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THE ROLE OF CELL BIOLOGY AND LEAFLET REMODELING IN THE PROGRESSION OF HEART VALVE DISEASE

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Introduction

Heart valves are complex tri-layered structures that ensure the unidirectional flow of blood. Scientists are actively investigating how characteristics of the two major cell types, valvular endothelial cells (VECs) and valvular interstitial cells (VICs), and their mechanical relationships with the valvular extracellular matrix promote structural integrity and age-related remodeling. Abnormal changes in VECs, VICs, and the extracellular matrix at the molecular level lead to gross tissue malformations and dysfunction. This review addresses current advances in the field of valve biology, mechanisms underlying valvular leaflet remodeling, and common pathological manifestations. Improving our understanding of heart valve biology, the impact of cardiovascular drugs, and remodeling changes will be critical to the development of novel therapies for heart valve diseases.

Overview

Heart valve diseases present a significant public health burden, with more than 100,000 Americans undergoing valve-related surgeries each year.[1] The heart valves are dynamic tissues that change with age according to both biological and mechanical stimuli. Advancements in tissue culture, computational biomechanics, and experimental systems that mimic *in vivo* conditions have enabled greater insight into normal and abnormal valve function from the organ to the cellular level.[2] These studies complement the growing field of valve biology, which is considered critical to our understanding of the function and pathology of heart valves. In this review, we highlight key concepts in valve biology and discuss leaflet remodeling within the context of normal turnover and disease. Finally, we will briefly discuss the implications of tissue engineering as a promising new strategy for heart valve diseases.

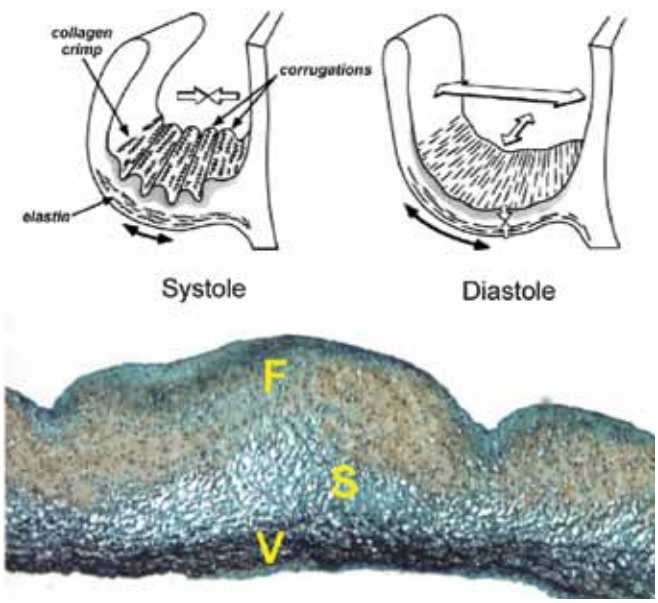

Figure 1. Layers of the aortic valve.
Above: Illustration of the histological layers of the aortic valve leaflet in the open valve (left) and closed valve (right) configuration. (Reprinted from Schoen FJ. Aortic valve structure-function correlations: role of elastic fibers no longer a stretch of the imagination. J Heart Valve Dis. 1997;6:2, with permission from ICR Publishers). Below: Movat stained aortic valve layers with yellow collagen (fibrosa), blue GAGs (spongiosa), and black elastin (ventricularis).

Microanatomy

The four heart valves (aortic, mitral, pulmonary, and tricuspid) are responsible for maintaining the unidirectional flow of blood. All valves consists of two or more leaflets, which are curved, flexible, layered structures well suited to stretch and bear cyclic high-pressure

Table 1. Extracellular matrix components of heart valves.

Component	Selected Types	Primary Location	Functional Role
Collagen	• Collagen I • Collagen III • Collagen V	Fibrosa layer	Imparts tensile and mechanical strength
Elastin		Ventricularis layer	Provides extensibility
Proteoglycans and glycosaminoglycans	• Chondroitin/Dermatan sulfate • Decorin • Versican • Hyaluronic acid	Spongiosa layer	Resists compression, provides hydration, regulates formation of other ECM

