Evolving Concepts of Cardiac Valve Dynamics
The Continuum of Development, Functional Structure, Pathobiology, and Tissue Engineering

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Abstract—Considerable progress has been made in recent years toward elucidating a conceptual framework that integrates the dynamic functional structure, mechanical properties, and pathobiological behavior of the cardiac valves. This communication reviews the evolving paradigm of a continuum of heart valve structure, function, and pathobiology and explores its implications. Specifically, we discuss (1) the interactions of valve biology and biomechanics (eg, correlations of function with structure at the cell, tissue, and organ levels and mechanical considerations, development, endothelial cell and interstitial cell biology, extracellular matrix biology, homeostasis, and adaptation to environmental change); (2) mechanisms of disease (eg, valve cell and matrix pathobiology in congenital anomalies, aortic valve calcification, and mitral valve prolapse); (3) considerations in replacement and repair (eg, cell/matrix biology of tissue valve substitutes and their degeneration and durability of repairs); and (4) the potential for tissue engineering approaches to therapeutic regeneration of the cardiac valves. Opportunities for research and clinical translation are highlighted. (Circulation. 2008;118:1864-1880.)

Key Words: aortic valve ■ mitral valve ■ pathology ■ prosthesis ■ tissue engineering
Figure 1. AV functional structure at both macroscopic and microscopic levels. a, Outflow aspect of AV in open (left) and closed (right) configurations, corresponding to systole and diastole, respectively. b, Tissue architecture, shown as low-magnification photomicrograph of cross-sectional cuspal configuration in the nondistended state (corresponding to systole), emphasizing 3 major layers: ventricularis (v), spongiosa (s), and fibrosa (f). The outflow surface is at the top. Magnification ×100; Movat pentachrome stain (collagen is yellow; elastin is black). From Schoen FJ, Edwards WD. Valvular heart disease: general principles and stenosis. In: Silver MD, Gotlieb AI, Schoen FJ, eds. Cardiovascular Pathology. 3rd ed. New York, NY: Churchill Livingstone; 2001:402–442. Copyright © 2001, W.B. Saunders.

Because they are sufficiently thin to be nourished by diffusion from the blood bathing the valves, normal leaflets and cusps have only scant and inconsistent blood vessels limited to the proximal portion; indeed, valvular angiogenesis is generally associated with disease. Although the valve leaflets and cusps also have nerves, and AV cusps have been shown to exhibit receptor-mediated contraction, probably modulated by valvular interstitial cells (VICs) (see The Role of VICs below), a functional role for neural elements and contractile responses has not yet been clarified.

The Functional Role of Valvular Extracellular Matrix

Healthy native heart valves maintain unidirectional blood flow via an extraordinarily dynamic functional structure with sufficient strength and durability to withstand repetitive and substantial mechanical stress and strain over many years. A highly responsive, compartmentalized internal microarchitecture of heart valves facilitates the substantial changes in size and shape of the valve cusps and leaflets that occur during the cardiac cycle (Figure 1). All 4 cardiac valves have a similar layered architectural pattern: a dense collagenous layer close to the outflow surface and continuous with valvular supporting structures, and which provides the primary strength component, a central core of loose connective tissue, and a layer rich in elastin below the inflow surface; for the AV, these are called the fibrosa, spongiosa, and ventricularis, respectively. The essential functional components of the heart valves comprise cells, including the valvular endothelial cells (VECs) at the blood-contacting surfaces and the deep VICs, and extracellular matrix (ECM), including collagen, elastin, and amorphous ECM (predominately glycosaminoglycans [GAGs]) (Table 1).

The AV (which is most frequently diseased, most frequently used in various modes of substitution, and most widely studied) provides a paradigm for valvular structural specialization and tissue dynamics across the cardiac cycle (Figure 2). In diastole, the back pressure (normally ≈80 mm Hg) stretches the valve cusps as they appose and seal the orifice to prevent backflow of blood. The rapid and reversible deformations of the cusps demand mechanical responses that are accommodated by the ECM components enumerated above. The major stress-bearing component is collagen. Individual collagen fibers can withstand high tensile
Collagen concentrated in glycosaminoglycans, elastin, and the endothelial cells lining inflow and outflow valve surfaces.

During systolic valve opening, the tissue of the cusps that was stretched during diastole becomes relaxed owing to recoil of the elongated, taut elastin. This decreases surface area, restoring the retracted configuration of the cusp, which is characterized by both a more random microscopic crimp and restored crimp of collagen fibrils. The GAGs-rich spongiosa facilitates the relative rearrangements of the collagenous and elastic layers during the cardiac cycle by both its high compliance and the bonds that link it to the adjacent fibrous layers. Moreover, the strains during closure and mechanical properties of the AV cusps are anisotropic (ie, different in the radial and circumferential directions), with compliance and stretching in the radial direction greater than that in the circumferential. Studies in which the AV fibrosa and ventricularis have been microdissected apart have demonstrated that not only are the mechanical properties of the several valve layers different, but also their properties have a layer-specific directionality; ie, the stiffer fibrosa dominates in the circumferential direction, whereas the more compliant ventricularis dominates in the radial direction. Moreover, there are regional differences in the mechanical properties of the cusps, ie, the cuspal belly region is substantially stiffer than the commissural region. Human valve cusps are ~43% to 55% collagen (predominantly type I but also some type III, as measured in bovine atrioventricular valve) and 11% elastin (dry weight ratio); together they comprise ~80% of total valvular protein.

