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Substrate Stiffness affects Sarcomere and Costamere Structure and Electrophysiological Function of Isolated Adult Cardiomyocytes

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Abstract

Introduction—The mechanical environment is a key regulator of function in cardiomyocytes. We studied the role of substrate stiffness on the organization of sarcomeres and costameres in adult rat cardiomyocytes, and further examined the resulting changes in cell shortening and calcium dynamics.

Methods—Cardiomyocytes isolated from adult rats were plated on laminin-coated polydimethylsiloxane substrates of defined stiffness (255 kPa, 117 kPa, 27 kPa, and 7 kPa) for 48 h. Levels of α -actinin and β 1 integrins were determined by immunofluorescence imaging and immunoblotting, both in the absence and presence of the phosphatase inhibitor calyculin A. Quantitative RT-PCR was used to measure message levels of key structural proteins (α -actinin, α 7 integrin, β 1 integrin, vinculin). Sarcomere shortening and calcium dynamics were measured at 2, 24, and 48 hours.

Results—Overall cardiomyocyte morphology was similar on all substrates. However, well organized sarcomere structures were observed on only the stiffest (255 kPa) and the most compliant (7 kPa) substrates. Levels of α -actinin in cells were the same on all substrates, while message levels of structural proteins were upregulated on substrates of intermediate stiffness. Inhibition of phosphatase activity blocked the degradation of contractile structures, but altered overall cardiomyocyte morphology. Shortening and calcium dynamics also were dependent on substrate stiffness, however there was no clear causative relationship between the phenomena.

Conclusions—Extracellular matrix stiffness can affect structural remodeling by adult cardiomyocytes, and the resulting contractile activity. These findings illuminate changes in cardiomyocyte function in cardiac fibrosis, and may suggest cardiac-specific phosphatases as a target for therapeutic intervention

Keywords

adult cardiomyocyte; substrate stiffness; fibrosis; sarcomere; costamere

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Introduction

Substrates with tunable stiffness provide crucial information about how cells sense and respond to the mechanics of their environment. Modulation of the mechanical environment has been used with substantial success for controlling stem cell fate [1], discerning the contractility of smooth muscle cells and other mesenchymal cell types [2,3], and observing the response of neonatal cardiomyocytes to the stiffness environment of the developing heart [4–12]. However, no studies have directly observed the effect of substrate stiffness on adult cardiomyocytes. In vivo, these cells secrete a basement membrane containing laminin and collagen IV that promotes integration with the myocardial extracellular matrix and provides a mechanically supportive network. There is growing interest in understanding how the extracellular microenvironment, and in particular the mechanical environment, affects cardiomyocyte function. A main goal of this research is to identify the mechanisms by which cell-matrix interactions malfunction in pathology, so that treatments can be developed to prevent progression to heart failure. The present study uses substrates of tunable stiffness to study the effects of extracellular matrix compliance on sarcomere and costamere organization, as well as contraction dynamics, in adult cardiomyocytes.

The question of how cardiomyocytes sense strain is crucial to understanding how these cells regulate their contraction/relaxation dynamics during systole and diastole, and multiple studies have identified proteins associated with a “stretch sensor” mechanism [13,14]. However it is likely that cardiomyocytes also have a “stress sensor” that can react to the changes in myocardial stiffness that occur as a result of pathology (myocardial infarction, hypertrophic cardiomyopathies, etc.) and aging. Atomic force microscopy measurements of the elastic modulus of fibrotic myocardium have yielded values between 20 and 55 kPa [15,16], compared to 2 and 8 kPa for non-fibrotic myocardium as determined by MRI [17]. Though it can be difficult to quantitatively compare elastic moduli determined by different methods [18], these data suggest that increased deposition of extracellular matrix in fibrotic tissue results in a mechanically stiffer microenvironment. A recent study indicated that substrate stiffness resembling a fibrotic scar inhibited beating of embryonic cardiomyocytes [19].

