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Cardiac myofibril networks induce shear stress

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Abstract

Myofibril arrangement is critical to cardiac muscle function in health and disease. Historically, analysis of the impact of myofibril organisation on force and cell contraction has relied on the assumption of uniaxial arrays. However, improvements in imaging indicate that myofibrils form complex networks, though how these networks modulate force has yet to be explored. Here, morphological analysis of sheep left-ventricular cardiomyocytes is utilised to inform a non-linear finite element model of cell contraction. Analysis of deep learning segmentations of z-discs demonstrate that myofibrils are oriented about the contraction axis (mean = 0.03°) but deviate locally by up to 30° (standard deviation = 6.56°). Simulations produce unique deformations for geometries informed by myofibril orientations, displaying internal rotation and off-axis deformations. Moreover, anisotropy generates shear stresses distinct from the uniaxial case, demonstrating spatial relationships that balance shear across the cell and a correlation between shear stress and z-disc orientation. These findings highlight the impact of myofibril networks on forces during cell contraction.

Introduction

Cardiac muscle cells produce the forces underpinning each heartbeat. These forces are a product of synchronous cell-shortening, facilitated by large structures called myofibrils^{1,2}. Myofibrils span the rod-like myocytes longitudinally in arrays, connecting 1.8-2 [μm] contractile proteins, sarcomeres, which are attached in series and shorten up to 20% during activation^{2,3}. Force is transmitted throughout the myofibril by z-discs which connect adjacent protein filaments, provide structural stability, and attach the myofibril to the cell membrane. Myofibril force production is facilitated by an influx of Ca^{2+} which enables interactions between actin and myosin filaments, producing shortening and decreasing the distance between adjacent z-discs^{2,4}.

However, shortening dynamics are non-ideal. How sarcomeres behave during contraction has been demonstrated as non-uniform^{5,6}. Experiments have indicated that during contraction some sarcomeres show no initial change, or even lengthen⁵, with pre-stretch increasing behavioural homogeneity^{5,6} in what may facilitate the Frank-Starling relationship⁷⁻⁹ at a cellular level. Further, the alignment of neighbouring sarcomeres, and thus, insurance of in-phase beating, is regulated by the local mechanical environment¹⁰⁻¹². Changes to mitochondrial-sarcomere contact during development modulates the expression of aligned sarcomeres¹⁰. These modulations may be due to strain¹¹, as researchers have found a correlation between the environmental elasticity and sarcomere alignment¹² which could help improve understanding of sarcomere assembly¹³. Nevertheless, while the fundamental mechanisms of force generation have long been understood, there is increasing appreciation of the complexities and physiological implications resulting from the intracellular directions along which these forces are generated^{14,15}.

Myofibril orientation has been historically presumed as uniaxial about the cell's longitudinal axis^{1,16,17}. These assumptions relied upon limited transmission electron microscopy (TEM) imaging, facilitating investigation in sarcomere structure and theories of contraction, but providing minimal 3D spatial context on global myofibril arrangement^{16,18}. Therefore, intracellular stress has been historically decomposed into an *active* (axial) component produced by myofibrils, and *passive* (off-axial) components attributed mostly to the cytoskeleton¹⁹⁻²¹. This assumption has been perpetuated in subsequent biophysical models²²⁻²⁷ and experimental studies^{20,28-30}, though deeper examinations of myofibrils, particularly in mammalian samples, may provide a more detailed understanding of intracellular stresses and their relevance to cardiac physiology and disease conditions.

Importantly, volumetric EM imaging has demonstrated that myofibrils may display splits^{31,32}, are asynchronously aligned^{33,34}, and form interconnected branches^{15,35}. Goldspink¹⁷ originally highlighted myofibril structure in mice bicep brachii, observing that distal sarcomeres 'split' into

smaller ‘daughter’ sarcomere along the myofibril length, later suggesting splitting acts as a modulator of tension development or proliferation in fast- and slow-twitch muscles^{32,36,36}. These splitting events were also observed by Tomanek³⁷ in kitten skeletal muscle. More recently, Jayasinghe et al.³³ applied laser scanning confocal microscopy to study z-discs as an indicator of myofibril structure, observing that myofibrils are helical over myocyte length. The images of rat, rabbit, and human cardiomyocytes similarly display an asynchronous alignment of z-discs, purporting that these structures may positively influence contraction synchrony. Further, with focused ion beam-scanning electron microscopy, Willingham et al.¹⁵ highlighted that myofibril networks form in fast-, slow-twitch, and cardiac myocytes through sarcomere branching events. The evolutionary conservation of these branching events was then demonstrated by Ajayi et al.³⁵ through selective gene-knockout of *Drosophila* myocytes, both groups hypothesising an impact on myocyte force production. Nevertheless, how such multiaxially aligned myofibrils impact intracellular stress has not been investigated.

An understanding of stress dynamics within the cell is critical for appreciating function in health, disease, and exercise. Cardiac myocytes respond to their mechanical environment, modulating membrane channels, calcium signalling, and force transmission^{38–40}. These regulatory behaviours may help myocytes respond to altered loading conditions in disease states⁴¹, as well as facilitate development^{10,42}, and accommodate demand²². Describing intracellular stresses is key to understanding the mechanosensitive behaviours of key organelles such as mitochondria, which have been shown to deform during contraction⁴³ and modulate calcium levels⁴⁴, and the nucleus, whose migration is impacted via myofibril force⁴². Still, while experimental designs incorporate off-axis stress behaviour^{45,46}, virtually all biophysical models assume myofibrils produce zero active shear stress.