The quantity, quality, and architecture of the valvular ECM, particularly collagen, elastin, and glycosaminoglycans, are the major determinants of not only the cyclical functional mechanics over the second-to-second periodicity of the cardiac cycle, as described above, but also the long-term (lifetime) durability of a valve. The macroscopic mechanical stimuli, both shear and solid stresses that occur during normal valvular function, are translated into microscopic forces that affect biological phenomena at the tissue and cellular levels. The cells of the heart valves sense the local tissue mechanical environment and, through complex cell-ECM interactions, transduce forces into molecular changes that mediate normal directional distribution and restored crimp of collagen fibrils. The GAGs-rich spongiosa facilitates the relative rearrangements of the collagenous and elastic layers during the cardiac cycle by both its high compliance and the bonds that link it to the adjacent fibrous layers. Moreover, the strains during closure and mechanical properties of the AV cusps are anisotropic (ie, different in the radial and circumferential directions), with compliance and stretching in the radial direction greater than that in the circumferential. Studies in which the AV fibrosa and ventricularis have been microdissected apart have demonstrated that not only are the mechanical properties of the several valve layers different, but also their properties have a layer-specific directionality; ie, the stiffer fibrosa dominates in the circumferential direction, whereas the more compliant ventricularis dominates in the radial direction. Moreover, there are regional differences in the mechanical properties of the cusps, ie, the cuspal belly region is substantially stiffer than the commissural region. Human valve cusps are ~43% to 55% collagen (predominantly type I but also some type III, as measured in bovine atrioventricular valve) and 11% elastin (dry weight ratio); together they comprise ~80% of total valvular protein.

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valve function and pathobiology. Indeed, through such mechanisms, healthy heart valves are able to maintain homeostasis, adapt to an altered stress state, and repair injury via connective tissue remodeling mediated by the synthesis, repair, and remodeling of the several ECM components. These critical processes that ensure valve health are themselves dependent on the viability and active function of valve cells. When environmental change becomes excessive, clinically significant valve pathology may result.

The Role of VICs
Crucial to function are VICs, the most abundant cell type in the heart valves and distributed throughout all of its layers. VICs are strongly attached to and synthesize the ECM; they express matrix-degrading enzymes (including matrix metalloproteinases [MMPs] and their inhibitors [tissue inhibitors of metalloproteinases]) that remodel collagen and other matrix components. Thus, VICs mediate matrix remodeling and continuously repair functional damage to collagen and the
other ECM components. VICs comprise a diverse and dy-
namic population of resident cells that can modulate along a
spectrum of phenotypes regulated by environmental
conditions.

Although most VICs in the normal valve are quiescent and
fibroblast-like, VICs are highly plastic and may transition
from one phenotypic state to another during valvular ho-
meostasis, response to injury adaptation, and pathology (Fig-
ure 3). The 5 distinct VIC phenotypes include embryonic
progenitor endothelial/mesenchymal cells (eVICs), quiescent
VICs (qVICs), activated VICs (aVICs), postdevelopmental/
adult progenitor VICs (pVICs), and osteoblastic VICs (ob-
VICs).24 The transition from a quiescent to an activated
phenotype may be reversible under some circumstances. The
characteristics of each of these phenotypes are summarized in
Table 2 and will be discussed below.

Adult heart valve VICs in situ have characteristics of
fibroblasts; they are quiescent (ie, are qVICs), with very low
levels of α-smooth muscle actin (α-SMA) and MMPs.

Indeed, we found that only 2% to 5% of normal adult VICs in
situ express α-SMA, as evidence of activation, and show
myofibroblastic differentiation (similar to the cells involved
in stereotypic physiological wound healing). In contrast,
previous studies demonstrate that 50% to 78% of cells isolated
from intact heart valves and cultured in vitro are α-SMA
positive. This suggests that removal of cells from the
environment of the intact valve or their manipulation may
stimulate/activate VICs.

