Valvular Heart Disease: Changing Concepts in Disease Management

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

Important conceptual advances, new data, and evolution in our understanding and application of the principles underlying the dynamic functional, biological, and mechanical behavior of the cardiac valves have occurred in recent years. Research in heart valve biology and disease has been enabled by the availability of cultures of heart valve cells, computational methodology, the design and use of in vitro and in vivo experimental systems that model elements of valve biological and pathobiological activity, and targeted study of normally functioning and pathological native and substitute human valves. Collectively, these developments have facilitated a growing understanding of how short- and long-term biomechanical valve function at the organ level relates to tissue and cell structure and normal valve function, elucidated the pathological anatomy and mechanisms of valvular dysfunction, fostered improvements in tissue heart valve substitutes and surgical repairs, and informed innovative approaches to heart valve tissue engineering and regeneration.1–4 In this communication, we highlight key recent insights into heart valve function and dysfunction and their implications for the prevention, diagnosis, and treatment of clinical heart valve disease, currently and in the future.

Dynamic Valvular Functional Macrostructure and Microstructure, Developmental Biology, and Postdevelopmental Changes

Normal heart valves ensure unidirectional blood flow throughout the cardiac cycle with minimal obstruction and without regurgitation. The semilunar valves (ie, the pulmonary valve [PV] and aortic valve [AV]) prevent retrograde flow back into the ventricles during diastole, and the atrioventricular valves (tricuspid valve [TV] and mitral valve [MV]) prohibit reverse flow from the ventricle to the atrium during systole. Heart valves open and close ~40 million times a year and 3 billion times over an average lifetime.

The heart valves are tissue structures whose motions are driven by mechanical forces exerted by the surrounding blood and heart. The ability of the valves to permit unobstructed forward flow depends on the mobility, pliability, and structural integrity of their leaflets (in the TV and MV) and cusps (in the PV and AV).

The individual AV cusps attach to the aortic wall in a crescentic (or semilunar) fashion, ascending to the commissures (where adjacent cusps come together at the aorta) and descending to the basal attachment of each cusp to the aortic wall. Behind the cusps are dilated pockets in the aortic root, called sinuses of Valsalva, which bulge with each ejection of blood. The AV cusps and their respective sinuses are named for their relationship to the coronary artery ostia that arise from them, normally a left, a right, and a noncoronary (cusp and associated sinus). In the middle of the free edge of each cusp on the ventricular surface is a fibrous mound called the nodule of Arantius. Coaptation of the 3 nodules ensures complete central closure of the valve during diastole. Located...
along the ventricular surface of each cusp, between the free edge and the closing edge, are 2 crescentic regions, each called a lunula; these areas contact the corresponding regions of both adjacent cusps in diastole to effect a competent seal. The remainder of the cusp (ie, the noncoapting portion) is called the belly. A defect in or damage to a cusp confined to the lunula will not promote regurgitation; however, damage to the cusp in the belly region will permit backflow when the valve is closed.

As blood decelerates in the aorta at the end of systole, vortices in the sinuses of Valsalva behind the AV cusps facilitate valve closure. The competency (ie, ability to prevent reverse flow) of the semilunar valves (PV and AV) depends on the stretching and molding of their 3 cusps to fill the orifice during the closed phase of the cardiac cycle, during which back pressure from the blood is present in the pulmonary artery or aorta, respectively. We will see shortly that diastolic coaptation of the AV cusps is maintained by a mechanism that depends on a complex, highly differentiated, dynamic tissue macrostructure and microstructure. The function of the semilunar valves also depends on the integrity and coordinated movements of the cuspal attachments and the dynamics of the aortic and pulmonary root structures. Thus, stiffening or dilation of the aortic root can hinder movement and/or proper coaptation of the AV cusps during closure and thereby promote regurgitation. The PV has structure and function analogous to but less robust than the AV.

Maintaining competency of the atrophicventricular valves (TV and MV) is different than described above and involves a broader array of anatomic structures. Leaflet free margins are tethered to the ventricular wall by many delicate tendinous cords (chordae tendineae), themselves attached to papillary muscles that are contiguous with the underlying muscular ventricular walls. Thus, normal apposition of MV leaflets and thus MV competency depend on the coordinated actions of the annulus (the outer edge of the valve orifice, where the leaflets attach), leaflets, cords, papillary muscles, and associated left ventricular wall—collectively, the mitral apparatus—acting to maintain leaflet coaptation. Left ventricular dilation or a ruptured or fibrotic cord or papillary muscle can interrupt or distort the tethering of the leaflets and thereby interfere with MV closure, resulting in regurgitation. TV function depends on structures largely analogous to those of the MV.

