforts that closely link valve mechanics with valve biology, and through continued investigation of the role that mechanics play not only in valve disease but also in valve development and regeneration. This knowledge can assist in the formulation of pharmacological and/or gene therapies that inhibit the inflammatory reaction and prevent cell phenotype changes. In time, such treatments could alleviate the need for open-heart surgery, which is currently the only option for patients with valve disease. Furthermore, insight into the relationship between mechanics and valve growth and regeneration will be critical for the development of a functional tissue-engineered valve.

References

1. Schoen FJ, Levy RJ. Founder's Award, 25th Annual Meeting of the Society for Biomaterials, perspectives. Providence, RI, April 28-May 2, 1999. Tissue heart valves: Current challenges and future research perspectives. J Biomed Mater Res 1999;47:439-465
2. Yoganathan AP, Woo YR, Sung HW, Jones M. Advances in prosthetic heart valves: Fluid mechanics of aortic valve designs. J Biomater Appl 1988;2:579-614
3. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. Arterioscler Thromb Vasc Biol 1996;16:523-532
4. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. Circulation 1994;90:844-853
5. Thubrikar MJ, Aouad J, Nolan SP. Patterns of calcific deposits in operatively excised stenotic or purely regurgitant aortic valves and their relation to mechanical stress. Am J Cardiol 1986;58:304-308
6. Freeman RV, Otto CM. Spectrum of calcific aortic valve disease: Pathogenesis, disease progression, and treatment strategies. Circulation 2005;111:3316-3326
7. Robicsek F, Thubrikar MJ, Fokin AA. Cause of degenerative disease of the trileaflet aortic valve: Review of subject and presentation of a new theory. Ann Thorac Surg 2002;73:1346-1354
8. Otto CM. Calcification of bicuspid aortic valves. Heart 2002;88:321-322

9. Olsson M, Thyberg J, Nilsson J. Presence of oxidized low density lipoprotein in nonrheumatic stenotic aortic valves. Arterioscler Thromb Vasc Biol 1999;19:1218-1222
10. O'Brien KD. Pathogenesis of calcific aortic valve disease: A disease process comes of age (and a good deal more). Arterioscler Thromb Vasc Biol 2006;26:1721-1728
11. Rajamannan NM, Subramaniam M, Rickard D, et al. Human aortic valve calcification is associated with an osteoblast phenotype. Circulation 2003;107:2181-2184
12. Rajamannan NM, Subramaniam M, Springett M, et al. Atorvastatin inhibits hypercholesterolemia-induced cellular proliferation and bone matrix production in the rabbit aortic valve. Circulation 2002;105:2660-2665
13. Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. J Biomech Eng 1985;107:341-347
14. Levesque MJ, Liepsch D, Moravec S, Nerem RM. Correlation of endothelial cell shape and wall shear stress in a stenosed dog aorta. Arteriosclerosis 1986;6:220-229
15. Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. FASEB J 1989;3:2007-2018
16. Chester AH, Misfeld M, Yacoub MH. Receptor-mediated contraction of aortic valve leaflets. J Heart Valve Dis 2000;9:250-255
17. Kershaw JD, Misfeld M, Sievers HH, Yacoub MH, Chester AH. Specific regional and directional contractile responses of aortic cusp tissue. J Heart Valve Dis 2004;13:798-803
18. Deck JD, Thubrikar MJ, Schneider PJ, Nolan SP. Structure, stress, and tissue repair in aortic valve leaflets. Cardiovasc Res 1988;22:7-16
19. Merryman WD, Huang HY, Schoen FJ, Sacks MS. The effects of cellular contraction on aortic valve leaflet flexural stiffness. J Biomech 2006;39:88-96