VIC phenotypes change with age and environmental con-
ditions in normal valves. For example, VICs are activated
during intrauterine valvar maturation, by abrupt changes in
the mechanical stress state of valves, and in disease states
such as MV prolapse (see Myxomatous Degeneration of the
MV [MV Prolapse] below). Cyclic stretch induces ex vivo
remodeling of AV tissue.28 Moreover, either induced me-
chanical stretch or transforming growth factor-β (TGF-β)
treatment of isolated VICs from mature valves increases their
synthetic activity, and the effects of stress and TGF-β on
Table 2. Characteristics of VIC Phenotypes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>eVICs</td>
<td>Embryonic cardiac cushions</td>
<td>Give rise to resident qVICs, possibly through an activated stage; EMT can be detected by the loss of endothelial markers and gain of mesenchymal markers</td>
</tr>
<tr>
<td>qVICs</td>
<td>Heart valve leaflet</td>
<td>Maintain physiological, normal valve structure and function and inhibit angiogenesis in the leaflets</td>
</tr>
<tr>
<td>pVICs</td>
<td>Bone marrow, circulation, and/or heart valve leaflet</td>
<td>Enter valve or are resident in valve to provide aVICs to repair the heart valve, may express CD34, CD133, and/or S100</td>
</tr>
<tr>
<td>aVICs</td>
<td>Heart valve leaflet</td>
<td>α-SMA containing VICs with activated cellular repair processes including proliferation, migration, and matrix remodeling; respond to valve injury caused by pathological conditions and abnormal hemodynamic/mechanical forces</td>
</tr>
<tr>
<td>obVICs</td>
<td>Heart valve leaflet</td>
<td>Mediate calcification, chondrogenesis, and osteogenesis in the heart valve; secrete alkaline phosphatase, osteocalcin, osteopontin, bone sialoprotein</td>
</tr>
</tbody>
</table>

CD34 and CD133 are stem cell markers; S100 is an intracellular calcium-binding protein. Modified from Liu et al. Copyright © 2007, American Society for Investigative Pathology.

cultured aortic VICs are synergistic. Because the macroscopic mechanical state of the valve is likely transmitted to the VICs through their interactions with the surrounding ECM, considerable interest exists in the effects of mechanical forces on VIC function, the mechanisms of response of VICs to their physical environment (mechanotransduction), and the forces on VIC function, the mechanisms of response of VICs to their physical environment (mechanotransduction), and the mechanical properties of isolated VICs. The remodeling potential of PV and AV interstitial cells appears to be different. Whether VICs in different regions of an AV have different functional properties is unknown, but recent evidence suggests regional heterogeneity of synthetic response in VICs from the MV.

The Role of VECs

The blood-contacting surfaces of the valves are lined by endothelial cells. At a basic structural and functional level, VECs resemble endothelial cells elsewhere in the circulation. Nevertheless, evidence is increasing that VECs are phenotypically different from vascular endothelial cells in the adjacent aorta and elsewhere in the circulation, which is consistent with the increasing recognition of more widespread endothelial heterogeneity across circulatory sites, and the possibility that VECs may interact with VICs to maintain the integrity of valve tissues. For example, in response to fluid shear stress, porcine aortic VECs align perpendicular to flow, whereas endothelial cells from the nearby aorta align parallel to flow, and the transcriptional gene expression profile of aortic wall and aortic VECs is different when these different cells are exposed to the same mechanical environment. Furthermore, recent evidence indicates that different transcriptional profiles are expressed by the endothelium on the opposite (ie, aortic and ventricular) faces of a normal adult pig AV, and some investigators have hypothesized that these differences may contribute to the typical predominant localization of pathological AV calcification near the outflow surface.

Development, Maturation, and Maintenance of the Cardiac Valves

Recent studies have clarified how valves form in the atrioventricular canal and ventricular outflow tracts, mature in the fetus, and adapt, maintain homeostasis, and change throughout life. Elegant studies in zebra fish, chickens, and mice have isolated key molecular pathways in normal cardiac development and demonstrated that disruption of key pathways lead to abnormal valves. Members of the TGF-β superfamily (including TGF-β and bone morphogenetic protein 2), vascular endothelial growth factor and its receptors, the nuclear factor of activated T cells (NFATc) transcription factor, Notch, Wnt/β-catenin, and other pleiotropic signaling pathways have been shown to be particularly important regulators. Moreover, a wide spectrum of human congenital heart disease, including abnormalities involving the inflow and outflow tracts of the heart and their respective valves, are clearly related to aberrant transcriptional events, signaling, and other molecular events in cardiac development, whose critical normal functions have been elucidated in animal models.

During normal development of the heart, the heart tube consists of endocardium and myocardium separated by an acellular ECM called cardiac jelly. After the completion of heart looping, the valve cusps/leaflets originate from mesenchymal outgrowths known as endocardial cushions, the precursors of valves and the cardiac septa. A subset of endothelial cells in the cushion-forming area, driven by signals from the underlying myocardium, change their phenotype to that of mesenchymal cells and migrate into the cardiac jelly to form VICs (ie, the aforementioned eVICs). This phenotypic/transitional transformation of embryonic progenitor endothelial/endocardial cells to mesenchymal cells is termed transdifferentiation or epithelial-to-mesenchymal transformation (EMT). During EMT, a complex process involving >100 genes, the activated endothelial cells lose cell-cell contacts, gain mesenchymal markers such as α-SMA, and reduce their endothelial markers as they invade into the cardiac jelly. Human cardiac morphogenesis is complete in 8 to 10 weeks.

