Side-specific mechanical properties of valve endothelial cells

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The side specificity of valve calcification might be attributed to the inherent differences in the ECs lining the aortic and ventricular sides of the valve, and indeed there is evidence supporting this hypothesis (18, 34). In a previous study from our laboratory, we demonstrated that the endothelium is a source of mediators that may serve to protect the valve from procalcific stimuli by regulating the mechanical stiffness of the valve (13). This could play a key role in regulating the calcification process due to the association between matrix stiffness and valve calcification (39). Several studies have shown that restoration of endothelial function mitigates aortic valve calcification. With the use of an in vitro model of porcine aortic valve interstitial cell calcification, it has been shown that supplementation of cultures maintained in osteogenic medium (containing transforming growth factor-β) with nitric oxide (NO) donors or agents that increase intracellular cGMP prevented an osteogenic response (19). Exercise training was also shown to simultaneously preserve endothelial integrity and limit progression of aortic valve calcification in a low density lipoprotein receptor-deficient mouse model (24). Considering the emerging evidence of side-specific differences in ECs from the aortic valve, we hypothesized that in the aortic valve, the ability of ECs to stiffen or soften occurs via the action of autacoids in a side-specific manner.

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The aim of this study was to investigate if the mechanical properties of the ECs differ on each side of the valve to establish the role of autocrine mediators in modulating the mechanical properties of the cells. Additional aims include determination of whether the mechanical properties of the valve ECs are affected by the flow to which they are exposed or if they are an inherent property of the cells and independent of flow. This information will aid in our understanding of how these cells contribute to the sophisticated function of the valve in physiological and pathophysiological conditions.

MATERIALS AND METHODS

Tissue Isolation

Porcine hearts (18–24 mo old) were obtained from a commercial slaughterhouse (Cheale Meats). Aortic cusps were excised under sterile conditions and placed immediately in DMEM and used within 12 h of death. A square-shaped section from the belly region of each cusp was cut using a scalpel blade.

Side-Specific Valve EC Isolation and Culture

Aortic valve cusps were obtained as described above and placed in a custom-made chamber. A Perspex plate with a series of holes 1 cm in diameter was then placed over the tissue and firmly tightened, creating a tight seal, so that either the aortic or the ventricular surfaces surface was exposed to enzymatic digestion. Collagenase type II solution (Sigma) diluted in PBS (600 U/ml) was added to the chamber and placed in an incubator (95% O2-5% CO2) at 37°C for 12 min. The liquid contents of the chamber were then transferred into tubes, and an equal volume of cold serum-free M199 solution (10 ml) was added. The tubes were vigorously vortexed for a period of 60 s to dislodge the ECs from the surface of the valve. The resultant medium was placed in a centrifuge at 1,000 g for 5 min. The supernatant was discarded, and the pellet was resuspended in 1 ml of endothelial growth medium (EGM 2; Promocell) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml (1%) penicillin (Life Technologies), 100 μmol/l (1%) streptomycin (Life Technologies), and 4 mmol/l (1%) L-glutamine (Invitrogen). Cells were then clonally expanded in culture plates coated with 1% gelatin and subsequently characterized by immunocytochemistry. Cells staining positive for platelet EC adhesion molecule (CD31) and von Willebrand factor while showing negative staining for smooth muscle α-actin were considered pure EC colonies.

Scanning Ion Conductance Microscopy

A scanning ion conductance microscope (SICM) is a scanning probe microscopy technique that uses a electrolyte-filled glass nanopipette with an electrode inside as a sensitive probe that detects ion current passing through the pipette tip (20). The probe, mounted on a three-axis piezo translation stage, is modulated vertically at a frequency of 200 Hz (hopping mode). In the hopping mode, the reference current is measured at each imaging point while the pipette is well away from the surface. The pipette then approaches until the current is reduced by a predefined amount, usually 0.25–1%. The position of z-dimension actuator when the current achieves this reduction is recorded as the height of the sample at this imaging point. Typically, even at a 1% reduction of the current, the pipette is still at a distance of about one inner pipette radius from the surface. The z-dimension actuator then withdraws the pipette away from the surface and the sample is moved laterally to the next imaging point. By continuously updating the reference current while the pipette is away from the surface, the method automatically adjusts for any slow drifts in the pipette current. The control/data acquisition electronics record both the lateral and vertical positions of the probe and generate the topographical image (26).