Proteins involved in sensing of stretch have been identified in both the sarcomere and the costamere complex [13]. The function of the costamere is to couple the z disk to the basement membrane, so that force generated by the sarcomere can be translated to the surrounding extracellular matrix. The costamere complex includes $\beta 1$ and $\alpha 7$ integrins that span the plasma membrane [20]. The $\beta 1$ integrins in particular are important surface receptors in cardiomyocytes, regulating cell matrix interactions [21] and playing a role in cytoprotection [22]. Integrins are anchored to the z-disk by structural proteins including vinculin, talin, desmin, and zyxin [14]. The z-disk complex is a complicated array of proteins that also includes candidates for the stretch sensing mechanism, with α -actinin forming the backbone of the structure. Recent studies have indicated that the z-disk is among the initial targets for deterioration during isoproterenol-induced adult cardiomyocyte apoptosis [23], suggesting that z-disk integrity is a crucial indicator of cardiomyocyte function.

In the present study, components of both costamere and sarcomere structure and function in culture were analyzed in response to changes in substrate stiffness. Adult cardiomyocytes in culture beat spontaneously and undergo a slow dedifferentiation process, which involves changes in the cytoskeleton and t-tubule structure [24,25]. The calcium handling in these cells also changes with time, possibly due to influx through protein kinase A-activated L-type Ca^{2+} channels [26]. To determine how the stiffness of the extracellular environment affects these properties, isolated cardiomyocytes were plated on laminin-coated substrates

with varying stiffness and cultured for 48 hours. The organization of α -actinin and integrin $\beta 1$ was observed by immunofluorescence, and message levels of proteins associated with the sarcomere and costamere were measured. The effect of phosphatase inhibition on cytoskeletal rearrangement was also examined using calyculin A, a general phosphatase inhibitor. Finally, cells were paced at different time points to determine how changes in cell structure affected the electrical function of the cardiomyocytes, as well as to characterize the time-dependence in the cellular response.

Methods

Cell Isolation and Culture

Adult rat ventricular cardiomyocytes were isolated as previously described [27]. Briefly, hearts from Sprague–Dawley rats were enzymatically digested in a modified Langendorff perfusion apparatus to isolate the cardiomyocytes. The protocol was approved by The University of Michigan University Committee on Use and Care of Animals (UCUCA) in accordance with University guidelines. Aliquots of 2.0×10^4 cells were plated on polydimethylsiloxane (PDMS) substrates coated with 40 $\mu\text{g/mL}$ of laminin, using M199 culture medium supplemented with 5% fetal bovine serum, 50 U/mL penicillin, and 50 $\mu\text{g/mL}$ streptomycin. After two hours, this media was replaced with serum-free M199 supplemented with 10 mM HEPES, 0.2 mg/ml bovine serum albumin, 10 mM glutathione, 50 U/mL penicillin, and 50 $\mu\text{g/mL}$ streptomycin. Cells were cultured in standard incubators with 5% CO_2 at 37°C. For experiments involving calyculin A, 0.5 $\mu\text{g/mL}$ of the phosphatase inhibitor was added to the media. Different cells on separate substrates were used for the immunofluorescence, sarcomere shortening, and calcium transient assays.

PDMS Substrate Fabrication and Validation

The base and curing agent (Sylgard 184) were mixed in ratios of 10:1, 20:1, 30:1, and 50:1 by weight, and 1.0 mL of the elastomer was cured in 6-well polystyrene plates, creating slab constructs approximately 1 mm thick. For experiments that required electric field stimulation, blocks of the elastomer were cut out of the 6-well plate and bonded to glass coverslips. Prior to coating with laminin, the PDMS was etched with 5 M sulfuric acid for 60–90 minutes, washed thoroughly in distilled water, and sterilized using a UV lamp for 30 minutes. To determine elastic modulus, the elastomer was molded in a rectangular mold with defined width and length. A uniaxial mechanical testing system was used to apply 20% total strain at a rate of 10 %/s. The elastic modulus was calculated as the slope of the stress/strain curve at approximately 10% strain, in order to keep within the limits of linear strain theory. PDMS mixed in ratios of 10:1, 20:1, 30:1, and 50:1 produced substrates with mean moduli of 255 kPa, 117 kPa, 27 kPa, and 7 kPa respectively.