Several lines of evidence suggest that VICs in adult valves may be continuously replenished via circulating endothelial or mesenchymal cell precursors derived from the bone marrow and subsequent EMT (ie, the aforementioned pVICs). These precursors contribute to vascular healing and remodeling under physiological and pathological conditions. For example, in recent experiments using green fluorescent protein expressing hematopoietic stem cells implanted into lethally irradiated congenic mice, green fluorescent protein–expressing cells found within the heart valves
demonstrated at least some synthetic functions characteristic of VICs.\(^47\) Moreover, bone marrow–derived myofibroblasts have been demonstrated in adult human heart valves.\(^48\)

The role of ECM components in mediating the creation and remodeling of the endocardial cushions into mature valves is poorly understood.\(^49\) Nevertheless, the glycosaminoglycan hyaluronan is recognized to have multiple functions in EMT and subsequently in valve development,\(^50\) and periostin, an ECM protein that influences matrix remodeling via cell migration, adhesion, and collagen formation, has recently been demonstrated to be an important mediator of post-EMT valvular maturation.\(^51\)

Moreover, several lines of evidence suggest that cardiac morphogenesis and function are closely linked and that the molecular pathways of embryonic heart valve development are regulated in part by mechanical forces.\(^52\)–\(^54\) For example, microdissection and implantation of polymorphic beads in the inflow or outflow tract of the zebra fish heart, which lowers the shear stress across the endocardial cushions and valves, leads to abnormal valve phenotypes,\(^55\) and a genetic mutation in the cardiofunk (cfk) gene that encodes a sarcomeric actin and causes poor contractility and blood flow in zebra fish leads to abnormal cushion/valve formation.\(^56\) Insights derived from the study of tissue mechanical properties during valve morphogenesis may inform studies of valve regeneration.\(^53,57\)

### Postdevelopmental Evolution and Adaptation of the Cardiac Valves

Dynamic changes in ECM architecture and VIC phenotype, proliferation, and apoptosis continue throughout human fetal and postnatal development and indeed throughout life and in response to altered environmental conditions (Figure 4). The effects of these changes on cyclical function and potentially valve degeneration are currently being explored.

Comparative studies of human valves obtained from second- and third-trimester fetuses, neonates, children, and adults have shown that valve structure evolves over a lifetime, reflecting both a progressive adaptation to hemodynamic conditions and ongoing synthesis and architectural changes in ECM (Figure 4a and 4b).\(^58\) Second- and third-trimester fetal valves have proliferating VICs, a nascent ECM, and α-SMA–positive cells, indicative of myofibroblasts. Fetal VICs show an activated myofibroblast-like phenotype (α-SMA expression), abundant embryonic myosin, and MMP collagenases, indicating an immature/activated phenotype engaged in matrix remodeling, and fetal VECs express intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, markers of an activated endothelial phenotype. VIC density, proliferation, and apoptosis are high in fetal valves and low in adult valves; indeed, cell density in adult valves is reduced to \(~10\%\) of that in fetal valves. In contrast to a largely myofibroblast-like aVIC phenotype engaged in matrix remodeling in fetal valves, adult valves have a fibroblast-like qVIC phenotype. At birth, the abrupt change from fetal to neonatal circulation is associated with increased aVIC (α-SMA–positive VICs), consistent with abrupt changes in the mechanical regimen stimulating VIC activation. Collagen content increases from early to late fetal stages. The trilaminar architecture characteristic of valves appears late in gestation. Moreover, collagen fibers became progressively more aligned with increasing age (ie, more characteristic of the diastolic phase of the cardiac cycle), suggesting that an ongoing “creep” of AV structure occurs during life, consistent with a measured progressive loss of mechanical compliance of the AV with increasing age.\(^59\)

Normal and pathological cardiac valves also respond to environmental conditions, such as mechanical loading, by cell activation and matrix remodeling. For example, in conditions of disease (eg, myxomatous MV [Figure 5a]),\(^60\) adaptation (early pulmonary-to-aortic autograft [Figure 5b]),\(^61\) or remodeling (tissue-engineered valves\(^62\)), VICs have an activated (ie, myofibroblast-like) phenotype (aVICs). Moreover, after return of a stable equilibrium mechanical state achieved by adaptive ECM remodeling, VICs return to their normal fibroblast-like quiescent phenotype (qVICs), as ex-
emplified by late PAV (>3 years postoperative) and tissue-engineered valves implanted in vivo. Therefore, heart valves can respond to environmental change via reversible phenotypic modulation of qVICs to aVICs; aVICs regulate repair, adaptation, remodeling, and potentially pathology. We have hypothesized that the regulatory principle is maintenance of a normal stress profile in the tissue, analogous to the putative regulatory principle for mechanical load–induced cardiac hypertrophy. It is possible, though not yet demonstrated, that bone marrow–derived VICs (ie, pVICs) could contribute to remodeling and potentially pathology of adult human heart valves.

