Inhibition of focal adhesion kinase increases myofibril viscosity in cardiac myocytes

Nilay Taneja1 | Matthew R. Bersi2 | Megan L. Rasmussen1 | Vivian Gama1 | W. David Merryman2 | Dylan T. Burnette1

1Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee
2Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee

Correspondence
Dylan T. Burnette, Department of Cell and Developmental Biology, Vanderbilt University, MCN Room T2209, 1161 21st Ave South, Nashville, TN 37232.
Email: dylan.burnette@vanderbilt.edu

Funding information
American Heart Association, Grant/Award Numbers: 18PRE33960551, 19PRE34380515; National Heart, Lung, and Blood Institute, Grant/Award Numbers: K99 HL146951, R35 HL135790; National Institute of General Medical Sciences, Grant/Award Numbers: R35 GM125028, R35 GM128915

Abstract
The coordinated generation of mechanical forces by cardiac myocytes is required for proper heart function. Myofibrils are the functional contractile units of force production within individual cardiac myocytes. At the molecular level, myosin motors form cross-bridges with actin filaments and use ATP to convert chemical energy into mechanical forces. The energetic efficiency of the cross-bridge cycle is influenced by the viscous damping of myofibril contraction. The viscoelastic response of myofibrils is an emergent property of their individual mechanical components. Previous studies have implicated titin-actin interactions, cell-ECM adhesion, and microtubules as regulators of the viscoelastic response of myofibrils. Here we probed the viscoelastic response of myofibrils using laser-assisted dissection. As a proof-of-concept, we found actomyosin contractility was required to endow myofibrils with their viscoelastic response, with blebbistatin treatment resulting in decreased myofibril tension and viscous damping. Focal adhesion kinase (FAK) is a key regulator of cell-ECM adhesion, and microtubules as regulators of the viscoelastic response of myofibrils. Here we found inhibition of FAK signaling altered the viscoelastic properties of myofibrils. Specifically, inhibition of FAK resulted in increased viscous damping of myofibril retraction following laser ablation. This damping was not associated with acute changes in the electrophysiological properties of cardiac myocytes. These results implicate FAK as a regulator of mechanical properties of myofibrils.

KEYWORDS
cardiac myocyte, focal adhesion kinase, laser ablation, myofibril, myosin II, viscoelasticity

1 INTRODUCTION
The functional unit of contraction within striated muscle is the myofibril, which is comprised of sarcomeres arranged in series. Sarcomeres are complex protein assemblies, the core of which consists of "thick" myosin-II muscle filaments interacting with "thin" actin filaments. The barbed ends of actin filaments from adjacent sarcomeres are cross-linked by α-actinin-2 to form the sarcomere borders known as Z-discs (Knöll, Buyandelger, & Lab, 2011). Interactions between the multiple components within the sarcomere ultimately determine its mechanical properties. For example, the giant protein titin spans half the length of the sarcomere, with its N-terminal domain binding the Z-disc and its C-terminal domain extending to the M-line (Maruyama, 1997). The complete list of...
factors controlling the mechanical properties of myofibrils continues to be elucidated.

Titin has long been established as a major contributor to the myofibrillar mechanical properties required for proper heart function. The PEVK domain of titin behaves like a molecular spring, generating passive tension within the myocardium against stretch, as experienced in the heart during peak diastole (Granzer & Irving, 1995; Granzer & Labeit, 2004). Furthermore, interactions between actin and the titin PEVK domain lead to viscous damping of myofibrils by impeding actin filament sliding (Kulke et al., 2003; Linke et al., 2002). At longer length scales, viscous damping in the myocardium is thought to be provided by collagen fibrils in the extracellular matrix (ECM) (Granzer & Irving, 1995). Previous studies have demonstrated that faster shortening of myofibrils has a higher energetic cost (Gibbs & Chapman, 1985; He, Bottinelli, Pellegrino, Ferenczi, & Reggiani, 2000). Thus, viscous damping reduces this energetic cost of contraction and contributes to an overall increased efficiency of energy consumption in the heart (Caporizzo, Chen, Salomon, Margulies, & Prosser, 2018).

Cardiomyocytes interact with the ECM at specialized sites called costameres, which anchor the Z-linesto closest to the cell membrane to the ECM (Samarel, 2005). We have previously established a role for cell-ECM adhesion in myofibril assembly, using human iPSC-derived cardiomyocytes (hiCMs) as a model system (Taneja, Neininger, & Burnette, 2020). Cell-ECM sites transmit mechanical forces from immature myofibrils (muscle stress fibers) to the substrate, promoting their maturation.

