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Simulation of the Mechanical Response of Cells on Micro-post Substrates

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Abstract

Experimental studies where cells are seeded on micropost arrays in order to quantify their contractile behaviour are becoming increasingly common. Interpretation of the data generated by this experimental technique is difficult, due to the complexity of the processes underlying cellular contractility and mechanotransduction. In the current study, a coupled framework that considers strain rate dependent contractility and remodelling of the cytoskeleton is used in tandem with a thermodynamic model of tension dependent focal adhesion formation to investigate the biomechanical response of cells adhered to micropost arrays. Computational investigations of the following experimental studies are presented: cell behaviour on different sized arrays with a range of post stiffness; stress fibre and focal adhesion formation in irregularly shaped cells; the response of cells to deformations applied locally to individual posts; and the response of cells to equi-biaxial stretching of micropost arrays. The predicted stress fibre and focal adhesion distributions, in addition to the predicted post tractions are quantitatively and qualitatively supported by previously published experimental data. The computational models presented in this study thus provide a framework for the design and interpretation of experimental micropost studies.
1 Introduction

Mechanotransduction is an essential component of cellular functions such as cell motility [1]; and the physical environment around the cell has been shown to direct stem cell lineage specification [2, 3]. The contractile response of cells to extra-cellular matrix stiffness has been shown to be an important factor in wound healing [4], atherosclerosis [5], and cancer progression [6, 7]. In order to further elucidate mechanotransduction, previous experimental studies have employed arrays of microposts, or micropillars, to quantify the traction generated by a cell and provide a tuneable mechanical environment [8, 9]. These microarrays have been used to study the adhesion and contractility of static cells [8, 10] cells subjected to local [11] and global applied deformation [12], cell migration [13, 14], and cell - cell adhesion [15]. Micropost arrays provide detailed quantitative data about the contractility of cells and measure tractions at a number of points on the cell [8], in contrast to whole-cell measurements obtained from atomic force microscopy or cell probing [16-18]. Numerous different designs of these arrays exist, with variations in the length and diameter of the pillars, the spacing between pillars, and the arrangement in square or triangular patterns. Experimental studies typically use the average force per post or the total cell force as a metric for cellular contractility. However, these quantities are significantly affected by the number of posts to which a cell adheres and by the design of the array. Therefore, interpretation of the data generated by these arrays is difficult in the absence of comprehensive modelling formulation that considers the key biochemical processes, i.e. the tension dependent formation of focal adhesions (FAs) and the remodelling and contractility of the actin cytoskeleton.

FAs provide a mechanical link between the stress fibres (SF) contractility and the physical environment around the cell, in this case the microposts. Previous studies of cells on microarrays have established a relationship between cell tractions and FA area [8, 10] and also the changes in
SFs and FAs on arrays with different post stiffness and geometry [19, 20]. Post spacing and stiffness has also been spatially varied in arrays to investigate the migration of cells over gradients in substrate stiffness [14, 21]. Micropost arrays have also been used to examine cell-cell adhesions by examining the tractions of two neighbouring cells and restricting the spread shapes of cells to only allow a single cell-cell adhesion to form [15]. Microarrays have also been used to apply deformations to a cell: Sniadecki et al. [11] use magnetic microposts to subject a cell to local loading, i.e. only one of the posts that a cell is adhered to is moved, observing that changes in FA area occur only on actuated posts; Mann et al. [12] subject a cell to a biaxial strain by stretching the membrane to which the base of the posts are attached, allowing the increase and subsequent decrease in post force to be captured. A robust SF and FA formulation should capture these experimentally observed phenomena, despite the differences in boundary conditions for each experiment.

Previously, the contractile cytoskeleton has been included in computational models as a network of pre-positioned passive filaments with prescribed shrinkage strains[22, 23] and, more recently, using models that consider SF remodelling based on fibre stretch [24]. Also, previous FA formulations have not included the role of cellular contractility [25, 26]. A number of studies by Deshpande et al. [27, 28] present a coupled framework that considers the signal dependent remodelling and strain rate dependent contractility of the cytoskeleton in tandem with a thermodynamic model of tension dependent FA formation. Previously, this formulation has been used to investigate SF formation in cells adhered to patterned substrates [29] and in cells adhered to different elastic substrates [30] and the SF component has been used to simulate SF formation in cells adhered to static micropost arrays[20]. However, the previous study of SF formation in cells adhered to microposts by McGarry et al. did not consider the formation of FAs or investigate the response of the cell to applied deformations. A preliminary study by Pathak et al [31]
investigated the behaviour of SFs and FAs on microposts arrays with different post stiffness. In the current study, these previous works are expanded to consider both SF and FA formation in cells adhered to static and dynamically loaded micropost arrays. Extensive quantitative validation of the simulations presented in the current study is performed based on previously published experimental observations.