**Pathobiology of Valvular Heart Disease**

Pathological changes of valves are largely of 4 types: (1) disruption of the formation of the functional valve architecture, as in congenital abnormalities; (2) damage to or inadequate collagen leading to weakness of the leaflets, exemplified by myxomatous valve degeneration (MV prolapse); (3) nodular calcification beginning in VIC, as in calcific aortic sclerosis/stenosis; and (4) fibrotic thickening either with neovascularization, the key feature in rheumatic heart disease, or owing to superficial intimal thickening (a process that likely involves proliferation and matrix production by VECs and VICs), stimulated by circulating serotonin 5-hydroxytryptamine levels in carcinoid heart disease or in serotonin agonist drug–induced valve changes, including fenfluramine (part of the fen-phen combination of appetite suppressants used for the treatment of obesity), some antiparkinsonian drugs, and methysergide or ergotamine therapy for migraine headaches. The important structural/functional changes and mechanisms of a spectrum of valve diseases are summarized in Table 3. This section summarizes data and concepts emerging for several common forms of valve disease that suggest that genetic, mechanical, and soluble factors play an important (and perhaps interactive) role in valve pathology and that valve pathology is often mediated through abnormal and complex interactions among VICs, VECs, ECM, and their environment.

Evidence is increasing that the pathogenesis of nonrheumatic AV and MV diseases has a prominent genetic component. For example, the genetic determinants of atherosclerosis may contribute to aortic stenosis in older individuals. In addition, bicuspid AV and other congenital deformities of the ventricular outflow tracts may be heritable in many cases, and MV prolapse may be related to aberrations of key remodeling events that are both involved in physiological valve homeostasis and genetically determined.

**Bicuspid AV**

With a prevalence of 1%, bicuspid AV is the most frequent congenital cardiovascular malformation in humans. Although usually uncomplicated in early life, bicuspid AV frequently eventuates in aortic stenosis or regurgitation, infective endocarditis, and aortic dilation and/or dissection later in life. Bicuspid AVs underlie >67% of aortic stenosis in children and >50% in adults.

Recent studies have confirmed previous reports of familial clustering of bicuspid AV, left ventricular outflow tract obstruction malformations, and other cardiovascular malformations, suggesting that these common valvular malformations are genetic defects leading to faulty valvulogenesis and/or cardiogenesis. Particularly interesting in this regard is the report that nonsense and frameshift mutations in the signaling and transcriptional regulator NOTCH1 caused a spectrum of developmental AV abnormalities and severe
calcification in 2 families with nonsyndromic familial AV disease.72

Calcific AV Stenosis

Acquired aortic stenosis is usually the consequence of calcification intrinsic to the cuspal tissue of either previously anatomically normal AVs or bicuspid AVs. Calcification of a bicuspid valve occurs approximately a decade earlier than in those with an anatomically normal valve. With the rising average age of the population, the prevalence of aortic stenosis, estimated at 2%, is increasing. Calcification of the AV restricts cuspal opening, thereby decreasing the effective valve orifice area. Nevertheless, aortic jet velocity is the most reliable predictor of clinical outcome.73 Calcific deposits in aortic stenosis typically occur in regions of highest functional valve stresses74; thus, mechanical factors are thought to potentiate valve calcification. The deposits predominantly grow from the outflow aspect distally, but because they extend deep into the cuspal matrix, they cannot be readily debrided.

Deposition of calcific deposits in AV disease is initiated in the VICs.75 AV calcification is traditionally believed to have a degenerative, cell damage–mediated, dystrophic mechanism with passive accumulation of hydroxyapatite mineral, distinct from the pathogenesis of atherosclerosis. However, several lines of evidence suggest that calcific aortic stenosis and atherosclerosis share some mechanistic features and that there may be active regulation of calcification in AVs similar to that in atherosclerotic arteries, with inflammation, lipid infiltration, and phenotypic modulation of VICs to an osteoblastic phenotype.76–78 For example, (1) male sex, hypertension, elevated serum low-density lipoprotein cholesterol, and smoking, which are classic atherosclerosis risk factors, are also risk factors for calcific aortic stenosis; (2) pathological studies of some early calcified valves show lesions that resemble those of early atherosclerosis; and (3) patients with familial hypercholesterolemia who have elevated low-density lipoprotein also have AV lesions. Additionally, animal models of hypercholesterolemia develop AV lesions.79,80 These findings have stimulated interest in the possibility that the statin drugs, which lower systemic cholesterol and decrease inflammation in atherosclerosis, may decrease the rate of aortic stenosis progression.81–83

VICs with an osteoblastic phenotype (obVICs) are found in calcifying valves. Such cells express markers that characterize osteoblasts in bone (eg, alkaline phosphatase, osteocalcin, osteopontin84). Heart valves and bone may share regulatory mechanisms for connective tissue formation and remodeling.85 Cartilaginous nodules and mature lamellar bone with maturing trilineage hematopoietic marrow and fat are frequently observed in surgically explanted degenerated human heart valves.86 Calcified AVs also have increased levels of specific protein markers of osteoblastic activity, such as osteopontin, bone sialoprotein, alkaline phosphatase, and bone morphogenetic protein 2 and 4, in at least some VICs.87–90 It is unknown whether obVICs evolve directly from resident qVICs or aVICs or whether they may be derived from circulating pVICs or other cells.