Assessment of EC Compliance and Data Analysis

To compare mechanical properties of cells plated on a cell culture dish and cells directly on the valve leaflets, we extended the SICM technology by a new method that allows probing cell surface compliance and determining mechanical properties such as the modulus of elasticity. The new modification profited from the fact

Fig. 1. The principle of measurements of membrane compliance of endothelial cells (ECs). A: schematic diagram of the experiment to probe cell mechanical properties. A jet of liquid is applied through the pipette while it remains keeping the same distance (d) to the membrane due to the feedback control mechanism. It results in the displacement of the pipette (z) that can be recorded. B: displacement as function of pressure (P) applied to the highest point in the middle of ECs in the cardiac valve and a typical image obtain from the surface of the valve (shown in the inset).
that we are probing cells with a glass pipette, and therefore, we can apply hydrostatic forces through the pipette to defined positions on the cell surface (33). As in the standard SICM, the distance between the pipette and cell surface was kept constant using ion conductance-based distance feedback (25). This prevented any contact of the pipette with the cell surface, thus eliminating contamination and permitting fast and accurate measurements. Importantly, as we could map individual cells after topographical scanning, we then could choose multiple areas of interest on each cell and successfully measure their membrane mechanical properties. To measure mechanical properties of cell membrane, the same SICM pipette was mounted in a patch-clamp electrode holder (Harvard Apparatus) and, after having acquired the scan image, a positive hydrostatic jet (0.1–40 kPa) was supplied through the pressure port of this holder via tubing as described previously (33). The pressure was monitored using a PM100D pressure manometer (WPI). The distance between the pipette and cell surface (≈200 nm) was controlled by the fast feedback mechanism of the “hopping” mode of the SICM to prevent any contact between the sample and the probe during the pressure application. Pipettes used for mechanical probing had resistance of ≈25 MΩ when filled with PBS. Each sample was first imaged and then probed for mechanical properties using the same pipette. The recorded topography image was used to position the pipette on a particular spot to perform the pressure application (Fig. 1).

**Effects of endothelial-derived mediators on EC compliance.** After an initial measurement of the mechanical properties of at least 20 cells on each specimen, cusps were incubated for 20 min with either the endothelium-derived vasoconstrictor peptide endothelin-1 (ET-1) or the NO donor sodium nitroprusside (SNP; both from Sigma-Aldrich). In addition, the effect of endogenously released NO was assessed by the addition of the NO synthase inhibitor N^G^-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich). Following incubation of each agent the mechanical properties of individual ECs on the cusp surface were reassessed.

**Fig. 2.** Topography of EC architecture demonstrating cell size, shape, and alignment of cell on the ventricular and aortic surface of aortic valve cusps, as seen in fresh samples of tissue using scanning ion conductance microscopy (SICM; A and C) and in glutaraldehyde-fixed samples using scanning electron microscopy (SEM; B and D).

**Fig. 3.** Comparisons of the compliance of ECs from the ventricular and aortic surface of aortic valve cusps. Compliance was measured with SICM of cell on freshly isolated valve cusps (A) or in cells that had been isolated selectively from the ventricular (V) and aortic (A) surface of valve cusps by collagenase digestion and maintained under standard culture conditions for up to 5 passages culture (B; *P < 0.05; n = 6).

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Scanning Electron Microscopy

The morphology of the valve endothelium on each side of the valve was also assessed with scanning electron microscopy (SEM). Specimens of porcine valves were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer for 2 h at room temperature. After two buffer washes, the secondary fixation with 1% osmium tetroxide in 0.1 M phosphate buffer was carried out at room temperature for 1 h. Then the specimens were washed twice in buffer, incubated with 1% tannic acid in 0.1 M phosphate buffer for 1 h and dehydrated in ascending ethanol series starting from 20% ethanol. Then, the specimens were treated with hexamethyl disilazane (Sigma) for three times 2 min in each solution, air-dried, and mounted on SEM stubs. The stubs were sputter coated with gold and viewed in JEOL JSM-5500 LV SEM.