Focal adhesion kinase (FAK) is a key regulatory protein present at cell-ECM sites which regulates adhesion formation and turnover (Parsons, Martin, Slack, Taylor, & Weed, 2000). A role for FAK in contributing to heart mechanics has also been well established in vivo. Cardiac specific knockout of FAK results in an ability to respond to increased load in the heart (DiMichele et al., 2006; Peng et al., 2006). On a mechanistic level, FAK performs both scaffolding and signaling functions (Sulzmaier, Jean, & Schlaepfer, 2014). FAK phosphorylates a variety of targets, including itself at Tyr397, which is required for adhesion disassembly (Sulzmaier et al., 2014). Inhibition of this autophosphorylation using the inhibitor PF-228 results in focal adhesion disassembly (Slack-Davis et al., 2007; Taneja et al., 2016). Treatment of hiCMs with PF-228 results in stabilization of focal adhesions, longer Z-line lengths and increased incorporation of titin (Taneja, Neininger, & Burnette, 2020). However, the effect of focal adhesion stabilization by FAK on the mechanical properties of myofibrils remains unknown.

In nonmuscle cells, FAK regulates actin cytoskeleton stability and cell stiffness (Fabry, Klemm, Kienle, Schäffer, & Goldmann, 2011; Mierke et al., 2017). Stress fibers that are attached on either end to the ECM through focal adhesions have higher tension compared to those that are not directly coupled to the ECM (Lee, Kassianidou, & Kumar, 2018). Given the role of FAK in regulating cellular mechanics, and its role in load adaptation in the heart, here we specifically investigate its role in modulating the mechanical properties of cardiac myofibrils at the cellular level. Using laser-assisted dissection of myofibrils, we show that inhibition of FAK results in increased myofibril viscosity. We further show that these changes in mechanics are not mediated by changes in electrophysiology.

2 | RESULTS AND DISCUSSION

Laser ablation is a widely used biophysical tool to assay the mechanical properties of cytoskeletal systems, such as stress fibers, epithelial cell–cell junctions and myofibrils (Fernandez-Gonzalez, Simoes, Röper, Eaton, & Zallen, 2009; Lee et al., 2018; Roman et al., 2017; Taneja, Neininger, & Burnette, 2020). We have previously used this technique to interrogate cortex mechanics during cell division, as well as testing mechanical coupling between cell-ECM adhesions and myofibrils (Taneja et al., 2020; Taneja, Neininger, & Burnette, 2020). Myofibrils are considered complex mechanical systems. Conceptually, they can be thought of as a series of springs and dashpots (sorcomeress), which lend both elasticity and viscosity to the mechanical response, respectively. We used an existing mechanical framework to estimate these properties from the dynamics of myofibril retraction following laser-assisted dissection (Lee et al., 2018).

We have previously described the dynamics of myofibril assembly using hiCMs as a model system (Fenix et al., 2018). hiCMs are transcriptionally similar to neonatal or embryonic cardiac myocytes (DeLaughter et al., 2016; Kuppusamy et al., 2015). As such, they lose their myofibrils upon trypsinization, and reform them within 24 hr after plating (Fenix et al., 2018). Thus, we plated hiCMs on glass coverslips coated with fibronectin and allowed them to spread overnight prior to performing laser-assisted dissection of preformed myofibrils (Figure 1a). Similar to the response of stress fibers, the newly created ends of the myofibrils retracted away from each other, reaching a steady-state separation in less than 100 s. Kymographs were used to track the retraction profile of each end of the ablated myofibril (Figure 1b). Note the hiCMs continued to beat during the duration of myofibril retraction (see horizontal lines in kymograph, Figure 1b) indicating the focused ablation did not disrupt normal hiCM function over the course of myofibril separation.

Using a custom MATLAB script based on edge detection, we segmented the retracting ends of the ablated myofibril and fit the trajectories to a standard Kelvin–Voigt viscoelastic solid model widely used for retracting stress fibers and cell–cell junctions in nonmuscle cells (Fernandez-Gonzalez et al., 2009; Kumar et al., 2006; Lee et al., 2018). Using this framework, we identified two material parameters that describe the viscoelastic behavior: (a) the asymptotic distance \( D_o \) is the maximal distance between the two retracting ends as they reach a steady-state separation and is proportional to stored elastic energy or tension within the myofibril and (b) the relaxation time constant \( \tau \) describes the shape of the time-dependent retraction profile and is proportional to myofibril viscosity; for the Kelvin–Voigt model, the relaxation time is mathematically equivalent to 0.632 \( D_o \) (Figure 1c).

