Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis

W. David Merryman,1 Inchan Youn,2 Howard D. Lukoff,2 Paula M. Krueger,3 Farshid Guilak,2 Richard A. Hopkins, and Michael S. Sacks1

1Engineered Tissue Mechanics Laboratory, Department of Bioengineering and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; 2Orthopaedic Research Laboratories, Departments of Surgery and Biomedical Engineering, Duke University Medical Center, Durham, North Carolina; and 3Collis CardioSacral Research Laboratory, Department of Cardiovascular and Thoracic Surgery, Brown University, Providence, Rhode Island

Submitted 18 May 2005; accepted in final form 19 August 2005

Merryman, W. David, Inchan Youn, Howard D. Lukoff, Paula M. Krueger, Farshid Guilak, Richard A. Hopkins, and Michael S. Sacks. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis. Am J Physiol Heart Circ Physiol 290: H224–H231, 2006. First published August 26, 2005; doi:10.1152/ajpheart.00521.2005.—It has been speculated that heart valve interstitial cells (VICs) maintain valvular tissue homeostasis through regulated extracellular matrix (primarily collagen) biosynthesis. VICs appear to be phenotypically plastic, inasmuch as they transdifferentiate into myofibroblasts during valve development, disease, and remodeling. Under normal physiological conditions, transvalvular pressures (TVPs) on the right and left side of the heart are vastly different. Hence, we hypothesize that higher left-side TVPs impose larger local tissue stress on VICs, which increases their stiffness through cytoskeletal composition, and that this relation affects collagen biosynthesis. To evaluate this hypothesis, isolated ovine VICs from the four heart valves were subjected to micropipette aspiration to assess cellular stiffness, and cytoskeletal composition and collagen biosynthesis were quantified by using the surrogates smooth muscle α-actin (SMA) and heat shock protein 47 (HSP47), respectively. VICs from the aortic and mitral valves were significantly stiffer (P < 0.001) than those from the pulmonary and tricuspid valves. Left-side isolated VICs contained significantly more (P < 0.001) SMA and HSP47 than right-side VICs. Mean VIC stiffness correlated well (r = 0.973) with TVP; SMA and HSP47 also correlated well (r = 0.996) with one another. Assays were repeated for VICs in situ, and, as with in vitro results, left-side VIC protein levels were significantly greater (P < 0.05). These findings suggest that VICs respond to local tissue stress by altering cellular stiffness (through SMA content) and collagen biosynthesis. This functional VIC stress-dependent biosynthetic relation may be crucial in maintaining valvular tissue homeostasis and also prove useful in understanding valvular pathologies.

Address for reprint requests and other correspondence: Michael S. Sacks, Dept. of Bioengineering, 100 Technology Dr., Rm. 234, Univ. of Pittsburgh, Pittsburgh, PA 15219 (e-mail: msacks@pitt.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(20), the MV demonstrated the highest level of hydroxyproline expression, whereas all the leaflets demonstrated comparable collagen contents. This finding suggests that the collagen of the MV has the highest rate of turnover. In a separate study, normal, floppy, and rheumatic MVs were labeled in an organ culture with \[^{14}C\]proline to determine whether there were appreciable differences in collagen biosynthesis between the VIC populations (12). The rate of collagen synthesis and total protein content were significantly increased in floppy and rheumatic leaflets compared with normal valves. There was no significant difference in DNA content in either abnormal state, suggesting that the VIC population was not larger but was, indeed, making more collagen. Hence, it appears that the VIC likely plays an important role in various valve pathologies according to their synthetic state.

Plated AVICs have been observed to deform silicone substrates in the presence of vasoconstricting drugs (19). Additionally, this population has been shown to generate small, but measurable, forces at the tissue level because of vasoconstriction during uniaxial tension (15). Recently, we reported the ability of AVIC to significantly alter the bending stiffness of circumferential porcine leaflet tissue strips as a result of elicited contraction (via KCl) and relaxation (via thapsigargin) of smooth muscle α-actin (SMA) and quantified the AVIC basal tonus for the first time (18). The AVIC basal tonus measurably influenced the bending stiffness of the leaflets, suggesting that the cells have an intrinsic stiffness due to the SMA component of the cytoskeleton (CSK).

