The mechanobiology of mitral valve function, degeneration, and repair

Jennifer M. Richards, BS [a], Emily J. Farrar, BS [a], Bruce G. Kornreich, DVM, PhD [b], N. Sydney Moïse, DVM, MS [b], Jonathan T. Butcher, PhD [a],*

[a] Department of Biomedical Engineering, Cornell University, Ithaca NY, USA
[b] Department of Clinical Sciences, Section of Cardiology, College of Veterinary Medicine, Cornell University, Ithaca NY, USA

Abstract   In degenerative valve disease, the highly organized mitral valve leaflet matrix stratification is progressively destroyed and replaced with proteoglycan rich, mechanically inadequate tissue. This is driven by the actions of originally quiescent valve interstitial cells that become active contractile and migratory myofibroblasts. While treatment for myxomatous mitral valve disease in humans ranges from repair to total replacement, therapies in dogs focus on treating the consequences of the resulting mitral regurgitation. The fundamental gap in our understanding is how the resident valve cells respond to altered mechanical signals to drive tissue remodeling. Despite the pathological similarities and high clinical occurrence, surprisingly little mechanistic insight has been gleaned from the dog. This review presents what is known about mitral valve mechanobiology from clinical, in vivo, and in vitro data. There are a number of experimental strategies already available to pursue this significant opportunity, but success requires the collaboration between veterinary clinicians, scientists, and engineers.
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Introduction

The mitral valve is a unique tissue exposed to a complex mechanical environment, and while pathogenesis of the mitral valve is caused by many factors, it has become increasingly apparent that changes in the mechanics of the valve or its environment have great impact on degeneration. Myxomatous mitral valve disease (MMVD) is the most common cardiovascular disease of the dog, and is also recognized as a disease of increasing importance in the human population.[1,2] This chronic degeneration is largely responsible for

  * Corresponding author.
    E-mail address: jtb47@cornell.edu (J.T. Butcher).

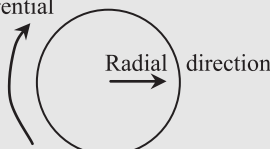| Abbreviations	
CT	chordae tendineae
ECM	extracellular matrix
MMVD	myxomatous mitral valve disease

mitral regurgitation, which can lead to congestive heart failure. Myxomatous mitral valves are characterized by a disorganization of the structural components of the leaflets and a weakening of the chordae tendineae (CT), which causes the valve to lose much of its mechanical ability.[3] This review addresses the mechanical complexities of the mitral valve and its environment, as well as the changes in leaflet composition and mechanics due to progressive degradation.

Mitral valve mechanics

The mitral valve consists of two leaflets, the anterior (septal) and posterior (parietal), with unique morphological and mechanical properties. Based on data from multiple mammalian species, the anterior leaflet is larger in surface area and thickness, has fewer CT attachments, and has a higher modulus of elasticity in response to both biaxial and uniaxial tensile tests. The modulus of elasticity is the tendency of a structure to be deformed and return to its original condition, that is, non-permanent change (Table 1). Reported tensile moduli values in sheep for both the anterior and posterior leaflets vary between 0.02 and 10 N/mm^2 in vitro[4–6] and 11–43 N/mm^2 in vivo.[7] The reason for this discrepancy is unknown, though hypotheses concerning contractile activation of the leaflet in vivo or variations in finite element modeling techniques have been posed.[7,8] Finite element modeling is a mathematical system for analyzing stresses and strains in material (e.g. heart valves) under certain conditions and numerically a method for solving nonlinear differential equations. A computer performs this analysis by breaking down a large, complex structure into a mesh of small, simplified components, and then applying forces to these components simultaneously. The posterior leaflet, in contrast, is smaller, thinner, and more compliant. Its greater number of chordal attachments are hypothesized to provide increased mechanical support, compensating for its comparatively delicate structure.[9] Both leaflets exhibit classical soft tissue biomechanical responses to stretch. Stretching tissue imparts deformation called strain, defined loosely as the change in length of a material with respect to a reference state. For a relatively large amount of strain, very little load is resisted by the valve leaflet, often called the "toe-region" of the stress-strain curve. After this region, the tissue dramatically stiffens, thus resulting in a highly nonlinear biomechanical response profile. At a microstructural level, collagen fibers of the valve become aligned and uncrimped under relatively small applied loads throughout the toe region of the response curve. After unfurling, fibers recruit neighboring filaments through their intertwined network, increasing the tissue's resistance to stretch thus raising its modulus. During the final linear phase of the curve, the fibers are at their maximum stretched length and fiber recruitment is