In the current study, the thermodynamically motivated adhesion model is used in tandem with the active SF contractility model to investigate the response of cells adhered to micropost arrays. An overview of these formulations is given below in section 2 and the reader is referred to the original publications for a full description of the SF [27] and FA [28] models. The simulations are presented in four sections and relevant experimental results are discussed in each section. Briefly: 1) Cells are simulated on different sized arrays with a range of post stiffness; 2) Realistic cell geometries are considered based on previously published experimental images; 3) The response of cells to deformations that are applied locally to individual posts is predicted; 4) Cells are simulated on micropost arrays subjected to equibiaxial stretch.

2 Methods

2.1 Stress fibre material formulation

Stress fibre (SF) formation consists of three coupled phenomena: an activation signal which triggers the formation of the SFs, dissociation of fibres due to a reduction in tension, and a Hill type law relating the contractility of a SF to strain rate.

The role of cellular signalling has been closely linked to cytoskeletal remodelling and mechanotransduction. In this study, the complete signalling pathway which triggers the SF formation is phenomenologically represented as an exponentially decaying signal [32]:
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\[ C = e^{\left(\frac{-t_1}{\theta}\right)} \]  

(1)

where \( \theta \) is a constant that controls the decay rate of the signal and \( t_1 \) is the time since the most recent signal.

Cytoskeletal tension is essential for sustaining SF bundles and a reduction below a defined isometric level leads to fibre dissociation [33, 34]. The contractile behaviour of assembled SF bundles is similar to that of skeletal muscle. The tension in the SF bundle, which is generated by cross-bridge cycling of actin-myosin pairs [35], is related to the bundle contraction rate using the following Hill-like equation:

\[
\frac{\sigma_f}{\sigma_0} = \begin{cases} 
0 & \frac{\dot{\varepsilon}}{\varepsilon_0} \leq -\frac{\eta}{k_v} \\
\frac{\bar{k}_v}{\eta} \frac{\dot{\varepsilon}}{\varepsilon_0} & -\frac{\eta}{k_v} \leq \frac{\dot{\varepsilon}}{\varepsilon_0} \leq 0 \\
1 & \frac{\dot{\varepsilon}}{\varepsilon_0} > 0 
\end{cases}
\]  

(2)

where \( \sigma_f \) is the stress in the SF bundle, \( \sigma_0 \) is the isometric tension, and \( \bar{k}_v \) is the reduction in stress upon increasing the shortening strain rate, \( \dot{\varepsilon} \), by \( \dot{\varepsilon}_0 \). The dimensionless activation level of a SF bundle, \( \eta \), at any orientation, also defines the isometric tension, \( \sigma_0 \), where \( \sigma_0 = \eta \sigma_{\text{max}} \). \( \sigma_{\text{max}} \) is the maximum tension in a fully activated bundle.

SFs are described by defining the dimensionless activation level \( \eta: \eta \ (0 \leq \eta \leq 1) \), where \( \eta = 1 \) corresponds to the maximum possible SF activation level allowed by the biochemistry. The signal induced formation and tension dependent dissociation of the actin cytoskeleton is captured using a first order kinetic equation [27]:

\[ \dot{\eta} = \left[ 1 - \eta \right] \frac{C k_f}{\theta} - \left( 1 - \frac{\sigma_f}{\sigma_0} \right) \eta \frac{k_b}{\theta} \]  

(3)

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The overdot denotes change with respect to time. The first term on the right of the equation governs the rate of formation of the SFs and is controlled by the dimensionless constant $\overline{k_f}$, the signal $C$, and decay constant $\theta$. The latter part of the equation gives the rate of dissociation and is governed by the dimensionless constant $\overline{k_b}$, the stress level $\sigma_f$, and the isometric tension $\sigma_0$.

The active stress is determined by summing the contribution from all possible fibre orientations. In a two-dimensional framework, an arbitrary fibre is considered at an angle $\phi$ with unit vector, $m_i = \cos \phi x_1 + \sin \phi x_2$. The strain rate in this fibre direction is given in terms of the material strain rate as

$$\dot{\epsilon} = \dot{\epsilon}_{11} \cos^2 \phi + \dot{\epsilon}_{22} \sin^2 \phi + \dot{\epsilon}_{12} \sin 2\phi$$  \hspace{1cm} (4)

The active stress is then calculated by summing the contribution of each fibre:

$$\sigma_{ij}^A = \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \sigma_f(\phi) m_i m_j d\phi$$