In this work we examine the intracellular stresses in a striated cardiomyocyte resulting from off-axis z-disc orientations. This process is highlighted visually in Fig. 1. Here, serial-block face scanning electron microscopy (SBF-SEM) images of sheep left ventricular myocytes were segmented to create a physiologically informed biophysical model. A U-Net++⁴⁷ was trained on manual segmentations to facilitate morphological analysis of z-discs as a proxy for measuring myofibril orientations. This analysis indicates that myofibrils permute at a distribution of angles within the network about the contraction axis (Fig. 1, red arrow; $\mu = 0.03^\circ$, $\sigma = 6.56^\circ$). Angles were then interpolated into a hyper-elastic Finite Element (FE) model using the FEniCSx^{48–51} open-source package. The FE model is parameterised to fit existing experimental trends for myocyte tension^{20,28,52–54}. Integration of myofibril multiaxial alignment results in shear behaviours that are not reproducible with a uniaxial assumption. Further, a correlation between shear stresses and orientation is found with spatial distributions of shear transitioning across the cell width. Taken

together, this study indicates that incorporating multiaxially alignment in biophysical models is vital for describing intracellular stresses in cardiac myocytes.

Results

Z-Disc segmentation reveals myofibrils are not uniaxially aligned

Isolated z-discs create varied patterns that do not predominantly align with the contraction axis. The larger 3D cell volume is partitioned into smaller volumes and regions (Fig. 2a, green, red and blue). The bottom layer (Fig. 2b) is characterised by a large longitudinal grouping of mitochondria that span between edges, interrupted only by a nucleus on the proximal end. The boundaries of this mitochondria region and the nucleus contort the neighbouring sarcomeres' orientation and shape, similar to results shown elsewhere⁵⁵. This structure is more pronounced in the top-down 2D render shown at the bottom of Fig. 2b, where the z-disc axial alignment is clearly impacted by the mitochondria and the nucleus.

In each volume the regular ~ 1.9 [μm] spacing of z-discs along myofibrils is apparent (see Fig 2b-d bottom row), although z-discs are not vertically aligned over width (see Supplementary Figure 1). Asymmetry in alignment between myofibrils has recently been explored³³, however the z-discs in this sample only exhibit alignment across width within myofibrils bundles, with a loss of this coherence between myofibrils that are spatially separated. Z-Discs do not appear aligned over the full cell width.

Differences in myofibrillar arrangement patterns are apparent near the cell's lateral periphery and nuclei. 2D visualisation of the slices (Fig. 2b-d bottom) highlight the z-discs at the cell periphery (all slices) and about the nuclei (Fig. 2b bottom). The cell periphery aligns myofibrils with the cell contraction axis, with a reduction of orientation changes particularly apparent in the second volume (Fig. 2c). Investigation into z-discs surrounding the nuclei, however, show a contouring of bodies about its border (Fig. 2b bottom). In both cases, statistical testing did not indicate any significant differences in orientations about these cell landmarks (see Supplementary Figure 2, Supplementary Figure 3, and Supplementary Figure 4).

Myofibrils vary as much as 30° from longitudinal axis

Principal component analysis (PCA) of segmented z-discs indicates that myofibrils throughout the cell are oriented between -30° and 30° of the contraction axis (Fig. 3; standard deviation $\sigma = 6.56^\circ$; mean $\mu = 0.03^\circ$), which was calculated from the cell's long axis (see Methods). This

distribution occupies a similar range to Feinberg et al.'s measurements upon cardiomyocytes⁵⁶, however the 'normal' shape here is more in line with expectations. To compute this angle the isolated z-discs were processed to determine the *normal* vector (Fig. 3a) through the third principal component. The z-disc is directly connected to the *actin*- and *titin*-filaments which run perpendicular to the z-disc body and parallel with contraction¹. The normal vector is then compared to the contractile axis of the cell to determine spherical deviation (see Fig. 3a middle illustration).

Myofibrils are oriented symmetrically about the major axis ($n = 1138$, $skew = -0.16$) with a standard deviation (σ) of 6.56° and mean (μ) of 0.03° (see Fig. 2b). The large kurtosis value ($\kappa = 3.49$) of this distribution indicates a concentration of values within the first standard deviation. Indeed, whilst a uniaxial model of myofibrils presumes complete alignment, only $\sim 12\%$ of instances had an orientation within 1° of the major longitudinal axis. The distribution was validated against another cardiomyocyte; a similar orientation profile was produced (see Supplementary Figure 5 and Supplementary Table 2).

Regional orientations exhibit significant deviations in distribution and mean with respect to the main axis. Compared to the global set with Mann-Whitney U test and Welch's t-test, 9 of the 18 test sets demonstrated significance (see Fig. 3c; see Supplementary Figure 2). Whilst region size impacts statistical relevance, these distributions nevertheless highlight the regional variability in myofibril arrangement. Further, of regions exhibiting a statistically significant orientation distribution, all but one (R17) were immediately adjacent to another significant region. Of the 10 significant regions, 5 included the cell periphery and 2 included the nucleus, though these landmarks did not significantly impact adjacent orientations when tested independently with the same Mann-Whitney U test and Welch's t-tests (see Supplementary Figure 3). Broadly, each region demonstrates strong contraction axis alignment with orientation profiles like the global cell (see Supplementary Table 3 and Supplementary Figure 6).

The median of myofibril orientation also shifts across the cell volume, where Fig. 4a and 4c indicate that fibre orientation is heterogenous. Interestingly, slicing through the x -axis (Fig. 4c, left) depicts a transition from a positive deflection to a negative deflection. This observation mimics Fig. 3c, where Regions 0-8 and Regions 9-17 exhibit a decreasing trend in median orientation, the former being a larger gradient. The largest median positive orientations (R0, R9, R12 – see Fig. 3c) all appear closer to the distal end ($0 \leq X < 11 [\mu m]$) of the cell, whereas the largest negative median orientations all occur on the proximal end ($22 \leq X \leq 33 [\mu m]$; R6, R8, R17). Observing slices through the y -axis (Fig. 4c, middle) confirms this behaviour. Therefore, the cell proceeds from positive- to negative- median orientation shift over its length.

Myofibril anisotropy introduces uneven deformation and rotation

A computational model of myofibril organisation and large nonlinear deformation mechanics was implemented using FEniCSx^{48–51} to investigate the influence of myofibril organisations on muscle contraction. Myofibril orientation was introduced into simulation meshes with an interpolation of orientation into mesh nodes. A Lagrangian framework was adopted following existing strategies for anisotropy⁵⁷. Due to the Lagrangian formulation, a tensor push-forward transformation was used to convert the local coordinate space.

