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Micromechanical Regulation in Cardiac Myocytes and Fibroblasts: Implications for Tissue Remodeling

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Abstract

Cells of the myocardium are at home in one of the most mechanically dynamic environments in the body. At the cellular level, pulsatile stimuli of chamber filling and emptying are experienced as cyclic strains (relative deformation) and stresses (force per unit area). The intrinsic characteristics of tension-generating myocytes and fibroblasts thus have a continuous mechanical interplay with their extrinsic surroundings. This review explores the ways that the micromechanics at the scale of single cardiac myocytes and fibroblasts have been measured, modeled, and recapitulated *in vitro* in the context of adaptation. Both types of cardiac cells respond to externally applied strain, and many of the intracellular mechanosensing pathways have been identified with the careful manipulation of experimental variables. In addition to strain, the extent of loading in myocytes and fibroblasts is also regulated by cues from the microenvironment such as substrate surface chemistry, stiffness, and topography. Combinations of these structural cues in 3D are needed to mimic the micromechanical complexity derived from the extracellular matrix of the developing, healthy, or pathophysiologic heart. An understanding of cardiac cell micromechanics can therefore inform the design and composition of tissue engineering scaffolds or stem cell niches for future applications in regenerative medicine.

Keywords

strain; stress; myocardium; hypertrophy; cell tracking; muscle model

Introduction

Cells of the myocardium are at home in one of the most mechanically dynamic environments in the body. Heart chamber filling and wall distention within diastole account for rapid changes in pressure and volume that are released by the wave of contraction that pumps blood through the body [72]. At the cellular level, these pulsatile stimuli are experienced as cyclic strains (relative deformation) and stresses (force per unit area). The heart consists predominantly of matrix-depositing fibroblasts by number and striated myocytes by volume [100]. In myocytes, contractile forces are produced through the sliding of actin and myosin filaments in sarcomeres, as triggered by action potentials and

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intracellular calcium signals. Actomyosin-based forces are also developed in fibroblasts from stress cables, though the structure, assembly, composition, and contractile profile of these differ from myofibers. Active tension in both cell types is also generated from microtubules, while passive tension can be established from cytoplasmic viscosity, intermediate filaments, muscle-specific titin filaments, and properties of the surrounding extracellular matrix (ECM) [52, 104]. Altogether, such mechanics determine the extent of myocardial cell loading, which for myocytes has been distinguished physiologically with clinical assessments of a preload (relating to end-diastolic volume or pressure) or afterload (left ventricular end-systolic wall stress). Though the use of these terms vary, preload and afterload are often defined at the single cell level as the strain subjected to myocytes before contraction and the resistance encountered during contraction, respectively [106].

For most of the body, the proliferative potential of cells contributes to organ growth. However, myocytes do not divide in the post-embryonic heart; instead a progenitor pool is thought to replace a limited number of dead myocytes with new ones [98]. Therefore, adaptation to meet new mechanical demands and maintain cardiac function is most readily achieved by myocyte hypertrophy, an increase in cell size and protein content due to the parallel or in-series addition of myofilaments. Such elongation or thickening of myocytes typically occurs in an attempt to normalize tissue stresses following pressure or volume overload or a segmental loss of the myocardium [119]. Apart from changes in contractile protein mass, mechanical loading can also affect cytoskeletal or sarcomeric organization to regulate cell shape and alignment [30, 51]. Collectively, these responses make up a large part of the pathological and physiological remodeling processes that exist in the heart, in which a mechanical balance is sought between cardiac cell architecture and the effective transmission of forces [31].

Cardiac myocyte function is governed by fundamental mechanisms of excitation, contraction, and energy consumption. For example, the Frank-Starling law describes the relationship between muscle length and active tension [2]. In the heart, as the myocardium is stretched by ventricular filling, the ability to develop pressure is altered. Such a mechanism illustrates the adaptive control built into myocytes for the recognition of mechanics-based loads [85, 117]. The intrinsic characteristics of myocytes have continuous interplay with the extrinsic dynamics and surroundings. Consequently, variables related to the tension, scale, topography, or rigidity of a cellular domain *in vitro* can be used to mimic a preload or afterload and explore how contractile machinery engages and remodels (see Fig. 1).