Cultured human AVICs have demonstrated increased proliferation and collagen synthesis in the presence of vasoconstrictors (11). This finding has led us to speculate that the contractile state of VICs is related to the corresponding biosynthetic levels and, ultimately, valve ECM homeostasis (18). However, it has not been shown that these two are indeed correlated. Moreover, the distinct TVPs experienced by the right- and left-side heart valves suggest different leaflet tissue stress levels, resulting in varying local VIC stress fields. Thus examination of the VICs from each leaflet provides an excellent scenario for determining the relation between tissue stress, VIC stiffness, and related biosynthetic activity.

We thus hypothesize that VICs respond to physiological tissue stresses in vivo, which are a direct function of TVP, by altering cell stiffness via CSK composition, and that this relation, in turn, affects collagen biosynthesis. This stiffness–synthesis relation presumably exists because higher tissue stresses in the left-side valves require synthesis of greater amounts of collagen for proper valve leaflet tissue homeostasis.

In the present study, cell stiffness was determined by micropipette aspiration, and CSK quantity and collagen production of the VICs were quantified with the surrogates SMA and heat shock protein 47 (HSP47), respectively. Our goal in the present work was to conduct an initial study to investigate VIC functionality by examining isolated cells from healthy hearts.

**MATERIALS AND METHODS**

VIC isolation and culture. Ovine hearts (N = 5) from young sheep (≈10 mo old, ≈150 lbs; Animal Technologies, Tyler, TX) were shipped in PBS containing antibiotics at 4°C and dissected within 24 h of euthanasia. Hearts were grossly dissected in a laminar-flow biological safety cabinet with use of sterile materials. From each valve, all leaflets were excised, and each leaflet was individually placed in a 35-mm tissue culture dish; MV leaflets, which are significantly larger than the other leaflets, were halved. Standard tissue culture medium consisting of DMEM, 10% FBS, and t-glutamine supplement (all from Mediatech, Herndon, VA) was used, and antibiotic concentrations were doubled while the leaflets remained in the culture wells to prevent infection (200 μg/ml penicillin-streptomycin and 2 μg/ml amphotericin B; Mediatech). The dishes were kept in an incubator at 37°C and 5% CO₂–95% O₂, and the medium was changed every 2 days. When VICs appeared, the leaflets were removed from the dishes, and cells from the same valve were combined and replated in culture medium with normal antibiotic levels (day 5). Endothelial cells were believed to be abolished after this duration of culture, because the medium was not optimal for endothelial cell survival (25, 36). VICs reached ≈80% confluence and, after 12 days, were trypsinized and cryopreserved at −80°C in DMEM with 10% DMSO. Cells from each valve were divided into two groups: one for mechanical testing and the other for protein quantification. The cells were thawed and plated simultaneously so that the plating times for mechanical testing and protein assay populations were the same (5 days).

**VIC stiffness measurements.** Micropipette aspiration has been used to determine the mechanical response of multiple cell types (4, 6, 9, 13, 28, 30), and the experimental setup has been described previously (35). Briefly, cells were trypsinized, pelleted (1,500 rpm, 5 min), and resuspended in medium before testing. For each population, 80 μl of cell-suspended medium were aspirated and placed in a chamber that allows entry of a pipette from the side (13). Capillary tubes (A-M Systems, Carlborg, WA) were fabricated into micropipettes with a pipette puller (David Kopf Instruments, Tujunga, CA) and then fractured with a microforge to achieve an inner diameter of 6–9 μm. These micropipettes were coated with Sigmacoat (Sigma, St. Louis, MO) to prevent cell adhesion.