We also obtained estimates for two additional parameters related to the retraction dynamics—linear velocity \( v_1 \) and instantaneous velocity \( v_i \) (Figure 1c). Instantaneous velocity was calculated as the
gressive retraction of the dissected myofibril ends until reaching a bent or buckled shape upon blebbistatin treatment (Figure 2a). Laser-assisted dissection to assess mechanical properties of myofibrils. (a) Representative control human iPSC-derived cardiomyocyte (hiCM) at 16 hr post replating. Yellow arrowhead shows ablation site. Dotted white rectangle was used to create montage of myofibril retraction over 60 s. (b) Kymograph showing retraction profile of myofibril shown in (a). (c) Schematic showing different parameters obtained from analysis of the kymographs using custom code in MATLAB [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 1 Laser-assisted dissection to assess mechanical properties of myofibrils. (a) Representative control human iPSC-derived cardiomyocyte (hiCM) at 16 hr post replating. Yellow arrowhead shows ablation site. Dotted white rectangle was used to create montage of myofibril retraction over 60 s. (b) Kymograph showing retraction profile of myofibril shown in (a). (c) Schematic showing different parameters obtained from analysis of the kymographs using custom code in MATLAB [Color figure can be viewed at wileyonlinelibrary.com]

dervative of the fitted retraction profile at the time of ablation and is equivalent to the ratio of asymptotic distance to relaxation time. Thus, instantaneous velocity is proportional to tension and inversely proportional to viscosity and represents a combined effect of the viscoelastic parameters. Linear velocity is an estimate of the average rate at which myofibrils reach steady-state separation following ablation.

As a proof-of-concept, we first turned to investigating the effect of the inhibition of actomyosin contractility by blebbistatin on the mechanical properties of myofibrils. Actomyosin contractility is known to be the primary driver of active tension generation in both muscle and nonmuscle systems (Gordon, Homsher, & Regnier, 2000; Murrell, Oakes, Lenz, & Gardel, 2015). Toward this end, we expressed Lifeact-mEmerald (actin filament marker) in hiCMs and allowed them to spread overnight prior to treatment with multiple doses of two different inhibitors of FAK activity, PF-228 and Defactinib (Kang et al., 2013; Slack-Davis et al., 2007). Expression of Lifeact tagged with mApple or mEmerald did not have any effects on nor Defactinib treatment resulted in a markedly decreased instantaneous retraction velocity (Figure 3d). Given no significant change in the asymptotic distance (or myofibril tension), the altered retraction dynamics and energy release upon ablation can be primarily attributed to the increase in myofibril viscosity observed following FAK inhibition. Linear velocity was similarly decreased highlighting the impact of viscosity on the average rate of separation (Figure 3d).

A previous study in nonmuscle cells has shown that PF-228 can activate BK Ca channels (So, Wu, Liang, Chen, & Wu, 2011). We therefore wanted to confirm that the observed changes in cardiac properties of myofibrils. We expressed Lifeact-mEmerald in hiCMs and allowed the cells to spread overnight prior to treatment with multiple doses of two different inhibitors of FAK activity, PF-228 and Defactinib. We next investigated the role of FAK inhibition of the mechanical properties of myofibrils. We expressed Lifeact-mEmerald in hiCMs and allowed the cells to spread overnight prior to treatment with multiple doses of two different inhibitors of FAK activity, PF-228 and Defactinib (Kang et al., 2013; Slack-Davis et al., 2007). Expression of Lifeact tagged with mApple or mEmerald did not have any effects on neither PF-228 nor Defactinib treatment attenuated hiCM beating, and we found no significant difference in the initial separation distance following dissection. While PF-228 treated myofibrils reached the steady-state retraction distance in a similar time as control (46.2 ± 3.4 s for control, 44.2 ± 3.0 s for 1 μM PF-228 and 54.2 ± 2.9 s for 3 μM PF-228), Defactinib-treated myofibrils took significantly longer to reach steady state (95.1 ± 4.6 s for 2.5 μM Defactinib and 78.9 ± 5.4 s for 5 μM Defactinib).

Analysis of separation distance profiles revealed no significant changes in the asymptotic distance between treatment groups, relative to control (Figure 3a–d). However, we observed a significant increase in the relaxation time with both concentrations of Defactinib treatment, suggesting an increase in the viscous damping experienced by the myofibril; PF-228 treatment only yielded increased relaxation times at the higher concentration (Figure 3c,d). Both PF-228 and Defactinib treatment resulted in a markedly decreased instantaneous retraction velocity (Figure 3d). Given no significant change in the asymptotic distance (or myofibril tension), the altered retraction dynamics and energy release upon ablation can be primarily attributed to the increase in myofibril viscosity observed following FAK inhibition. Linear velocity was similarly decreased highlighting the impact of viscosity on the average rate of separation (Figure 3d).

A previous study in nonmuscle cells has shown that PF-228 can activate BK Ca channels (So, Wu, Liang, Chen, & Wu, 2011). We therefore wanted to confirm that the observed changes in cardiac

Consistent with the idea that myosin II ATPase activity is required for active tension generation, we found a significant decrease in the asymptotic distance $D_a$ of separated myofibrils following blebbistatin treatment. As $D_a$ is proportional to myofibril tension, this suggests blebbistatin reduces active tension generation and elastic energy dissipation following ablation. Linear velocity was also significantly reduced indicating a slower average separation speed. We also observed a significant reduction in the relaxation time constant $\tau$, suggesting blebbistatin reduces viscous damping within the myofibril. That the reduction in asymptotic distance is more substantial than the reduction in relaxation time following treatment (92% reduction vs. 81% reduction, respectively), suggests the change in instantaneous velocity and energy dissipation immediately after ablation is primarily driven by reduced myofibril tension (Figure 2c,d). Taken together, these data indicate that our laser mediated dissection approach recapitulates current models of myofibril contractility.