Numerous temporal and biochemical properties play a part in the mechanics of contractile cells [32]. The following review examines some of the dimensional or structural properties involved in the micromechanics within myocytes and fibroblasts to regulate cardiac cell behavior and function. The literature covered, however, is by no means complete. Rather, this work focuses on how micromechanical stimuli have been measured, sensed, and recapitulated in cardiac myocytes and fibroblasts in order to study their effect on cellular and subcellular organization. The basic approaches discussed are aimed at understanding which micromechanical aspects are important to principles of cardiac cell and tissue engineering *in vitro* and myocardial remodeling under various pathophysiological conditions *in vivo*.

Measurement of Cellular Micromechanics

Innovative methods are needed to measure contractile dynamics, given that the micromechanics of cardiac myocytes and fibroblasts can vary under certain stimuli or loads. Such techniques frequently rely on sensitive detection of motion or force, whether a direct or indirect result of muscle activity. In the intact heart, the mechanical response of functioning myocardium has been successfully measured in clinical settings through noninvasive uses of magnetic resonance imaging, echocardiography, or more specialized derivations of each [35, 99, 125, 151]. Recent advances in these techniques have permitted detailed recording of radial, longitudinal, and circumferential motions of the heart with high sampling rates to arrive at near real-time assessments of strain amplitude, strain rate, and local elasticity that approach the microscale [3, 25, 84]. The scale of these data have diagnostic implications, as regional differences in strain can be used to identify areas of ischemia, diseased tissue, or impaired contractile function [27, 127]. In all, such types of studies may guide experimental approaches *in vitro*, where analyses can be more precise.

The micromechanics measured at a cellular or subcellular level can act as a reasonable estimation of tissue-wide properties due to a conservation of elastic behavior. Fortunately, myocytes isolated from their native collagen-rich ECM may retain their passive stresses, as the mechanics of relaxation were reported to be similar between single and multicellular samples of a given sarcomere length [101, 152]. Yet within and around individual cells there often exist more complex distributions of stress and strain. Local nonuniformity of stress and strain has long been recognized in strips of contracting papillary muscle [65, 111]. As an analysis moves closer to the level of the sarcomere, there is a need to make meaningful measurements at or near this scale.

Micromechanics in cardiac myocytes have been studied most often in precisely-controlled conditions *in vitro*, but inevitably some of the actual complexity found in the whole heart is lost. The tracking of muscle cell contraction (or fractional shortening) alone serves as a simple index of cross-bridge activation and force development. However, by simplifying the structural environment and restricting contractile parameters, one can begin to separate intrinsic and extrinsic contributions of myocyte micromechanics.

Some of the more established methods to measure the mechanical responses of single cells have involved a variety of transducer-based systems (briefly summarized in Fig. 2). Atomic force microscopy (AFM), cell poking, micropipette suction, and parallel plate devices have all been used to gauge cellular forces through various forms of translated movement or deflection [38, 107, 143]. A few of these techniques are specialized for the probing of passive cell mechanics, whereas others are well-suited for the recording of tension as a function of cell length. Nevertheless, these approaches share an inherent limitation in that their measurements require direct physical contact with isolated cells. For example, efforts to attach free ends of myocytes to transducers such as glass beams or carbon fibers have long struggled with issues of preserving the fragile sarcolemma and maintaining stress-bearing cell edges [19, 68, 136]. Recent improvements in the preparation of intact myocytes and carbon fiber transducers have, however, allowed for more precise axial relationships between sarcomere length and load to be explored [68, 73]. Conversely, nanoindentation

analysis with AFM has been largely confined to the surface of cells and in non-skinned myocytes has shown poor sensitivity to micromechanics generated within the cell interior [36]. Such contact-dependent methods also tend to relegate the contractile motion of cells to the same axis of the transducer, though systematic probing has allowed for highly-localized mapping of certain submembranous characteristics [161]. To that end, experiments with spontaneously beating myocytes have shown that mechanical and spatial heterogeneity overlaps at sarcomeric structures in the cells [6].

