The cytoskeleton of cardiac myocytes is a complex network of many interacting protein filaments and associated proteins with multiple functions: it forms structures responsible for maintaining cell shape and contractile filament registration, structural integrity, internal transport and cell division, and scaffolding for several putative signaling cascades. The tight coupling between the extracellular matrix and components of the cytoskeleton at the cell membrane suggests that, in addition to transmitting contractile forces generated by the myofilaments to the matrix and the cardiac chambers, the cytoskeleton may also be important for propagating external physical signals into the cell.

Mutations in a growing list of cytoskeletal genes are associated with cardiomyopathies. Disruption of desmin, plakoglobin, N-cadherin, plectin, and vinculin all produce a dilated cardiac phenotype with impaired function, either in fetal development or after birth. These are elements of the cytoskeleton that connect intracellular structures with the extracellular matrix, and thus are likely force-transmitting components in the myocyte. Originally, Chien proposed that defects in the cytoskeletal component of the myocyte result in a dilated cardiomyopathic phenotype, whereas mutations in the sarcomeric proteins typically generate a pattern of hypertrophic cardiomyopathy with preserved ventricular systolic function. But as more data have accumulated, the picture has changed, and the contributions of cytoskeletal structures, remains sparse.

Cytoskeletal proteins have been implicated in several load-sensing pathways, and there is evidence that cytoskeletal proteins play a critical role in biomechanical signaling and may be involved with ventricular dilation and heart failure. These studies have led to the hypothesis that there may exist a common pathway to cardiac dilation and failure, and the critical components of this “failure transition” are likely to be structural themselves or closely related to force transmitting elements. The complex structure and multiple functions of the cytoskeleton make it inherently difficult to study on a quantitative scale. Its physical and signaling properties rely not only on its molecular structure but its dynamic three-dimensional organization. Experimental interventions that disrupt components of the cytoskeleton can alter the distribution of stresses within the other components. Separating the “inside-out” force transmission functions of the cytoskeleton from the “outside-in” mechanotransduction and signaling roles is challenging, especially in vivo. Yet it is likely that both play a significant role in cardiac remodeling and heart failure. Defects in the actin-associated cytoskeleton causing dilated cardiomyopathy, such as the deletion of muscle LIM protein (MLP)—a Z-disc-associated structural protein interacting with actin, titin, and alpha-actinin—have been shown to result in alterations both to biomechanical signal transduction and to diastolic mechanical properties. Understanding the precise molecular mechanisms of these alterations will require a comprehensive theoretical framework for the biophysics and mechanical properties of the cytoskeletal network within the context of the intact myocyte.

Both from a molecular viewpoint and a biophysical and mechanotransduction perspective, the microtubule network is among the better-studied components in the cytoskeleton. A substantial body of evidence implicates microtubule polymerization and depolymerization in myocyte dysfunction in some models of ventricular hypertrophy, though not all. Studies have shown that microtubules contribute significantly to the viscoelastic properties of myocytes, especially their passive viscosity.

With few exceptions, studies of the mechanical properties of myocytes and the contributions of the microtubules have used a single uniaxial mechanical test, most commonly measuring the longitudinal elastic stiffness or viscosity of the cell. However, the resting and active myocardium is well known to be anisotropic. Even the mechanotransduction responses of micropatterned cultured myocytes to stretch in vitro are anisotropic, with differential induction of hypertrophic gene expression and protein synthesis by longitudinal versus transverse stretch. One isolated cell study measured the axial and transverse mechanical properties of single myocytes, but the intact myocardium also experiences substantial shearing stresses and strains in vivo. Tagawa and colleagues used magnetic twisting rheometry to probe the viscoelastic properties of the myocyte cytoskeleton. However, information on the anisotropic material properties of myocytes under multi-axial tension, compression and shear, and the contributions of cytoskeletal structures, remains sparse.

In this issue of Circulation Research, Nishimura and coworkers describe a novel combination of single cell mechanical tests that they have used to probe the elastic properties of isolated adult rat ventricular myocytes under axial tension, transverse compression, as well as axial and longitudinal shearing. Using a combination of flexible and rigid carbon microfibers, latex microspheres and thin glass plates together with piezoelectric translators, these authors...
constructed an ingenious suite of micromechanical tests for stretching single myocytes, indenting them transversely with a 10-μm microsphere, and shearing them two glass plates displaced axially or transversely.

When normal rat ventricular myocytes were treated with colchicine to depolymerize microtubules or paclitaxil to hyperpolymerize them, there were no significant alterations in elastic stiffnesses for axial tension, transverse compression, or transverse shear. However, longitudinal shearing stiffness was significantly decreased by colchicine and increased by paclitaxil. Although viscous properties were not measured as comprehensively, the comparative specificity of this result is what makes it most interesting, because it affords the opportunity to distinguish the contributions of different compartments of the cell skeleton to specific mechanical responses. Ultimately, this will require the development of structurally and biophysically detailed computational models of myocyte micromechanics based on the macromolecular structure and interactions of the cytoskeletal filaments systems. In an early step toward this goal, the authors have developed a finite element model of intramyocyte stress distributions that includes elements for the microtubule network, the actin cytoskeleton, membrane, and myofilaments.