Pressures were applied to the surface of a VIC through the micropipette via a custom-built water reservoir with an in-line pressure transducer having a resolution of 1 Pa (model DP15-28, Validyne Engineering, Northridge, CA). While pressure was applied, digital images of the cell aspiration were recorded to a DVD-R with a charge-coupled device camera (COHU, San Diego, CA) through a bright-field microscope (Diaphot 300, Nikon, Melville, NY) with a ≈40 or ≈60 oil-immersion objective and a ×10 wide-field eyepiece (Edmund Scientific, Barrington, NJ). Applied pressure and time were displayed on a video monitor using a digital multiplexer (Vista Electronics, Ramona, CA). Pipette inner diameter, cell diameter, and aspiration length were determined with single-frame digital images analyzed after testing (SigmaScan, Systat Software, Point Richmond, CA).

Aspiration of VICs was achieved in a three-step process: 1) initial tare pressure (∼50 Pa, 60 s; Fig. 1A) to ensure that a seal was formed between the micropipette and the cell, 2) the first instantaneous pressure step increase to ∼250 Pa for 120 s (Fig. 1B), and 3) the second instantaneous pressure step increase to ∼500 Pa for 120 s (Fig. 1C). At the end of each step, the applied pressure and aspirated length of the cell were recorded. The effective stiffness (E) of the cell was determined with a half-space model, referred to as the punch model, which assumes that the cell is an isotropic, elastic, incompressible, half-space material (34). This model assumes the VIC to be a homogeneous material while ignoring discontinuities (e.g., organelles and nucleus) and viscous effects from the cytosol. Hence, as an elastic model, it was chosen to demonstrate the intrinsic stiffness of cell populations and was not intended to fully characterize the mechanical behavior of the cells. With this model, E of the cell is given by Eq. 1.

\[
E = \frac{\phi(\eta(3r)/(2m)\Delta P/L)}{1}
\]

where \(\phi(\eta)\) is the wall function and is set equal to 2.1 (dimensionless parameter calculated from the ratio of the pipette inner radius to the wall thickness), \(r\) is the micropipette inner radius, and \(\Delta P/L\) is determined from the slope of the applied pressure vs. aspirated length of the cell.
Quantification of VIC protein levels. SMA and HSP47, which interacts with procollagen in the endoplasmic reticulum, were quantified by ELISA for CSK and synthesized collagen protein levels, respectively. HSP47 is believed to bind to procollagen after it enters the endoplasmic reticulum and dissociates before secretion (32), and it has been shown that type I collagen synthesis is dependent on HSP47 levels (24). Rocnik et al. (24) demonstrated that selective transfection of smooth muscle cells with a retrovirus containing HSP47 cDNA increased intra- and extracellular steady-state type I collagen production. Furthermore, Northern blots of total RNA from their cells showed a tandem increase in HSP47 and procollagen (24). Hence, HSP47 was deemed a suitable surrogate for type I collagen in this study.

A 96-well plate was prepared by coating the entire surface of each well with 100 μl of primary antibody. The primary antibodies were prepared as 2 ng/ml of monoclonal mouse anti-HSP47 (clone M16.10A1) or monoclonal mouse anti-SMA (both from AbCam, Cambridge, MA). The plate was wrapped in Parafilm and incubated for 24 h at 4°C. Before use, each well was washed four times with 400 μl of wash buffer (0.01% PBS + Tween 20 (PBS-T)). Blocking buffer (400 μl), consisting of 1% BSA and 5% sucrose in 0.01% PBS-T, was added to each well and incubated for 1 h at 20°C. Wells were washed again four times with wash buffer, allowed to air dry, and sealed in Parafilm.