Other means to evaluate cardiac myocyte activity are optically-based measurement methods. Early work with myocytes exploited the diffraction of light by striations, in which the spacing and dynamics of sarcomeres could be revealed instantaneously as interference patterns [56, 66, 77]. Older imaging methods have taken advantage of the light scattered along the cell periphery during contraction to build semi-automated edge detection procedures; this approach has been applied to one or both ends of myocytes to assess rapid changes in cell or sarcomere length [131, 133]. Also performed with high temporal resolution are linear arrays (or line scanning), which have been used under a range of conditions to acquire the axial displacement of myocytes as they transition between their resting and contracting states [55, 132]. However, many of these one-dimensional (1D) techniques have confirmed that, depending on how they are cultured or supported, isolated myocytes can exhibit nonuniform cell geometry, irregular mechanics at cell ends, bulk lateral and rotational movement, and uneven regional periodicity of sarcomeres [34]. Consequently, the spatial compensations of 1D capture methods have fueled a steady transition towards more quantitative high-speed two-dimensional (2D) imaging as technological improvements have been made.

To reveal how micromechanics vary within an entire cell, contractile motion is analyzed with *post hoc* computation over an area that contains distinct particles or objects with traceable positions. Several strategies have used indirect tracking, wherein the effect of actomyosin forces can be measured through deformation of engineered landmarks on a compliant culture surface [7, 70, 95, 140]. The same concepts are in fact widely used in the broader field of traction force microscopy to map the passive tensions that develop in adherent cells [148]. For example, fluorescent spots patterned on a 2D surface have shown that focal adhesions in myocytes and fibroblasts correlated with substrate deformation and, hence, the localization of static stresses [7]. Such tracking is not restricted to flat domains, as the bending of elastomeric micropillars affected by contractile cells has been used to identify a minimum threshold size of focal adhesions (1 μm^2) to correlate with substrate stresses [140]. A similar arrangement of flexible micropillars with isolated cardiac myocytes demonstrated how the transmission of active forces can vary within the cell thickness [160]. Strides have also been made with indirect tracking in full 3D, as new work has combined fibroblasts and submicron-sized fluorescent beads in 3D matrices to map the principal strains exerted along the contours of cell membranes [81].

Optical methods recently gaining popularity are those that have incorporated direct tracking, in which subcellular activity is monitored by entirely non-destructive and non-contact means through fluorescently labeled molecules or simple fiduciary particles on or within cells [10, 63, 71, 118]. These approaches (sometimes referred to as digital image correlation or

videomicroscopy analysis) rely on the software-assisted correlation of captured images to track changes in position of visible cell morphology [26]. Cell displacement data can then be paired with the deformation of the culture surface to evaluate both the transfer of forces and the viscoelastic losses at cell-substrate interfaces [49, 80]. Such methods have also been used to calculate in-plane values of work, power, and strain energy density of contracting myocytes [42, 116]. Similarly, one of the most sophisticated applications of the technique to date has used direct tracking of adult myocytes to help reconstruct the time-varying stresses in beating cells down to a submicrometer resolution [115, 145]. This integrative form of analysis therefore not only permits the faithful reproduction of contractile events, but also allows for the digitized cell to provide the framework for advanced continuum mechanics models. As the interest in combining experimental data and iterative modeling takes root, an entirely new set of considerations becomes important for the study of cardiac myocyte micromechanics.

Modeling of Cardiac Myocyte Micromechanics

Cell models built from known biophysical relationships can be used to pose new physiological questions. This approach of computational biology is based on the mathematical integration of structural and molecular processes within cells, which in cardiac myocytes has included equations relating ionic currents, calcium handling, cross-bridge interactions, metabolism, neurohormonal regulation, and other signaling events [92]. The mechanical function of myocytes is controlled by the interplay of all of these systems, and therefore often requires some broad “black box” interpretations of cell processes to begin modeling. The simplest mechanical models have treated myocytes as simple circuits with four components to represent muscle properties, including analogues for a force generator (actomyosin tension), a parallel damping element (viscous resistance of cytoplasm to shortening), nonlinear springs both in series with and parallel to the generator (active and passive elastic cell characteristics, respectively) [44]. These latter components that act secondary to actomyosin forces may be tempting to overlook during modeling, and their implementation has been continuously refined over the years [22, 57]. The modeling of such components has become increasingly dynamic, given that the relative contribution of elasticity to restore resting tension in myocytes is known to be length-dependent (via the Frank-Starling law), and that the viscosity of cells can alone contribute to nearly 70% of an intrinsic load at high shortening velocities [33, 103].