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, 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
forces when taut, but collagen cannot be compressed (ie, buckling occurs, in contrast to the ability of elastin to stretch and contract). Thus, (1) the changes in shape and size of the cusps during the cardiac cycle must involve changes in collagen structure beyond simple stretching and shortening (such as directional realignment and crimping); (2) the limit to cuspal stretching and potential prolapse of the cusps into the left ventricle during diastole is taut, aligned collagen, particularly in the fibrosa layer; and (3) the relative orientation of collagen fibers in regions of the cusps determines the directions in which the tissue has the greatest compliance (ie, orthogonal to the collagen fiber orientation) or can withstand the greatest tensile stresses (parallel to the collagen fiber orientation) (Figure 2a). Moreover, the cyclical internal rearrangements in collagen (ie, progressive rotational alignment of fibers from random to oriented and extension of microscopic crimp) are extremely sensitive to the instantaneous mechanical stresses; the diastolic pattern of collagen alignment in the plane of the valve tissue is virtually complete early after closing. Indeed, most collagen alignment occurs as the back pressure increases from 0 to 4 mg Hg during the onset of cardiac diastole (Figure 2b). Moreover, the collagen crimp decreases (ie, collagen is flattened) rapidly as pressure is applied and is nearly completely (90%) lost at a back pressure of 20 mg Hg; little further rearrangement occurs from 4 to 80 mm Hg (Figure 2c).12–15

When the valve is closed, the fully unfolded, taut, and aligned collagen not only maintains apposition of cusps without prolapse but also helps to shift the load from the cusps to the aortic wall. 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 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.16 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.17,18 Moreover, there a regional differences in the mechanical properties of the cusps, ie, the cuspal belly region is substantially stiffer than the commissural region.19 Human valve cusps are ≈43% to 55% collagen (predominantly type I but also some type III, as measured in bovine valve)20 and 11% elastin (dry weight ratio); together they comprise ≈80% of total valvular protein.21

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
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

Figure 2. AV cuspal internal tissue dynamics across the cardiac cycle. a, Schematic representation of architecture and configuration of collagen and elastin in systole and diastole. b, Diagram of the cusp showing the locations of the belly, commissure, and nodulus, regions of coaptation, and small-angle/light-scattering results for the AV cusp at 0, 4, and 90 mm Hg transvalvular pressures. The color fringes represent the local degree of fiber alignment. At back pressures beyond ~4 mm Hg, no further changes in fiber alignment are observed. c, Rapidly diminishing collagen creep as the transvalvular pressure increases. Less than 10% creep remains beyond ~20 mm Hg transvalvular pressure. a, Modified from Schoen.11 Copyright © 1997, the Journal of Heart Valve Disease. b and c, From Sacks and Yoganathan.15 Copyright © 2007, the Royal Society.
other ECM components. VICs comprise a diverse and dynamic 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 homeostasis, response to injury adaptation, and pathology (Figure 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 (obVICs). 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 conditions in normal valves. For example, VICs are activated during intrauterine valvular 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. Moreover, either induced mechanical 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
cultured aortic VICs are synergistic.39 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). 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.40

Table 2. Characteristics of VIC Phenotypes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Location</th>
<th>Function</th>
</tr>
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<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>a-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>
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CD34 and CD133 are stem cell markers; S100 is an intracellular calcium-binding protein. Modified from Liu et al.24 Copyright © 2007, American Society for Investigative Pathology.

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,36 and the possibility that VECs may interact with VICs to maintain the integrity of valve tissues.37 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,38 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.39 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.40

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.41-42 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.43

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.44,45 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/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 a-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.46 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. Moreover, bone marrow–derived myofibroblasts have been demonstrated in adult human heart valves.

The role of ECM components in mediating the creation and remodeling of the endocardial cushions into mature valves is poorly understood. Nevertheless, the glycosaminoglycan hyaluronan is recognized to have multiple functions in EMT and subsequently in valve development, and peristatin, 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.

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. 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, 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. Insights derived from the study of tissue mechanical properties during valve morphogenesis may inform studies of valve regeneration.

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). Second- and third-trimester fetal valves have proliferating VICs, a nascent ECM, and a-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.