VIC populations were lysed with RIPA buffer (100 mM Tris·HCl, 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, and 1% SDS-distilled H2O), scraped, and placed on ice for 10 min. The solution was pelleted for 10 min at 14,000 rpm at 4°C. Then the DNA was sonicated and homogenized. This key validation step was intended to acquire and assay for CSK and synthesized collagen protein levels, respectively after no difference was found between VICs from the same valve source between hearts (i.e., AVICs were not appropriate after no difference was found between VICs from the four valves of two hearts (H1 and H2) were successfully tested, along with additional AVICs and TV interstitial cells (TVICs) from a third heart (H3). Stiffness results of VICs were pooled to make up the total cell populations as follows: n = 21 for TVICs, n = 17 for PV interstitial cells (PVICs), n = 18 for MV interstitial cells (MVICs), and n = 25 for AVICs; pooling was deemed appropriate after no difference was found between VICs from the same valve source between hearts (i.e., AVICs were not different between H1 and H2). Average ΔP/L values for the VIC populations demonstrated two distinct mechanical responses (Fig. 2A). E values for AVIC and MVIC populations (0.449 ± 0.024 and 0.479 ± 0.052 kPa, respectively) were significantly greater (P < 0.001, Fig. 2B) than those for PVIC and TVIC populations (0.276 ± 0.023 and 0.285 ± 0.030 kPa, respectively). No differences were found between AVIC and MVIC stiffness or between PVIC and TVIC stiffness. Additionally, there were no differences between cell or micropipette diameters (Fig. 3A) or the ratio of cell diameter to micropipette diameter (Fig. 3B). This indicates that the testing geometries did not influence the results.

VIC protein quantification. VICs from H1 and H2 were assayed for SMA and HSP47. Leaflets were acquired and assayed as described above, except these leaflets were sonicated and homogenized. This key validation step was intended to simultaneously show the difference between in vitro and in situ cell states while also demonstrating that any changes resulting from in vitro isolation occurred proportionally among all the valves.

Statistics. Cell stiffness, dimensional measurements, and ELISA absorbance values are expressed as means ± SE. Comparisons were determined by Tukey’s one-way ANOVA, with statistical significance at P < 0.05. Data correlations were determined from mean values using a Pearson’s product-moment correlation and are reported with the correlation coefficient (r) and the associated P value (SigmaStat). Variable pairs with positive correlation coefficients and P < 0.05 tend to increase together.

RESULTS

VIC stiffness. VICs from the four valves of two hearts (H1 and H2) were successfully tested, along with additional AVICs and TV interstitial cells (TVICs) from a third heart (H3). Stiffness results of VICs were pooled to make up the total cell populations as follows: n = 21 for TVICs, n = 17 for PV interstitial cells (PVICs), n = 18 for MV interstitial cells (MVICs), and n = 25 for AVICs; pooling was deemed appropriate after no difference was found between VICs from the same valve source between hearts (i.e., AVICs were not different between H1 and H2). Average ΔP/L values for the VIC populations demonstrated two distinct mechanical responses (Fig. 2A). E values for AVIC and MVIC populations (0.449 ± 0.024 and 0.479 ± 0.052 kPa, respectively) were significantly greater (P < 0.001, Fig. 2B) than those for PVIC and TVIC populations (0.276 ± 0.023 and 0.285 ± 0.030 kPa, respectively). No differences were found between AVIC and MVIC stiffness or between PVIC and TVIC stiffness. Additionally, there were no differences between cell or micropipette diameters (Fig. 3A) or the ratio of cell diameter to micropipette diameter (Fig. 3B). This indicates that the testing geometries did not influence the results.