VICs extracted from intact valves do not normally promote calcification spontaneously; however, VICs undergo osteoblastic differentiation (ie, express chondrogenic and osteogenic proteins) and promote calcification when cultured in osteogenic culture medium. The bone matrix protein osteopontin, detected in calcified human AVs and MVs, may be an important inhibitor of valvular calcification.91 Moreover, observations on an in vivo animal model of AV disease and
Myxomatous Degeneration of the MV (MV Prolapse)

MV prolapse is the displacement of enlarged, thickened, redundant mitral leaflet(s) into the left atrium during systole; potential serious complications include heart failure, mitral regurgitation, bacterial endocarditis, thromboembolism, and atrial fibrillation. MV prolapse is the most common indication for surgical repair or replacement of the MV.

The underlying pathological process in MV prolapse is called myxomatous degeneration. Histologically, the essential change is attenuation of the collagen-rich fibrosa layer of the valve, on which the structural integrity of the leaflet depends, accompanied by focally marked thickening of the spongiosa layer with deposition of myxomatous material rich in GAGs. MV prolapse is associated with weakening of the valve cusp to calcification in calcific aortic stenosis.

Also, VECs of porcine AV bioprostheses (themselves devitalized) are largely denuded by handling, thereby increasing the permeability of the tissue to fluid. In addition, the

Bioprosthetic Valve Structural Degeneration

The processes responsible for structural deterioration of bioprosthetic heart valves are logical sequelae of the specific chemical, mechanical, and morphological changes that occur during tissue processing, fabrication, and insertion of bioprosthetic valves. Indeed, the clinical success, failure modes, and mechanisms of deterioration depend largely on the type, source, preservation, and handling of the tissue and the method of tissue attachment to and support by a stent (which determines the stress state of the tissue during cyclical function). During the preparation of bioprosthetic valves, chemical fixation with glutaraldehyde destroys the viability of the VECs (of porcine AV bioprostheses) or fibroblasts (of bovine pericardial valves), and therefore the mechanical properties and durability of the tissue depend primarily on the quality of the collagen in the fabricated valve. Because the fixed, nonviable cells are incapable of remodeling collagen, ongoing repair of the ECM by the cells endogenous to the transplanted tissue is impossible, and any damage to the ECM is cumulative. Moreover, the fragments of the devitalized VECs that remain in the tissue serve as nuclei for calcification. Also, VECs of porcine AV bioprostheses (themselves devitalized) are largely denuded by handling, thereby increasing the permeability of the tissue to fluid. In addition, the
collagenous network is mechanically locked into a single configuration, inhibiting the usual internal cuspal structural rearrangements of the ECM accompanying normal valvular function. Thus, buckling of the cuspal tissue occurs during opening and closing of a fixed bioprosthesis because the coordinated internal rearrangements involving collagen crimp and alignment are not possible.\textsuperscript{110,111}

Pathological analysis of tissue valve explants from patients and animal models with the use of bioprosthetic heart valve tissue implants has elucidated the pathophysiology of valve mineralization and facilitated the testing of hypotheses for preventive approaches. Experimental models have employed isolated tissue samples implanted subcutaneously in very young, rapidly growing rats or orthotopic valve replacements in large-animal models, especially sheep.\textsuperscript{112–114} Indeed, the normal extrusion of calcium ions is disrupted in these nonviable cells. Normally, the plasma/extracellular calcium concentration is 1 mg/mL ($\approx 10^{-7}$ mol/L); because the membranes of healthy cells pump calcium out, the concentration of calcium in the cytoplasm is normally 1000 to 10 000 times lower ($\approx 10^{-7}$ mol/L). In these experimental models, bioprosthetic tissue calcifies progressively with a morphology similar to that observed in clinical specimens but with markedly accelerated kinetics. Mineralization in the cusps of bioprosthetic heart valves is initiated predominantly at the cell membranes and other intercellular structures high in phosphorus (as phospholipids) of the devitalized connective tissue cells (Figure 6b and 6c).\textsuperscript{115} The essential reaction is between the phosphates of the devitalized cells with calcium in the surrounding fluid to yield calcium phosphate mineral. Collagen and elastic fibers can also serve as nucleation sites for calcium phosphate mineral, independent of cellular components.\textsuperscript{116} Initial calcific deposits eventually enlarge and coalesce, resulting in grossly mineralized nodules that stiffen and weaken the tissue and thereby cause prosthesis malfunction.

Collectively, these studies have confirmed the key determinants of bioprosthetic valve mineralization: (1) biochemical environment, (2) implant structure and chemistry, and (3) mechanical factors. Calcification is accelerated by young recipient age, likely a result of age-related biochemical differences in systemic calcium and phosphorus metabolism, and glutaraldehyde fixation, which devitalizes the cells. Furthermore, mineralization of a bioprosthetic tissue is generally enhanced at the sites of intense mechanical deformations, such as the points of flexion in heart valves. Moreover, many lines of evidence suggest that no causal immunologic basis exists for bioprosthetic valve calcification or failure.