loads; the mitral and tricuspid valves also contain numerous chordae tendineae. In turn, the leaflet is composed of three distinct layers: the collagen-rich fibrosa, the glycosaminoglycan-rich spongiosa, and an elastin-rich layer (Table 1, Figure 1). The dominant layer of the leaflets is the fibrosa, which is located towards the outflow side and is a thick corrugated structure that mainly contains highly aligned collagen fibers. On the other side of the leaflet (the inflow side) is a thinner layer primarily containing elastic fibers. This layer is termed the *ventricularis* in the aortic and pulmonary valves and the *atrialis* in atrioventricular valves. The circumferentially aligned collagen fibers in the fibrosa are responsible for bearing most of the transvalvular pressure load when the valve is closed, whereas the elastic fibers in the opposite layer permit great extension when the valve is closing and then recoil to return the leaflet to its undeformed state when the valve is opening. These two layers are separated by a middle layer termed the *spongiosa*, which is rich in proteoglycans (PGs) and glycosaminoglycans (GAGs). The presence of highly hydrated GAGs and PGs in the spongiosa is speculated to provide compression resistance and to lubricate the outer layers as they shear and deform relative to each other during leaflet bending and pressurization.[3] The unique layered assembly imparts considerable fatigue resistance to the overall structure and ensures proper valve function. Consequently, the disruption of the extracellular matrix (ECM) and the layered structure can negatively impact leaflet geometry and material behavior and lead to valve dysfunction. Furthermore, the composition and thickness of each layer is known to change with load, age, and pathology.[4]

In normal adult cardiac valves, microvasculature is minimal except in the proximal third.[5] In contrast, pathological valves (with the exception of myxomatous mitral valves) exhibit significant neovascularization, although the mechanism for this is not completely clear. Normal valves are also innervated, although this innervation is greatly reduced with age.[6] These nerves are suspected to control the release of vasoactive factors such as nitric oxide by valvular endothelial cells through the secretion of neurochemicals.

Cell Biology

Cell groups found in cardiac valves include smooth muscle, cardiac muscle, valvular interstitial cells (VICs), and valvular endothelial cells (VECs); this review will focus on VICs and VECs (Figure 2). The subendothelial VICs are the most abundant cell type in valves and can be found throughout the three layers. These

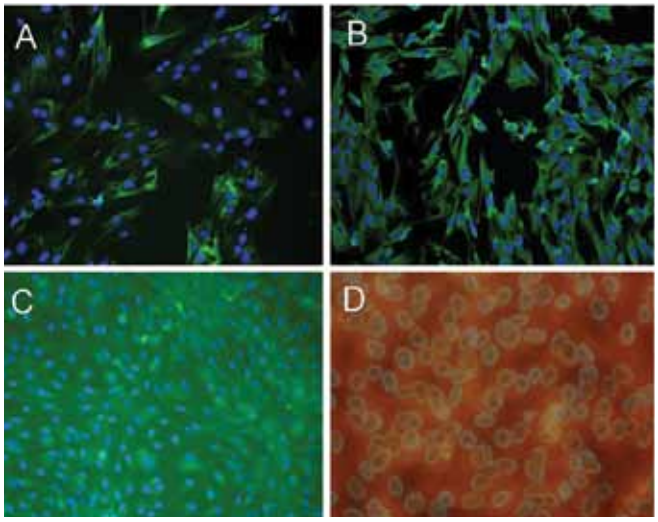

Figure 1. Common phenotypic characteristics of valvular interstitial and endothelial cells
In all images, cell nuclei are stained blue and magnification is 200 times. A small proportion of VICs stain for smooth muscle alpha-actin (green in A), but all VICs show strong staining for the intermediate filament vimentin (green in B). VECs demonstrate uniform staining for von Willebrand factor (green in C) and live cells can uptake acetylated low-density lipoprotein (red in D).

cells are considered to be responsible for the maintenance, structural integrity, and function of the valve in response to mechanical forces. VICs demonstrate characteristics of both fibroblasts and smooth muscle cells, exhibiting both cuboidal and spindle-shaped, elongated morphologies *in vitro* and expressing either prolyl 4-hydroxylase, an enzyme involved in early collagen synthesis, or smooth muscle α-actin.[7] These cells' adherence to ECM via focal and fibrillar adhesions presumably provides a basis for mechanotransduction of leaflet strain to the cell level, which could elicit a variety of cell responses. These adhesions primarily involve beta1 integrins in conjunction with $alpha_1$, $alpha_2$, $alpha_3$, $alpha_4$, and $alpha_5$ integrin subunits.[8] VICs also produce matrix-degrading proteases necessary for ECM turnover,[9] a response that is greater in diseased conditions. VICs are generally quiescent and proliferate slowly, even in the presence of growth factors.[10] The accelerated proliferation of VICs leads to pathological characteristics such as angiogenesis and increased ECM production, altered ECM synthesis, or excess ECM degradation.[11]