Immunofluorescence Staining

Aortic valve cusps were dissected, the middle region of each leaflet was cut into two adjacent square pieces, and each was placed in a well of a 12-well plate containing DMEM. Cusps were incubated with either $10^{-8}$ M ET-1 or $10^{-6}$ M SNP, with the adjacent half of each leaflet acting as the corresponding control. Identical procedures were carried out with either the aortic or ventricular surface of the cusps facing uppermost. All samples were incubated at 37°C for 30 min, after which samples were then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 30 min and then washed in PBS. All the specimens were permeabilized and stabilized in 5% normal goat serum in 0.5% Triton X for 4 h. Fluorescein isothiocyanate-conjugated CD31 (Serotec) at 1:100 and rhodamine-conjugated phalloidin at 1:500 (Millipore) were added to the specimens and incubated overnight at room temperature. Specimens were washed in PBS (Sigma), and DAPI was added at 1:20,000 was added for a further 10 min and then mounted on cavity slides using Permafluor (Invitrogen). Slides were viewed and 8–10 images were taken from each sample using the same settings on Zeiss LSM 500 confocal microscope. CD31 staining was used as a guideline to determine the presence of endothelium. Z-stacks were constructed, and maximum intensity projection was used to quantify the images using ImageJ software. The intensity of phalloidin staining was measured as a percentage of the total area.

Exposure of Valve ECs to Aortic and Ventricular Flow Patterns

To replicate the oscillatory and laminar shear stress at the same magnitude of flow as the physiological condition of aortic valve, a purpose built flow chamber was used that is mainly composed of a spinning cone made with 179°C one angle and a cylindrical plate containing nine equi-angularly spaced shallow wells where valve ECs seeded onto coverslips can be mounted. Isolated cells or intact valve tissue can be exposed to the different shear stress patterns that can be then generated by a different pattern of rotation of the cone as previously described (36). During exposure to flow, valve tissue or ECs were maintained with 0.4% FBS DMEM in a bottle that allows O$_2$ and CO$_2$ exchange. Nine segments of tissue (with either surface exposed to the flow) or nine coverslips of either aortic surface ECs or ventricular surface ECs were placed in the flow chamber and exposed...
Fig. 6. Concentration-dependent effect ET-1 on compliance of ECs on the aortic surface of fresh aortic valve cusps. The compliance of the cells is reduced as the concentration of ET-1 increased (*P < 0.05 compared with control; n = 6).

RESULTS

Topographical Imaging of the Aortic Valve Surface Using SICM

The topography of live ECs was first probed in situ in the preparations of the porcine cardiac valve specimens. Comparing SICM images with identical SEM photographs of the same tissues validated the use of SICM for further imaging purposes. Live images depicted better details of protrusions on the surface of cells than SEM images, which may be reflecting the artifacts of fixation and processing required for the SEM (Fig. 2). Having examined cell topology on ventricular and aortic parts of the cardiac valve, we observed fundamental differences in morphology (Fig. 2). Figure 2A represents an 80 × 80 μm topographical SICM image of ventricular surface of the valve. Individual ECs of varying shape can be seen with a clearly visible elevated area above the nucleus in many cells and cell-cell contacts. It is clear that some cells are not fully connected and gaps between cells are apparent. Figure 2B shows a SEM image of the ventricular part of the valve. Similarly to the SICM image, in these fixed preparations cell-cell contacts can be seen as well as individual cells with variable morphology and shape. Figure 2C shows 80 × 80 micrometer topographical SICM image of aortic surface of the valve. Cell borders are obscured in this image, and therefore, it is difficult to distinguish individual cells. Cells on the aortic part of the valve are characterized by a more elongated shape than those on the ventricular surface, and the cells are aligned along one axis. The image itself does not have the same sharpness as the image of the ventricular surface of the valve. Figure 2D represents a SEM image of the aortic surface of the valve. It shows similar pattern of cell alignment as shown on the SICM image of the corresponding region.

Side-Specific Assessment of EC Compliance

Intact tissue samples. As shown on Fig. 3, ECs on ventricular and aortic surface of the valve differed significantly in their compliance. Cells on the ventricular surface of the valve were relatively stiffer with a mean membrane compliance of 0.012 ± 0.002 μm/kPa, whereas cells on aortic surface deformed more for the same applied pressure and were significantly softer with a membrane compliance of 0.032 ± 0.001 μm/kPa.

Cultured cells. A similar pattern and magnitude of cell compliance was observed in ECs that were selectivity isolated and cultured from each side of the valve. Cells cultured from the ventricular side gave a membrane compliance of 0.02 ± 0.001 μm/kPa, which was significantly less than the ones seen in cells from the aortic surface of the valve (0.032 ± 0.002 μm/kPa).