...
myofibril mechanics were due to alterations in the intrinsic material properties of the myofibril rather than to any potential effects of FAK inhibition on the electromechanical properties of hiCMs. That is, we wanted to evaluate whether FAK inhibition alters myocyte excitability to an extent which could affect sarcomere contraction through excitation–contraction coupling and ultimately lead to the observed differences in retraction dynamics. To that end, we performed electrical recordings of hiCMs using the Microelectrode Array (MEA) technology (Figure 4a). This noninvasive label free approach allows the measurement of field potentials in thousands of cells (Asai, Tada, Otsuji, & Nakatsuji, 2010). Using this technique, we found that inhibition of FAK activity by treatment with either PF-228 or Defactinib had no effect on the beat period, spike amplitude or field potential duration (Figure 4b–d). Taken together, our results suggest that FAK inhibition alters the viscoelastic response of myofibrils through altered biophysical properties rather than changes in the electrophysiological properties of the cells.

In this study, we used laser-mediated myofibril dissection as a tool to assess the viscoelastic properties of myofibrils. The interpretation of such data requires mechanical descriptions of myofibrillar contraction. As such, we have employed a standard Kelvin–Voigt viscoelastic solid model commonly used to describe stress fibers or cell–cell junctions in nonmuscle cells (Fernandez-Gonzalez et al., 2009; Kumar et al., 2006). Using this framework, we found a good agreement between model fits and measured retraction dynamics of control and treated hiCMs ($R^2 > .97$ for all groups).

We further validated our approach by performing myofibril dissection experiments on blebbistatin-treated hiCMs. We found a marked decrease in both myofibril tension and viscosity, as indicated by significantly decreased asymptotic distances and relaxation times. Thus, blebbistatin treatment attenuated active tension generation by actomyosin contraction and significantly reduced the viscous damping of the myofibril. As such, the myofibril showed minimal instantaneous velocity and end-to-end separation following the initial ablation.
Together, this would indicate actomyosin contractility endows myofibrils with their viscoelastic mechanical response; however, additional techniques would be necessary to fully assess the impact of myofibril viscosity in the absence of actomyosin contractility.

Interestingly, we also found that blebbistatin treated hiCMs displayed bent conformations of myofibrils that relaxed immediately following laser-assisted dissection. This relaxation correlated with an increase in the initial separation distance $D_0$ following...
myofibril dissection. It is well appreciated that myofibrils have passive elasticity that is independent of actomyosin contractility and largely dependent on titin (Linke & Fernandez, 2002). The instantaneous relaxation of the myofibril upon dissection, followed by a lack of any subsequent retraction, is suggestive of this passive elastic response.

In this study, we have identified a role for FAK signaling in determining the viscoelastic properties of the myofibril. Using multiple concentrations of two known FAK inhibitors—PF-228 and Defactinib—we found that ablated myofibrils showed no difference in asymptotic distance but a significant increase in relaxation time. This would suggest FAK inhibition does not alter active tension generation of the myofibril, but instead increases viscous damping of the retraction. Indeed, the observed decrease in instantaneous velocity and energy release following ablation is driven largely by the increased myofibril viscosity. However, the precise mechanism by which FAK inhibition increases myofibril viscosity remains unclear.

Previous studies have implicated actin–titin interactions, collagen fibrils, and microtubule tyrosination as potential regulators of myofibril viscosity (Caporizzo et al., 2018; Granzier & Irving, 1995; Kulke et al., 2001). We have previously shown that FAK inhibition results in stabilization of cell-ECM adhesions, which leads to increased incorporation of titin (Taneja, Neininger, & Burnette, 2020). Therefore, one possible mechanism could be due to increased actin–titin interactions mediated by increased cell-ECM adhesion. Alternatively, FAK inhibition may be altering microtubule stability by altering tyrosination, as has been reported previously in fibroblasts (Palazzo, Eng, Schlaepfer, Marcantonio, & Gundersen, 2004). These hypotheses are not mutually exclusive and future studies will be required to elucidate the exact mechanism(s) by which FAK regulates myofibril mechanics.