Several recent attempts to model cardiac myocyte behavior have focused on ways to identify the micromechanical conditions that drive cell growth and reorganization [50, 64, 76]. Simulations have related local myofiber orientations to optimally minimized shear strains, increased stroke work density, and more homogeneous peak stresses within the modeled myocardium [76]. In addition, the mechanical response of myocardial tissue has been accurately approximated with a model defined in three orthogonal directions; this minimal framework showed conformity with myofiber direction and myocyte alignment [59]. Predictions of mechanical-induced changes in cell or tissue shape has overlapped with a large and fascinating field known as finite growth modeling [78, 94]. For cardiac muscle, such concepts have been used to represent myocytes as theoretical volumes with competing

elastic and growth components, the configuration of which can ultimately account for pathological dilation and thickening of the myocardium [50].

Intrinsic Loading of Cardiac Myocytes and Fibroblasts

The oscillatory contraction of sarcomeres acts as an intrinsic mechanical load for myocytes in addition to performing external work. Indeed, electrical stimulation of myocyte contraction over several days causes increases in the size and myofibrillar organization of isolated cells [93]. Conversely, arrest of contraction in cultured myocytes results in a more rounded morphology with less alignment of Z-discs; more prolonged contractile arrest leads to widespread myofibril disassembly [128]. Further studies have verified that sustained excitation-contraction coupling alone is adequate to promote both the synthesis and addition of sarcomeric proteins in anchored myocytes – hallmarks of a hypertrophic response [21, 40, 93, 128]. However, other aspects of hypertrophy are difficult to attribute to overall beating activity, as myocyte contraction involves a number of overlapping kinetic and biochemical events. Some aspects of sarcomeric organization, gene expression, and protein accumulation and activation appear to coincide differently with actomyosin cross-bridge cycling or calcium signaling [21, 40].

The intrinsically-generated micromechanics of cardiac myocytes and fibroblasts can also have an effect on the development or behavior of neighboring cells. For example, in zebrafish embryos, cells of the mesoderm sort according to actomyosin-dependent cell tension rather than cell-cell adhesivity [75]. Such work has reinforced the biological principle that form follows function, and for the various cell types of the myocardium, contractile function is clearly the main evolutionary determinant [120]. Computations have suggested that the endogenous forces of contractile cells, when transmitted throughout the local environment, can produce a mechanical feedback that results in cell polarization [159]. However, this relatively slow-acting phenomenon is posited to be significant only in static states, and would thus be overridden with any moderate cyclic stimulus experienced within a beating heart. Experiments with fibroblasts in 3D matrices have shown that a change in neighboring cell tension corresponds less with the overall alignment of contractile stress cables than with their size and number [53]. Thus, the net micromechanics generated and experienced by a group of cells seem to be more critical than the cytoskeletal organization of individual cells.

Extrinsic Loading and Mechanosensing in Cardiac Myocytes and Fibroblasts

Application of Strain

The most thoroughly investigated micromechanical responses of cardiac myocytes and fibroblasts have been those affected by externally-applied strain, which is an imposed length change relative to a reference state (ΔL in Fig. 1). Strain is easily manipulated *in vitro* by the controlled stretching (tensile strain) of culture substrates or scaffolds, which can simulate a preload at the end of diastole as it relates to the resting length of myocardial cells prior to contraction [106]. While it is straightforward experimentally to introduce strains as high as 20% of initial cell length, such strains can actually be quite complex as perceived at the level

of a single cell. Any stretch administered to cells in a single direction will, by conservation of Poisson's ratio, produce a compression in an orthogonal direction [89]. Some discretion must thus be used when these stimuli are employed *in vitro*, especially when the layered, anisotropic strains of the native myocardium are often approximated with uniaxial or isotropic conditions [51]. Furthermore, many studies have been performed with cells on flat, relatively rigid surfaces subjected to flexing, where the resulting changes in cell orientation may have more to do with the stabilization of cell anchorage than with strain-dependent remodeling [91, 124, 141].