The change in coordinate space is mapped with the deformed contravariant, g^{ij} , and covariant, g_{ij} , metric tensors, where the convention of superscript (\cdot^{ij}) and subscript (\cdot_{ij}) is adopted for contravariant and covariant indices respectively. From here, the Christoffel symbol of the second kind, Γ_{ij}^k , is calculated

$$\Gamma_{ij}^k = \frac{1}{2} g^{kl} (g_{jl,i} + g_{il,j} - g_{ij,l}), \quad (1)$$

which characterises the change in coordinate space over a manifold. Here, a comma separated index ($\cdot_{,i}$) indicates differentiation by that component. The Christoffel symbol then allows calculation of the covariant derivative, $\nabla_j \cdot$,

$$\nabla_j v^\alpha = \frac{\partial v^\alpha}{\partial x_j} + \Gamma_{kj}^a v^k. \quad (2)$$

The covariant derivative adjusts the regular derivative to the deformed space and forms a necessary part of variational form of the biophysical model with incompressibility restraint

$$R = \int_{\Omega} T^{\alpha\beta} F_{\beta}^j \nabla_j v^\alpha d\Omega + \int_{\Omega} q (J - 1) d, \quad (3)$$

where T is the second Piola-Kirchhoff tensor, F is the deformation gradient tensor, and J is the third invariant, while v and q are the field variables. Contraction was simulated with stepwise displacement boundary conditions on the maximal and minimal faces of the mesh.

Despite rigid displacement boundary conditions and incompressibility, myofibril anisotropy introduced asymmetrical and region-specific deformation. Comparison of the boundary y -displacement across mesh segments with the uniaxial deformation indicates that all test regions increase variance in deformation alongside a median shift (see Fig. 5a-b).

Four regions were randomly selected to more closely investigate how boundaries deform with anisotropy. Fig. 5a and 5c plot the simulation mesh boundaries on y - and z -faces overlaid onto the boundaries of the uniaxial case (Fig. 5a, 5c; black solid lines). The simulation boundaries are displayed with three shaded sections depicting the nodal displacement in thirds of the volume due to their rapid changes over the surface. These regions depict how anisotropy deflects the volume's maximum and minimum boundary irregularly in both the y - and z -axis. z -displacement in Regions 6 and 14 further indicate that despite a minimal median shift from the uniaxial case (Fig. 5d) there is notable deviation across the region's profile; R14 begins with a predominantly negative deflection before progressing positively (see shading in Fig. 5c bottom right). Region 11 demonstrates the largest deflection in z -displacement, whilst also producing the lowest global alignment (see Supplementary Table 3) and subsequent large boundary deflections (see Supplementary Figure 7 and Supplementary Figure 8). Therefore, despite volume constraints, introducing anisotropy creates boundary displacement deflections.

Myofibril anisotropy further introduces internal rotation. Measurement of rotation about the screw axis in the YZ -plane in our physiological sample exhibits unique profiles for all test cases (Fig. 5e). In contrast, the uniaxial case produces 0° of rotation in the YZ -plane or across streamlines in the displacement field (see Supplementary Figure 9). Internal rotation varies predominantly between -25° and $+25^\circ$, increasing with a larger median shift in displacement; direct comparison of scale of violin distribution (Fig. 5e) matches median shifting in displacement boxplots (Fig. 5d; for example, Regions 3, 4, 9, 10, and 11). Whereas more uniformly displaced tests that align medially with the uniaxial example demonstrate less rotation (i.e. Regions 6 and 16). Larger changes in deformation profiles due to anisotropy result in increased rotation of nodes within the mesh (see Supplementary Figure 9).

Anisotropy creates shear stress and unique spatial profiles

Anisotropic myofibrils produce unique shear stresses not reproducible by a uniaxial model. The Cauchy stress tensor, σ , (Fig. 6a; diagrammatic), calculated through conversion from the first Piola Kirchoff tensor, P , is decomposed and plotted for each displacement component in Fig. 6b.

$$P = J\sigma F^{-T} + pJG^{-1}F^{-T} \quad (5)$$

Where F is the deformation gradient tensor, p is hydrostatic pressure, G is the undeformed metric tensor and indices with a subscript T , (\cdot^T) , indicating transpose.

The average normal stress components in each case are similar in response to the identical displacement boundary conditions for each simulation (Fig. 6b, left) and optimised to be within

the bounds of experimental values^{20,28,52–54} (see Supplementary Figure 11). However, all anisotropic simulations produce unique shear responses (Fig. 6b, middle and right). These profiles are most pronounced after 12% displacement, suggesting that anisotropy may be less relevant for early stages of contraction. Full contraction³, 20% (Fig. 6b right), demonstrates variability between all cases. Clearly, anisotropic regions, even with minimal orientation mean-shift, produce shear stress.

Shear stress in the xz - and yz -components is distributed between positive and negative inflection for simulations (Fig. 6b, right). In contrast, xy -shear was decidedly negative for all but one test, R12, which had a large positive skew in orientation distribution (see Fig 3c); xy -shear was also the only non-zero shear for the uniaxial case. This non-zero shear for the uniaxial simulation is likely due to the artificial degree of freedom constraint on the x -axis faces. Normalising for the uniaxial case results in four simulations with positive stress (see Fig. 7a), though this may still be artificially constrained. Implementation of deformation with active contraction, however, shows that the produced shear profiles are comparable with the artificially constrained boundaries (see Supplementary Figure 11). xz - and yz -shear, alternatively, produce null shear in the uniaxial case with notable distribution ratios of positive and negative shear stress for all simulations (xz : 1: 1, yz : 8:6, *positive: negative* for tests with noticeable shear).

Shear stress exhibits a spatial relationship over the cell volume like that observed in the orientation behaviour. The balance of shear stress displayed by the xz -component, visualized by the bar charts in Fig. 6b, corresponds with the spatial shear distributions (Fig. 6c). σ_{xz} is noticeably negative for all upper regions of the cell slices (Fig. 6c left), with a positive value for all lower slices. This effect is most apparent for Regions 0-2 and 9-11 which oppose sides of a nucleus.