**FIGURE 4** Human iPSC-derived cardiomyocyte (hiCM) beating properties following FAK inhibition. (a) Schematic showing experimental workflow. See Methods for further details. (b) Beat period, spike amplitude, and field potential duration upon treatment of hiCMs with PF-228 or Defactinib measured using Axion MEA system. N = 3 independent experiments with three technical replicates (wells) per experiment for PF-228 and two technical replicates for Defactinib. Each technical replicate comprises recordings from 16 electrodes from 20,000 cells. Error bars show SEM [Color figure can be viewed at wileyonlinelibrary.com]
3 | MATERIALS AND METHODS

3.1 | Cell culture

hiCMs were purchased fully differentiated from Cell Dynamics International (Cat. CMM-100-012-000.5). hiCMs were thawed in proprietary Plating Medium according to the manufacturer’s instruction in 96-well tissue culture plates at 50,000 cells per well. Cells were subsequently maintained using manufacturer-provided proprietary Maintenance Medium in a cell culture incubator at 37°C and 5% CO₂. Media was exchanged every 2 days as recommended by the manufacturer. Exogenous expression was performed using Viafect (Promega, Cat. E4981). Then, 200 ng of plasmid DNA was used to transfect one well of a 96-well culture plate according to the manufacturer’s instructions.

For ablation experiments, one well of hiCMs (50,000 cells) was replated onto a 35 mm dish with a 10 mm glass bottom well (CellVis, Cat. D35-10-1.5-N), as described in detail previously (Fenix et al., 2018). The growth substrate was coated with 10 μg/ml fibronectin (Corning, Cat. 354,008) for one hour prior to use. Briefly, cells were washed twice with 100 μl washes of PBS, followed by incubation in 40 μl of 0.1% Trypsin for 2 min at 37°C. Cell detachment was confirmed using a bright-field microscope. Following trypsinization, 160 μl of Maintenance Media was added to the well and cells were collected for centrifugation. Cells were spun at 200g for 3 min. The supernatant was removed, and the pellet was resuspended in 200 μl of Maintenance Media and plated on the fibronectin-coated coverslip. Cells were allowed to spread for 16 hr prior to ablation. At this time point, myofibrils are on the dorsal surface of the cell (Fenix et al., 2018).

For experiments using the Axion bioanalyzer, hiCMs were thawed onto a 48-well CytoView MEA plate (Axion Biosystems, Cat. M768-tMEA-48B) coated with 50 μg/ml fibronectin at a concentration of 20,000 cells per well. Cells were cultured as outlined above for 10 days prior to experimental treatments.

3.2 | Chemicals

FAK inhibitor PF-228 (PZ0117) and blebbistatin (B0560) were purchased from Sigma. Defactinib (S7654) was purchased from Selleckchem. For all chemicals, treatment was performed for 2 hr prior to laser-assisted dissection.

3.3 | Plasmids

Plasmids used in this study are available from Addgene. mApple-Lifeact-7 (Addgene plasmid #54747) and mEmerald-Lifeact-7 (Addgene plasmid # 54148) were gifts from Michael Davidson.

3.4 | Laser-assisted dissection

Focused laser mediated myofibril dissection was performed as described previously (Taneja, Bersi, et al., 2020). Myofibril dissection was performed on a Nikon Spinning Disk confocal microscope equipped with a x60 1.4 NA objective and an Andor iXON Ultra EMCCD camera, provided by the Nikon Center of Excellence at Vanderbilt University. Dissection was performed using a 100 mW UV laser (Coherent technologies) at 50% power, using a dwell time of 500 μs for a total period of 1 s using a Stimulation Line ROI of 2 μm length. Cells were maintained at 37°C and 5% CO₂ using a Tokai Hit stage incubator. Three preablation images were acquired at 2 s intervals. Following ablation, images were acquired using continuous acquisition at a net acquisition rate of 138 ms per frame. For the Defactinib experiments, the frame rate was 1 s. Images were acquired until retraction reached a steady-state distance as determined by visual inspection.

3.5 | Myofibril retraction analysis

To quantify myofibril mechanical properties, individual myofibrils were subjected to laser-assisted dissection to induce separation. Following visible separation, kymographs were drawn along the separation axis to visualize the time-varying end-to-end distance. Using a custom edge detection algorithm based on the Canny filter, edge profiles were defined based on gradients in the kymograph image and the separation distance (L(t)) was computed as one-half of the distance between the upper and lower edge profiles at each image frame. The half-distance profile was used to measure the average retraction of one of the severed ends of the myofibril. Separation profiles were then fit to a standard Kelvin–Voigt viscoelastic solid model of the form,

\[ L(t) = D_\alpha + D_\gamma \left(1 - \exp \left( -\frac{t}{\tau} \right) \right) \]

to identify two viscoelastic parameters: the asymptotic distance \(D_\alpha\) and the relaxation time constant \(\tau\). Note that \(D_\alpha\) is related to elastic energy dissipation, whereas the relaxation time constant is representative of the ratio of viscosity to elasticity in the material. Indeed, for a given myofibril stiffness, \(D_\alpha\) is proportional to myofibril tension and \(\tau\) is proportional to myofibril viscosity. For the Kelvin–Voigt model, \(\tau\) can also be mathematically expressed as 0.632 \(D_\gamma\). Additionally, any initial damage induced by the laser was accounted for by defining an initial separation distance \(D_\gamma\) that was measured at the time of ablation and subtracted from the measured retraction curve prior to fitting.