Table 1 Glossary of mechanical terms and definitions.

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Strain	The deformation of an object in terms of the ratio of current size over original size. Denoted by "ε". $\varepsilon = \Delta L / L$
Stress	Average force per unit area. Denoted by "σ". $\sigma = F/A$
Linear Modulus	Measure of the stiffness of an elastic material, equal to the slope of the linear portion of a material's stress-strain curve. Denoted by "E". $E = \sigma/\varepsilon$
Tensile	A force related to the stretching of an object; the opposite of compression
Circumferential Radial	
Finite element analysis	A mathematical system for analyzing stresses and strains in material (e.g. heart valves) under certain conditions. A computer performs this analysis by breaking down a large, complex structure into a mesh of small, simplified components, and then applying forces to these components simultaneously.
Inverse finite element analysis	The process of working backwards from a computer model to the implied properties of the material being modeled.
Extensibility	The degree to which an object undergoes deformation under an applied load.
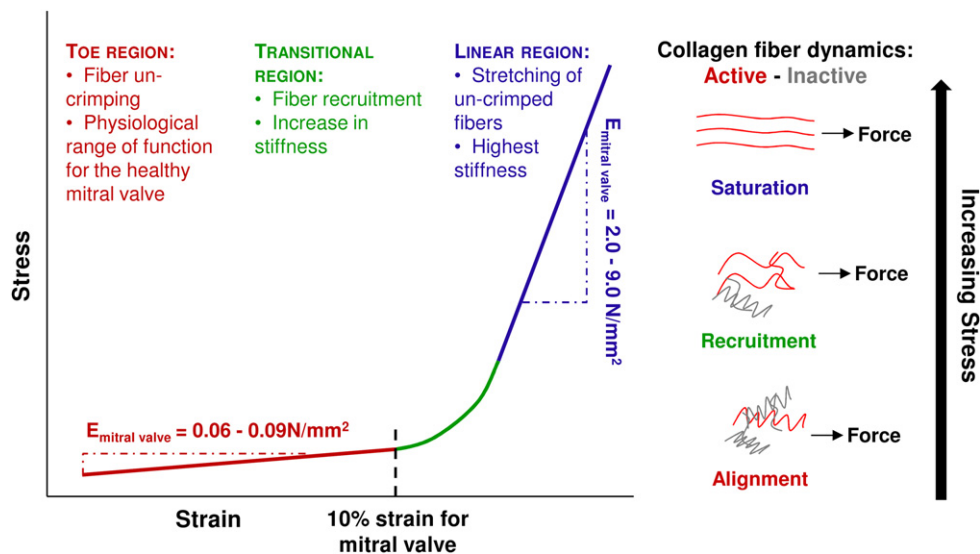
Figure 1 Representative stress-strain curve of a soft tissue under uniaxial tension with values relevant to the mitral valve, with uncrimping and aligning of collagen fibers as stress increases.

saturated. In a recent sheep study, Krishnamurthy et al. showed that during physiological function, the valve operates linearly in the toe or pre-transitional region, with strains less than 0.10 mm/mm (Fig. 1).[10] The same group has demonstrated that the mitral valve stiffens transiently throughout the cardiac cycle, having a stiffness in contraction that is 1.7 times its stiffness in relaxation.[11] As finite element modeling and in vivo mechanical monitoring techniques continue to improve, more nuances of the intricacy of the mitral valve's mechanical function will arise.