Analysing the relationship between orientation and shear stresses demonstrates a positive correlation between spherical deviation and shear (Fig. 7). All shear tests present a positive correlation, however, for whole-group analysis the strongest positive relationship is observed for xy -shear ($R = 0.76$, Fig. 7a left). xz -shear, however, produces two separate clusters of points (Fig. 7a middle) which match the positive and negative shear group described above for upper regions ($y > 9.9$ [nm]) and lower regions ($y < 9.9$ [nm]). Regression analysis of spatial clusters is present in Table 1. The largest correlation between xz -shear and mean orientation was found for the highest spatial cluster ($z > 10$ [nm]). This cluster exhibits myofibrils which are uninterrupted by large mitochondrial groups or nuclei, unlike lower clusters (see Fig. 2b-d and Fig. 4c). Similarly, the group produces a stepwise increase in both mean orientation angle (see Fig. 3c) and average shear stress (see Fig. 7c) along its length, with R8 and R11 displaying the largest pairwise difference in xz -magnitude. Variance is also positively correlated between stress and orientation.

To further investigate the spatial relationship of xz -shear, simulations were run on the overlap between upper and lower regions (Fig. 7c). Clustering shear by upper, overlap, and lower groups, clearly indicates the spatial relationship of xz -shear direction. All upper regions producing positive shear, and all lower regions producing negative shear. If the simulated shears could be physically coupled, then it was hypothesised that overlapping regions should produce shear profiles that demonstrate a transition between these extremes. Accordingly, xz -shear for all overlap regions was significantly lower in magnitude. The direction of shear is also directly related to whichever (upper or lower) adjacent region has a larger magnitude. Similarly, overlaying the average shear between the two clusters onto the overlap simulations (Fig. 7c middle) indicates further that despite being simulated in isolation the shear behaviour can be related between adjacent regions.

Investigating the orientation behaviour of the upper, overlap, and lower regions indicates significant difference in profiles. Fig. 7d shows the orientation distributions of z -discs within each cluster for spherical, azimuthal, and elevation angles. Whilst azimuthal angles show no significant difference between the groups, spherical deviation of the lower cluster is different (t-test, $p < 0.05$) from both the overlap region and the upper region. Similarly, elevation angle shows the same differentiation with a much larger change in median and distribution (Fig. 7d bottom). Elevation angle (x - z) would also directly influence xz -shear, with the significant difference in median angle corresponding with the opposing shear profiles.

Table 1 | Comparisons of grouped trends and correlations for shear stress (σ_{xy} , σ_{xz} , σ_{yz}) versus orientation (θ).

Group	σ_{xy}		σ_{xz}		σ_{yz}	
	$\bar{\sigma}_{xy}(\bar{\theta})$	R	$\bar{\sigma}_{xz}(\bar{\theta})$	R	$\bar{\sigma}_{yz}(\bar{\theta})$	R
All regions	$0.12\bar{\theta} - 0.86$	0.76	$0.11\bar{\theta} - 0.30$	0.33	$0.02\bar{\theta} - 0.03$	0.46
Across length						
$x \geq 22$ [nm]	$0.10\bar{\theta} - 0.87$	0.86	$0.25\bar{\theta} - 0.13$	0.62	$0.04\bar{\theta} - 0.04$	0.76
$11 \geq x < 22$ [nm]	$0.14\bar{\theta} - 0.87$	0.80	$0.14\bar{\theta} - 0.55$	0.41	$-0.01\bar{\theta} + 0.01$	-0.31
$x < 11$ [nm]	$0.11\bar{\theta} - 0.87$	0.60	$0.03\bar{\theta} - 0.09$	0.09	$0.02\bar{\theta} - 0.02$	0.66
Across width						
$y > 9.9$ [nm]	$0.19\bar{\theta} - 1.00$	0.80	$0.02\bar{\theta} - 0.44$	0.17	$0.00\bar{\theta} + 0.03$	-0.08
$y < 9.9$ [nm]	$0.08\bar{\theta} - 0.83$	0.84	$-0.04\bar{\theta} - 0.86$	-0.33	$0.03\bar{\theta} - 0.04$	0.70
Across depth						
$z \geq 10$ [nm]	$0.14\bar{\theta} - 0.73$	0.76	$0.39\bar{\theta} + 0.64$	0.81	$0.04\bar{\theta} + 0.01$	0.74
$5 \geq z < 10$ [nm]	$0.07\bar{\theta} - 0.93$	0.40	$0.35\bar{\theta} + 0.15$	0.66	$0.00\bar{\theta} - 0.00$	-0.11
$z < 5$ [nm]	$0.16\bar{\theta} - 0.96$	0.92	$0.05\bar{\theta} - 0.34$	0.18	$0.03\bar{\theta} - 0.09$	0.67

Note: R is Pearson's correlation coefficient, $\bar{\sigma}$ represents mean stress, $\bar{\theta}$ represents mean orientation

Discussion

This study reveals that cardiac muscle cell myofibrils create a distribution of orientations and produce unique intracellular shear stress distributions. This finding challenges long-standing assumptions based on a uniaxial model, which has treated muscle contraction as occurring purely under axial loading since Huxley's foundational work in the early 1900s¹⁶, as well as more contemporary ultrastructural imaging work¹, biophysical simulations of fibre force^{23,27}, and experimental measurements^{19,58}.

Understanding the production of shear stresses has the potential to elucidate the impacts of multi-axial force trajectories; existing biophysical models have so far limited active stress dynamics to uniaxial forces. However, lateral force transmission is an important feature of musculoskeletal physics^{25,59} and has burgeoning appreciation in cardiac constitutive models^{60,61}. Recent work has demonstrated that lateral forces change in disease states⁶² and theorise its role in inter-cellular transmission^{59,63}. Further, Rothermel et al.⁶⁴ demonstrated that transverse length-tension relationships in neonatal cardiomyocytes follow the same trend as axial tension, which they hypothesise is a result of myofilament spacing. Similarly, the shear stress trends calculated here (Fig. 6) follow the same exponential behaviour as axial measurements^{19,20,53,65} which may represent a combination of the radial myofilament spacing Rothermel et al.⁶⁴ proposed and cardiomyocyte passive viscoelastic properties⁶⁶. While historical models of myofibril arrangement do not characterise these dynamics, our physiologically informed biophysical model demonstrates the presence and magnitude of shear stresses that would contribute to multi-axial force.