Regulation of EC Compliance by ET-1 and NO Donor

Having observed a significant difference in the compliance of the cells on either side of intact valves, we wanted to try and influence this parameter by exogenous application of endothelial-derived vasoactive agents. Representative recordings from 7 individual cells under control conditions and 7 cells following treatment with 10⁻⁶ M SNP (Fig. 4A) and 13 cells in the absence and 9 cells in the presence of 10⁻⁸ M ET-1 (Fig. 4B) show the shifts in the displacement of the cell membrane in...
response to applied pressure. It is apparent that SNP significantly increases compliance of the cells on the ventricular side of the valve, while ET-1 has the opposing effect on cells on the aortic surface.

The average changes in cell compliance in response to the addition of vasoactive agents are shown in Fig. 5. Following application of $10^{-6}$ M SNP, ECs on the ventricular surface of the valve became softer and their compliance was significantly increased as witnessed by a steep increase in membrane compliance from 0.011 ± 0.002 to 0.032 ± 0.001 μm/kPa (Fig. 5A). The compliance of the ventricular ECs was then comparable with the displacement seen by cells on the aortic surface. In contrast, $10^{-6}$ M SNP was unable to alter the compliance of the cells on the aortic surface of the valve (Fig. 5B). Inhibition of endogenous NO by $10^{-4}$ M L-NAME also affected the compliance of ECs on the valve. Inhibition of endogenous NO with L-NAME was able to reduce the compliance of the cells on the aortic surface as seen by a reduction in the displacement observed to 0.01 ± 0.001 μm/kPa. In contrast, L-NAME had no effect on the displacement of the cell membrane on the ventricular surface (Fig. 5, A and B). The vasoconstrictor peptide ET-1 had no effect on the compliance of the ECs on the ventricular surface of the valve but did reduce the compliance of the cells on the aortic surface from 0.033 ± 0.001 to 0.012 ± 0.03 μm/kPa. In the presence of ET-1 the compliance of the ECs on the aortic surface resembled those from the ventricular surface (Fig. 5, A and B). This effect of ET-1 on the aortic side of the valve was shown to be concentration-dependent between $10^{-10}$, $10^{-8}$ M (Fig. 6).

Expression of the Actin Cytoskeleton in ECs on Valve Surfaces

Actin filaments were clearly visible in the ECs of both the ventricular and aortic surfaces of the valve in en-face sections stained with phallloidin and an anti-CD31 antibody. The intensity of the actin filaments was significantly greater in ECs on the ventricular surface compared with those on the aortic surface (Fig. 7). Following treatment with $10^{-6}$ M SNP, the intensity of the actin filaments was reduced in cells on the ventricular surface, but unaltered on the aortic side. Conversely, $10^{-8}$ M ET-1 increased the intensity of the actin filaments in the ECs on both sides of the valve (Fig. 7). Western blot analysis showed that the levels of actin in ECs from the ventricular surface of the valve were significantly greater than that seen in ECs from the aortic surface (Fig. 8).

Effect of Flow Pattern on the Compliance and the Expression of Actin in ECs from Each Surface of the Valve

ECs isolated and cultured from the ventricular and aortic surface of the valve maintained their differential expression of actin after 24 h exposure to the physiological pattern of flow experienced by each type of cell. When the patterns of flow were reversed, such that that aortic side ECs were exposed to...
the ventricular pattern of flow and the ventricular ECs were exposed to the aortic pattern of flow for 24 h, there was no significant difference in the expression of actin in ECs from each side of the valve compared with the expression seen when they were exposed to their physiological pattern of flow (Fig. 9).

When tissue was placed in the flow bioreactor for 72 h, and exposed to either the physiological pattern of flow or the flow pattern usually seen by the opposing surface, we did not observed any changes of the relative compliance of ECs on either surface of the valve (i.e., compliance for the EC on the aortic surface was significantly greater than that of the EC on the ventricular surface). This pattern did not change when the aortic surface was exposed to ventricular flow and when the ventricular surface was exposed to aortic flow (Fig. 10). It was noted, however, that the levels of compliance for ECs on both surfaces were both reduced after 72 h exposure to flow compared with fresh tissue.

**DISCUSSION**

This study demonstrates marked differences in the mechanical properties of ECs from either surface of the aortic valve. We were able to show that the cells on the ventricular surface, which are exposed to high unidirectional shear stress, are significantly stiffer (less compliance) than those on the aortic surface (greater compliance), which are exposed to low oscillatory shear stress. In addition, we have illustrated the capacity of ECs to modulate their own compliance by an autocrine mechanism.