VIC protein quantification. VICs from H1 and H2 were assayed for SMA and HSP47 protein levels. ELISA results for each showed that, consistent with the stiffness results, the AVIC (616.77 ± 2.26 and 411.57 ± 2.35 pg/ml for SMA and HSP47, respectively) and MVIC (612.68 ± 2.66 and 395.62 ± 2.21 pg/ml for SMA and HSP47, respectively) protein levels were significantly greater (P < 0.001) than the PVIC (495.63 ± 2.37 and 317.63 ± 4.86 pg/ml for SMA and HSP47, respectively) and
TVIC (367.18 ± 4.91 and 244.52 ± 3.58 pg/ml for SMA and HSP47, respectively) protein levels (Fig. 4A). No differences were found between the AVIC and MVIC protein levels or between the PVIC and TVIC protein levels. Additionally, the protein levels of in situ VICs (H4 and H5) revealed similar differences, with the left-side valve proteins significantly greater (P < 0.05) than the right-side valve proteins (Fig. 4B). There were also clear similar trends, with increasing protein levels from the TV to the AV in the in vitro and in situ VICs (Fig. 4). However, HSP47 and SMA levels were threefold greater for in situ than for in vitro VICs. PVL and MVL HSP47 values were not statistically different. Results from the CD31 ELISA (63.33 ± 12.29, 95 ± 16.28, 68.33 ± 23.30, and 76.50 ± 16.80 for TVIC, PVIC, MVIC, and AVIC, respectively) revealed very few contaminating endothelial cells in any of the cultured VIC populations compared with the manufacturer’s positive control (9,608 ± 154); hence, the tested cells were almost certainly fibroblasts or myofibroblasts.

**DISCUSSION**

* Measured differences between right- and left-side VICs. This is the first known study to report VIC mechanical properties, as well as the possible implications of these measurements on valvular collagen biosynthesis. The findings of this study revealed significantly greater cell stiffness, CSK protein content, and collagen production, as determined by SMA and HSP47 surrogates, respectively, for AVICs and MVICs than for PVICs and TVICs. These results represent an initial attempt to elucidate the differences in VIC function between the four heart valves. Although these in vitro measurements on isolated VICs provide an indirect examination of VIC functionality, they serve to support the hypothesis that local stress affects VIC mechanical and biosynthetic properties in vivo.

* VIC phenotypic plasticity. The VIC phenotype has been observed to be plastic and reversible, depending on the remodeling state of the tissue due to development, disease, and adaptation (23). Rabkin-Aikawa et al. (23) found that 2.5% of the VIC population in normal adult human and ovine valves was SMA positive, whereas in the “developing/activated/diseased” states, the percentage was significantly higher: 19–62% were SMA positive. Additionally, the phenotypic characteristics of AVICs have been shown to be distinct from those of aortic smooth muscle cells, in that they have increased ability to synthesize matrix components in collagen gels (3). Although it has long been known that the VIC phenotype is dualistic in nature (7, 19, 21), the implications of this characteristic in terms of valve function and biosynthesis have remained unclear.

It has been speculated that the contractility of the VIC population may, in some biomechanical capacity, affect valve function (15). However, the contractile forces generated by the cells are many orders of magnitude less than the forces im-
posed on the leaflets during normal valve function (18). In addition, the delivery of endothelin-1 or KCl levels needed to elicit a measurable contraction within the leaflets is unrealistic in vivo. Although VIC contractile forces are too small to affect valve function, it has been demonstrated that the vasoactive agent 5-hydroxytryptamine induces mitosis and collagen synthesis in cultured VICs (11). Taken together, these studies reveal that the VIC population is phenotypically dynamic, contractile when remodeling is necessary, and mitogenic and secretory when contractility is induced by vasoconstrictors.

**VIC functional correlations.** The local stress-strain fields in the vicinity of a cell are highly dependent on a number of factors, including cell shape, orientation, and the relative properties of the cell and ECM (8). Thus it is not clear how the pressures imposed on different sides of the heart translate into local stress on the VICs. To answer this question, a multiscale mechanical analysis of many factors, including leaflet dynamic geometry (14), leaflet mechanical properties (2), tissue layer thickness and composition (31), and VIC-ECM connectivity, is necessary. Clearly, the progression from the organ to the cell and subcellular levels is a complex process (Fig. 5). Hence, predictions of the effects of TVP on cellular level stress remain largely speculative, and sophisticated computational models and experimental validation data are needed to further describe this relation.