The leaflets of the mitral valve express wide biomechanical anisotropy and heterogeneity, directed by the complex underlying micro-structure as recently reviewed by Grande-Allen and

Figure 2 Atrial view of the closed mitral valve, showing gradations of tissue stiffness. Valve has highest stiffness (least deformation under applied load) at the annulus and lowest stiffness at the free edge, where the valve tissue experiences largest deformations.

Liao.[12] Both the anterior and posterior leaflets are stiffer in the circumferential direction than in the radial, with a decrease in modulus from the annulus to the free edge[13] (Fig. 2). Under normal physiological conditions, strains are uniform in the circumferential direction with a magnitude of approximately 5% and variable in a gradient across the radial direction with an average of 8% and a maximum in the belly region of the anterior leaflet[14] and throughout the thickness of the valve.[15] The annular region is stiffer than the free edge, and the fibrosa is less pliable than the atrialis or the ventricularis.[16] These properties correspond with the micro-structure of each area, which is influenced by the presence or absence of the CT.[4]

The CT are columnar structures unique to the mitral and tricuspid valves. During the cardiac cycle, the CT interface mechanically with the valve, papillary muscles, and (indirectly) the ventricular wall in response to intrinsic and extrinsic stimuli.[17,18] Mitral valve CT have been classified into three subtypes based on locale and function: strut, primary, and secondary.[19] The CT modulate the transmission of strain to the valve leaflets, as evidenced by the heterogeneity of mechanical properties and micro-structure of the chordal insertion regions within the leaflet. The clear zone in each valve leaflet, which has no CT insertion regions,[20] is stiffer circumferentially than the rough zone, where the CT attach to the leaflet.[6] Collagen fiber orientation is complex throughout the insertion region, allowing for three dimensional transmission of strain through the CT. Recently, it has been shown that the insertion region of the anterior leaflet strut CT experiences
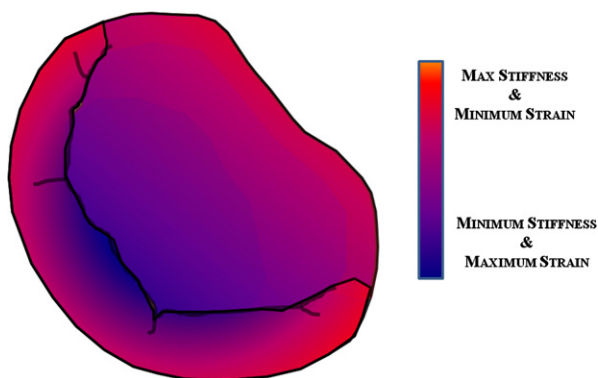
stretch throughout all phases of the cardiac cycle. Heterogeneous forces varying from radial stretch in early systole to coupled radial-circumferential deformation in mid-systole continue dynamically throughout the process of a single heart beat.[21]

Elongated CT are a common early-stage marker of degenerative canine mitral valve disease, in conjunction with leaflet thickening and prolapse.[22] The importance of the CT structure in mechanical transmission of strain between the valve and ventricular wall, the demonstrated surgical relevance of preserving the subvalvular structures, and the implication of the insertion region as the pathological "ground zero" for degenerative valve diseases suggest that dysregulation of the biomechanical signaling accomplished through the CT is a critical link in propagation of degenerative mitral valve disease in dogs.