Independent variables of the direction or rate of strain can, along with the structure of the cell or tissue environment itself, influence how strain is distributed within cells. Extensive studies with fibroblasts, in particular, have shown that both cell and actomyosin filament alignment is readily controlled by such aspects of applied strain [88]. Interestingly, while externally-applied strain is known to trigger stress cable alignment and turnover, focal adhesion kinase (FAK) is required only in the alignment of whole fibroblasts, not cables [29, 61]. When strain rate is varied, fibroblasts and their stress cables are known to align parallel to the direction of strain when presented in the form of static or very low-frequency strain [138]. At higher frequencies of cyclic strain (or those more analogous to the rates experienced in the beating heart), fibroblasts adopt a perpendicular orientation; theoretically, a high rate of depolymerization of stress fibers in line with an anisotropic load results in an accumulation of all other fiber orientations away from the direction of strain [30, 150]. This dependence on strain frequency also extends to cells grown in 3D, as fibroblasts seeded in collagen matrices were reported to align parallel with a static strain vector and perpendicular to a cyclic strain vector [8, 62]. Apart from strain rate, it has been established that cells respond specifically to the direction of principal strain, identified as an axis of pure extension or contraction [15]. Yet in a 3D milieu, other seeding conditions unrelated to strain such as cell number, matrix density, or bulk architecture can affect where this principal strain exists relative to the applied strain axis [112]. In one study, the shape and aspect ratio of a 3D construct was found to alter the principal strain direction perceived by fibroblasts, as thick, cuboidal constructs yielded principal strains slightly perpendicular to the applied load but long, thin constructs confined principal strains parallel to the load [39]. Though many discrepancies remain as to which characteristics of strain dominate in the regulation of fibroblasts, it is clear that all forms of external strain can elicit changes in the cytoskeleton that guide cell orientation and morphology.

Strain of Myocytes and Related Mechanosensors

By concentrating focal adhesions at points of distortion in cells, an external mechanical stimulus can be transmitted throughout the cell for detection [147]. In muscle, externally-applied strain and internally-generated stress are summed and sensed at specific locations. Cardiac myocytes possess specialized forms of focal adhesions at the circumference of the Z-disc known as costameres, where protein complexes anchor cells by connecting filamentous proteins of the ECM to the actin-based cytoskeleton [119, 123, 129]. It is not known how many proteins are mechanically deformed to sense the mechanical input, but transmembrane integrin receptors, talin, vinculin, α-actinin, FAK, and paxillin are all part of the adaptor complex. Signaling results when these proteins themselves translocate or trigger

traditional pathways like small GTPases of the Rho family [24, 60, 156]. Once signaling cascades are activated by strain stimuli, some proteins can shuttle to the nucleus to act as strain-sensitive transcription factors. Another mechanical sensor is titin, a very long, spring-like protein that links the M-line and Z-disc of half-sarcomeres [52]. Regions of varying elasticity in titin have been correlated to distinct interactions with muscle-specific LIM protein (MLP), telethonin, myopalladin, and cardiac ankyrin repeat protein upon stretching [9, 60, 74, 153]. The duration, frequency, and amplitude of strain experienced in myocytes are integral to the activation of such signals and the onset of hypertrophic responses [1, 144]. Thus, the detection of strain triggers signaling pathways, gene reprogramming, protein synthesis, and other hallmarks of hypertrophy in cardiac myocytes independent of any contractile activity or actin polymerization [119, 121].

It has long been known that the whole heart and the individual myocytes have anisotropic contributions to longitudinal or transverse strain (relative to the long axis of cells) [16, 134]. Efforts to understand the sensitivity of myocytes to strain direction has led to ways of restricting cell alignment with substrate patterning or grooved topography in order to compare distinct longitudinal and transverse regimes [51, 126]. Through these approaches, it has become evident that the mechanosensors have the ability to detect and respond to the vector of an applied strain. Longitudinal strain of myocytes is less effective on myofibrillar orientation and contractile protein turnover than transverse strain [51, 130]. The situation is complex because some mechanosensors show subcellular redistribution in response to the direction of strain. For example, applied cyclic strain of a given direction alters both MLP shuttling to the nucleus and the subsequent myofibrillar location of this mechanosensor [17]. A static strain applied longitudinally to myocytes leads to the insertion of new sarcomeres all along the cell length; this adaptive remodeling progresses steadily and is complete within 4 hours, adding one sarcomere per hour [90]. The transduction of longitudinal strain stimuli in myocytes is known to involve certain highly-expressed isoforms of the enzyme protein kinase C (PKC), which are required for such stretch-induced hypertrophy [90, 157]. The ends of the myocytes show extensive remodeling to longitudinal strain of the intercalated discs and gap junctions [41, 122, 155, 158].