Immunofluorescence

After 48 hours in culture, cells were fixed, permeabilized, and stained using previously described protocols [3]. Immunostains included fluorescent DAPI (1:50 dilution), phalloidin (1:50) conjugated to Alexa Fluor 488, and monoclonal mouse antibodies for α -actinin (1:500) and integrin $\beta 1$ (1:50). A secondary Texas Red-tagged anti-mouse antibody was used to visualize these proteins. Cells were visualized using confocal microscopy. Projection image Z-stacks of approximately 10 μm were collected using a confocal microscope.

Immunoblotting

After 48 hours in culture, cells were lysed in ice cold sample buffer (24.77% glycerol, 1.63% SDS, 0.1 M Trizma stacking buffer, 3.31% Bromophenol Blue stock solution, 24.88 mM DTT, 1.78 mM leupeptin), and then stored at -20°C . In preparation for protein

separation, samples were boiled for 3 min and then sonicated for 10 min. Protein separation was performed as previously described [27] using 12% or 4–12% gradient SDS-polyacrylimide 12-well gels (Bio-Rad), and then transferred onto a PVDF membrane overnight. The PVDF membrane was then blocked in either 5% dry nonfat milk or BSA, washed in Tris buffered saline (TBS), and incubated overnight at 4 °C with a mouse monoclonal antibody against α -actinin (1:5000). The next day, blots were rinsed with TBS and incubated for 45 minutes at room temperature with HRP-linked anti-mouse secondary antibodies (1:2000). Gels were silver stained and a representative protein band was used to normalize for protein loading on the blot. Immunodetection was visualized with chemiluminescence using Pierce ECL Western Blotting substrate (Thermo-Fisher).

qRT-PCR

A guanidium thiocyanate-phenol-chloroform extraction protocol (TRIzol) was used to isolate mRNA from the cells after 48 hours in culture. Briefly, cells were dissolved in TRIzol, buffer, and reverse transcription of mRNA was performed with a high-capacity cDNA Archive Kit and a C-1000 Thermocycler. The complete quantitative PCR protocol is described in a previous publication [3].

Cell Shortening Assay

At 2, 24, and 48 hours, PDMS coverslips were transferred to a stimulation chamber mounted on a microscope stage and perfused with M199 culture media at 37 °C throughout the assay. A video-based detection system (Ionoptix) was used to determine sarcomere shortening in response to 40V, 0.2 Hz electrical shortening.

Calcium Transient Assay

At 2, 24, and 48 hours, cells on PDMS coverslips were loaded with 5.0 μ M Fura-2 AM for 4.5 minutes at 37 °C followed by a 4 minute wash in media without Fura-2 AM for de-esterification. Coverslips were again transferred to a stimulation chamber and paced at 0.2 Hz frequency. Calcium transients were measured by taking the ratio of emission at 510 nm from excitations at 360 nm and 380 nm.

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean. For comparisons of immunoblotting and message levels, an unpaired Student's t test was used to determine statistical significance for comparisons between two groups, with $p < 0.05$ considered significantly different. For grouped comparisons of the contractility and calcium transients, a two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test ($P < .05$) was used to compare multiple groups using GraphPad Prism 5 software.