The differences in the compliance of the ECs in intact cusps observed in the current study could be contributed to by differences in the stiffness of the underlying tissue. It has been shown that matrix stiffness can affect EC behavior (28, 45). Indeed, the mechanical properties of different parts of the tri-layered structure have shown the fibrosa is significantly stiffer than the ventricularis (35, 37, 40). Hence, we repeated our observations with ECs selectively cultured from either surface of the valve and maintained on a plastic surface. While there was some difference in the compliance of ECs on the ventricular side of the valve compared with when the cells were grown on a plastic substrate, the difference in compliance between cultured aortic and ventricular ECs persisted. The fact that these differences were present in cultured cells that had not been exposed to flow also suggests that the difference in cell compliance is not conferred on the cells just by the different patterns of flow over their surface. This observation is supported by the data showing that the difference in compliance of ECs on both side of the valve is still preserved when the EC are exposed to the pattern of flow associated with the reverse side. It is known that ECs have epigenetic pathways that regulate the

**Fig. 8.** Analysis of actin expression in ECs from either side of aortic valve cusps under static conditions. A: Western blots of actin expression the valve ECs (VECs) culture from the aortic surface (lanes 1–6) and the ventricular surface (lanes 7–12). B: semiquantitative analysis of actin expression in cells from the aortic and ventricular surfaces (*P < 0.05; n = 12). OD, optical density.

**Fig. 9.** Analysis of actin expression in response to specific patterns of flow in ECs from either side of aortic valve cusps. A: representative Western blots of actin expression from VECs in culture from the aortic surface and the ventricular surface and exposed to aortic (a) or ventricular (v) flow for 24 h. B: semiquantitative analysis of actin expression in cells cultured from the aortic surface of the valve exposed to either the physiological pattern of flow (aortic flow on the aortic side and ventricular flow on the ventricular side) or the reverse pattern of flow (ventricular flow on the aortic side and aortic flow on the ventricular side; *P < 0.05, compared with aortic VECs under the same conditions of flow; n = 6).
expression of gene such as NO type III (38). It is possible that such epigenetic pathways confer differences to the ECs on either side of the valve, which determines their mechanical properties.

Valve ECs have been shown to be different from other ECs in the vascular system (3, 34). Analysis of gene expression in ECs from either side of the valve has revealed that the aortic surface of the valve was associated with greater expression of genes implicated in valve calcification (34). In addition, it has recently been shown that regions of the vasculature prone to atherosclerosis ECs in areas, such as the lesser curvature of the aortic arch, are prone to inflammation and have a less elongated morphology and increased compliance compared with cells on the outer curvature of the same vessels (16, 31). However, in the study by Potter et al. (31) exposure of ECs cultured from the aorta were able to regulate their compliance in response to flow. Thus vascular ECs appear to also have differential mechanical properties according to the levels of shear stress they experience. Differences also exist in the morphology of valve and vascular ECs, in that under conditions of high shear stress vascular ECs have a more aligned elongated morphology, while the ECs on the aortic aspect of the valve, which has low shear interrupted flow were more aligned that those on the ventricular surface of the valve that experience higher shear stress than the aortic side. It has been suggested that valve EC alignment is influenced more by pressure stress that that of shear stress (12). While it has been shown that, in canine aortic valves and cultured porcine aortic valve ECs (3, 12), ECs tend to align perpendicular, rather than parallel, to the direction of flow, more recent observations using human cultured aortic valve ECs have shown this is not the case, suggesting that the alignment of valve ECs is species dependent (18).

In general the valve endothelium is associated with a protective role against valve calcification. It has been shown that NO is capable of inhibiting the calcification process in an in vitro model using cultured cells (19). NO and ET-1 are also able to modulate the mechanical properties of valve cusps, whereby the relaxant effect of NO can reduce valve stiffness and the vasoconstrictor effects of ET-1 makes the valve stiffer (13). These data suggest that ECs are capable of modulating their own compliance. However, to show this, a concentration-response relationships on each side of the valve will need to be demonstrated for molecules that stimulate the release of NO and ET-1, as well as for the molecules themselves. The data presented with increasing concentrations of ET-1 show that this is true for the effect of this peptide on EC the aortic surface of the valve; however, the additional studies suggested warrant their own investigation. An autocrine effect of NO and ET-1 suggest that there is possibly a balance that exists in maintaining the deformation of the valves by the flow of blood, which is achieved by the cells themselves.

