Editorial: Special Issue on Heart Valve Mechanobiology

New Insights into Mechanical Regulation of Valve Disease and Regeneration

Heart valve diseases—both “degenerative” etiologies associated with aging and rheumatic disease in developing countries—affect ~2–3% of the world’s population.¹⁰ Medical treatments for the most common valvular diseases are largely nonexistent, and as such valvular surgeries now make up more than 20% of all cardiac surgeries.¹⁰ While there have been significant advances in minimally-invasive surgical interventions for valvular diseases in the past decade,¹⁴ there remain unmet needs for alternative therapies that are broadly applicable, enable early intervention before significant deterioration in quality of life, and address the needs of pediatric patients.

To address these unmet needs, two main alternative approaches have emerged: (1) pharmacological treatments that arrest or reverse the disease process before the need for surgery, and (2) tissue engineering of living replacement valves as alternatives to mechanical or bioprosthetic valves, which cannot integrate and grow with the host tissue in pediatric patients.

The interest in medical therapies has sparked increased research activity over the past 10 years to better understanding the etiology of valvular diseases. Calcific aortic valve disease (CAVD) has been a particular focus, as it is the most common heart valve disease in the aging population in the developed world, with a projected disease burden of 4.5 million people by 2030.¹ Valve dysfunction in CAVD results from tissue stiffening due to scarring and calcification of the valve leaflets, with nodules often localized to the fibrosa layer on the aortic side of the valve. Progression to aortic stenosis, in which outflow from the left ventricle is obstructed, occurs in up to 5% of elderly patients^{12,24} and carries an 80% 5-year risk of progression to heart failure, valve replacement, or death.¹⁶ The maladaptive remodeling of the leaflet extracellular matrix (ECM) is now recognized not to be simply due to degeneration with age, but to be an actively regulated cell-mediated process.^{19,31} This implies that there are opportunities to intervene, and thus there is a pressing need to better understand the cellular and molecular underpinnings of CAVD as targets for its treatment.

For pediatric patients with congenital disease affecting their valves, tissue engineering holds great potential to provide replacement valves that can integrate, grow, and adapt with the child. Although recent reports show early promise for tissue engineered valve replacements in sheep^{3,27} and evidence of somatic growth of vascular grafts in lambs,²⁶ clinical translation has yet to be achieved. To this end, better understanding of how multiple regulatory factors individually and integratively influence cell function, tissue formation and organization, and mechanical function is likely to inform design criteria for engineered heart valves.²⁸

Notably, valve biology is exquisitely sensitive to biomechanical forces. This is observed in development, where disruption of blood flow-induced shear stresses leads to malformed valves,⁸ and is exploited in *in vitro* tissue engineering strategies that use bioreactors to condition nascent valves prior to implantation.¹⁷ Similarly, in diseases like CAVD, the spatial correlation between lesions and distinct local biomechanical environments with disturbed shear stress, increased stretch, and stiffer matrices suggests that valvular diseases are also mechanobiologically-regulated. Thus, insights into both valve disease mechanisms and tissue regeneration strategies will come from interdisciplinary approaches that span biology and engineering to dissect the roles of mechanics in valve biology.

In this special issue of *Cardiovascular Engineering and Technology*, mechanobiological regulation of valve biology is explored in a variety of contexts relevant to valve disease and tissue engineering. The issue begins with a masterful introduction to valve health and disease by Schoen.²² His review provides comprehensive clinical, structural, biological, and pathological perspectives on valvular biology, disease, repair, and regeneration, with consideration given to a variety of valvular diseases and valve substitutes. Of particular relevance to this special issue, key structural, functional, and biological features of the aortic valve that contribute to its unique mechanobiology are highlighted, and mechanisms by which mechanobiological feedback, in which mechanics both contributes to and is altered by CAVD, are proposed. Finally, Schoen nicely identifies priorities for ongoing research and development in native and substitute valve patholo-

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gies, many of which are linked to the mechanobiology of the valve.

Links between biomechanics and valve biology introduced in Schoen's review are explored in the five original research articles that comprise the remainder of this special issue. Two related articles by Heath *et al.*⁶ and Mongkoldhumrongkul *et al.*¹⁵ explore the novel question of how fluid flow-induced shear stresses on the fibrosa side of the aortic valve regulate matrix synthesis and remodeling by valvular endothelial cells (VECs). These studies are motivated by the pathological observation that aortic valve lesions form preferentially in the fibrosa layer facing the aorta, where the endothelium experiences low magnitude, oscillatory shear stress. In contrast, lesions rarely form in the ventricularis layer, facing the left ventricle, which experiences high magnitude, pulsatile, unidirectional, laminar shear stress. Transcriptional²³ and microRNA^{7,20} profiling previously identified side-specific endothelial phenotypes, and subsequent *in vitro* studies demonstrated that side-dependent shear stresses can regulate endothelial function consistent with side-dependent disease susceptibility.^{20,21,25}

In this special issue, Heath *et al.*⁶ build on their previous work to determine the role of one particular microRNA, miR-181b, which they previously identified to be abundant in VECs and upregulated by oscillatory shear stress *in vitro*, suggesting that it may promote CAVD. Through *in silico* analysis and functional assays *in vitro*, they showed that increased miR-181b expression, as is observed in the fibrosa, increases metalloproteinase (MMP) activity *via* downregulation of TIMP3, an inhibitor of ECM degradation through MMP inhibition. Elevated MMP levels and matrix degradation and remodeling are characteristic of CAVD pathology, and thus this work identifies a shear stress-dependent miRNA and TIMP3 as potential targets for CAVD treatment.