Results

Effect of substrate stiffness on α -actinin and β 1 integrin organization

To assess the effect of substrate stiffness on z-disk integrity, α -actinin was visualized using immunofluorescence. As shown in Figure 1, adult cardiomyocytes seeded on substrates of 255, 117, 27, and 7 kPa had a similar and uniform morphology, exhibiting predominantly binucleated, rod-shaped cells. However, the organization of α -actinin into uniform, periodic z-disks was substantially affected by substrate stiffness. On substrates of 255 kPa and 7 kPa (Fig. 1A, 1D) α -actinin was organized in a clearly defined sarcomeric pattern that was less evident in cells seeded on 117 kPa substrates (Fig. 1B), and was completely absent for cells on 27 kPa substrates (Fig. 1C). However even at the intermediate stiffness, the antibody did

produce positive staining, suggesting that α -actinin, or at least an immunoreactive degradation product, was still present in the cytoplasm.

Immunoblotting was used to quantify the amount of α -actinin present within the cells, and to determine whether immunoreactive degradation products were being produced by the cells seeded on substrates with stiffness values of 117 and 27 kPa. As shown in Figure 2, the amount of α -actinin within cells was constant for all the substrate stiffness values tested, even though Figure 1 indicates a substantial effect on α -actinin organization. Furthermore, the blot did not reveal the presence of degradation products. These data suggest that the effect of substrate stiffness on α -actinin organization is not due to decreased overall levels of the protein.

To determine the effect of substrate stiffness on the costamere complex, β 1 integrins were visualized using immunofluorescence. In cells seeded on 255 kPa and 7 kPa substrates, β 1 integrin colocalized with the z-disk of the sarcomere (Fig. 3A, 3D). As for the results of the α -actinin staining, this pattern was not observed in cells on 117 kPa and 27 kPa substrates (Fig. 3B, 3C). In cells on intermediate stiffness substrates, the β 1 integrin appeared to be distributed homogeneously throughout the cell as small blotches of positive staining. It is not possible to determine conclusively if these areas of positive staining correspond to the location of z-disks. However, in cells plated on 255 kPa and 7 kPa substrates, β 1 staining was seen to span essentially the entire z-disk. Again, the immunofluorescence images indicate that the general cell morphology was unchanged despite the cytoskeletal alterations.

Effect of substrate stiffness on message levels of structural proteins

The observation that substrate stiffness affects the organization of key proteins in the sarcomere and costamere complex led us to further examine its effect on message levels of key proteins using qRT-PCR, as shown in Figure 4. Expression of α -actinin and integrin were examined because of the immunofluorescence and immunoblotting results. Additionally, the message levels of α 7 integrin and vinculin, two other costamere-related proteins were measured. For cells plated on 117 kPa substrates, expression of α 7 integrin and vinculin were significantly increased compared to the levels at 255 kPa (Figure 4B, 4D). For cells on 27 kPa substrates, message levels for all four proteins were significantly increased (Figure 4), relative to the 255 kPa substrate. In contrast, cells on 7 kPa substrates showed no significant differences in expression levels of these key proteins, relative to cells on 255 kPa substrates. Hence, although cells on intermediate stiffness exhibited substantially different organization both a sarcomere-related protein (α -actinin) and a costamere-related protein (integrin β 1), the message levels of these and other related proteins actually increased. These data provide insight into the effects of substrate stiffness at both the transcriptional and protein levels.

Effect of phosphatase inhibition by calyculin A

The general phosphatase inhibitor, calyculin A, was used to determine if the sarcomere and costamere disorganization caused by plating on intermediate stiffness involved protein dephosphorylation, which is linked to enhanced proteolytic activity [28]. The α -actinin immunofluorescence was repeated for cells cultured in calyculin A-containing media. As Figure 5 indicates, cells plated on all substrates presented well organized z-disks, though the presence of calyculin did have a marked effect on overall cell morphology. This effect was especially apparent in cells on 255 kPa substrates, which displayed actin fibers of varying orientation and a loss of the usual rod-shaped morphology (Fig 5A). Cells plated on the other substrates also showed varying levels of actin assembly and rod-shaped morphology. These data suggest that inhibition of protein dephosphorylation affected various cell

processes in addition to blocking the degradation of the sarcomere and costamere structures at intermediate substrate stiffness.