We also calculated two additional parameters—instantaneous velocity and linear velocity—to characterize the dynamics of myofibril retraction. Instantaneous velocity \(v_i\) represents the initial release of stored energy upon ablation and was calculated as the local derivative of the retraction (i.e., \(dl/dt\)) at the time of ablation (\(t = 0\)). Defined in this way, the instantaneous velocity is equivalent to the ratio of the asymptotic distance to the relaxation time \(\tau\). The linear velocity \(v_l\) represents a measure of the combined mechanical effect of the two fit parameters on the retraction response. Finally, the linear velocity \(v_l\)
is calculated as the slope of the line connecting the first and last points of the retraction profile and represents the average rate at which myofibrils retract toward the steady-state separation distance.

### 3.6 | Impedance assays

The Axion Biosystems analyzer was used to measure contractility and impedance in hiCMs as described previously (Rasmussen et al., 2020). Recordings were taken for 5 min at baseline and after 2 hr of drug treatment. Cells were maintained at 37°C and 5% CO₂ during recording. Cells were assayed using the standard cardiac analog mode setting with 12.5 kHz sampling frequency to measure spontaneous cardiac beating. The Axion instrument was controlled using Maestro Pro firmware version 1.5.3.4. Cardiac beat detector settings were defined as follows: beat detection threshold 300 µV; min. beat period 250 ms; max. beat period 5 s; synchronized beat maximum propagation delay 30 ms; minimum active channels ratio 50.00%; and running average beat count 10.

### 3.7 | Statistical analysis

Statistical analysis was performed in GraphPad and Excel. The comparison of retraction parameters was performed using the Student’s t test while comparison of electrophysiological properties was performed using two-way analysis of variance. All error bars represent SEM.

**ACKNOWLEDGMENTS**

The authors thank Nikon Center of Excellence at Cell Imaging Shared Resource at Vanderbilt University for access to the Nikon Spinning Disk microscope and technical support, and Dr Kevin Ess for access to the Axion Biosystems MEA analyzer. This work was funded by a MIRA (R35-HL135790) to W. D. M. R., a MIRA (R35-GM128915) to V. G., K99-HL146951 to M. R. B., and an American Heart Association Predoctoral Fellowship (18PRE33960551) to N. T., an American Resource at Vanderbilt University for access to the Nikon Spinning Disk microscope and technical support, and Dr Kevin Ess for access to the Axion Biosystems MEA analyzer. This work was funded by a MIRA (R35-GM128915) to V. G., K99-HL146951 to M. R. B., and an American Heart Association Predoctoral Fellowship (18PRE33960551) to N. T., an American Heart Association Predoctoral Fellowship (19PRE34380515) to M. L. R., a MIRA (R35-GM128915) to V. G., K99-HL146951 to M. R. B., and a MIRA (R35-HL135790) to W. D. M.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**REFERENCES**

Asai, Y., Tada, M., Otsuji, T. G., & Nakatsuji, N. (2010). Combination of functional cardiomyocytes derived from human stem cells and a highly-efficient microelectrode array system: An ideal hybrid model assay for drug development. Current Stem Cell Research & Therapy, 5 (3), 227–232. https://doi.org/10.2174/157488810791824502

Caporizzo, M. A., Chen, C. Y., Salomon, A. K., Margulies, K. B., & Prosser, B. L. (2018). Microtubules provide a viscoelastic resistance to myocyte motion. Biophysical Journal, 115(9), 1796–1807. https://doi.org/10.1016/j.bpj.2018.09.019

DeLaughter, D. M., Bick, A. G., Wakimoto, H., McKean, D., Gorham, J. M., Katohriya, I. S., ... Seidman, C. E. (2016). Single-cell resolution of temporal gene expression during heart development. Developmental Cell, 39 (4), 480–490. https://doi.org/10.1016/j.devcel.2016.10.001

DMichele, L. A., Doherty, J. T., Rojas, M., Berggs, H. E., Reichardt, L. F., Mack, C. P., & Taylor, J. M. (2006). Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. Circulation Research, 99(6), 636–645. https://doi.org/10.1161/01.RES.0000240498.44752.d6

Fabry, B., Klemm, A. H., Kienle, S., Schäffer, T. E., & Goldmann, W. H. (2011). Focal adhesion kinase stabilizes the cytoskeleton. Biophysical Journal, 101(9). 2131–2138. https://doi.org/10.1016/j.bpj.2011.09.043

Fedorov, V. V., Lozinsky, I. T., Susovun, E. A., Anyukhovsky, E. P., Rosen, M. R., Balke, C. W., & Efimov, I. R. (2007). Application of blebbistatin as an excitation-contraction uncoupler for electrophysiological study of rat and rabbit hearts. Heart Physiology, 4(5), 619–626. https://doi.org/10.1016/j.jhrthm.2006.12.047