Although we do not know the precise local VIC stress state, we believe that the higher left-side pressures translate into some increase in local stress on the VICs. For instance, when the VIC stiffness measured in this study is plotted vs. the maximum TVP (10) experienced by the respective valves (Fig. 6A), there appears to be a strong dependence on the stress level imposed on the cells \( (r = 0.974, P = 0.026) \). Moreover, the normal AVL is \(~700~\mu\text{m} \) thick, whereas the PVL is \(~400~\mu\text{m} \) thick. This twofold difference in thickness between the PV and the AV is small compared with the eightfold difference in their resting TVPs, further suggesting that left-side VICs are subjected to elevated stresses.

The strong relation between the CSK protein (SMA) and the collagen biosynthetic protein (HSP47) \( (r = 0.996, P = 0.077; \) Fig. 6C) implies that VICs are responsive to their mechanical environment for maintenance of proper tissue homeostasis. The increased SMA in left-side VIC populations suggests that the cells are adapted for the stresses imposed on them, whereas the increased HSP47 is necessarily expressed to synthesize larger amounts of collagen, the primary structural component of heart valves. Furthermore, SMA and HSP47 quantified from VICs in situ reveal a similar, although not as strong, correlation. More importantly, although the in situ VICs contain higher levels of both proteins, their relative levels remained

---

**Fig. 4.** ELISA results for smooth muscle α-actin (SMA) and heat shock protein 47 (HSP47). A: SMA and HSP47 absorbance from in vitro VIC populations. Significant difference was found between AVIC and MVIC populations compared with PVIC and TVIC populations \( (P < 0.001) \). Protein levels were significantly greater in PVICs than TVICs. B: SMA and HSP47 absorbance from in situ VICs of explanted heart valve leaflets: tricuspid (TVL), pulmonary (PVL), mitral (MVL), and aortic (AVL). Note difference in data range \((y\text{-axis})\) between in situ and in vitro VICs. As with in vitro VICs, left-side valve protein levels were significantly greater \( (P < 0.05) \) than right-side valve levels, and PVL levels were greater than TVL levels. HSP47 was not statistically different between MVL and PVL. *\( P = 0.786 \).

**Fig. 5.** Hypothesized mechanism by which transvalvular pressure translates into local tissue stress \((\sigma)\) on VIC and resulting response. ECM, extracellular matrix.
consistent after the cells were isolated from the tissue. This result validates retention of the majority of the in situ characteristics of VICs in the cells tested in vitro.

Although our data show a strong correlation between SMA and HSP47 of VICs, the mechanism underlying this relation is not clear. It can be speculated that SMA (or HSP47) expression is the by-product of HSP47 (or SMA) expression, or the two may be upregulated simultaneously. The correlation coefficient for this relation simply indicates that the two are correlated but does not assign one as the dependent variable.

A full explanation of the mechanisms behind these correlated protein expressions is beyond the scope of this study; however, other groups have reported findings that shed light on this collagen-CSK relation. Berry et al. (1) found that, in human wound closure, myofibroblasts were associated with thicker collagen fibers exclusively and that wound contraction, presumably facilitated by the SMA-positive cells, contributed to 88% of closure. In another study (5), human dermal fibroblasts expressed SMA upon confluence when plated in monolayer culture at low density; however, this expression was reduced by 50% when the cells were covered with a collagen lattice for 24 h. This finding was believed to be due to inhibition of cell-cell communication via cadherins, which was speculated to interrupt the communicative tension from cell to cell needed to maintain a contractile phenotype. Collagen fibril formation in vascular smooth muscle cells has been observed to be dependent on integrins and actin filaments (16). Forming fibrils were found parallel to actin microfilament bundles. Additionally, collagen fibril assembly was inhibited in cells incubated with cytochalasin D, whereas cells transduced with dominant-negative RhoA developed minimal collagen fibril assemblies. These findings using different myofibroblast cells support our results of VIC collagen synthetic activity as functionally linked to SMA content.