Effect of substrate stiffness on cell contractility

Cardiomyocytes were paced at specified times in culture to understand how the described changes in sarcomere and costamere organization affected cross bridge cycling. These tests were also used to examine the relationship between varying substrate stiffness and the degree of cell stretch, and the consequent preload on the cardiomyocytes. If cardiomyocytes exerted the same level of force regardless of substrate stiffness, the cells on softer substrates would always have higher levels of strain, and the observed changes could be caused solely by levels of stretch. However, the results of the contraction assay indicate that after 2 hours all the cells contracted roughly the same amount (Figure 6). At 24 hours, cells on the intermediate stiffness substrate (27 kPa) developed the highest shortening amplitude, even higher than cells plated on 7 kPa substrates. These results suggest that varying substrate stiffness affected the stress generated within the cardiomyocytes, and not just the levels of strain. The temporal changes in the observed effects furthermore indicate that the cell response is an adaptive, time-dependent process.

For cells plated on the stiffest substrate (255 kPa) the shortening amplitude decreased and the rates of shortening and re-lengthening slowed significantly over the first 24 hours in culture. The amplitude of shortening recovered by 48 hours, but this recovery was not mirrored in shortening or re-lengthening rates. In contrast, cardiomyocytes cultured on 27 kPa substrates developed a significant increase in shortening amplitude between over the first 24 hours, and this increase was followed by diminished shortening amplitude and slowed rates of shortening and re-lengthening by 48 hours. Cells on the 7 kPa substrate exhibited significant increases in shortening amplitude and shortening and re-lengthening rates at 48 hours. Therefore, even though cells plated on 255 kPa and 7 kPa substrates both exhibited normal α -actinin and β 1 integrin organization, their shortening dynamics followed opposite trends over the 48 hours in culture.

Effect of substrate stiffness on calcium dynamics

Having shown that the shortening trends produced by substrate stiffness is an adaptive response, the calcium transients were measured to determine if the contractile response was directed by changes in the calcium transient. Changes in the calcium transient often are directly associated with the force and shortening response in myocardium. However, there is evidence that increased Ca^{2+} transient precedes diminished shortening during α -adrenergic induced apoptosis in cardiomyocytes [23]. Interestingly, in the present study the calcium transient of cardiomyocytes generally did not follow the shortening response and elevated Ca^{2+} did not develop prior to diminished contractile function, as shown in Figure 7. Myocytes cultured on the 27 kPa substrate exhibited significant decreases in the peak Ca^{2+} transient ratio and slowing of the rates of Ca^{2+} rise and re-uptake at the 24 hour time point (Fig. 7), while at the same time there was enhanced shortening amplitude but no significant change in shortening or re-lengthening rates (Fig. 6). Even though higher calcium gradients are often associated with cardiomyocytes undergoing apoptosis [29], calcium signaling was impaired in the cells displaying sarcomere and costamere degradation at the 24 hour time point.

The reductions in the rates and amplitude of shortening detected in myocytes cultured on 255 kPa substrates also were not reflected in the Ca^{2+} transient. Instead, the amplitude and rate of Ca^{2+} rise and re-uptake did not significantly change over the 48 hour culture period. Moreover, the Ca^{2+} transient was relatively constant over the testing period and did not mirror the enhanced shortening amplitude and accelerated re-lengthening rates observed in

myocytes cultured on 7 kPa substrates. Only the increase in the rate of Ca^{2+} rise matched the direction of shortening rate in these cardiomyocytes. Collectively, these data suggest that the adaptive response of cardiomyocytes to substrate stiffness is not initially mediated through changes in the calcium transient, and instead depends on adaptations in the myofilament.