Fenix, A. M., Neininger, A. C., Taneja, N., Hyde, K., Visetsouk, M. R., Garde, R. J., ... Burnette, D. T. (2018). Muscle specific stress fibers give rise to sarcomeres in cardiomyocytes. eLife, 7, e42144. https://doi.org/10.7554/eLife.42144

Fernandez-Gonzalez, R., Simoes, S. d. M., Rüper, J. C., Eaton, S., & Zallen, J. A. (2009). Myosin II dynamics are regulated by tension in intercalating cells. Developmental Cell, 17(5), 736–743. https://doi.org/10.1016/j.devcel.2009.09.003

Gibbs, C. L., & Chapman, J. B. (1985). Cardiac mechanics and energetics: Chemomechanical transduction in cardiac muscle. American Journal of Physiology - Heart and Circulatory Physiology, 259(2), H199–H206. https://doi.org/10.1152/ajpheart.1985.249.2.h199

Gordon, A. M., Homsher, E., & Regnier, M. (2000). Regulation of contraction in striated muscle. Physiological Reviews, 80(2), 853–924. https://doi.org/10.1152/physrev.2000.80.2.853

Granzier, H. L., & Irving, T. C. (1995). Passive tension in cardiac muscle: Contribution of collagen, titin, microtubules, and intermediate filaments. Biophysical Journal, 68(3), 1027–1044. https://doi.org/10.1016/S0006-3495(95)80278-X

Granzier, H. L., & Labelt, S. (2004). The Giant protein titin. Circulation Research, 94(3), 284–295. https://doi.org/10.1161/01.RES.0000117769.88862.F8

He, Z. H., Bottinelli, R., Pellegrino, M. A., Ferenczi, M. A., & Reggiani, C. (2000). ATP consumption and efficiency of human single muscle fibers with different myosin isoform composition. Biophysical Journal, 79(2), 945–961. https://doi.org/10.1016/S0006-3495(99)076349-1

Kang, Y., Hu, W., Ivan, C., Dalton, H. J., Miyake, T., Pecot, C. V., ... Sood, A. K. (2013). Article role of focal adhesion kinase in regulating YB-1-mediated podilaxal resistance in ovarian cancer. Journal of National Cancer Institute, 105(19), 1485–1495. https://doi.org/10.1093/jnci/djt210

Knöll, R., Buyandelger, B., & Lab, M. (2011). The sarcomeric Z-disc and Z-discopathies. Journal of Biomedicine and Biotechnology, 2011, 569628. https://doi.org/10.1155/2011/569628

Kulke, M., Fujita-Becker, S., Rostkova, E., M., A.., Lele, T. P., Labeit, D., Manstein, D. J., ... 105–1044. https://doi.org/10.1016/j.bpj.2011.09.1495. https://doi.org/10.1161/01.RES.0000117769.88862.F8

Kumar, S., Maxwell, I. Z., Heisterkamp, A., Polte, T. R., Lele, T. P., Salanga, M., ... Ingber, D. E. (2006). Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization,
and extracellular matrix mechanics. *Biophysical Journal*, 90(10), 3762–3773. https://doi.org/10.1529/biophysj.105.071506

Kuppusamy, K. T., Jones, D. C., Sperber, H., Madan, A., Fischer, K. A., Rodriguez, M. L., ... Ruohola-Baker, H. (2015). Let-7 family of micro-RNA is required for maturation and adult-like metabolism in stem cell-derived cardiomyocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 112(21), E2785–E2794. https://doi.org/10.1073/pnas.1424042112

Lee, S., Kassianidou, E., & Kumar, S. (2018). Actomyosin stress fiber subtypes have unique viscoelastic properties and roles in tension generation. *Molecular Biology of the Cell*, 29(16), 1992–2004. https://doi.org/10.1091/mbc.E18-02-0106

Linke, W. A., & Fernandez, J. M. (2002). Cardiac titin: Molecular basis of elasticity and cellular contribution to elastic and viscous stiffness components in myocardium. *Journal of Muscle Research and Cell Motility*, 23(5–6), 483–497. https://doi.org/10.1023/A:1023462507254

Linke, W. A., Külke, M., Li, H., Fujita-Becker, S., Neagoe, C., Manstein, D. J., ... Fernandez, J. M. (2002). PEVK domain of titin: An entropic spring with actin-binding properties. *Journal of Structural Biology*, 137(1–2), 194–205 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12064946

Maruyama, K. (1997). Connectin/titin, giant elastic protein of muscle. The *FASEB Journal*, 11(5), 341–345. https://doi.org/10.1093/fasebj.11.5.9141500