Microenvironmental Factors Affecting Cardiac Myocyte and Fibroblast Loading

Regulation by Substrate Surface Chemistry

It has been established that anchorage-dependent cells on flat culture surfaces tend to strike a functional balance between cytoskeletal tension and adhesive spreading [67]. Much *in vitro* work has explored such spreading by constraining morphology to ECM protein-micropatterned surfaces of defined size and shape [23, 102]. This geometric control of cells has allowed for the prediction of micromechanical forces, which have been shown to correlate with the area, concentration, and orientation of adhesion sites in fibroblasts and myocytes [7, 37]. On circular micropatterned islands, fibroblasts were found to assume higher tractional stresses and a lower variance of shear moduli than cells on unpatterned substrates [135]. For myocytes, the introduction of corners within these islands (*i.e.* a change in micropattern shape from circles to rectangles) was reported to induce a regional formation

of sarcomeres with aligned Z-discs [48, 109]. Furthermore, as the aspect ratio of rectangular islands was increased, the sarcomere formation was seen to expand throughout the myocyte to result in a more *in vivo*-like uniaxial anisotropy during contraction [20]. The same group also demonstrated that different micropatterned shapes of similar surface areas did not alter the overall myocyte volume, but rather the striated architecture of cells [48]. Similarly, the mechanical properties of fibroblasts have shown a biphasic dependence on relative micropattern size, an effect thought to be related to the volume fraction of polymerized actin within the cell [135]. Beyond a certain size threshold, however, there is evidence that the distribution of tension in contractile cells on a substrate surface is conserved at the tissue level [13].

Regulation by Substrate Stiffness

If an afterload is defined as the effective resistance against which a cardiac myocyte must work, perhaps the clearest link to an afterload *in vitro* is through modification of domain stiffness, or the resistance of a substrate to deformation. Cardiac myocytes are mechanically anisotropic, but their passive stiffness has widely been approximated with a single Young's modulus between 10 and 50 kPa [6, 11, 19, 86]. In order to reproduce this range of native stiffnesses presented in the study of isolated cells, a number of groups have employed polymeric substrates of tunable crosslinker content. Neonatal myocytes on 10 kPa collagen-coated elastic surfaces displayed well-organized sarcomeres, while cells on 1 kPa or 50 kPa surfaces had poorly-defined or less-aligned striations, respectively [69]. Furthermore, myocytes on 50kPa surfaces were spread into irregular shapes, but on a 10kPa substrate they adopted an axially-aligned shape that corresponded with higher developed forces. A continuous tensional balance thus exists between external stiffness and internal remodeling of the contractile filaments, which determines the state of cytoskeletal "prestress" at any given time [149]. This concept can account for the general finding that both myocytes and fibroblasts exhibit more spreading with increasing substrate stiffness [42]. Likewise, individual stress fibers have been shown to contribute more to intracellular tension and overall cell shape on softer surfaces than on relatively stiffer ones [79]. For myocytes, the matching of stiffnesses between cells and their local surroundings appears to be especially crucial for maximizing the efficiency of the actomyosin power stroke [42, 69, 137]. In all, these trends have suggested that the ideal rigidity for sarcomeres to organize, mature, and optimize a mechanical capability is within the 10-50 kPa passive stiffness range of resting myocytes. However, many studies continue to culture cells on very hard surfaces (*e.g.* polystyrene, MPa, or glass, GPa) that are considerably stiffer than physiologic or pathophysiologic tissues.