The material properties assigned to each component are oversimplified and somewhat arbitrary. For example, the properties of the myofilaments are modeled using a constitutive law for the whole myocardium, whose resting properties are also dependent on the extracellular matrix. Nevertheless, the model did show that alterations in microtubule density had a significant influence on longitudinal shear stiffness but not on transverse shear stiffness or axial elastic properties consistent with the experimental observations. The authors did not report the changes in the model on transverse compressive stiffness. Although changes in transverse compressive stiffness did not reach statistical significance in the myocyte experimental measurements, the mean values were 40% to 50% greater for paclitaxil-treated cells than those incubated in colchicine. More cells or a larger range of doses measured as comprehensively, the comparative specificity of this new, more comprehensive multi-axial systems. In an early step toward this goal, the authors have developed a finite element model of intramyocyte stress distributions that includes elements for the microtubule network, the actin cytoskeleton, membrane, and myofilaments.

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These observations and the computational analysis are in keeping with the "tensegrity" hypothesis of cell mechanics proposed by Ingber. When the famed architect Buckminster-Fuller coined the term, he characterized a tensegrity structure as one that derives stability (especially in shear) from continuous tension and discontinuous compression. In the present model, the continuous tension arises from pre-tension in the actin cytoskeleton and the microtubules are the discontinuous compressive members. Although the model is clearly a simplification, the new experimental findings suggest that these new, more comprehensive multi-axial studies of single myocyte mechanics have the potential to elucidate specific structural roles of individual cytoskeletal compartments.

It is difficult to extrapolate the significance of these new findings in isolated myocytes directly to ventricular mechanics in vivo. Yet there is ample reason to believe they are important. It is well known that the myocardium undergoes complex three-dimensional deformations during the cardiac cycle. In particular, transverse and longitudinal shearing comprise significant components of three-dimensional myocardial stress and strain. Shear is also associated with ventricular torsion during contraction and relaxation, which is important for maintaining transmural uniformity of fiber stresses and strains during ejection and filling. Studies also suggest that alterations in systolic torsion are sensitive indicators of systolic dysfunction in ischemia and other pathological conditions.

Transverse shearing between myocardial laminae or "sheets" has also been shown to play a significant role in wall thickening. Left ventricular torsion during relaxation is driven by lengthening of myofibers in the epicardial layers, and is associated with rearrangement of myolaminar sheets in the endocardial layers. Although most of the transverse shearing strains are probably accommodated by sheet rearrangement and the extracellular matrix, shear stresses must also be transmitted to the myocytes within the myocardial laminae. The first intervention reported to dramatically alter transverse myocardial shears was ectopic pacing. Subsequently, studies of chronic ventricular pacing demonstrated asymmetric ventricular remodeling associated with the decreased wall stress in early activated myocardium and increased systolic stresses in late-activated regions. The prevalence of ventricular conduction delays in dilated cardiomyopathy (especially associated with left bundle branch block) and the potential to partially restore systolic function with cardiac resynchronization, have focused interest on structural remodeling associated electromechanical dysynchrony in the failing heart.

Changes in myocardial laminar orientations during dilation and remodeling contribute to decreased myocyte number across the wall of the failing heart and may decrease the efficiency by which fiber shortening and transverse shears can be converted into systolic wall thickening.

In this issue of Circulation Research, Helm and coworkers investigated whether electromechanical heterogeneity in a canine model of the dysynchronous failing heart may lead to regional alterations in myocardial fiber or sheet architecture. They performed diffusion tensor MRI in isolated perfusion-fixed dog hearts to estimate the distributions of ventricular myofiber and sheet orientations at sub-millimeter resolution without the need for laborious dissection and histology. The resulting large data sets were compared statistically between normal and dysynchronous failing groups using novel techniques from computational anatomy. Although myofiber angles were not significantly altered independent of gross geometric changes in the dysynchronous failing hearts, early-activated sites showed significant reorientation of myocardial laminae whereas there was no change in late-activated sites. This remodeling may affect regional wall mechanics and electrical conduction. Although the study of Helm and colleagues focused on the variability of regional mean fiber and sheet orientations between normal and failing subjects, there is also substantial local dispersion, especially of subendocardial sheet orientations. This should be reflected in the degree of diffusion tensor anisotropy and would be interesting to examine using these new analytical methods.
On November 18th, 2005, the National Heart, Lung, and Blood Institute released a new Request for Applications (RFA-HL-06-004) for exploratory research in systems biology. The program emphasizes the use of new computational modeling techniques and experimental measurements to develop integrative analyses of complex phenotypes in heart, lung, blood, and sleep disorders. The articles in this issue of Circulation Research by Nishimura and colleagues and Helin and coworkers represent progress at the cellular and whole organ scales toward the development of predictive multiscale models of ventricular mechanics in the normal and failing heart.

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References

Myocyte Shearing, Myocardial Sheets, and Microtubules
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