Local shear may influence mechanosensitive processes. Recent work by Rog-Zielinska et al.^{43,67} has demonstrated that both the mitochondria and t-tubules deform during contraction with the latter experiencing an increase in metabolite diffusion during contraction. Both structures are local to myofibrils⁵⁵ and the spatial shear (Fig. 6) and deformation patterns (Fig. 5) simulated here would likely influence their behaviour. Similarly, shear behaviour may contribute to the motility of nuclei during development⁴² which was suggested by previously reported helical myofibril structures³³. Bavi et al.⁶⁸ simulated the influence of local membrane curvature on mechanosensitive channels and found that these structures are pro-activation. Caveolae on mitochondrial membranes⁴⁰ may therefore be an interesting recipient of local-level shear. Likewise, titin is known to respond to stress and modulate myocyte hypertrophy⁶⁹. Converting the simulated axial and shear stresses to force per titin molecule, similar to Granzier and Irving²⁰, demonstrates these values fall within the expected range for sarcomere strain (ranges 2-4 [pN] and 0.05-0.4 [pN])^{20,70}. How shear impacts hypertrophic signalling remains unclear and warrants investigation. Piezo1 similarly modulates

hypertrophy and is known to be mechanosensitive⁷¹. Previous reports have demonstrated that activation can occur on locally deforming membranes at a pico-Newton ($[pN]$) scale^{68,72}, though the shear forces here are likely too low to activate Piezo1⁷³, further testing may help elucidate the coupling between mechanosensitive channels and shear.

Visualisation of the spatial shear stress relationship over isolated cell regions, and observations of internal rotation, indicates that myofibrils produce local torsions under deformation. As displayed in Fig. 5e, all simulations experienced internal rotation due to anisotropy. Similarly, in Fig. 6c shear in the xz -component indicates that lateral sides of the cell experience opposing shear. This behaviour was then explored by simulating the overlapping regions between the lateral sides and observing that shear stress transitions from peak negative and positive magnitudes across the cell centre, with overlap regions producing shear approximating the average of either adjacent region (Fig. 7c). Whilst the regions are still physically insulated, this transition suggests that local shear simulations can provide inference on adjacent cell sections. Further, comparison with active contraction (see Supplementary Figure 11) demonstrates similar shear behaviour, though revealing that passive deformation may underestimate the magnitude. However, a larger scale analysis is still required to understand whole-cell shear behaviour within its tissue context.

Shear stress demonstrates a correlation with orientation angle (Fig. 7a). As z -disc spherical deviation angle increased, all shear responses similarly increased, with the strongest correlation being xy -shear (Table 1). When clustering the lateral sides and comparing their z -disc elevation orientation, the two sides were shown to differ significantly in spherical and elevation angles. Whilst the cell has no discrete axis for defining elevation or azimuth, measurements relative to the cell's fixation during imaging highlight that the lower and upper regions differ significantly in elevation which may explain their opposing shear profiles. This bilateral shear and orientation behaviour suggests that anisotropy would produce internal rotation in isolated regions during contraction, and more broadly that shear stress may produce torsion about the cell's major axis. Cardiomyocyte torsion would interestingly mimic ventricular twisting during systole⁷⁴ suggesting further multiscale mechanical coupling and warranting further investigation.

Reports have demonstrated that muscle cell architecture is impacted by fibre type^{15,34}. Cardiomyocytes require a constant provision of energy dissimilar from skeletal muscle, impacting the size, orientation, and connectivity of mitochondria and sarcomeres³⁴. Similarly, Willingham et al.¹⁵ displayed that branching events in myofibrils are increased in slow-twitch muscles compared to cardiac and fast-twitch variants, whereas Ajayi et al.³⁵ indicated that myofibril branching is impacted by gene knock-out in *Drosophila*. This work demonstrates that non-axial orientation produces shear stresses significantly different from the uniaxial case in a physiological model of a cardiomyocyte regions, future work, therefore, may focus on the whole cell, or how other muscle

cell architectures impact shear stress. Further, recent studies have indicated that multiscale models can inform how physics at a cellular level influences bulk tissue^{75,76}, pivotal to observing remodelling in disease. Myocyte architecture is known to change in pathology^{77,78}, therefore modelling the impacts of these changes on shear and its compounding effects on tissue should be investigated.

Characterisation of myofibrils in other cells indicate the applicability of these findings beyond the single cardiomyocyte. Quantification of networks has indicated that branching^{15,35} and orientation⁵⁶ distributions are standard in cardiomyocytes. The orientation data calculated here (see Fig. 3) is similar to distributions previously reported⁵⁶. Similarly, analysis of another cell section indicated that orientation distribution, mean, and standard deviation, all fall within the current distribution (see Supplementary Figure 5). Therefore, it is anticipated that the local shear behaviour explored here is representative of local region in other cardiomyocytes. Jayasinghe et al.³³ showed that myofibrils create helices that form over the cell nucleus, a feature that would complement the centripetal forces required for nuclei migration described by Roman et al.⁴², for this reason it is expected that these regions experience shear and torsion behaviours similar to described here. Further, the cytoskeleton has been shown to modulate shear stiffness in cardiomyocytes^{79,80}. Nishimura et al.⁷⁹ demonstrated that microtubule stiffness, increasing with disease, produces shear stresses during experimentation and in FE studies with uniaxial myofibrils. It is likely that cytoskeleton and multi-axial stress present here couple during contraction.