20. Vesely I. Aortic root dilation prior to valve opening explained by passive hemodynamics. J Heart Valve Dis 2000;9:16-20
21. Yacoub MH, Kilner PJ, Birks EJ, Misfeld M. The aortic outflow and root: A tale of dynamism and crosstalk. Ann Thorac Surg 1999;68(3 Suppl.):S37-S43
22. Karau KL, Krenz GS, Dawson CA. Branching exponent heterogeneity and wall shear stress distribution in vascular trees. Am J Physiol Heart Circ Physiol 2001;280:H1256-H1263
23. Nandy S, Tarbell JM. Flush mounted hot film anemometer measurement of wall shear stress distal to a tri-leaflet valve for Newtonian and non-Newtonian blood analog fluids. Biorheology 1987;24:483-500
24. Weston MW, LaBorde DV, Yoganathan AP. Estimation of the shear stress on the surface of an aortic valve leaflet. Ann Biomed Eng 1999;27:572-579
25. Kilner PJ, Yang GZ, Wilkes AJ, Mohiaddin RH, Firmin DN, Yacoub MH. Asymmetric redirection of flow through the heart. Nature 2000;404:759-761
26. Deck JD. Endothelial cell orientation on aortic valve leaflets. Cardiovasc Res 1986;20:760-767
27. Muller AM, Cronen C, Kupferwasser LI, Oelert H, Muller KM, Kirkpatrick CJ. Expression of endothelial cell adhesion molecules on heart valves: Up-regulation in degeneration as well as acute endocarditis. J Pathol 2000;191:54-60
28. Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. Arterioscler Thromb Vasc Biol 2004;24:1429-1434
29. Butcher JT, Tressel S, Johnson T, et al. Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences: Influence of shear stress. Arterioscler Thromb Vasc Biol 2005;26:69-77
30. Mohler ER, III. Are atherosclerotic processes involved in aortic-valve calcification? Lancet 2000;356:524-525
31. Mohler ER, III, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. Circulation 2001;103:1522-1528
32. Mohler ER, III. Mechanisms of aortic valve calcification. Am J Cardiol 2004;94:1396-1402, A6
33. Simmons CA, Grant GR, Manduchi E, Davies PF. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res 2005;96:792-799
34. Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. Circ Res 1995;77:1-6
35. Drake CJ. Embryonic and adult vasculogenesis. Birth Defects Res C Embryo Today 2003;69:73-82
36. Taylor PM, Batten P, Brand NJ, Thomas PS, Yacoub MH. The cardiac valve interstitial cell. Int J Biochem Cell Biol 2003;35:113-118
37. Mulholland DL, Gotlieb AI. Cell biology of valvular interstitial cells. Can J Cardiol 1996;12:231-236
38. Sappino AP, Schurch W, Gabbiani G. Differentiation repertoire of fibroblastic cells: Expression of cytoskeletal proteins as marker of phenotypic modulations. Lab Invest 1990;63:144-161
39. Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells. Synthesis of

chemokines and regulation of inflammation. Am J Pathol 1997;151:317-322
40. Rabkin E, Farber M, Aikawa M, Schoen FJ. Dynamic and reversible changes of interstitial cell phenotype during remodeling of cardiac valves. J Heart Valve Dis 2004;13:841-847
41. Yperman J, De Visscher G, Holvoet P, Flameng W. Molecular and functional characterization of ovine cardiac valve-derived interstitial cells in primary isolates and cultures. Tissue Eng 2004;10:1368-1375
42. Rabkin E, Hoerstrup SP, Aikawa M, Mayer JE, Jr., Schoen FJ. Evolution of cell phenotype and extracellular matrix in tissue-engineered heart valves during in vitro maturation and in vivo remodeling. J Heart Valve Dis 2002;11:308-314
43. Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation 2001;104:2525-2532