In parallel to the active SF behaviour described above, the passive material surrounding the SFs in the cell cytoplasm is modelled using a compressible neo-Hookean hyperelastic formulation, whereby the passive stress tensor is given as:

$$\sigma_{ij}^p = 2 \int C_{10} \left( \frac{B_{ij}}{j^{2/3}} - \frac{1}{3} \frac{B_{ij}}{j^{2/3}} \delta_{ij} \right) + \frac{2}{D_1} (J - 1)$$  \hspace{1cm} (5)

where the left Cauchy-Green tensor $B$ is determined from the deformation gradient $F$:

$$B_{ij} = \frac{F_{ik} F_{jk}}{\left(\epsilon_{imn} F_{1i} F_{2m} F_{3n}\right)^{2/3}}$$  \hspace{1cm} (6)

The elasticity constants are given in terms of Young’s modulus, $E$, and Poisson’s ratio, $\nu$, as:
The complete stress state at any point in the cell cytoplasm is then given as:

\[ \sigma_{ij} = \sigma_{ij}^A + \sigma_{ij}^P \]  

(8)

A viscoelastic cytoplasm is also considered and implemented using a standard Prony series formulation where the bulk \( K(t) \) and shear \( G(t) \) relaxation functions are defined individually in terms of a series of exponentials [36]:

\[ K(t) = K_\infty + \sum_{i=1}^{n_k} k_i e^{-t/\tau_i} \]  

(9)

\[ G(t) = G_\infty + \sum_{i=1}^{n_g} g_i e^{-t/\tau_i} \]  

(10)

where \( K_\infty \) and \( G_\infty \) are the long term bulk and shear moduli, \( \tau_i \) is the relaxation time. In the current study \( n_k = n_g = 1 \), therefore the instantaneous shear modulus can be written as \( G_0 = G_\infty + G_1 \). By setting the term \( \alpha = G_1/G_0 \) to 1 or 0.5 a Maxwell model or a Standard Linear Solid (SLS) model can be simulated. The bulk moduli are determined similarly.

### 2.2 Focal adhesion formulation

Binding integrins on the cell surface exist in two conformational states: high affinity, or “straight”, integrins with a high reference chemical potential, \( \mu_L \), and low affinity, or “bent”, integrins with lower reference chemical potential \( \mu_H \). Only the high affinity integrins form bonds and low affinity integrins remain unbound. Low affinity integrins with a concentration \( \xi_L \) have a chemical potential:

\[ \chi_L = \mu_L + kT \ln \left( \frac{\xi_L}{\xi_0} \right) \]  

(11)
where $\mu_L$ is the internal energy and the last term accounts for the configurational entropy. $\xi_0$ is the total concentration of integrins, and $k$ and $T$ are the Boltzmann constant and the absolute temperature.

High affinity integrins form bonds and undergo stretching, therefore the potential energy stored in the bond and the mechanical work done are accounted for in the chemical potential as:

$$\chi_H = \mu_H + kT \ln \left( \frac{\xi_H}{\xi_0} \right) + \Phi(\Delta_i) - F_i \Delta_i$$

where $\Phi$ is the stretch energy and $F_i \Delta_i$ is the mechanical work due to the stretch $\Delta_i$ of the bond by the force $F_i$. The force $F_i$ is related to the stretch by:

$$F_i = \frac{\partial \Phi}{\partial \Delta_i}$$

The kinetics of bond formation and diffusion of low affinity integrins along the cell membrane are considered fast compared with other time scales involved. Therefore diffusive fluxes are neglected and the concentrations of the integrins are given by thermodynamic equilibrium: $\chi_H = \chi_L$.

Therefore the concentrations of high and low affinity integrins are determined as[28]:

$$\xi_H = \frac{\xi_0}{\exp \left[ \frac{\mu_H - \mu_L + \Phi - F_i \Delta_i}{kT} \right] + 1}$$

$$\xi_L = \frac{\xi_0}{\exp \left[ -\frac{\mu_H - \mu_L + \Phi - F_i \Delta_i}{kT} \right] + 1}$$

The stretch energy $\Phi$ is expressed as a piecewise quadratic potential:

$$\Phi = \begin{cases} 
\frac{\kappa_s \Delta_e^2}{\Delta_n} & \Delta_e \leq \Delta_n \\
-\kappa_s \Delta_n^2 + 2\kappa_s \Delta_n \Delta_e - (\kappa_s \Delta_n^2) & \Delta_n < \Delta_e \leq 2\Delta_n \\
\kappa_s \Delta_n^2 & \Delta_e > 2\Delta_n
\end{cases}$$