Methods for assessment of mitral valve mechanics

Geometry, dynamic motion, and mechanical environment of the mitral valve have been of interest to researchers for more than 40 years. The most common method of in vivo imaging of the valve employs a system of radiopaque markers, which are opaque to the transmission of X-rays. The subject is then imaged using a bi-plane video angiogram, or a system of two perpendicular X-ray source and video cameras, over a series of cardiac cycles. This technique has progressed primarily in the addition of smaller markers more intentionally placed on regions of interest on the mitral valve, in reduction of surgical complications, and in more advanced processing of the resulting data. A large animal model such as sheep, cow, or pig is required to obtain the necessary surgical manipulation and imaging resolution of the mitral valve using this technique.[24–26] Additional mechanical data has been collected via implantation of force transducers on the mitral valve CT,[27–29] MRI, or echocardiography.[30–35] The latter approaches are more difficult to analyze due to the complexity of motion of the left side of the heart and valve; however, advances in 3-D echocardiography continue to improve the available resolution.[36,37]

After geometric data is acquired in vivo, inverse finite element analysis is used to infer the dynamic mechanical environment of the valve throughout the cardiac cycle. Additional necessary inputs include material properties of the valve, fluidic and structural boundary conditions, and a function for interpolation of results between discrete markers and time points. Limitations to finite element analyses involve the resolution of the inputs mentioned above. As measurement techniques become more sophisticated, we gain greater insight into complex mitral valve mechanical properties. Krishnamurthy et al. recently showed in an ovine model that the elastic modulus of the anterior valve leaflet, previously supposed to vary geometrically but not temporally, changes more than 70% throughout the cardiac cycle.[11] It is proposed that this change is due to contractile force development in the cardiac myocytes of the leaflet, later shown to act in porcine mitral valves through $\alpha2\beta1$ integrins coupling valvular interstitial cells with structural collagen.[38] Geometric heterogeneity is informed by both in vitro studies using finely controlled uni- and biaxial testing devices, and by histological evaluation of valve tissue corresponding with known mechanical properties of structural components. As recently reviewed by Weinberg et al,[39] these studies are limited by their choice of length scale (molecular, cellular, tissue, or organ) and a lack of investigation concerning mechanical interaction between the levels. Emerging technology platforms will undoubtedly enhance our ability to tie together insights from these different length scales,[40] where the nexus of these problems likely lie.

Mechanobiology of mitral valve degeneration

Degeneration of the mitral valve can refer to a number of conditions including ischemia, regurgitation, and myxomatous mitral valve disease, all of which include structural changes in the valve that impair normal function. While many of these pathologies are often used interchangeably, each disease does in fact have important differences in their mechanical and biological consequences (Table 2).

Typically, mitral valve degeneration is characterized by leaflet enlargement and annular dilation,[41] which often leads to prolapse and/or mitral regurgitation, as summarized in Fig. 3. The single most common acquired cardiovascular disease in dogs is myxomatous mitral valve disease. Accounting for nearly 75% of all cardiovascular disease in canines,[23,42] myxomatous valve disease in dogs is a much more prevalent pathology than in humans. It is becoming increasingly important for veterinary science to understand the biological and mechanical impacts of this disease.

Myxomatous mitral valve disease is often manifested by thickened chordae, which become elongated over time.[43] The valve is permeated

Table 2 Mechanical and biological effects of mitral valve pathologies.

Pathology	Mechanical effect	Biological effect
Ischemia[1–3]	• Septal-lateral dilation • AML stresses inc by 8% • PML by 5% • Increases nonplanarity angle (less curved) from 127 to 138°	• Raises MR 300%
Post-infarction[3–7]	• Flattened annulus • LV volume increases 30–40% • Increases wall shear stress • Loss of contractility • Papillary muscle rupture	• Regurgitation, ventricular remodeling
Dilated cardiomyopathy[8,9]	• Both leaflets increase in length Anterior: 2.11–2.43 cm Posterior1.14–1.33 cm) • Annular dilation	• Greater cell density, loss of leaflet layered structure, collagen and elastic fiber turnover and myofibroblasts was greater
Regurgitation[4,10]	• Annular area increases 115% • Interestingly, no change in annular shape	• Type III collagen, MMP, proteoglycan increase
Annular dilatation[11]	• Overall stress increases >100% • CT tension increased • Changes leaflet interface during coaptation	• Regurgitation is the only studied effect
Chordal dysfunction[12–14]	• Stresses increase in adjacent chords, can improve coaptation by loosening tension in CT	• Mixed, cause remodeling at the MV-CT junction
Myxomatous MVD[15–18]	• Stretch of CT and leaflets (prolapse), inc nonplanarity angle from 127 to 133, inc'd LV internal diameter in diastole, lower CT modulus and tensile failure strength	• Glycosaminoglycans, collagen disruption, leaflet thickening