Discussion

The results of this study show clearly that the mechanical stiffness of the extracellular environment influences adult cardiomyocyte contractile function. The present study also demonstrates that cellular re-modeling in the myofilament z-band and costameric complexes accompanies structural adaptation in response to the extracellular environment, and that phosphatase inhibition attenuates these adaptations. Most importantly, our work identifies the myofilament as a potential target for mediating functional adaptation to the extracellular environment.

The stress sensor in the adult cardiomyocytes used in this study appears to be sensitive to a specific range of substrate stiffness, and our results suggest that this mechanotransduction mechanism exhibits nonlinear behavior. In many other cell types, there is a specific range of substrate stiffness that produces normal cell function [1,19]. The results described in this study suggest there is a range of substrate stiffness that promotes degradation of sarcomeres and costameres, both crucial structures in the myocyte. This finding has potentially important implications for the study of pathological fibrosis and scar growth following myocardial infarction, as well as for the understanding of cell-matrix interactions in both cultured and native cardiomyocytes.

Our results using substrates of defined stiffness indicated that on both the stiffest (255 kPa) and the most compliant (7 kPa) substrates, α -actinin was well organized into sarcomeres and was colocalized with β 1 integrins. However on the intermediate stiffness substrates (27 and 117 kPa), α -actinin was not well organized, and β 1 integrin did not colocalize to the z-disk. Immunoblotting showed equal amounts of α -actinin within cardiomyocytes plated on different substrates, but cells upregulated expression of α -actinin as well as β 1 and α 7 integrins and vinculin for cells on intermediate stiffness substrates. These changes in transcription suggest that information about the cellular environment is transduced and transmitted to the nucleus in a feedback loop. Moreover, the steady level of α -actinin protein levels despite mRNA upregulation suggests there may be increased cellular protein turnover, possibly resulting from enhanced proteolytic activity in the cells. Interestingly, the detrimental effects of the intermediate stiffness substrates on cellular structures were attenuated by the phosphatase inhibitor calyculin A. Further work is needed to determine whether dephosphorylation of the stress sensor(s) may directly mediate remodeling and/or protein turnover, or instead works via less direct mechanisms.

Assays for cardiomyocyte contractility and calcium transients indicated that even though cells on 255 kPa and 7 kPa substrates both retained their sarcomere and costamere structure, the contractile function dynamics in response to pacing produced quite different functional outcomes. This conclusion is best represented by the significantly increased sarcomere shortening in myocytes cultured on 7 kPa and diminished shortening in cells on the 255 kPa substrate at the 48 hours time point. Taken together, these data suggest that there is a preferred range(s) of stiffness for optimal cardiomyocyte function, and that when the extracellular environment is too stiff or too elastic the cells begin to structurally and functionally remodel. Moreover, the fact that cardiomyocytes on soft substrates did not sustain larger contractions demonstrates that these cells can be modeled as a dynamically controlled system with some element of sensing, actuating, and feedback information. If the cardiomyocyte were an open loop system, it would exert the same amount of shortening

regardless of substrate stiffness. However, our contractility and calcium transient data indicate a complex, time-dependent response to extracellular substrate stiffness. The data further suggest that this stress sensor is located in the sarcomere, costamere, and/or cytoskeleton. Additionally, the message level response suggests that this mechanical signal feeds back to the nucleus of the myocyte. The trends in calcium transients and contractility clearly show that these mechanisms cannot be explained solely by changes in calcium handling.

The results of this study have potentially important clinical implications in the treatment of myocardial infarction. Although the majority of cardiomyocytes die immediately in response to ischemia in the infarction zone, myocytes in the border zone are more likely to remodel and/or die over time, during the initial remodeling phase of scar formation. This remodeling response in cardiomyocytes and the later cell death facilitates scar growth, but may eventually lead to heart failure. The stiffness in and surrounding the scar is increased due to increase matrix deposition, and the tissue may reach stiffness values that promote the structural deformations described in the present study. If the mechanism by which these cells sense extracellular stiffness can be discerned, then the pathways responsible for the malfunction can be targeted to create a cytoprotective environment and retain more functional myocytes and overall cardiac function after infarction. Further work is needed to identify the key steps and candidate target(s) for mechanosensing and matrix remodeling by adult cardiac myocytes during the early and later stages of pathological remodeling following myocardial infarction.