The hyperplastic anisotropic biophysical model presented here is a foundational model which has the further potential to incorporate other cell physiology. First, myofibril orientation is established perpendicular to z-discs and then smoothed between subsequent z-discs (see Methods) to approximate myofilaments splines. Whilst myofilaments are anchored at the z-disc structurally⁸¹ and have been used as a proxy for myofibril architecture in cardiomyocytes previously³³, it is the myofilaments which dictate force. Myofilaments have been shown to curve over the sarcomere, though this is minimal in cardiac muscle⁵⁵. Further, this model does not consider splitting events which, if originating at the z-disc as suggested³², would likely increase local shear as myofilaments diverge from the z-disc. Myofilaments also form complex binding structures^{55,82}, producing helical and triangular lattices over the length which may influence force dynamics. Active contraction behaviour, and calcium dynamics, are also important contributions that have been incorporated into other multi-dimensional cell models^{26,27,83}; incorporation of these components could be used to produce a more detailed physiological model. Further, changes to the rigid boundary conditions considered here may impact how extreme anisotropy diverts from the uniaxial model, with emphasis on coupling physics to expand insight across simulated regions and local organelles.

In this study we investigated, to our knowledge, the largest and most detailed z-disc segmentation of a cardiomyocyte and produced the first fibre-orientation informed non-linear myocyte deformation model. Deep learning segmentation of the cardiac cell confirmed that z-disc produce a distribution of orientations values concentrated about the major axis. These values varied up to 30° and were not significantly impacted by organelles. However, fibre orientation did display characteristic differences across the cell geometry with a gradient of positive to negative mean-shift over the cell length.

Myofibril anisotropy produces shear stresses that are not reproducible with the uniaxial model. Whilst normal stresses were maintained, physiologically informed simulations of cell regions created arrays of shear values. All simulations produced shear components of greater magnitude than the uniaxial case, the latter resulting in no shear in the xz - and yz -components. This study indicates that incorporating off-axis myofibril orientation is necessary to account for local shear stresses produced during cardiac muscle cell contraction.

Methods

Z-Disc Segmentation.

The ultrastructure of sheep left ventricular cardiomyocytes were captured with scanning-block-face electron microscopy (SBF-EM) as previously described⁷⁵. Within this volume ($6000 \times 6000 \times 359$ [pixels]; X and Y : $0.11 \frac{\mu m}{pixel}$, Z : $0.5 \frac{\mu m}{pixel}$), three cardiomyocytes were present and 19 random regions ($1024 \times 1024 \times 100$ [pixels]) were isolated for manual annotation of z-discs in Napari⁸⁴. These annotations were then used to automatically segment the largest cardiomyocyte. The chosen cardiomyocyte was a full cell depth and half-length of similar cardiomyocytes (~ 94 [μm])⁸⁵.

A smaller initial subset of these patches were annotated and used to train a 2D U-Net++⁴⁷ with a ResNet-50⁸⁶ weights using unified focal loss⁸⁷. The output segmentations from this original model were used to create a segmentation on the remaining patches which were manually corrected before creating the full set to train the final model. During each epoch smaller patches from the 19 annotated 3D blocks were extracted to introduce training set variability. Sampled 2D patches were 384×384 pixel covering 70% area, resulting in 1,648 patches per epoch.

Output segmentation voxels were generated through 2D smoothing tiling of overlapping predictions. Manual corrections were made to erroneous segmentations after inspection and before utilization in further analysis. In total 1138 z-discs were segmented and included in this study, with manual annotation regions comprising $\sim 14.9\%$ of the whole volume and automatic segmentation comprising $\sim 59.9\%$.

Morphological Analysis

For morphological analysis, orientations were normalised to the cell's contraction axis. The contraction axis was determined as the first principal component of the cardiomyocyte, its long axis. The contraction axis was validated via the average orientation angle of the z-discs (see Fig. 3); the average orientation aligned with the contraction axis. This is reminiscent of similar orientation measurements⁵⁶. From here, instances of z-discs were analysed to inform on centroid position, pixel-size, and orientation. Centroid data, pixel quantities, size, and elliptical axis were calculated with the scikit-image⁸⁸ *measure* module.

Fibre orientation was defined through the z-discs' normal vector in direction of cell's major axis. To determine the normal vector *Principal Components Analysis* (PCA)⁸⁹ was utilised; the first two components characterizing the structure of the segmentation, as the major and minor axis of length and width. The third component is, by definition, perpendicular to the first two, and normal to the face. PCA was implemented with scikit-learn⁹⁰.

The spherical orientation was calculated as the angle between the vectors of the third principal component and contraction axis (see Fig. 3a). To achieve this all third components were required to be pointing in the positive x-axis. The resulting distribution of values is displayed in Fig. 3b with a bin size of 1.5°. Bin sizes larger than this are insufficient for appreciating variance, smaller sizes increase noise.

Mesh

A prism mesh defined on the dimensions of cell regions was created in Gmsh⁹¹. The geometry (11000 × 11000 × 5000 [nm]) was defined with second-order tetrahedral elements. Mesh refinement was tested to ensure convergence of solution and minimization of adjacent node difference. The resulting test mesh had 358,656 elements and 463,120 nodes.

Constitutive Equation

The Guccione type constitutive equation (see Eq. 1) for orthotropic behaviour was implemented⁶¹.

$$\Psi = \frac{c_1}{2} * (e^Q - 1)$$

$$Q = b_f E_{ff}^2 + b_t (E_{tt}^2 + E_{ss}^2 + E_{ts}^2 + E_{st}^2) + b_s (E_{fs}^2 + E_{sf}^2 + E_{tf}^2 + E_{ft}^2) \quad (1)$$

Where Ψ is the strain energy density function, and E is the green Lagrange strain tensor with components f, t, s are the tensor components.

Here, b_t and b_s were set as equal reducing the equation to transverse isotropic as previously explored^{21,92}. The other constants (b_f , b_t , c_1) were calculated via optimisation against experimental and model trends of tension development in cardiomyocytes. The literature presents a range of values so $9 \left[\frac{kN}{mm^2} \right]$ (or $[kPa]$) was set to balance the range of experimental measurements of cardiac tension^{20,28,52–54}. Solutions converged to $b_f \approx 12.68 [kPa]$, $b_t \approx 11.04 [kPa]$, $c_1 \approx 2.81$.