with myxoid lesions that damages the tri-layered architecture as well as altering the collagen and elastin composition within the leaftlet.[41] The spongiosa increases in size while the structure of the fibrosa layer degenerates.[44,45] The myxoid lesions are most pronounced at the free margins of the valve, extending in some cases to the base of the leaflet.[42,44] Unlike myxomatous mitral valve disease, fibroelastic deficiency does not exhibit tissue leaflet or CT thickening other than in the prolapsed area.[41]

Not surprisingly, alterations in the mechanical environment and structural composition of myxomatous mitral valves lead to changes in the mechanical properties of the leaflets and CT. Barber et al reported higher extensibility in human myxomatous leaflets for both circumferential and radial strain, although no great difference in overall leaflet strength.[46] The mechanical changes of the leaflets, while significant, are not as drastic as the mechanical changes in the CT. Whether this is true in dogs has not been studied. Myxomatous valves are characterized by enlarged leaflets and expanded CT that become permanently stretched, which accounts for the documented decrease in extensibility. Increased stiffness in the leaflet, combined with the enlargement of the mitral annulus, prevents the valve from completely covering the orifice[5,47] which in turn instigates mitral regurgitation. Finite element analysis reveals that increased stiffness results in increased stress in the CT and leaflets, and additionally
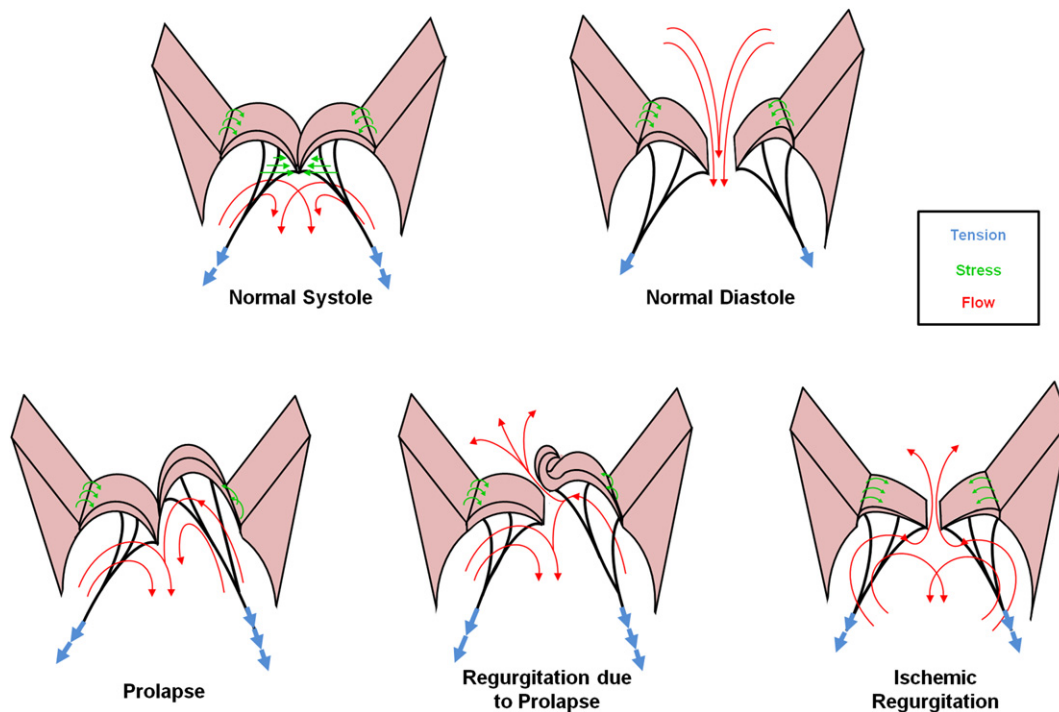
Figure 3 Mechanical environment of the mitral valve under normal and pathological conditions. The mitral valve experiences a complex mechanical loading profile that changes dramatically during disease conditions. Red: general blood flow patterns, green: regions of elevated tissue stress, blue: degree of chordal tension. The combination of these forces exacerbates structural remodeling, but it is not yet known whether the underlying mechanisms for each component are similar.