44. Walker GA, Masters KS, Shah DN, Anseth KS, Leinwand LA. Valvular myofibroblast activation by transforming growth factor-beta: Implications for pathological extracellular matrix remodeling in heart valve disease. Circ Res 2004;95:253-260
45. Schneider PJ, Deck JD. Tissue and cell renewal in the natural aortic valve of rats: An autoradiographic study. Cardiovasc Res 1981;15:181-189
46. Thubrikar MJ. Mechanical stresses in the aortic valve. The Aortic Valve. CRC Press, Inc., Boca Raton, FL, 1990:97-127
47. Thubrikar MJ, Aouad J, Nolan SP. Comparison of the in vivo and in vitro mechanical properties of aortic valve leaflets. J Thorac Cardiovasc Surg 1986;92:29-36
48. Merryman WD, Youn I, Lukoff HD, et al. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: Implications for collagen biosynthesis. Am J Physiol Heart Circ Physiol 2006;290:H224-H231
49. Ku CH, Johnson PH, Batten P, et al. Collagen synthesis by mesenchymal stem cells and aortic valve interstitial cells in response to mechanical stretch. Cardiovasc Res 2006;71:548-556
50. Xing Y, Warnock JN, He Z, Hilbert SL, Yoganathan AP. Cyclic Pressure affects the biological properties of porcine aortic valve leaflets in a magnitude and frequency dependent manner. Ann Biomed Eng 2004;32:1461-1470
51. Xing Y, He Z, Warnock JN, Hilbert SL, Yoganathan AP. Effects of constant static pressure on the biological properties of porcine aortic valve leaflets. Ann Biomed Eng 2004;32:555-562
52. Ikhumetse JD, Konduri S, Warnock JN, Xing Y, Yoganathan AP. Cyclic aortic pressure affects the biological properties of porcine pulmonary valve leaflets. J Heart Valve Dis 2006;15:295-302
53. Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: New perspectives and therapeutic strategies. Nat Med 2002;8:1249-1256
54. Yacoub MH, Cohn LH. Novel approaches to cardiac valve repair: From structure to function: Part I. Circulation 2004;109:942-950
55. Agmon Y, Khandheria BK, Meissner I, et al. Aortic valve sclerosis and aortic atherosclerosis: Different manifestations of the same disease? Insights from a population-based study. J Am Coll Cardiol 2001;38:827-834
56. Agno FS, Chinali M, Bella JN, et al. Aortic valve sclerosis is associated with preclinical cardiovascular disease in hypertensive adults: The Hypertension Genetic Epidemiology Network study. J Hypertens 2005;23:867-873
57. Boon A, Cheri, E, Lodder J, Kessels F. Cardiac valve calcification: Characteristics of patients with calcification of the mitral annulus or aortic valve. Heart 1997;78:472-474
58. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. J Am Coll Cardiol 1997;29:630-634
59. Warnock JN, Burgess SC, Shack A, Yoganathan AP. Differential immediate-early gene responses to elevated pressure in porcine aortic valve interstitial cells. J Heart Valve Dis 2006;15:34-42
60. Jono S, Peinado C, Giachelli CM. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. J Biol Chem 2000;275:20197-20203
61. Speer MY, McKee MD, Guldberg RE, et al. Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: Evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. J Exp Med 2002;196:1047-1055
62. Steitz SA, Speer MY, McKee MD, et al. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. Am J Pathol 2002;161:2035-2046
63. Wada T, McKee MD, Steitz S, Giachelli CM. Calcification of vascular smooth muscle cell cultures: Inhibition by osteopontin. Circ Res 1999;84:166-178
64. Hoerstrup SP, Sodian R, Sperling JS, Vacanti JP, Mayer JE, Jr. New pulsatile bioreactor for in vitro formation of tissue engineered heart valves. Tissue Eng 2000;6:75-79