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where \( \kappa_s \) is the stiffness of the bond; \( \Delta_e = \sqrt{\Delta_1 + \Delta_2} \) is the stretch magnitude and \( \Delta_n \) is the peak bond length. The bond stretch \( \Delta_i \) is related to the displacement of the membrane relative to the substrate as:

\[
\Delta_i = \begin{cases} 
  u_i & \Delta_e \leq \Delta_n \\
  0 & \frac{\partial \Phi}{\partial \Delta_e} \Delta_e < 0 
\end{cases} \quad (17)
\]

The tractions on the cell surface depend on the force \( (F_i) \) on each bond and the concentration of bound high affinity integrins \( (\xi_H) \) such that:

\[
T_i = -\xi_H F_i \quad (18)
\]

These tractions are balance by stresses in the cell caused by cellular contractility such that:

\[
T_i = \sigma_{ij} n_j = -\xi_H F_i \quad (19)
\]

where \( \sigma_{ij} \) is the Cauchy stress in the cell, and \( n_j \) is the surface normal. In the current study, detachment of the cell from the substrate is not considered. Therefore, the cell is constrained so that points that are in contact on the cell surface remain in contact, i.e. only tangential displacements are allowed.

The focal adhesion parameters are chosen based on previous calibrations of this model [28, 29] as shown in Table 1.

### 2.3 Microposts and finite element models

Microposts are simulated as vertical cantilevers; i.e., it is assumed that the post is completely constrained at the base. The traction force applied by the cell is assumed to be a point load, therefore the deflection of the beam tip \( \delta_p \) as a function of the post force \( F_p \) is:
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\[ F_p = \left( \frac{3E_p I}{L^3} \right) \delta_p \]  

(20)

where \( E_p \) is the Young’s modulus of the post material, \( L \) is the length of the beam, and \( I \) is the second moment of area of the post cross-section. The behaviour of the posts is simulated using a linear spring element where the stiffness of the spring is the term in parentheses in Eqn 20.

Finite element models include the microposts, which are simulated using linear springs attached to rigid circular surfaces, and the cell, which is simulated as a membrane with a uniform thickness, as shown in Figure 1. The active material model is implemented as a FORTRAN user-defined material using the commercial finite element code Abaqus (Dassault Systemes, RI, USA). Contact is defined between the rigid circular surfaces, which represent the top post surfaces, and the cell membrane using the FA formulation described above, which is implemented as a FORTRAN user-defined interface in Abaqus.

Material and interface parameters are chosen based on previous implementations of these formulations [20, 37] and are identified in Table 1. Three different cell phenotypes are considered: mesenchymal stem cells (MSCs), fibroblasts (FBs), and smooth muscle cells (SMCs), reflecting the cell types used by Fu et al. [10]; Sniadecki et al. [11]; and [8] and Mann et al. [12], respectively. The contractility of each cell type is represented by altering the maximum stress in a fully activated fibre (\( \sigma_{\text{max}} \)).

2.4 Interpretation of results

In order to visualise the predicted SF distributions and compare the predictions to experimental observations of SFs, it is necessary to define an output variable that describes the SF formation at each calculation point in the model. Experimental imaging techniques for the cytoskeleton typically stain for actin, and these images show areas with dominant bundles of SFs. In order to
show comparable plots of SF formation, a SF variance is defined that shows the location of dominant SFs. In order to calculate this variance, we first consider the average level of SF formation at a point, which is calculated by integrating the level of SF formation over every possible direction:

\[
\bar{\eta} \equiv \frac{1}{\pi} \int_{-\pi/2}^{\pi/2} \eta d\phi
\]

The variance \( \Pi \) is then calculated by subtracting this average from the maximum level of SF formation in any direction:

\[
\Pi = \eta_{\text{max}} - \bar{\eta}
\]

Finally, vector plots are presented to illustrate the direction in \( \eta_{\text{max}} \) occurs at each point.
3 Results and discussion

3.1 Simulation of fibroblasts adhered to static micropost arrays.

Figure 2 shows SF and FA formation in cells adhered to 5x5 and 13x13 arrays of microposts after 600 s of signal driven SF formation. Cells are simulated on arrays of microposts with 1μm post radii, 4μm centre-to-centre post spacing, and a post stiffness of 18.16 nN/μm. Spread areas are ~485 μm$^2$ and ~2920 μm$^2$ for cells adhered to 5x5 and 13x13 arrays, respectively. In both cases, high levels of SF formation are computed at the cell periphery, particularly in the region around the post adhesion site. Large FAs are predicted to form near the cell periphery in regions containing high levels of SF formation as a result of increased traction on peripheral posts. A large area in the centre of the cell on the 13x13 array contains low levels of SF formation; this is also seen on larger arrays of 25x25 posts where SF formation is confined to the 4 rows closest to cell periphery (as shown in supplementary Figure S1).