reduces likelihood of coaptation.[47] While increased tissue thickness in this model can reduce chordal and leaflet stress, coaptation is also reduced.

Myxomatous valves exhibit abnormal cell phenotypes. Normal mitral interstitial cells display a fibroblastic phenotype; however, progressively severe myxomatous valves shift to a myofibroblastic phenotype.[48,49] A recent canine study documented interstitial cells that progressively stained positive for α-actin in severely degenerated valves.[48] Matrix metalloproteinases are present in increasing quantities with progressively degenerate valves. Rabkin et al. found that activated interstitial cells in human myxomatous valves expressed collagenolytic enzymes, but no increase in collagen itself.[50] This altered enzymatic activity changes the ECM, which leads the valve down a degenerative path. In myxomatous mitral valves, the endothelial cells lining each leaflet are also affected by the constantly changing leaflet environment. Hemodynamic changes due to leaflet thickening as well as altered ECM contribute to altered endothelial function. Endothelial cells will exhibit atypical phentotypes or even be lost completely in more advanced stages of mitral valve disease.[51]

Myxomatous mitral valves are characterized with significant collagen and glycosaminoglycan accumulation.[52] An early human study identified 59% increase of proteoglycan content, as well as a 62% increase in the elastin content in diseased valve leaflets, which corroborates with recent findings.[53] Despite an increase in ECM, there is no significant increase in mature collagen or elastic fibers.[44] Indeed, myxomatous areas of the valve are often only sparsely arranged with collagen fibrils, which frequently have a spiraling appearance.[45] Alterations in ECM may result from the changed mechanical environment in which the enlarged valve leaflets reside.[45,54] A recent canine study characterized ECM content of normal and chronically diseased mitral valves and found basement membrane components such as laminin and fibronectin were also increased.[55] Myxoid infiltrating lesions in the marginal region were mainly comprised of collagen I and V, whereas lesions in the central region were found to be mainly collagen I and III.[55] Changes in ECM composition, specifically those that modulate stiffness, have been shown to effect both cell motility and adhesion by decreasing the ability of the cell to bind to its substrate and exert

contractile forces.[56] Substrate stiffness has also been shown to influence cell signaling, specifically in the context of endothelial cell network formation[57] and tissue assembly.[58] The changes in ECM stiffness corresponding to mitral valve degeneration feasibly changes the ability of the resident cells to communicate, migrate, and influence the valvular mechanical environment, thereby propagating the dysregulation of valve phenotype associated with mitral valve disease. Combining functional studies of the effects of ECM composition on cell behavior with the existing descriptions of diseased mitral valves will lead to increased understanding of the mechanisms of valve degeneration.