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Summary

Cardiomyocytes isolated from adult rats are plated on substrates of varying stiffness to demonstrate that a specific range of substrate elastic moduli causes z-disk and costamere disorganization and excitation/contraction coupling. The study uses an in vitro model to elucidate mechanisms by which altered matrix mechanical properties can contribute to pathological cardiac remodeling.

Figure 1.

Immunofluorescence images of z-disk organization in adult cardiomyocytes after 48 hours in culture on different substrate stiffness. Actin is stained by a conjugated phalloidin-FITC antibody (green), cell nuclei with DAPI (blue), and α -actinin of the z-disk with a monoclonal antibody (red). Whole images are $150 \times 150 \mu\text{m}$. Inset shows magnification of cellular structures of interest for clarity.

Figure 2.

Immunoblot of cell homogenates from adult cardiomyocytes seeded on substrates with elastic moduli ranging from 255 kPa to 7 kPa for 48 hours. The blot in (A) shows representative lanes for three of the stiffness levels and (B) displays the quantification of the immunoblots.

Figure 3.

Immunofluorescence images of $\beta 1$ integrin colocalization with the z disk after 48 hours in culture on different substrate stiffness. Actin is stained by a conjugated phalloidin-FITC antibody (green), cell nuclei with DAPI (blue), and integrin $\beta 1$ with a monoclonal antibody (red). Inset shows magnification of cellular structures of interest for clarity.

Figure 4. Message levels of key proteins associated with the sarcomere (α -actinin) and costamere complexes (α 7 integrin, β 1 integrin, and vinculin) for adult cardiomyocytes seeded on substrates with different elastic moduli at 48 hours. * = significant difference from the 255 kPa sample at $p < 0.05$.

Figure 5.

Immunofluorescence images of z-disk organization in adult cardiomyocytes after 48 hours in culture on different substrate stiffness with the addition of 0.5 μ g/mL of calyculin A. Actin is stained by a conjugated phalloidin-FITC antibody (green), cell nuclei with DAPI (blue), and α -actinin of the z-disk with a monoclonal antibody (red). Inset shows magnification of cellular structures of interest for clarity.

Figure 6.

Composite traces (left panels) and quantitative analysis (right panels) of contractile shortening and re-lengthening in cardiomyocytes plated for 2, 24, and 48 hours on substrates with elastic moduli of 255, 27, and 7 kPa. The left column shows averaged traces of sarcomere shortening at 2, 24, and 48 hours during electrical stimulation. The right column presents quantification of peak shortening amplitude, shortening rate, and re-lengthening rate. A + denotes significance ($p < 0.05$) compared to the 2 hour time point for each stiffness value, and a * denotes significance ($p < 0.05$) compared to the 255 kPa stiffness value for each time point, as determined by Bonferroni posttests following a two-way ANOVA.

Figure 7.

Intracellular calcium transient traces (left panel) and quantitative analysis of the transient (right panel) in cardiomyocytes plated for 2, 24, and 48 hours on substrates with elastic moduli of 255, 27, and 7 kPa. The left column shows averaged traces of the calcium transients for electrically paced cells at 2, 24, and 48 hours. The right column presents quantification of total shortening amplitude, rate of Ca^{2+} rise, and rate of Ca^{2+} re-uptake in these myocytes. A + denotes significance ($p < 0.05$) compared to the 2 hour time point for each stiffness value, and a * denotes significance ($p < 0.05$) compared to the 255 kPa stiffness value for each time point, as determined by Bonferroni posttests following a two-way ANOVA.