Regulation by Substrate Topography

Cells cultured on conventional two-dimensional dishes generally display an irregular, flattened morphology. This morphology is uncharacteristic of the stellate to fusiform shape of fibroblasts or the rounded cross-sectional area (~15 μm thickness) of myocytes common *in vivo* [87, 114]. Given that the micromechanics of stress fibers and myofibrils are otherwise confined within a single thin plane on two dimensions, the engineered recapitulation of a cellular environment into three dimensions is more lifelike. For example, myocytes grown on elastomeric substrates containing regularly-spaced 5 μm-high

topographical structures were shown to terminate with sarcomeric striations over a greater thickness than those cell ends on untextured surfaces [96, 97]. Fibroblasts seeded on similar substrates exhibited greater adhesive interactions and trailing edge lengths than cells on uniformly flat substrates [110, 142]. In these environments, the local dynamics in fibroblast contractility were linked to global changes in cell proliferation and RhoA expression. In a much larger scale, 1.5 mm-high elastomeric posts have been used to guide cell alignment and the spatial arrangement of tension in cardiac muscle tissues [12]. Cell contact with a vertically-oriented substrate therefore appears to provide conditions for optimal anchoring of tension-generating elements and the ability to transmit forces. This is likely to carry over into full 3D environments, as cell-populated collagen gels are known to develop a net mechanical stress from the collective actomyosin constituents of myocytes or fibroblasts alone [44]. In addition, cardiac fibroblasts have been shown to convert to a more contractile myofibroblast phenotype with distinct differences in ECM protein production when maintained in 3D matrices [113].

Combinations of Substrate Cues in 3D: Mimicking ECM Complexity

There have been efforts to explore how physical cues can overlap or compete to affect the micromechanics in cardiac myocytes and fibroblasts. For instance, with discontinuities of stiffness in 3D environments, fibroblasts assemble the cytoskeleton to polarize in the direction of the greatest effective stiffness, orienting perpendicular to the stiffer boundary [14]. Fibroblasts that encounter local resistance in 3D matrices change their morphology from dendritic to fusiform, indicative of resting and contractile states, respectively [139]. The cell–matrix interactions at such regions of high stiffness in 3D also mature from punctate complexes to larger clusters of focal adhesions [139]. Mechanical heterogeneity has also been introduced to fibroblasts through the use of discrete polymeric microstructures of variable stiffness suspended in pliant 3D gels [5, 105]. Even a low concentration of relatively stiff microstructures (50 kPa) causes a decrease in fibroblast proliferation and the expression of the contractility marker alpha-smooth muscle actin compared to 3D cultures containing soft (2 kPa) or no microstructures [5]. Embryological development of the heart and its contractile function are accompanied by similarly complex changes in stiffness and 3D architecture. The formation of the heart tube depends on local forces, and mechanical loads can influence gene expression patterns during development [146, 154]. Recapitulation of some of these features in tube-like 3D organoid constructs using immature, proliferative ventricular myocytes from 15-day old fetuses show a transition from the quiescent to contractile phenotype [43]. Neonatal cardiac myocytes respond to microstructures in 3D with both hypertrophic growth and spontaneous contractile activity [28]. Myocytes and fibroblasts have also been combined in a 3D matrix with underlying mesoscale topography to show that muscle tissue develops greater tension with an increased stiffness than of either scaffold component alone [82].

The filamentous proteins of the ECM in the heart provide a common anchor for myocytes and fibroblasts, thereby stabilizing the transmission of forces parallel to the myocyte direction [18, 108]. Specifically, the network of collagen, laminin, elastin, and fibronectin serves to enmesh densely-packed myocytes in the myocardium as 50 μm-thick laminar sheets, a layout that allows for the high shear strains associated with ventricular wall

shortening to be maintained with minimal shear stresses [4, 83]. The overall composition of the ECM is one suspected reason for differences in elastic anisotropy at various myocardial depths [25]. Yet many studies have focused on the mechanical contribution of fibroblast-deposited collagen alone, particularly its role in scar tissue formation to fill the physical void left by diseased or damaged myocytes. The triple-helical structure of collagen is known to be relatively stiff, and the scar-dependent increase in collagen content following a myocardial infarction can therefore cause an increase in local tissue stiffness from about 25 kPa up to 500 kPa within 2 weeks [19, 46]. The quantity, alignment, and degree of crosslinking of collagen have also been shown to alter the mechanical properties of a myocardial scar, and thus significantly influence the load of adjacent myocytes and the total heart function [45, 54, 58].