The effect of post stiffness on predicted SF and FA formation is shown in Figure 3. Simulations are performed for a high post stiffness of 1556 nN/μm and for a low post stiffness of 1.9 nN/μm for cells adhered to a 13x13 micropost array (note that the cell in Figure 2 is adhered to 18.16 nN/μm posts). The cell on the stiffer posts contains higher levels of dominant SF formation. Furthermore, on the stiffest posts, SFs are confined to the 2 rows of posts adjacent to the cell periphery. FAs are predicted to form only in areas of high SF formation; therefore, large FAs are computed at the cell periphery for very stiff arrays. In contrast to predictions for stiffer posts, significantly less SF formation is predicted in the cell on the compliant posts, as there is not enough support for stress fibre tension, leading to stress fibre dissociation. Furthermore, FAs on the compliant posts are small, but are not confined to the outer two rows of posts as a small degree of FA formation is also computed posts in the central region of the cell.
The individual post forces for cells adhered to 13x13 arrays of microposts are shown in Figure 3E for post stiffness of 1.90 nN/μm, 18.16 nN/μm, and 1556 nN/μm. A linear increase in force from the cell centre to the cell periphery can be observed for the compliant posts, with a maximum force of ~2.2nN being computed at the cell periphery. In contrast, for stiffer posts, negligible forces are computed near the cell centre, with extremely high forces being computed near the cell periphery. For posts of stiffness 1556 nN/μm negligible forces are computed within a radius of 15 μm from the cell centre, with forces of ~10 nN being computed at the periphery.

The average force magnitude per post is shown in Figure 4(A) for cells adhered to arrays consisting of between 5x5 and 25x25 posts with stiffness in range 1.90 nN/μm to 1556 nN/μm. On compliant posts (1.9 nN/μm and 3.8 nN/μm) the average force increases with increasing cell area. In contrast, the average force on very stiff posts (1556 nN/μm ) decreases with increasing cell area. As shown in Figure 4B, the total force, i.e., the sum of the magnitudes of each post force, increases approximately linearly for each level of post stiffness. These predictions are in very good agreement with the experimental observations of Han et al. [19] for endothelial cells adhered to arrays of microposts, where an approximately linear increase in total force and a non-linear decrease in average force is observed with increasing cell area. The predicted average force for cells seeded on 11 nN/μm are in very good agreement with the experimental observations of Han et al. [19] as both show a slight increase in average post force for an increase in cell area from ~500 to ~1000 μm² followed by a decrease in average force for larger areas. This trend was also observed in the study of Tan et al. [8] for smooth muscle cells spread on arrays of 2 – 25 posts and was also captured in the study of McGarry et al. [20]. The post arrangement of these two experimental studies have different post spacing, and the study of Han et al. [19] does not include data for very low spread areas or for very low post stiffness. Future experimental studies should investigate this trend for lower areas and stiffness using the smaller post spacing as Han et al. [19].
The total FA area for each cell was calculated by summing the areas of the cell membrane where the concentration of high affinity integrins ($\xi_h/\xi_0$) above a threshold level. Figure 5A shows that the total FA area per cell depends only on the cell size and is largely independent of post stiffness, except for very large cells on very stiff arrays. Fu et al. [10] observed a similar trend experimentally for fibroblasts, with an approximately linear dependence on cell size and no clear dependence on post stiffness, as shown in Figure 5C. The changes in FA area with post stiffness in Figure 3 show that cells on stiffer posts contain a small number of large FA. In contrast, cells on compliant posts contain a large number of small FAs. Therefore, these competing trends result in an overall independence of FA area on post stiffness despite significant changes in the distribution of FAs across the cell.

The relationship between the total force and total adhesion area for different sized cells on microposts with different stiffness is shown in Figure 5B. For each micropost stiffness, total force increases approximately linearly with FA area and also with post stiffness. A linear trend line is fitted to the predicted results for each post stiffness and the slope of each line is shown in Figure 5E as a function of post stiffness. This slope has previously been referred to as the “FA Stress” [10]. Similar to the experimental observations of Fu et al. [10], this slope is predicted to double over the range 1.9 nN/μm to 18.16 nN/μm, as shown in Figure 5E.