The surfaces of valve leaflets and chordae are covered with a single layer of VECs. In vascular biology, it has been well established that endothelial cells regulate inflammation, remodeling, tone, and coagulation. It is speculated that VECs perform similar roles in the heart valves; however, additional work must be done to elucidate these functions. Although VECs share some similarities with endothelial cells from other sections of the circulatory system, VEC studies have shown differential expression of hundreds of genes compared to vascular endothelial cells.[12] In pathological conditions, VECs exhibit alterations in shape, microvilli, and cell borders as well as desquamation.[13] These changes lead to increased thrombogenicity and have been implicated in myxomatous degeneration, rheumatic fever, and senile valve disease. Growing evidence from three-dimensional co-culture models also indicates valve-specific roles for VECs through their interactions with VICs;[14] communication within and between these two cell types appears essential to valve homeostasis. VICs communicate through extensive gap and adherens junctions[14] and are signaled by VECs via nitric oxide.[15,16] Mechanical and biological signaling between VECs and VICs has been shown to be critical to valvular function, remodeling, and response to injury.[17] In co-culture, VECs suppress VIC proliferation and stimulate a more quiescent phenotype, in which there is reduced expression of smooth-muscle α-actin.[14] As these investigations of VEC-VIC interactions are very recent and limited in number, further work in this area will be critical for elucidating the mechanisms of valve disease and remodeling.

The ECM appears to play critical roles in valvular biology and disease, although ECM-cell interactions within valves have only begun to be characterized. VICs cultured on ECM components such as collagen and fibronectin exhibited greater resistance to calcification than VICs grown on standard tissue culture plastic, even after the application of mineralization-inducing growth factors.[18] Distinct ECM components can also differentially modulate how certain growth factors induce the activation of VICs towards a more myofibroblastic phenotype (indicated by smooth muscle α-actin expression).[19] The inherent mechanical properties of ECM can also influence VIC biology and phenotype. When cultured on stiffer materials, VICs exhibited fibroblastic

Table 2. Classification and description of heart valve pathologies.

Type	Descriptions	Relevant Conditions
Congenital	Improper development leads to anatomical deformities preventing the valves from maintaining unidirectional flow of blood	• Valvular stenosis • Valvular regurgitation • Valvular atresia • Bicuspid aortic valve
Collagen disruption	Altered expression of PGs and matrix degradation enzymes; leads to mechanical weakness and thickened valve leaflet	• Myxomatous mitral valve degeneration
Calcification	Changes in VIC phenotype along the osteogenic pathway leads to pathological deposits of calcium	• Aortic calcification • Aortic stenosis
Fibrotic thickening	Characterized by excess collagenous ECM production by VICs and marked by neovascularization	• Rheumatic heart disease
Serotonergic drug induced	Paired with an increase in serum serotonin, heart valves exhibit superficial plaques and an increase in cellular proliferation	• Valvular regurgitation • Valvular stenosis

characteristics whereas more compliant substrates cause VICs to differentiate along the osteoblastic pathway.[20] Mechanotransduction of valve tissue strains are transmitted from the ECM to valvular cells via integrins and strain-activated ion channels. This activates signaling pathways and often leads to changes in protein expression and reciprocal remodeling of the ECM.[21]

Valve Remodeling

During life, human valves exhibit significant changes in terms of ECM, VEC, and VIC phenotype. VIC density, activity, and proliferation decreases significantly from fetal development to adulthood. In terms of cell density, adult heart valves exhibit only 10% of fetal valves. With increasing age, more collagen fibers align themselves with the direction of mechanical loads.[22] The most common valve pathologies are summarized in Table 2.[23] Genetic defects in key ECM components are seen as major contributors to heart valve pathologies.[24]

Children with congenital heart valve defects face serious health challenges. The success of valve repair and replacement surgeries for children is significantly less predictable than surgeries in adults and depends heavily on age.[25] Furthermore, the replacements for children are often improperly sized and cannot remodel with age or grow with the child. Bicuspid aortic valve disease is a congenital defect that affects 1-2% of the population.[26] Instead of the normal three leaflets, the aortic valve exhibits only two, leading to aortic stenosis and poor hemodynamic function. Highly heritable, patients with bicuspid aortic valve disease are 10 times more likely to suffer an aortic dissection.[27] Genetic defects in key ECM components like collagen type III and tenascin, an ECM glycoprotein, are implicated as major contributors to valvular pathologies that also cause abnormalities in other connective tissues.[28] These defects have the potential to initiate signaling cascades that exacerbate ECM abnormalities and resemble earlier developmental stages.[27]