65. Hoerstrup SP, Sodian R, Daebritz S, et al. Functional living trileaflet heart valves grown in vitro.

Circulation 2000;102(19 Suppl.3):III44-III49
66. Perry TE, Kaushal S, Sutherland FW, et al. Thoracic Surgery Directors Association Award. Bone marrow as a cell source for tissue engineering heart valves. Ann Thorac Surg 2003;75:761-767
67. Shinoka T, Ma PX, Shum-Tim D, et al. Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model. Circulation 1996;94(9 Suppl.):II164-II168
68. Sutherland FW, Perry TE, Yu Y, et al. From stem cells to viable autologous semilunar heart valve. Circulation 2005;111:2783-2791
69. Lester WM, Gotlieb AI. In vitro repair of the wounded porcine mitral valve. Circ Res 1988;62:833-845
70. Durbin A, Nadir NA, Rosenthal A, Gotlieb AI. Nitric oxide promotes in vitro interstitial cell heart valve repair. Cardiovasc Pathol 2005;14:12-18
71. Rothenburger M, Volker W, Vischer JP, et al. Tissue engineering of heart valves: Formation of a three-dimensional tissue using porcine heart valve cells. Am Soc Artif Intern Organs J 2002;48:586-591
72. Flanagan TC, Wilkins B, Black A, Jockenhoevel S, Smith TJ, Pandit AS. A collagen-glycosaminoglycan co-culture model for heart valve tissue engineering applications. Biomaterials 2006;27:2233-2246
73. Butcher JT, Nerem RM. Valvular endothelial cells regulate the phenotype of interstitial cells in co-culture: Effects of steady shear stress. Tissue Eng 2006;12:905-915
74. Lester WM, Damji AA, Tanaka M, Gedeon I. Bovine mitral valve organ culture: Role of interstitial cells in repair of valvular injury. J Mol Cell Cardiol 1992;24:43-53
75. Marron K, Yacoub MH, Polak JM, et al. Innervation of human atrioventricular and arterial valves. Circulation 1996;94:368-375
76. Rozanski GJ, Jalife J. Automaticity in atrioventricular valve leaflets of rabbit heart. Am J Physiol 1986;250(3 Pt.2):H397-H406
77. Brewer RJ, Deck JD, Capati B, Nolan SP. The dynamic aortic root. Its role in aortic valve function. J Thorac Cardiovasc Surg 1976;72:413-417
78. Miller G. Developmental biology. Nerves tell arteries to make like a tree. Science 2002;296:2121-2123
79. Weind KL, Ellis CG, Boughner DR. The aortic valve blood supply. J Heart Valve Dis 2000;9:1-7
80. Weind KL, Ellis CG, Boughner DR. Aortic valve cusp vessel density: Relationship with tissue thickness. J Thorac Cardiovasc Surg 2002;123:333-340
81. Lehoux S, Tedgui A. Cellular mechanics and gene expression in blood vessels. J Biomech 2003;36:631-643
82. Weston MW, Yoganathan AP. Biosynthetic activity in heart valve leaflets in response to in vitro flow environments. Ann Biomed Eng 2001;29:752-763
83. Engelmayr GC, Jr., Hildebrand DK, Sutherland FW, Mayer JE, Jr., Sacks MS. A novel bioreactor for the dynamic flexural stimulation of tissue engineered heart valve biomaterials. Biomaterials 2003;24:2523-2532
84. Jensen MO, Lemmon JD, Gessaghi VC, Conrad CP, Levine RA, Yoganathan AP. Harvested porcine mitral xenograft fixation: Impact on fluid dynamic performance. J Heart Valve Dis 2001;10:111-124
85. Dumont K, Yperman J, Verbeken E, et al. Design of a new pulsatile bioreactor for tissue engineered aortic heart valve formation. Artif Organs 2002;26:710-714
86. Hildebrand DK, Wu ZJ, Mayer JE, Jr., Sacks MS. Design and hydrodynamic evaluation of a novel pulsatile bioreactor for biologically active heart valves. Ann Biomed Eng 2004;32:1039-1049
87. Warnock JN, Konduri S, He Z, Yoganathan AP. Design of a sterile organ culture system for the ex vivo study of aortic heart valves. J Biomech Eng 2005;127:857-861
88. Konduri S, Xing Y, Warnock JN, He Z, Yoganathan AP. normal physiological conditions maintain the biological characteristics of porcine aortic heart valves: An ex vivo organ culture study. Ann Biomed Eng 2005;33:1158-1166