Regions Orientation Interpolation

Myofibril orientation was interpolated into the mesh by first mapping the nodal positions of the mesh to the z-disc pixel data from segmentation regions. This was supported with SciPy's⁹³ KD-tree. Once mapped, angle data was interpolated into second-order (10-node) tetrahedral Lagrange functions with the in-built FEniCSx^{48–51} architecture. Nodes within the z-disc boundaries were assigned to the same angles as those which were directly mapped to the nearest-neighbour tree.

Force production in muscle cells occurs in the direction of the myofilaments. This model assumes that myofibril orientation can be tracked between subsequent z-discs. Imaging has demonstrated that cardiac muscle myofilaments diverge minimally from a linear path compared to other fibres⁵⁵. To ensure continuity over the sarcomere, gaussian smoothing of orientation was applied between z-discs. Smoothing boundaries were based on the diameters of z-disc (disc-axis) and length of sarcomeres (fibre-axis). Reports suggest cardiac z-disc range from $100 [nm]$ to $140 [nm]$ ^{94,95}, and sarcomere slack length of $1.8 - 2 [\mu m]$ ^{1,3,96}; these values corresponded to the standard deviations provided to the smoothing function.

Fibre Field

Anisotropy was incorporated into the variational calculus similarly to Nash and Hunter⁵⁷ and Guccione et al.⁶¹, following the Lagrangian formulation. First, undeformed basis vectors (A_i) and metric tensors (G_{ij}) are calculated via rotation of cartesian coordinates to orient with the myofibril data in each region. Here, G_{ij} is the inner product of the basis vectors,

$$G_{ij} = A_i \cdot A_j.$$

Where A represents the Lagrange basis vectors. This is interpolated into Quadrature points within native FEniCSx^{48–51}. FEniCSx provides the Lagrangian form of variational calculus, therefore the Euclidean metric covariant and contravariant tensors are also determined. Lower case g_{ij} and a_i are used as convention to indicate deformed tensors.

$$g_{ij} = a_i \cdot a_j \tag{2}$$

Subsequentially, the Christoffel Symbols of the second kind,

$$\Gamma_{ij}^k = \frac{1}{2} g^{kl} (g_{jl,i} + g_{il,j} - g_{ij,l}), \quad (3)$$

and covariant derivatives,

$$\nabla_j v^\alpha = v_{,j}^\alpha + \Gamma_{kj}^\alpha v^k, \quad (4)$$

are calculated to update the variational form

$$R = \int_{\Omega} T^{\alpha\beta} F_\beta^j \nabla_j v^\alpha d\Omega + \int_{\Omega} q (J - 1) d. \quad (5)$$

The First-Piola Kirchoff is calculated per a push-forward transform on the Cauchy stress tensor.

$$P = J\sigma F^{-T} + pJG^{-1}F^{-T} \quad (6)$$

Boundary Conditions and Simulation

Contraction was simulated with discrete displacement of the regions at maximum and minimum x -position. The planes were fixed in y - and z -axes with the x -axis displaced 20%. All other degrees of freedom move freely.

An iterative non-linear solver native to FEniCSx⁴⁸⁻⁵¹ was employed. The solution was incremented at 2% displacement intervals. Convergence was determined with an incremental tolerance calculation, requiring a tolerance of 10^{-5} and a maximum of 50 iterations per Newton Solver. Simulations were run on the University's High Performance Computer and were allocated 512GB per node and run serially to avoid node-mismatch in parallel.

Displacement and Rotation

Boundary displacements for each region were compared by taking the edges of the deformed shape. To compare z -displacement, values along x -axis were plotted for three regions through the y -axis. Maximum and minimum displaced values on the upper and lower edge of these regions were overlaid (see Fig. 5). Similarly, y -displacement was displayed across the x -axis for three regions across the z -axis.

Rotation data were extracted from the centre region and calculated in the y - z plane about x -axis. Two vectors were produced once each datapoint was centralized to the origin: the first being a

vector to the undeformed point, the second being to the deformed point. The angle difference between the two with the y -axis was provided as the angle of rotation.

Stress and Strain Calculation

Stress values were extracted from an internal 90% volume. This bounding allowed for the reduction of boundary artefact in the solutions and overestimations of stress. Cauchy stress was calculated with the inversion of Eq. 6 yet omitting hydrostatic pressure. Hydrostatic pressure was interpolated with first-order (4-node) Lagrange tetrahedra.

Each tensor component was then calculated by taking the mean stress over the internal volume in each direction. The stresses displayed in Fig. 6 correspond with the principal directions and relevant shear terms. All spatial visualisations were achieved in Paraview⁹⁷.

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Conflict of interest declaration

The authors declare no competing financial or non-financial interests.

Author Contributions

L.A.M. wrote the manuscript, code, created figures, and performed analysis. L.A.M. and V.R. investigated scope and planned investigation. C.P. performed and provided EM imaging. A.Q. created machine learning model and applied model to segmentation. L.A.M. segmented raw EM data. V.R., D.J.C., C.P., A.Q. contributed to editing of manuscript.

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Fig 1 | Visual abstract demonstrating the workflow from cardiomyocyte segmentation through to morphological analysis and mechanical simulation. (a) Z-Discs (red in EM image) are isolated from portion of sheep left-ventricular cardiomyocyte ($33 \times 19.8 \times 15 \mu\text{m}$; one $5 \mu\text{m}$ volume indicated here) through U-Net++ Machine Learning model instance segmentation. (b) Isolated segmentations of z-disc are analysed with principal component analysis to quantify orientation of fibre in reference to contraction axis of cell. (c) Fibre orientation interpolated into non-linear mechanical model for contraction simulation. Fibre-informed simulations and uniaxial tests are contracted and shear stress quantified. Quantification indicates that shear stress in fibre-informed cases produce a distribution not reproduced by uniaxial model.