### 3.2 Simulation of irregularly shaped SMCs

SF contractility and FA formation is simulated in an irregularly shaped smooth muscle cell (SMC) adhered to a micropost array, as reported in the study of Tan et al. [8]. Microposts have a radius of 1.5 μm and a centre-to-centre spacing of 10 μm and a post stiffness of 32 nN/μm. SF formation is shown following 600s of signal driven formation in Figure 6A. Dominant fibre bundles are predicted to form near the cell periphery, particular near concave edges. In areas of high SF
formation, the dominant fibre direction is predicted to be parallel to the cell edge, as shown by the vector plot in Figure 6B. Such long fibres parallel to the concave edges have been observed experimentally [8, 20]. The computed FA distribution for the same cell is shown in Figure 7A. Adhesions are predicted to form distinctive “horseshoe” type shapes, similar to those observed by Tan et al. [8], (reproduced in Figure 7B for comparison). The predicted reaction forces exerted by the cell on each post are superimposed as vectors over the FA plot and are in good agreement with the experimental observations. Magnified views of two regions are presented in Figure 7C to illustrate further the strong agreement between the computational and experimental results, particularly the orientation of traction vectors and the FA shape. It should also be noted that the computed post force acts in the direction of the open end of the FA “horseshoe” shape, and that the direction of the computed post forces is in good agreement with the experimental observations. Finally, an approximately linear relationship between the computed post force and post FA area is shown in Figure 7(D). This prediction is in very good agreement with the experimental observations of Tan et al. [8] (reproduced in Figure 7(E).

3.3 FA response to localised cell loading

In order to investigate the effect of localised loading on FAs, simulations are performed whereby fibroblasts are attached to an array of microposts and a displacement is applied to a single post. These simulations mimic the experimental study of Sniakecki et al. [11], which investigated the response of fibroblasts adhered to arrays with a 10 µm spacing, 1.5 µm post radius, and 32 nN/µm post stiffness. In the current study, simulations are performed for square cells adhered to a 5x5 array. Four cases are considered, as shown in Figure 8B: outwards movement of a corner post; inward movement of a corner post; outwards movement of a post at the midpoint of a cell edge; and inwards movement of the post at the midpoint of a cell edge. In all cases, a displacement...
magnitude of 1 µm is applied to the actuated post after the cell has reached a steady state level of SF and FA formation. The FA areas following a post movement relative to the FA areas immediately prior to the actuation are shown in Figure 8A. Simulations reveal that the change in area is highly dependent on both direction and post location. In the case of the corner post moving outwards, the actuated posts is acting against the SF tension and the increased tractions lead to an increase in FA area. In contrast, the inwards movement of the corner and edge posts lead to a reduction in traction and, consequently, lower SF tension, which leads to fibre dissociation (as shown in supplementary Figure S2) and a decrease in FA area. It should be noted that changes in area for all simulations are predicted to occur only on the moved post; the total change in area on the other posts is negligible. These observations are supported by Sniadecki et al. [11], where an increase in area was observed only on the moved post for single post actuation. The computed results presented in Figure 8 highlight the importance of considering both the location and direction of any applied deformation in single post actuation experiments.

3.4 Cells on microarrays subjected to biaxial stretch

A finite element model of a cell adhered to a 2x2 array was used to investigate the response of a cell to biaxial stretch. The micropost array in the current section of the study is the same as that used in the first results section and all microposts have a stiffness of 4.6 nN/µm. Initially, cells were simulated using the active and passive model parameters previously used to simulate SMCs, as shown in Table 1. Simulations consist of two steps: an equilibrium step lasting 600s, where SF formation is driven by an exponentially decaying signal; and a loading step of 3600s, where a biaxial strain of 7% is applied at a rate of 0.3 s\(^{-1}\) and the strain is then held constant over the remainder of the step. It should be noted that the prescribed strain is applied to the base of the posts and that the post is free to deform as the cell contracts or relaxes, as shown in Figure 9(A). In
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order to determine the effect of cellular signal during stretch, three stretch activated signalling regimes were considered during the second step of the analysis: a constant, fully activated signal; an exponentially decaying signal; and no signal.

The post force (normalized by the force at the end of the equilibrium step) is shown in Figure 9(B) for each simulation. In the second step of the simulation, for the case without a stretch activated signal, the post force rapidly increases and then decays down to value ~20% higher than the equilibrium value computed prior to stretching. In contrast, the simulations that have a stretch activated signal show a significant increase in the post force. In the case of the decaying signal, a higher equilibrium force is reached; approximately 2.7 times the original pre-stretching value. In the case of a constant signal during the second step, a continuous contraction of the cell and very large post deformations are computed. A recent study by Mann et al. [12] subjected SMCs on micropost arrays to 6% and 15% biaxial stretch and noted a decrease in the equilibrium post force after stretching. This observation is most closely captured by the simulation that does not contain a stretch activated signal. Comparing this experimental result to the computed forces in the current study suggest that a significant increase in cellular signalling does not occur with the onset of stretching. Furthermore, the observation of Mann et al. [12], whereby the post stretch force is lower than the pre-stretch equilibrium force cannot be captured using the active SF model in parallel with a passive hyperelastic component.