Collagen is largely responsible for the mechanical strength of the leaflets and CT. Collagen accounts for about 60% of the dry weight of normal sheep mitral valve leaflets, which decreases dramatically in conditions such as ischemia and infarctions.[47] During myxomatous degeneration, collagen fibrils undergo changes in organization and lose their ability to provide mechanical stability and flexibility.[59] A canine study using X-ray diffraction to analyze collagen fibril organization found parallel alignment to the free edge in healthy valve tissue, while collagen fibrils were arranged in a more orthogonal position closer to the annulus.[59] However, diseased portions of the valve lose any fibril order or orientation, which could have important mechanical consequences. While there is a significant reduction in the number of organized collagen fibrils in myxomatous valves, it was shown in a separate canine study that the overall collagen content does not decrease dramatically.[60] As the primary structural component of the valve leaflet, collagen fiber orientation determines the distribution and magnitude of strain within the valve leaflet as it undergoes the deformations imposed by the cardiac cycle. Strain is known to influence a wide variety of cellular functions, including remodeling,[61] apoptosis,[62] proliferation,[63] migration,[64] and calcyclin gene expression[65] in fibroblasts, cell cycle arrest in vascular smooth muscle cells,[66] and stress fiber formation in endothelial cells.[67] Studies using valvular cell types and loading conditions specific to the previously described changes in stretch-state associated with degenerative mitral valve disease are necessary to delineate which of these effects are involved in the mechanobiology of mitral valve disease.

There is a demonstrated feedback effect between ECM composition and strain, in which ECM changes effect strain distribution and magnitude and imposed strains influence cellular synthesis of ECM components. For example, stretch has been shown to modulate collagen production in pulmonary arterial smooth muscle cells[68] and to increase proteoglycan expression in porcine mitral valve interstitial cells.[69] The same mechanisms are likely at work within the degenerative mitral valve, leading to a two-way effect of pathological changes in stress-strain state on cellular and valvular function.

The evidence of ECM changes in degenerative mitral valves, the impact of collagen on strain distribution, and the demonstrated impact of strain on cellular function in many cell types which are similar in phenotype to valve cells combine to introduce a compelling case for expanded examination of the role of mechanics in mitral valve disease.

Surgical repair of degenerated mitral valves

Surgical intervention in mitral valve degeneration represents the most profound change in mechanics that can be applied to a native valve. Replacement strategies are limited to mechanical or bioprosthetic implant; whereas, repair can constitute any combination of leaflet resection, annuloplasty, edge-to-edge repair, and/or modification of CT. The mechanical and biological effects of these strategies are outlined in Tables 2 and 3. Options for mitral valve replacement include mechanical valves and bioprosthetics, usually reinforced porcine tissue. The former is rarely used in the canine context due to the high potential for thrombogenicity and the challenge of consistent anticoagulation therapy in veterinary patients.[70,71] Bioprosthetics, especially porcine valves, are gaining in popularity, with recent reports showing maintenance of antithrombogenicity out to twelve months post-operatively.[72]

Long-term studies have indicated that mitral valve repair offers increased survival and durability versus mitral valve replacement.[73] The preservation of native cellular and molecular structures compensates for the trauma of current operative procedures such as leaflet resection, annuloplasty, or neochordae implementation. These techniques aim to restore the native architecture of the valve at the macro-level, primarily assessed by coaptation area, annulus diameter, and regurgitation.[74] Common procedures include annuloplasty, edge-to-edge repair, and implementation of artificial CT.[71]

Annuloplasty adjusts the diameter of the atrioventricular canal annulus using a "purse-string" surgical technique to reduce the annulus diameter,

often with the addition of reinforcing material at the annular circumference.[74,75] From a mechanical perspective, annuloplasty necessarily changes the distribution of stresses both in the valve annulus and leaflets.[76] Edge-to-edge repair attaches the free edge of the prolapsed leaflet to the opposing leaflet free edge, resulting in a double orifice flow pattern.[77] When added to annuloplasty, edge-to-edge repair changes the leaflet stress distribution from a focal region of high stress at the suture site to a lesser, more uniform stress distributed across the leaflet free edge.[78] Post-operative evidence suggests that maintaining the integrity of the CT is critical to successful surgical outcomes,[79] primarily because loss of chordal function results in lesser radial deformation throughout the cardiac cycle, with a corresponding increase in circumferential stretch.[15] This is achieved either through adjustment of the native CT and adjoining papillary muscles or with the insertion of artificial CT (ACT).[80]

There are many gaps in the current understanding of interactions between valve mechanics and biology. For example, although various correlations between a change in valve annulus boundary conditions via annuloplasty or dilation and cellular remodeling have been presented, the causal relationships are lacking. Evaluating current surgical procedures and pharmacological regimes

Table 3 Mechanical and biological effects of mitral valve repair strategies.