Fig 2 | Z-Disc segmentation from cardiomyocyte volume and split into test regions. (a, top-left) Key indicating dimensions of full cell segment ($33 \times 19.8 \times 15 \mu\text{m}$), broken into three stacks (z-discs in red). (middle) Dimensions of test regions ($11 \times 11 \times 5 \mu\text{m}$) displayed. (right) Extraction from Region 0 with key cell landmarks indicated (mitochondria in green, nucleus in light blue, z-discs in red, and periphery in pink). (b-d) Visualisation of z-disc instance segmentations, electron microscopy (EM) images, and test regions for (b) $0 \leq z < 5 \mu\text{m}$, (c) $5 \leq z < 10 \mu\text{m}$, and (d) $10 \leq z < 15 \mu\text{m}$. (b-d) Row 1 (top), Orthogonal render of z-disc segmentations with contraction axis indicated. Row 2, orthogonal view of EM slices. Row 3, 2D top-down view of z-disc segmentations aligned left-to-right with contraction axis. Row 4 (bottom), 2D top-down view of EM slices (nucleus in light blue, z-discs in red, and periphery in pink) with overlay of numbers of regions for identification; dimensions as indicated in (a).

Fig 3 | Isolation of Spherical Angle distributions from Principal Components Analysis (PCA) of segmentation data. (a) Top left, Region 7 rendered in orthogonal view. Top middle, diagrammatic indication of relationship between z-disc, Fibre-Axis, and Contraction Axis; the spherical angle is computed through the vector angle between the z-disc normal vector and contraction axis. Top right, 2D top-down view of PCA computed normal vectors to segmentations from Region 7 shown top left. (b) Histogram of global orientation values for all segmented z-discs, bin size: 1.5° . (c) Violin plots with quartiles shown for orientation data per test region. Purple regions indicate statistical significance between regional orientation values and global set. Significance was determined with Mann-Witney U test and Welch's t-test $p < 0.05$. Vertical dash (--) and dotted (:) lines indicating global quartiles.

Fig 4 | Interpolation of fibre orientation into cell volume. (a) Whole cell volume render with interpolation of z-disc orientations throughout lengths of sarcomere sections. Colour spectrum is limited to two standard deviations of global orientation values. Overlay of z-disc segmentations onto orientation render displayed in lower left. (b) Glyph render of cartesian vector components for each z-disc from top-to-bottom: x -component, y -component, z -component. x -component demonstrates homogeneity in principal direction as anticipated, y and z show larger variability. (c) Axial slices through the rendered volume. Each slice is a third of the dimension's length. Note void in x -slices (left) which correspond with the large mitochondria group and nucleus observable in top-right z -slice. Slicing passes through z-discs and sarcomere unevenly, therefore z-disc patterning is less obvious.

Fig 5 | Region displacements and in-plane rotation at 20% displacement. (a-b) y -displacement visualized as select region shaded boundaries (a) and all regions boxplot distributions (b). (a) Boundary displacements for Region's **0-4** are overlaid onto displacements of uniaxial test. Displacements are plotted for thirds of the dimension to illustrate transition of deformation over length. Darkest shade indicates first slice, closest to origin ($y = 0$). (b) Boxplots of each Region's displacement values throughout volume. Dashed (--) and dotted (:) lines indicate quartiles of uniaxial test. (c-d) z -displacements visualized to similar capacity as (a-b). (e) yz -plane rotation produced by displacement displayed as violin plots with quartiles. Plane rotation determined at inner plane to reduce impacts of rigid boundaries. Violin plots show the distribution of common rotation values for each region, see Region 4 indicating frequent rotation values at $-25^\circ, 0, 25^\circ$.

Fig 6 | Stress trends and spatial shear for simulations at 20%. (a) Left, diagrammatic visualization of stress tensor components. Top-right, render of uniaxial deformation for each simulation step change, bottom-right, test label key for stress trends and bar charts. (b) Left, normal stress components plotted over displacement; nearly all test cases show minimal deflection from the uniaxial test, this is likely an artifact of rigid boundary displacement. Shear stress trend (middle) and bar charts (right) for each test condition. Shear trends demonstrate variance in shear profile as deformation reaches 20%. Bar charts are ordered numerically with region numbers, dashed (--) line indicates uniaxial test value. Values shown are final deformation stresses at 20%. (c) Spatial shear profiles for each region test case aligned with EM images of slices (z-discs in red). All distributions are displayed with the same colour bar range. Some pattern is evident in xz -shear which corresponds with bar chart (b, middle).

Fig 7 | Trends for shear stress and z-disc orientation. (a) Mean shear stress versus mean z-disc orientation per region. (left) x - y shear stress produces strong positive correlation ($R = 0.76$), increased positive mean orientation increases positive shear. (middle) x - z shear demonstrating a separation between shear in two groups with a weak positive correlation. Dashed circle (-) indicates upper regions ($y > 9.9 \text{ [nm]}$), double dash (--) circle indicating group of lower regions ($y < 9.9 \text{ [nm]}$), their correlations can be seen in Table 1. (right) y - z shear with moderate positive correlation. (b) Variance of shear stress versus variance of z-disc orientation. (left) x - y shear with moderate positive correlation. (middle) x - z shear with weak positive correlation. (right) y - z shear with moderate positive correlation. (c) Bar charts and trends of shear stress (x - z) for each region. (top) Upper region ($y > 9.9 \text{ [nm]}$) shear shows positive behaviour in both final value bar charts and trends. (middle) Overlap region simulations (solid) demonstrates that shear behaviour transitions over space. Average shear between upper and lower simulations (dashed, [--]) overlaid on bar chart. (bottom) Lower region ($y < 9.9 \text{ [nm]}$) shear stress is negative for all simulations and during trend. (d)

Comparison of angle orientations across Upper, Lower, and Overlap regions, pairwise t-test significance is indicated with asterisk (*, $p < 0.05$). (top) Spherical deviation, as previously reported, indicates lower regions are more negatively oriented than other regions. (middle) Distributions of azimuthal (lateral, x-y) angle indicates no significant differences between regions. (bottom) Elevation (x-z) angle demonstrates that lower regions have significantly more positive orientation.

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