In a related paper in this special issue, Mongkoldhumrongkul *et al.*¹⁵ investigated the effect of side-specific shear stresses on endothelial production of collagen, glycosaminoglycans (GAGs), and elastin, key ECM components of the aortic valve. Using a cone-and-plate bioreactor *in vitro*, they measured ECM protein levels in leaflet tissue when the aortic or ventricular side of the leaflet was subjected to oscillatory or laminar shear stress. Their results suggest that physiological flow helps to maintain ECM components relative to static culture, but that some of these effects are side-dependent: for example, oscillatory flow promoted collagen and GAG accumulation when applied to the aortic side of the leaflet (which normally experience oscillatory flow), but not the ventricular side. These interesting findings add to the growing evidence that shear-sensitivity of the valvular endothelium is

side-dependent,^{20,21,25} with has important implications not only for understanding disease mechanisms, but also for tissue engineering, as it implies that "side-appropriate" endothelial cells will be required to mimic native side-dependent endothelial functions in engineered heart valves.

In addition to shear stress, VECs may also be mechanically regulated by the material properties of their underlying ECM. Matrix stiffness regulation of cell biology has emerged as a key player and therapeutic target in cancer, atherosclerosis, and other diseases.¹¹ Similar studies in the valve have been motivated by (1) the fact that CAVD lesions form in the natively stiffer fibrosa layer,³² and (2) that the hallmark of CAVD is matrix remodeling and associated changes in tissue stiffness that may feedback to promote disease progression.⁵ Matrix stiffness regulation of valvular interstitial cell (VIC) function has been reasonably well-studied, particularly in the context of pathological differentiation of VICs to myofibroblasts and osteoblasts.^{2,4,13,30} ECM stiffness has been shown to influence vascular endothelial function,⁹ but its role in valvular endothelial biology had not been explored until the paper by Zhong *et al.*³³ in this issue. In their paper, Zhong *et al.* studied the effect of matrix stiffness on transforming growth factor (TGF)- β -induced transformation of VECs to myofibroblasts. This process, known as endothelial-to-mesenchymal transformation (EndMT), is essential to normal valve development and is thought to be re-activated in CAVD, with the endothelial cells transformed to the mesenchymal population contributing to remodeling of the valve interstitium. Using *in vitro* models, Zhong *et al.* found that EndMT by adult VECs is enhanced on stiffer, fibrotic tissue-like substrates compared with softer, normal tissue-like substrates. They also found that while initiation of EndMT by TGF- β did not depend on β -catenin signaling, complete transformation to activated myofibroblasts did. These studies provide new insights into the mechanobiological role of the matrix stiffness in regulating the valvular endothelium and identify the Wnt/ β -catenin pathway as a potential target to prevent the detrimental formation of activated myofibroblasts in CAVD.

Whether they derive from the resident VIC population or by EndMT, activated myofibroblasts contribute to many of the processes that result in failure of both native and engineered valves, including dysregulated matrix remodeling (leading to leaflet thickening and stiffening) and increased contractility (leading to leaflet retraction and regurgitation).¹⁷ The final two papers in this special issue consider the role of the activated myofibroblast in these contexts. In the first, Perez *et al.*¹⁸ used a combination of mouse models and novel *in vitro* approaches to report for the first time the

effects of serotonin (associated with drug-induced valve disease and known to be mechanosensitive) in a context of hypertension (induced by angiotensin-II (Ang-II) treatment) on valve disease and VIC function. They found that combined serotonin and Ang-II treatment resulted in the worst cardiac function, thicker leaflets with more collagen, and greater VIC activation, as indicated by increased contractility and collagen gene expression. An important implication of this study is that valve cell responsiveness to drugs or other soluble factors can be mechanosensitive, and thus a mechanism by which biomechanical forces may influence valve (patho)biology is to modulate responses to other regulatory cues in the cell microenvironment.

The final paper in this special issue by van Loosdregt *et al.*²⁹ considers myofibroblast contraction, not in the context of disease, but in the context of engineered cardiovascular tissues where one has the opportunity to define scaffold architecture and other properties to potentially influence cell function and tissue functionality. As in the native valve, cell contractile stress is important for engineered valves: too much stress can lead to leaflet shortening and too little stress can cause leaflet elongation, both of which will compromise valve function. In their paper, van Loosdregt *et al.* cleverly used microcontact printing on thin films to investigate if organization of cell monolayers, from strongly anisotropic to isotropic, influenced the intrinsic mechanical stress generated by individual myofibroblasts. Surprisingly, they found that the intrinsic stress generated by an individual cell in the monolayer was independent of the organization of the monolayer. This has important implications for valve tissue engineering, as it implies that the *total* intrinsic stress distribution in a cell-seeded scaffold can be prescribed by changing the architecture of the scaffold, since the individual cell stresses will remain unchanged.

In total, this special issue highlights both the opportunities and challenges inherent in the field of heart valve research. As outlined in Dr. Schoen's review, this is a relatively young field with many more outstanding questions than there are answers. But as demonstrated in the papers in this special issue, significant insights into the mechanisms of valve disease and strategies to develop novel therapeutics and to engineer living replacement valves can come from understanding the mechanobiology of the valve. While this approach is not without its challenges—including the general lack of *in vivo* models with which to study valve mechanobiology (particularly as it relates to CAVD) and the need for greater integration between engineers, biologists, and clinicians—we feel that this approach will continue to prove fruitful and therefore is worth pursuing.

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CONFLICT OF INTEREST

Craig Simmons and Hanjoong Jo declare that they have no conflict of interest.

ETHICAL APPROVAL

No human or animal studies were carried out by the authors for this article.

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