Implications for Tissue Remodeling

This review has attempted to cover the vast and rapidly growing literature concerning the micromechanical cues that guide the structure and function of fibroblasts and myocytes *in vitro*. Undoubtedly, the pathophysiological environment in the whole heart during remodeling is determined in part by these same micromechanical cues. The knowledge gained through the measurement, modeling, and application of micromechanical stimuli therefore provides a framework for meeting the physical requirements of cardiac myocytes and fibroblasts. With this understanding, new strategies may arise in the design of 3D tissue engineering scaffolds to employ appropriate structural cues that differentially direct cellular phenotype. Furthermore, the systematic mechanical breakdown of an artificial cell niche for stem and progenitor cell survival, engraftment, and maturation may ultimately enhance the success of cell therapy and regenerative medicine.

Abbreviations

1D	one dimension
2D	two dimensions
3D	three dimensions
AFM	atomic force microscopy
ECM	extracellular matrix
FAK	focal adhesion kinase
MLP	muscle LIM protein
PKC	protein kinase C

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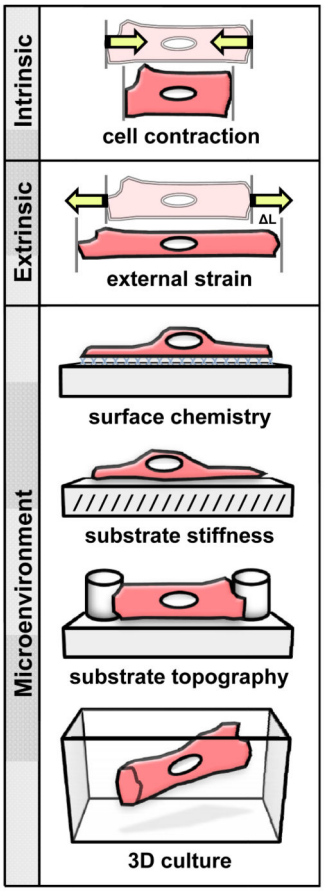


Fig. 1.

Contributions to micromechanical loading in cardiac myocytes. Contractile activity creates an intrinsic load in cardiac myocytes. Extrinsic loading may be applied through external strain (ΔL) to simulate a preload. The microenvironment influences the afterload of tension-generating cells *in* vitro and can be controlled through the surface chemistry, stiffness, topography, or three-dimensionality of a culture substrate

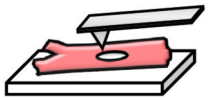
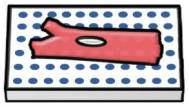
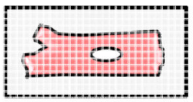
	Method	Advantage	Limitations	Ref.
Transducer	 **indentation** atomic force microscopy cell poker	live cells can be simultaneously probed and mapped with microscale or nanoscale precision	measurements confined to the surface of cells; only mechanical properties perpendicular to substrate plane are assessed	[6] [36] [38] [161]
	 **force transducers** micropipettes carbon fibers	cells are tested in a configuration that preserves their natural architecture	measurements are one-dimensional descriptions; some difficulty with attachment of cells	[68] [107] [136] [143]
Optical	 **indirect tracking** traction force microscopy elastic micropillars fluorescent spots	subcellular mechanical properties can be deduced with high accuracy and a minimal set of mathematical assumptions	system resolution constrained by distribution of tracking markers; deformable substrates must be mechanically defined and reproducibly fabricated	[7] [70] [81] [95] [140] [160]
	 **direct tracking** digital image correlation videomicroscopy light diffraction	recording of true cell morphology allows for activity to be analyzed from any imageable environment	non-aliased capture requires high-speed optical processing; continuum assumptions of intracellular properties necessary for calculations of cell kinetics	[10] [26] [42] [56] [63] [71] [116]

Fig. 2.

Methods for the analysis of micromechanics in isolated cardiac myocytes or fibroblasts. Techniques to study the active or passive micromechanical properties of cardiac cells rely on measurements that use either transducers or optical microscopy with image capture. Some methods have components that span multiple categories, but each comes with its own advantages and limitations