New prostheses pretreated with anticalcification agents (particularly those that remove or alter the cell-based phospholipid substrate) are being used in several commercial valves.\textsuperscript{108} However, because of progressive collagen damage, which cannot be repaired in a devitalized valve, degradation of the valvular collagenous skeleton would likely become the ultimate limiting factor in durability of valves protected from calcification.\textsuperscript{117,118} The role of degradation of GAGs in limiting bioprosthetic valve durability is less well characterized, but evidence suggests that development of improved GAG cross-linking techniques may improve valve longevity.\textsuperscript{119,120}

**AV Allografts**

Valvular allografts/homografts are AVs or PVs derived from cadavers (although occasionally obtained from diseased hearts removed at transplantation) and transplanted from one individual to another. They are preserved without chemical...
Thus, implanted cryopreserved allograft valves show an (in the absence of viable VICs) is incapable of renewing. As in chemically preserved bioprosthetic valves, the collagenous network is initially present but without long-term anticoagulation, and a low infection rate. Although cryopreserved allograft valves are free of degeneration limits their long-term success. The mode of failure of these valves when implanted on the left side of the heart is generally incompetence due to cuspal stretching or fibrosis. In contrast, right-sided valves in children who have right ventricle–to–pulmonary autograft valve transplanted to the aortic position generally yields good to excellent hemodynamic performance and may permit growth of the autograft proportional to the somatic growth of a child or young adult. PAV provide an opportunity to study the effects of a virtually instantaneous change in pressure regimen. Pulmonary autograft valves in place for up to 6 years showed near-normal trilaminar and ECM architecture, viable VECs and VICs, and absence of significant pathology (Figure 7, left). VICs of short-term explants (3 to 6 months) demonstrated activation with strong collagen remodeling (likely due to mechanical adaptation); in contrast, long-term explants (3 to 6 years) had quiescent fibroblast-like VICs, similar to normal valves (recall Figure 5b).

Heart Valve Tissue Engineering and Regeneration

The evolution in the scientific knowledge of heart valves has stimulated, and indeed has been stimulated by, the goal of generating a living heart valve replacement that would have healthy cells, repair ongoing ECM damage, adapt to changing environmental conditions, and potentially grow with a growing recipient. Innovative work toward this objective, often called tissue engineering, is active in many laboratories and may eventually lead to clinical application. Clearly, as emphasized in the preceding sections of this communication, the long-term success of an engineered tissue or regenerated heart valve will depend on the ability of its living cellular components (VECs and VICs) to function normally, maintain homeostasis, and repair structural injury to the ECM.2,3

One widely studied paradigm of tissue engineering to facilitate valve regeneration uses cells that are preseeded on a synthetic, biodegradable polymer scaffold fabricated in the shape of a trileaflet valve and matured in vitro in a controlled metabolic and mechanical environment (in a bioreactor121). The intent is for the cells to differentiate, proliferate, and produce ECM to form a living tissue model called a construct. Subsequently, the construct is implanted orthotopically as a valve prosthesis, and further remodeling in vivo is intended to recapitulate the normal tissue functional architecture. Key processes occurring during the in vitro and in vivo phases of tissue formation and maturation are (1) cell proliferation, sorting, and differentiation; (2) ECM production and organization; (3) degradation of the polymer scaffold; and (4) remodeling and, potentially, growth of the tissue commensurate with the growth of the individual. Essential requirements for the in vivo phase are biocompatibility and near-constant mechanical properties of the evolving tissue as the scaffold is resorbed.

Tissue-engineered heart valves grown as valve conduits from autologous cells (derived from vascular wall or bone marrow) seeded on biodegradable synthetic polymers and matured in vitro have functioned in the pulmonary circulation of growing lambs for up to 5 months (Figure 8). In this location, implanted constructs generated in vitro evolve in vivo to a complex, functionally appropriate structure that resembles that of native semilunar valve described earlier in

Figure 7. Comparative morphological features of autografts and homografts obtained from the same patient, who had both a pulmonary autograft valve transplanted to the aortic position and an allograft (replacing the PV) and later had heart transplantation or died. In all 3 patients, autograft valves had near-normal structure and cellular population (a, c, and e). In contrast, homografts from the same patients (b, d, and f) had progressive collagen hyalinization and loss of cellularity. Magnification ×400; hema-toxylin and eosin stain. Reproduced from Rabkin-Aikawa et al.61

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Tissue-engineered heart valves in vitro and in vivo. a, Gross photograph of tissue-engineered heart valve after 14 days of conditioning the cell-seeded polymeric scaffold in a bioreactor (in vitro). b, c, and d, Photomicrographs of heart valve cusps in vivo, demonstrating evolution to near-normal structure after 16 to 20 weeks. b, At 6 weeks, early organization of tissue is present predominantly in outer (outflow) layer (top) (magnification ×50). c, Cross section of leaflet at 16 weeks shows layered cellular fibrous tissue, which is more dense near outflow surface (top) (magnification ×100). d, Cross section of leaflet at 20 weeks demonstrates collagen (yellow), glycosaminoglycans (blue), and elastin (arrow, inflow surface; magnification ×100). b and c, Hematoxylin and eosin. d, Movat stain. Reproduced from Hoerstrup et al. Copyright © 2000, The American Heart Association.