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]), adaptation (early pulmonary-to-aortic autograft [Figure 5b]), or remodeling (tissue-engineered valves), 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 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 approximately 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

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**Figure 5.** Plasticity of fibroblast-like valvular interstitial cells to myofibroblasts. **a,** Expression of catabolic enzymes in VICs of myxomatous valves. Strong expression of MMP-1, MMP-13, MMP-2, and MMP-9 in interstitial cells of spongiosa of myxomatous valves compared with normal valves, demonstrated via semiquantitative analysis of MMPs (especially collagenases MMP-1 and MMP-13) in specimens stained by immunohistochemistry. **b,** Cellular activation of VIC in pulmonary autograft valves (PAV). In early (3 to 6 months) autograft explants, 19% of cuspal interstitial cells were myofibroblasts expressing α-actin. In contrast, myofibroblasts comprised only 6% of cells in late (3 to 6 years) explants and 3% and 5% of cells in normal PVs and AVs. The structure of PVs transplanted to the systemic circulation also evolved toward that of normal AVs. **a,** Reproduced from Rabkin et al. Copyright © 2001, the American Heart Association. **b,** Reproduced from Rabkin-Aikawa et al. Copyright © 2004, Elsevier.
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 stresses; 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 valvar calcification.91 Moreover, observations on an in vivo animal model of AV disease and

### Table 3. Key Structural/Functional Changes in Heart Valve Components With Homeostasis, Aging, Disease, and Replacement Types

<table>
<thead>
<tr>
<th>Situation</th>
<th>Structural/Functional Changes</th>
<th>Putative Mechanism</th>
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<tbody>
<tr>
<td>Homeostasis</td>
<td>Healthy, responsive VECs, VICs, and ECM</td>
<td>VECs of normal valve maintain thromboresistance; healthy VICs in the normal valve are obVIC; mediate slow, ongoing turnover of matrix</td>
</tr>
<tr>
<td>Aging</td>
<td>Valve stiffening and decreased matrix turnover</td>
<td>Progressive collagen alignment; loss of VIC number and functional capacity</td>
</tr>
<tr>
<td>Congenital valve abnormalities, including bicuspid AV</td>
<td>Incomplete cusp/commissure formation; secondary abnormal biomechanical function</td>
<td>Aberrant development/morphogenesis</td>
</tr>
<tr>
<td>AV calcification</td>
<td>Inadequate AV opening owing to gross calcific nodules</td>
<td>Calcification of individual VIC (as obVIC) and coalescence into macroscopic nodules</td>
</tr>
<tr>
<td>Myxomatous MV disease</td>
<td>Weakening/stretching of leaflet; disrupted fibrillar ECM; deposition of excess glycosaminoglycans</td>
<td>Inadequate ECM remodeling, in some cases possibly owing to genetic or acquired abnormal TGF-β signaling</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>Cuspal/leaflet thickening, with transmural fibrosis/disruption of layers; neovascularization</td>
<td>Immunologically mediated, postinfectious, inflammatory destruction of cells and ECM</td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>Destruction of tissues, fibrosis/disruption of layers; neovascularization</td>
<td>Direct inflammatory destruction of valve architecture (cells and ECM)</td>
</tr>
<tr>
<td>Carcinoid/drug-induced heart disease</td>
<td>Accumulation of subendothelial fibrous tissue</td>
<td>Endothelial injury inducing proliferation and ECM production by VECs and VICs</td>
</tr>
<tr>
<td>Bioprosthetic heart valves</td>
<td>Collagen degradation, calcification</td>
<td>Loss of viable VECs and VICs during preparation; precipitation of calcium-phosphate mineral, predominantly in cells</td>
</tr>
<tr>
<td>PV to AV autograft (PAV)</td>
<td>Near-normal valve structure</td>
<td>VIC viability maintained; transient activation of effective remodeling</td>
</tr>
<tr>
<td>Valve allograft</td>
<td>Original collagenous structure largely maintained; cells lost</td>
<td>Absence of viable cells prevents any ECM remodeling; degradation of structural elements is cumulative; role of immunological processes uncertain</td>
</tr>
</tbody>
</table>
in vitro calcification models support the hypothesis that AV stenosis is mediated by osteoblastic differentiation of VICs.92

The basis of the observed vulnerability of the aortic side of the valve cusp to calcification in calcific aortic stenosis is poorly understood, but VECs may regulate VIC function. An emerging hypothesis is that the inflow-to-outflow side differences in gene expression are a result of the different hemodynamic waveforms experienced by the inflow and outflow faces of the valve cusps, similar to the flow-dependent phenotypic modulation of cultured human endothelial cells.93 In a parallel to vascular atherosclerosis, a potential mediator of this effect is Kruppel-like factor 2 (KLF2), a transcription factor regulated by shear stress profile and selectively induced in endothelial cells exposed to a biomechanical stimulus characteristic of regions of the arterial tree protected from atherosclerosis.94 Indeed, endothelial cells exposed to shear profiles predicted (by finite element analysis modeling) for the inflow surface of the valve (below which the valve is usually free of calcification) upregulate KLF2 relative to the outflow surface (Eli Weinberg, PhD, unpublished data, 2008).