In order to predict the trends observed in the experimental study of Mann et al. [12], whereby the post stretch force is lower than the pre-stretch equilibrium force, the hyperelastic description of the passive cytoplasm component in the material formulation is replaced with a viscoelastic component. Two different viscoelastic models are used: a Maxwell model with no long-term modulus, and a SLS (standard linear solid) with a long term modulus that is half the instantaneous
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modulus. Figure 9(C) shows the post force before stretching, the peak immediately following stretch, and the force 3600s following stretch. The equilibrium force and peak force are not affected by the addition of the viscous component. The post force 3600s after loading decreases to 90% and 65% of the pre-stretch equilibrium value for the SLS and Maxwell models respectively.

The effect of strain magnitude is investigated in Figure 9(D) using the active model in parallel with the passive Maxwell cytoplasm. Peak force increases linearly with increasing strain and the force computed 3600s after stretching decreases with increasing applied strain. In the case of the smallest applied strain (1.5%), the post force after 3600s is ~15% above the equilibrium value. The larger applied strains of 7% and 15% lead to a reduction in force after 3600s to 65% and 40% of the pre-stretch equilibrium value. During this deformation, certain fibre orientations are shortening and therefore SF dissociation occurs, leading to a drop in cellular tension. This drop in tension leads to a decrease in post force and continued deformation of the viscoelastic cytoplasm, resulting in a continuous process of fibre shortening, tension reduction and SF dissociation. The change in SF formation between the equilibrium condition immediately prior to stretch and 3600s after stretch is shown in supplementary Figure S3 for different stretch magnitudes. Altering the loading rate was not found to alter the computed forces, as the elastic posts, which can be considered as acting in series with the active cell model, are the dominant factor in the instantaneous response (data not shown). Similar to the predictions in the current study, the experimental study of Mann et al. [12] observes a larger drop in post force 3600s after loading with increased applied strain, as shown reproduced in Figure 9(D). However, Mann et al. [12] observed that the peak force immediately after stretch was significantly smaller for 15% strain compared to 6% strain. A possible explanation for this is that the FA bonds rupture and reform during the application of the stretch, thus reducing the peak force. However, such bond kinetics are not presently considered in the FA formulation.
4 Concluding remarks

In the current study, a thermodynamically based focal adhesion model [28], combined with an active stress fibre contractility model [38], were used to successfully capture a number of experimentally observed phenomena for cells adhered to micropost arrays. The relationship between micropost stiffness, post tractions, and SF and FA formation, as observed by Fu et al. [10] and Han et al. [19], is successfully captured. In particular, the distinctive horse-hose shapes of FAs and the direction and magnitude of post tractions observed by Tan et al. [8] is correctly predicted. The computational framework is also used to simulate the response of the cell to both local and global externally applied loading. Following the movement of a single post, the FA area is seen to increase only on the moved post, similar to the observations of [11] and the direction of the movement is found to significantly alter the response. For cells subjected to biaxial stretch, the immediate increase and subsequent decrease of post force below the original equilibrium level, as seen by Mann et al. [12], is successfully simulated.

Acknowledgments

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Table 1 Material and interface properties for smooth muscle cells (SMCs), mesenchymal stem cells (MCSs), and fibroblasts (FBs).
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Figure 1 Schematic diagram showing simulation approach. The cell is simulated using the active SF material formulation that predicts SF formation in every direction at each point in the cell. Microposts are modelled as linear springs attached to rigid circular surfaces. Contact between the cell and posts is simulated using the FA interface model. (Online version in colour.)

Figure 2 Predicted stress fibre (SF) formation (A,C) and focal adhesion (FA) formation (B,D) in cells adhered to 5x5 and 13x13 arrays of microposts. Microposts have a 1 µm radius and 4 µm centre-to-centre spacing and a stiffness of 18.16 nN/µm. SFs and FAs are shown have reached a steady state following 600s of signal driven formation. (Online version in colour.)

Figure 3 Predicted stress fibre (SF) formation (A,C) and focal adhesion (FA) formation (B,D) in cells adhered to 13x13 arrays of microposts. Microposts have a 1 µm radius and 4 µm centre to centre spacing and a stiffness of 1556 nN/µm (A,B) and 1.9 nN/µm (C,D). SFs and FAs are shown having reached a steady state following 600s of signal driven formation. Individual post forces are shown for different levels of post stiffness as a function of distance from cell centre (E). (Online version in colour.)

Figure 4 Average force per post (A) for and total force per cell (B) for individual cells adhered to micropost arrays of different stiffness as a function of both cell area (top axis) and number of posts (bottom axis). Experimental observations by [19] of average and total force for endothelial cells (C,D), reproduced with permission. (Online version in colour.)