Repair strategy	Mechanical effect	Biological effect
Mechanical valve[19–21]	• Drastically changes mechanical conditions of the atrioventricular canal • Restricts annular deformation • More turbulent hemodynamics	• Increases thrombogenicity • Complex plasma–prostheses interactions • Incomplete re-endothelization
Bioprosthetic valve[20,22–24]	• Decreased prosthetic tissue extensibility • Reduction of failure strain over time (11 yr study)	• Long-term leaflet calcification and tearing • Increased collagen deposition • Progressive delamination of layers
Leaflet resection[25–28]	• Reduces leaflet area • Restores normal hemodynamic patterns • Introduces suture and scar tissue interference to the coaptation surface • Increases leaflet stiffness (unquantified)	• Reduces systolic pulmonary artery pressure (no bio data so far) • Abundant collagen fiber deposition • Calcified deposits in basal region at 4 weeks (dogs) • Slower wound healing at free edge than in belly region
Annuloplasty[2,29–32]	• Reduces peak stress • Increases bending stress • Lowers PM/CT tension • Flat ring configuration reduces peak strain on posterior leaflet by 78%	• Thin fibrous sheath (Type III collagen) encapsulates ring • Re-endothelization of the capsule surface • Elicits mild inflammatory reaction and neovascularization
Edge-to-edge repair[28,33–37]	• Changes flow pattern to double orifice valve • Eliminates leaflet coaptation • Doubles frequency of hemodynamic loading	• Short term: inflammation, wound healing • Long-term (1 yr): encapsulates device by organized, fibrous growth • Type I collagen rich matrix deposition, some calcified deposits
Chordae modification (shortening or ACT)[26,38,39]	• Optimally, restores native tension • Does not restore mechanical properties of native chordae • Changes direction of applied force, depending on attachment point	• Increases stress on native chordae • 3% increase in leaflet area • 2.8x increase in leaflet thickness • Increased volume of spongiosa layer • Possible endothelial to mesenchymal transformation

using the imaging techniques described above in combination with in vitro cellular studies such as that recently performed by Throm Quinlan et al[81] will progress the field toward a mechanistic explanation of why and how certain treatments of canine degenerative valve disease affect long-term patient outcomes.

The way forward

The mitral valve is a complex biomechanical structure that undergoes a multitude of mechanical inputs in normal physiology including cyclic bending, hemodynamic shear stresses, and heterogeneous strain. Current surgical, radiographic, and computational methods for quantification of these forces continue to expand understanding of its function and abilities. Pathological and surgical changes acting on mitral valve mechanics in concert with biological effects represent an important facet of clinical understanding. The successful development of a comprehensive therapeutic strategy will necessitate the quantification of the biomechanical consequences of surgical and pharmacological treatment in order to manage the feedback cycle between biomechanics and cell, tissue, and system-level responses. Increased focus on the synergistic effects of mechanobiological interactions within the mitral valve at all length scales will continue to introduce new perspectives on degenerative mitral valve disease. To move forward, we need a more detailed quantification of the local mechanical environment of the dog mitral valve, which is possible with current imaging technologies. Next, we need to establish mechanisms between these key mechanical signals and biological changes in valve cells. Here is where 3D cell culture models and in vitro bioreactor technologies can lead the way as has been initiated in other species. Once this information is known, we can begin to tie together the disparate clinical pathogeneses of the multiple MMVD pathways into common testable hypotheses. Moreover, the same in vitro models can then become high throughput test beds for screening new molecular strategies that target the specific degeneration pathways. The dog provides an ideal animal model to investigate and advance these scientific goals, the results of which will be a win–win for both species.

Acknowledgments

Conflict of interest

The authors have no financial interests to disclose.

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