Limitations of the study. VICs in the present study may have functional and biosynthetic properties different from those of VICs in situ because of the plating technique used to isolate the cells. Previously, in VIC populations isolated in a similar fashion, SMA-positive cells increased with longer culture times (37). To quantify the biosynthetic changes that occurred due to isolation of the cells, we compared the in vitro and in situ VIC protein levels. Our results demonstrate that, with respect to SMA and HSP47, the VICs were performing at a relatively normal, although diminished, capacity in vitro (Figs. 6, B and C), and changes were proportional among all valves. Finally, the indirect methodology of quantifying collagen biosynthesis via the HSP47 surrogate was utilized to avoid use of radioisotopes, which are costly and potentially harmful. Although previous work showed a dependence of collagen biosynthesis on HSP47 quantities at the RNA level (24, 32), the exact constitutive dependence of the relation has not been determined to the authors’ knowledge.

Cell stiffness may also be altered as the CSK filaments are relieved of stress at their focal adhesions when the cells are removed from the tissue. Additionally, the monolayer culture time used to expand the VICs (22 days) likely had an effect on the properties measured here. However, all VICs were obtained and cultured under identical conditions, which allowed for comparison between properties of the cells, and significant differences were apparent. Although we hypothesized that these properties were different between the valves, we do not believe that the in vivo VIC can be fully described by these results. Ultimately, it would be most appropriate to measure cellular stiffness in situ; however, mechanical testing of intact cells is much more complex, inasmuch as boundary conditions are difficult to define. Therefore, although we are confident that
our results demonstrate actual differences in VIC mechanical behavior, one must be cautious to use these values for a comprehensive model of valve mechanics or remodeling capabilities.

Role in valvular physiology, pathology, and tissue engineering. On the most fundamental level, our results suggest the physiological differences of VICS isolated from healthy ovine hearts. It is feasible that the stiffness-synthesis relation that persists for VICS may be applicable to cells of other tissues exposed to cyclic loads (e.g., blood vessels, tendons, ligaments, and myocardium). With regard to valve physiology and pathology, this normal VIC functional relation is likely not conserved in degeneratively diseased or genetically malformed valves (e.g., bicuspid aortic leaflets), inasmuch as they typically present with collagen fibrosis and altered collagen architectures, respectively. Overexpression of HSP47 has been found in multiple fibrotic diseases and is associated with fibrosis following myocardial infarction (29). This finding has led to the suggestion that HSP47 is a potential biomarker for a number of diseases, and we speculate that heart valve cells may appropriately be added to this repertoire of aforementioned tissues that are prone to fibrotic disease.

Defining the functional end points of engineered tissues on the basis of the properties and function of native tissues has been understudied. The relations shown here may be useful as an index of VIC functionality for tissue-engineered heart valves (TEHVs). The source of most TEHVs is nonvalvular cells, and monitoring their adaptation during development is critical. Inasmuch as this study involved young healthy sheep, the in vitro development of a TEHV could be compared with this relation to determine whether TEHV cells are experiencing proper local tissue stress. For instance, a tissue-engineered PV and a tissue-engineered AV would not require the same developmental point before implantation. Hence, this SMA-HSP47 relation could prove beneficial for assigning homeostatic cellular end points and tracking VIC destiny under different loading regimens.

In summary, this work is the first to report VIC stiffness and the correlation with TVP and the subsequent implications in biosynthesis of collagen proteins. These results suggest a conserved homeostatic VIC state from the right- to the left-side heart valves, which are exposed to largely different TVPs. Additionally, differences between the in vitro and in situ VIC biosynthetic function reveal the effect of isolation and extended culture on VICS. Future work is needed to understand the in vivo local stress environment of the VIC, with special attention to the tensile loading strain rate, which is germaine to valves only (27), and the cell-ECM junctions via integrins. It is believed that this information will add to the growing body of knowledge concerning VICS and will be of value for the fields of valve physiology, pathology, and tissue engineering.

REFERENCES


24. Rocnik EF, van der Veer E, Cao H, Hegele RA, and Pickering JG. Functional linkage between the endoplasmic reticulum protein Hsp47 and...