Moreover, the progression of the cell and ECM changes are analogous to those occurring during development and physiological valve remodeling described earlier, suggesting that the dynamic and chemical mechanical environment in vivo provides signals that induce functional organization of the tissue construct to a heart valve. This approach has been used to produce pulmonary arterial wall replacements to repair complex congenital heart disease in children, and a recent experimental study showed that pulmonary arterial wall replacements fabricated from vascular wall cells (predominantly vascular smooth muscle cells) seeded onto a biodegradable polymer and implanted into very young lambs enlarged proportionally to overall animal growth over a 2-year period. Whether “functional growth” can permit valve cuspal and supporting conduit enlargement in a valve is not yet known.

Variations on the theme of cellular tissue formation in vitro under investigation include fibroblast- or VIC-seeded natural degradable scaffolds, such as hyaluronan or fibrin gel, and the creation of a cellularized graft by maturation of tissue formed in association with either a microporous polyurethane valve assembled implanted into the subcutaneous space in rabbits or a photo-oxidized bovine pericardial valve implanted intraperitoneally in sheep. Cell-free collagen constructs fabricated by directed collagen gel contraction are also being investigated.

An alternative tissue-engineering strategy, called guided tissue regeneration, uses an implanted scaffold of a naturally derived biomaterial or decellularized valve designed to attract circulating endothelial and other precursor cells and provide a fertile environment for their adherence, growth, and differentiation. In this approach, the materials are not aldehyde-fixed or otherwise chemically preserved, as are conventional bioprosthetic heart valves. Natural tissue-derived valve scaffolds possess desirable 3-dimensional architecture, mechanical properties, and potentially adhesion/migration sites capable of promoting cell attachment and ingrowth. Decellularized tissue scaffolds derived from valve (in some cases with in vitro “reendothelialization” performed before implantation of the valve) have been used in clinical studies in the pulmonary position, but decellularized porcine valves implanted in humans as AV replacements elicited a strong inflammatory response and suffered structural failure, which has inhibited further use. Cell-free porcine small intestinal submucosa has been investigated experimentally as a valve cusp material. The specific patient and implant variables accounting for the spectrum of outcomes are not yet understood, and the long-term fate of these implants, the role of endothelial cell seeding, and the extent of cellular ingrowth into decellularized tissue in vivo are not yet known.

Accumulating evidence suggests that circulating endogenous cells can be recruited in vivo to adhere to intravascular sites of injury or prosthetic material via a pathway that likely mimics the adherence of inflammatory cells to the endothelium during physiological inflammation. For example, endothelial progenitor cells are bone marrow–derived cells that circulate in the blood, have the ability to differentiate into endothelial cells, express a number of endothelial- and stem cell–specific surface markers (eg, CD34, CD133, and vascular endothelial growth factor R2), and exhibit numerous endothelial properties. Various cytokines, growth factors, and hormones cause them to be mobilized from the bone marrow and into the peripheral circulation, where they ultimately are recruited to regions of angiogenesis. Endothelial progenitor cells are thought to participate in pathological angiogenesis such as that found in retinopathy and tumor growth, and they may play a role in the physiological repair of damaged blood vessels, such as after myocardial infarction. Recruitment and incorporation of endothelial progenitor cells require a coordinated sequence of adhesive signaling events including adhesion and migration, chemotraction, and differentiation.

Thus, a potential strategy may be to coat a degradable polymer scaffold in the configuration of a valve with appropriate cell-signaling molecules (or use a biological matrix already containing such information, as discussed above) in an effort to encourage and direct endothelial progenitor cell and other cell adhesion and differentiation. An experiment utilizing decellularized porcine AVs containing fibronectin and hepatocyte growth factor suggested that the growth factor enhances early endothelial cell recruitment to and coverage of the grafts, and attempts to attract endothelial progenitor cells from peripheral blood onto grafts via antibodies directed at proposed endothelial progenitor cell markers, such as anti-CD34 antibodies and kinase insert domain receptor, are
under investigation.142–144 Although such therapy is conceivable, a greater understanding of the regulatory mechanisms of endothelial progenitor cell mobilization, homing migration, adhesion, and (trans)differentiation will be necessary to realize such a strategy.

The possibility of therapeutic regeneration of the heart valves is indeed exciting; however, it is clear that immense adhesion, and (trans)differentiation will be necessary to achieve, a greater understanding of the regulatory mechanisms of regeneration.

The key concepts that unify the dynamic pathobiological basis of heart valves and the mechanisms of heart valve disease can be used to improve biological valve substitutes and potentially enable heart valve regeneration.

Disclosures
Dr Schoen is a paid consultant to Direct Flow Medical Inc, Medtronic Inc, Mitral Solutions Inc, Sadra Medical Inc, and St Jude Medical Inc.

References


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