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.60 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 valvular connective tissue, characterized biomechanically by a decrease in stiffness and an increase in extensibility,66 associated with increased GAGs97 and abnormal fibrillar ECM organization98,99 in both leaflets and chordae. The prevailing concept is that a defect in the mechanical integrity of the leaflet results from altered ECM synthesis and/or remodeling by VICs of the essential structural proteins and GAGs, which together with normal wear and tear leads to stretching, elongation, and other features of the clinical phenotype of MV prolapse. The connective tissue weakening may have as yet poorly understood implications for the durability of surgical procedures to repair MV prolapse. Furthermore, VICs in this disorder are activated, suggesting that a state of chronic mechanical disequilibrium exists because the adaptive ECM remodeling potential is exceeded (recall Figure 5a).

MV prolapse is associated with some heritable disorders of connective tissue, including Marfan syndrome, in which it is usually associated with mutations in fibrillin-1. However, most cases of MV prolapse are unassociated with fibrillin-1 abnormalities; indeed, it is unlikely that >1% to 2% of patients with MV prolapse have associated clearly identifi-
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.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.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 ($\approx 10^{-3}$ 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).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.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.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.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.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

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**Figure 6.** Initiation of bioprosthetic valve calcification in structural tissue elements. a, Gross photograph of explanted porcine bioprosthetic valve that failed by calcification and cuspal tearing. b, Photomicrograph of the edge of a calcific deposit in an experimental porcine AV bioprosthesis demonstrating both round and streaklike calcific densities, representing calcification initiated in cells (large arrow) and collagen (smaller arrow), respectively. Magnification $\times$400; von Kossa stain; calcium phosphates are black. c, Transmission electron microscopy photomicrograph of cell fragment–associated calcific deposits (arrows) (bar=1 $\mu$m). a and b, Reproduced 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. c, Reproduced from Schoen et al.113
cross-linking (freezing in dimethyl sulfoxide, followed by storage at \(-196^\circ\mathrm{C}\)), before thawing and implantation into the aortic root. Allograft valves have good hemodynamic profiles, a low incidence of thromboembolic complications without long-term anticoagulation, and a low infection rate. Although cryopreserved allograft valves are free of degeneration for periods comparable to those of conventional porcine bioprosthetic valves, progressive degeneration limits their long-term success. The mode of failure of these valves when inserted on the left side of the heart is generally incompetence caused by cuspal stretching or fibrosis. In contrast, right-sided valves in children who have right ventricle-to-pulmonary artery conduits usually suffer stenosis as a result of somatic growth of the recipient, with or without calcification of the cusps or distal aortic wall.

The pathological changes in allograft valves are in large part analogous to those of the aforementioned bioprosthesis valves. Owing to preparation ischemia and/or cryopreservation and handling damage, the cells of allograft valves (VECs and VICs) are nonviable. As in chemically preserved bioprosthetic valves, the collagenous network is initially present but (in the absence of viable VICs) is incapable of renewing. Thus, implanted cryopreserved allograft valves show an absence of cells, a loss of distinct normal structural features, and progressive collagen degeneration (Figure 7, right).

### PV-to-AV Autografts

Pulmonary autograft replacement of a diseased AV by surgical transfer of an individual’s PV to the aortic site 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).\(^{58}\) 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 bioreactor\(^{121}\)). 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 valved 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).\(^{62,122,123}\) 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
studies in the pulmonary position, but decellularized before implantation of the valve have been used in clinical ingrowth. Decellularized tissue scaffolds derived from valve migration sites capable of promoting cell attachment and architecture, mechanical properties, and potentially adhesion/differentiation. In this approach, the materials are not provide a fertile environment for their adherence, growth, 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 assembly 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 and signaling events including adhesion and migration, chemotraction, and differentiation.

Figure 8. 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.
under investigation. 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 endothelial progenitor cell mobilization, homing migration, and genetic aspects of common valvular lesions; and novel approaches to engineered tissue valve repair and therapeutic regeneration. Such progress exemplifies the integrated functional roles of valvular matrix, resident cells, and their mechanical and chemical environment. The key concepts that unify the dynamic pathological biology of heart valves and the mechanisms of heart valve disease can be used to improve biological valve substitutes and potentially enable heart valve regeneration.

Conclusions

There has been considerable and ongoing progress in understanding the dynamic pathophysiological basis of heart valve function and adaptation; the pathological basis, pathobiology, and genetic aspects of common valvular lesions; and novel approaches to engineered tissue valve repair and therapeutic regeneration. Such progress exemplifies the integrated functional roles of valvular matrix, resident cells, and their mechanical and chemical environment. The key concepts that unify the dynamic pathological biology 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.

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