Mierke, C. T., Fischer, T., Puder, S., Kunschmann, T., Soetje, B., & Ziegler, W. H. (2017). Focal adhesion kinase activity is required for actomyosin contractility-based invasion of cells into dense 3D matrices. *Scientific Reports*, 7(1), 1–18. https://doi.org/10.1038/srep42780

Murrell, M., Oakes, P. W., Lenz, M., & Gardel, M. L. (2015). Forcing cells into shape: The mechanics of actomyosin contractility. *Nature Reviews Molecular Cell Biology*, 16(8), 486–498. https://doi.org/10.1038/nrm4012

Palazzo, A. F., Eng, C. H., Schlaepfer, D. D., Marcantonio, E. E., & Gundersen, G. G. (2004). Localized stabilization of microtubules by integrin- and FAK-facilitated rho signaling. *Science*, 303(5659), 836–839. https://doi.org/10.1126/science.1091325

Parsons, J. T., Martin, K. H., Slack, J. K., Taylor, J. M., & Weed, S. A. (2000). Focal adhesion kinase: A regulator of focal adhesion dynamics and cell movement. *Oncogene*, 19(49), 5606–5613. https://doi.org/10.1038/sj.onc.1203877

Peng, X., Kraus, M. S., Wei, H., Shen, T. L., Pariaut, R., Alcaraz, A., ... Guan, J. L. (2006). Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis in mice. *Journal of Clinical Investigation*, 116(1), 217–227. https://doi.org/10.1172/JCI24497

Rasmussen, M. L., Taneja, N., Neininger, A. C., Wang, L., Robertson, G. L., Rifffe, S. N., ... Gama, V. (2020). MCL-1 inhibition by selective BH3 mimetics disrupts mitochondrial dynamics causing loss of viability and functionality of human cardiomyocytes. *iScience*, 23(4), 101015. https://doi.org/10.1016/j.isci.2020.101015

Roman, W., Martins, J. P., Carvalho, F. A., Voituriez, R., Abella, J. V. G., Santos, N. C., ... Gomes, E. R. (2017). Myofibril contraction and crosslinking drive nuclear movement to the periphery of skeletal muscle. *Nature Cell Biology*, 19(10), 1189–1201. https://doi.org/10.1038/ncc3605

Samarel, A. M. (2005). Costameres, focal adhesions, and cardiomyocyte mechanotransduction. *American Journal of Physiology. Heart and Circulatory Physiology*, 289(6), H2291–H2301. https://doi.org/10.1152/ajpheart.00749.2005

Slack-Davis, J. K., Martin, K. H., Tilghman, R. W., Iwanicki, M., Ung, E. J., Autry, C., ... Parsons, J. T. (2007). Cellular characterization of a novel focal adhesion kinase inhibitor. *The Journal of Biological Chemistry*, 282(20), 14845–14852. https://doi.org/10.1074/jbc.M606695200

So, E. C., Wu, K. C., Liang, C. H., Chen, J. Y., & Wu, S. N. (2011). Evidence for activation of BK Ca channels by a known inhibitor of focal adhesion kinase, PF573228. *Life Sciences*, 89(19–20), 691–701. https://doi.org/10.1016/j.jfis.2011.08.013

Straight, A. F., Cheung, A., Limouze, J., Chen, I., Westwood, N. J., Sellers, J. R., & Mitchison, T. J. (2003). Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor. *Science*, 299(5613), 1743–1747. https://doi.org/10.1126/science.1081412

Sulzmaier, F. J., Jean, C., & Schlaepfer, D. D. (2014). FAK in cancer: Mechanistic findings and clinical applications. *Nature Reviews Cancer*, 14(9), 598–610. https://doi.org/10.1038/nrc3792

Taneja, N., Bersi, M. R., Bailargeon, S. M., Fenix, A. M., Cooper, J. A., Ohl, R., ... Burnett, D. T. (2020). Precise tuning of cortical contractility regulates cell shape during cytokinesis. *Cell Reports*, 31(1), 107477. https://doi.org/10.1016/j.celrep.2020.03.041

Taneja, N., Fenix, A. M., Rathbun, L., Mills, B. A., Tyska, M. J., Hearn, H., & Burnett, D. T. (2016). Focal adhesions control cleavage furrow shape and spindle tilt during mitosis. *Scientific Reports*, 6, 29846. https://doi.org/10.1038/srep29846

Taneja, N., Neininger, A. C., & Burnett, D. T. (2020). Coupling to substrate adhesions drives the maturation of muscle stress fibers into myofibrils within cardiomyocytes. *Molecular Biology of the Cell*, 31, 1273–1288. https://doi.org/10.1091/mbc.E19-11-0652

How to cite this article: Taneja N, Bersi MR, Rasmussen ML, Gama V, Merryman WD, Burnett DT. Inhibition of focal adhesion kinase increases myofibril viscosity in cardiac myocytes. *Cytoskeleton*. 2020;77:342–350. https://doi.org/10.1002/cm.21632