The balance between a variety of potentially opposing stimuli need to be considered in defining the true relationship between autocrine and paracrine mechanisms and EC mechanics. Membrane potential has recently been shown to affect the stiffness of vascular ECs (5). This is achieved by the ability of membrane depolarization, caused by changing the electrochemical driving force for K⁺ and Cl⁻ ions across the plasma membrane to affect the cortical actin cytoskeleton. In addition, Na⁺ and K⁺ ions are able to change the stiffness of ECs and reduce or increase the amount of NO release, respectively (27, 28). While the precise mechanisms that regulate EC membrane compliance and thereby the response of the cell to shear stress warrants further investigation, the implications of situations of EC damage or impairment are that there would be a loss of the autoregulatory function of ECs that could alter the response of the cells to shear stress. Reductions in NO release, or increased levels of ET-1, which have both been associated with aortic stenosis (7, 15, 23, 29, 30), could in turn affect the regulation of the mechanical properties of the whole leaflet. Changes to the distensibility of the valve would affect valve coaptation and functional integrity, as previously suggested (13). A point of major focus for future investigations will need to be evaluation of the effect of various clinical risk factors for aortic valve disease such as hypertension (increased pressure and strain), smoking/dyslipidemia (endothelial dysfunction) or high circulating 5-HT levels (cardioid disease) on the mechanical properties of ECs and how changes to their stiffness may alter modulating the fidelity and speed of intracellular mechanical signaling responses.

The data presented suggest that there is a defined range of compliance that the ECs can adopt. This effect most likely reflects the boundaries of the structure of the cytoskeleton. The stiffness of eukaryotic cells is mainly determined by the cytoskeleton. The cytoskeleton is a highly dynamic and adaptive structure whose components are constantly rearranging. It has been shown in studies with fibroblasts that disaggregation of actin filaments resulted in a decrease in the elastic modulus of the cell (32). Our results suggest that the differences in actin expression and mechanical properties of the aortic and ventricular ECs is not regulated by flow. While the time course for these experiments could be extended, the current data suggest that the difference observed in the actin cytoskeleton are regulated by forces other than shear stress or that they are inherent differences in the cells themselves. We were able to identify differences in the expression of actin in cells on either surface of the valve. In addition, changes seen with the addition
of ET-1 and SNP largely paralleled the changes in compliance seen with these agents, with the exception of an increase in expression induced by ET-1 in the ECs on the ventricular surface. It has previously been shown that NO can cause the dissociation of actin filaments in aortic smooth muscle cells and myocytes, whereas ET-1 is capable of restoring actin organization in astrocytes and can increase actin polymerization in pulmonary arteries in response to hypoxia via the action of the Ras homolog gene family, member A (RhoA) and its associated kinase, Rho kinase (2, 8, 11, 21).

While the displacement/pressure relationships measured from the cells appear to be linear, this is not actually the case. We have reported before that the curve has two slopes, one at the first 100–200 nm of displacement and a second beyond this (33). We found that with pressures >40 kPa strong deformation is produced indicating damage to the cell; we, therefore, did not use pressure above this level. Interestingly we have previously observed similar nonlinear relationship with most of the other cell types studied including neurons, cardiomyocytes, and epithelial cells as well as intact valve tissue (33).

There are a number of limitations to the current study, which may be resolved by further study. The link between changes in the actin cytoskeleton needs to be more directly established by using pharmacological agents that will disrupt the actin filaments and then determining their effect of cell compliance. One such target would be RhoA/Rho kinase and its downstream target Rho-associated, coiled-coil containing protein kinase 1 (ROCK1), which are known to regulate organization of the actin cytoskeleton (22). With such an approach it should then be possible to determine how the actin cytoskeleton, cell mechanics, and the response of EC to flow are linked. The present study is also limited by the fact it relies on porcine tissue. The principal observations in this study should be confirmed used human tissue. While ambitious, studies of ECs from human valves affected by different degrees of calcification would be invaluable to determine if there is a correlation between changes in EC cell biomechanics and the ability to communicate with valve interstitial cells via the release of paracrine mediators that serve to protect the valve from calcific changes.

We have shown that there are side-specific differences in the mechanical properties of the valve endothelium. Studies in vascular tissue and heart valves have illustrated that the endothelium is involved in the homeostasis of normal function as well as in disease processes (30). The specific biomechanics of aortic valve ECs described here could have important implications in understanding the sophisticated function of the aortic valve and to the pathology of aortic stenosis.

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