Figure 5 Computed focal adhesion (FA) area for each cell for different post stiffness as a function of cell size (area – top axis, number of posts – bottom axis) (A). The experimental observations of FA area as a function of cell size by Fu et al. [10] are reproduced for comparison (C). The total force (i.e., the sum of the magnitudes of the individual post forces) is shown as a function of the total adhesion area for a range of cells adhered to micropost arrays with different post stiffness (B). A linear trend line is fitted to each group of cells on a given micropost stiffness (solid lines). The slope of these trend lines, which is also known as the “FA stress”, is presented as a function of post stiffness (D). Finally, the experimental results of Fu et al. [10] (reproduced with permission) are presented for comparison (E); note that the experimentally observed FA stress is computed in the same way. (Online version in colour.)

Figure 6 Predicted stress fibre formation (A) in an irregularly shaped cell geometry. The orientation of the dominant, or most activated, fibre at each point is shown as a vector plot. The length of each vector corresponds to the SF activation level in the dominant direction. (Online version in colour.)

Figure 7 Comparison of computed FA formation (A) and experimental observations of focal adhesion formation (B). Predictions show the dimensionless concentration of high affinity integrins ($\xi$). Experimental images are reproduced from Tan et al. [8]. In both the computed and experimental FA images, the reaction force exerted by the cell on each post is shown as a vector superimposed over the image. In order to highlight the distinctive horseshoe shaped adhesions, magnified views (150% of original size) of two regions identified in purple in (A) are shown in (C) for both the experimental and predicted results. For each individual post adhered to the cell, the
computed force and FA area are shown in (D) together with the corresponding experimental forces and FA areas observed by Tan et al. [8] (reproduced with permission). (Online version in colour.)

Figure 8 Focal adhesion areas for a cell on an array of microposts where a single post is subjected to an applied displacement (A). FA areas are normalized by the steady state area immediately prior to stretch are presented for the moved post and the remaining unmoved posts. In four separate simulations, a post at either the corner of the cell or midway along a cell edge is moved either inwards or outwards, as shown in (B). For comparison, the experimental results of Sniadecki et al. [11] are reproduced with permission (C). (Online version in colour.)

Figure 9 Cells are simulated adhered to a micropost array which is subjected to biaxial stretch, as shown in (A). Post forces are present for three separate simulations with different stretch activated signals (B): a constant signal triggered by the applied stretch at 600s; an exponentially decaying signal, also initiated at 600s; and with no stretch activated signal. Post forces are normalised by the equilibrium post force immediately prior to the application of the 7% strain at 600s. Simulations are also performed for a 7% strain using different material formulations to represent the passive cytoplasm (C). Forces are presented for the equilibrium force prior to stretch, the peak force following the stretch, and the force 3600s after the stretch is applied. For the case of the Maxwell model viscoelastic cytoplasm, simulations are performed for three different strain magnitudes (D). The experimentally observed post forces following biaxial strain are also adapted and reproduced for comparison [12]. (Online version in colour.)
### Tables

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Figures

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Figure2.tif
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Figure 3.tif

A

B

C

D

E

Post stiffness
- 1556 nN/μm
- 18.16 nN/μm
- 1.9 nN/μm

Individual post force (nN)

Distance from cell centre (μm)

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Figure 4.tif

A

Cell spread area (μm²)

Average force per post (nN)

Number of posts - N

Post stiffness (nN/μm)
- 1556
- 18.16
- 11.49
- 3.8
- 1.9

B

Cell spread area (μm²)

Total force (nN)

Number of posts - N

Post stiffness (nN/μm)
- 1556
- 18.16
- 11.49
- 3.8
- 1.9

C

Total Force (nN)

Area (μm²)

D

Stiffness (nN/μm)

Avg Force (nN)

Area (μm²)
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Figure 5: A) Cell spread area (μm²) vs. FA Area per cell (μm²) vs. Number of posts - N. B) Total force (nN) vs. FA Area (μm²) vs. Post stiffness (nN/μm). C) Total FA area per cell (μm²) vs. Cell spread area (μm²). D) FA Stress (nN/μm²) vs. Post stiffness (nN/μm). E) FA Stress (nN/μm²) vs. Post stiffness (nN/μm).
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Figure 9.tif

A Posts remain free to deform during and after applied strain
B Biaxial strain applied to base of each post

Signal:
- Constant
- Decaying
- None

C Normalised post force

D Normalised traction force

Passive cytoplasm component

Applied Biaxial Strain

Hyperelastic (SLS) Viscoelastic (Maxwell)
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