Calcific stenosis is associated with the transition of VICs from quiescent fibroblast behavior to an osteoblast-like phenotype.[29] Animal studies and *in vitro* culture work suggest that calcific stenosis is caused directly by VIC-related osteoblast expression,[29] causing calcium deposition in regions of highest stresses *in vivo*.[30] A recent study highlighted the contribution of the decreased expression of the antiangiogenic protein, chondromodulin, in calcific valve disease.[31] When silenced, the absence of chondromodulin led to increased VEGF-A expression, neovascularization, and calcification.[31] In calcific valves, increased expression levels of Sox9 and Cbfa1, classic transcriptions factors for the osteoblast pathway, were coupled to an increase in Wnt3 and its receptor (Lrp5) expression.[32]

Up to 5% of the population is thought to be affected by myxomatous valve disease.[33] Generally, myxomatous valve disease is clinically benign, but it has been indicated as a risk factor for regurgitation and thrombosis.[34] In myxomatous valves, the normally collagen-rich valvular fibrosa becomes infiltrated by proteoglycans and an increased abundance of matrix-degrading enzymes. Transforming growth factor-β (TGF-β), decorin, and fibrillin have also been implicated in pathological ECM remodeling leading to myxomatous valve disease.[35] The *wnt* signaling pathway also plays a role in the development of myxomatous valve disease but, unlike calcific valves, does not proceed to an osteoblastic pathway.[34]

Although rheumatic heart valve disease has become less significant in industrialized nations, it remains a significant burden to developing countries, causing 233,000 deaths annually.[36] In this condition, cross-reaction between streptococcal antigens and heart valve tissue proteins is caused by an overt immune response.[37] Calcification and neoangiogenesis are again present in rheumatic heart valve disease; Rajamannan et al. demonstrated significant upregulation of osteopontin, vascular endothelial growth factor, and von Willebrand factor in rheumatic valves compared with normal and degenerative valves.[38]

Serotonergic valve disease is characterized by elevated serum levels of the neurotransmitter serotonin and the presence of a superficial plaque on the valve surface, although the underlying valve microstructure is generally preserved. VICs are sensitive to serotonin evidenced by the presence of serotonin receptors (5-HT_{2B}) in diseased human heart valves.[39] Serotonin stimulates VICs to upregulate TGF-β through G-protein signal transduction via Src kinase and extracellular regulated kinase (ERK) phosphorylation.[40] The resulting superficial plaque is comprised of a collection of myofibroblasts within a matrix consisting mainly of collagen, GAGs, and elastin.[41] Comparable valve dysfunction is elicited by the diet drug fenfluramine-phentermine[42] and the dopamine receptor agonist pergolide[43] used to treat Parkinson's Syndrome, which also activate the 5-HT_{2B} receptor, leading to the formation of the characteristic plaque, increased myofibroblast proliferation, and valve tissue thickening.

Current Treatments and Future Outlook

The surgical treatments for heart valve diseases are well understood and characterized. Indeed, over the past 40 years heart valve surgery has enjoyed dimin-

ishing operative mortality and improved long-term survival.[44] However, current bioprosthetic replacements have a limited lifetime, while mechanical valves require indefinite anticoagulation therapy and increase the risk of thromboembolism. A patient who undergoes aortic valve replacement still exhibits a five-year decrease in life expectancy.[45] For these reasons, tissue engineering approaches to develop living heart valve replacements hold great promise. Unlike current treatments, tissue-engineered valves could grow with pediatric patients, better reproduce the hemodynamic and biological roles of native valves, and eliminate the need for anticoagulants. An additional, more recent alternative is the percutaneously implantable bioprosthetic aortic valve. These valves offer a less invasive means of valve replacement and are currently under evaluation in several clinical trials.[46] Both the tissue engineering heart valve field and the percutaneously implantable valve field are rapidly growing and numerous reviews are available for the interested reader.[1, 46]

Conclusion

The biology of VICs, VECs, and ECM plays a critical role in normal heart valve function and the progression of disease. Despite its importance, the current understanding of heart valve biology and leaflet remodeling appears to be at least a decade behind our understanding of the analogous field of vascular biology. Deeper investigations into cellular communications, the regulation of matrix remodeling, and the effects of medications on valvular cells will be instrumental in developing novel therapies that benefit hundreds of thousands of patients.

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