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<td><strong>Author(s)</strong></td>
<td>Ronan, William</td>
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<td><strong>Publication Date</strong></td>
<td>2012-10-21</td>
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<td><strong>Item record</strong></td>
<td><a href="http://hdl.handle.net/10379/3718">http://hdl.handle.net/10379/3718</a></td>
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The Response of Cells to the Mechanical Environment:
A Numerical Investigation of the Actin Cytoskeleton and Cell Adhesion

By

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B.E. National University of Ireland, Galway, 2008

A thesis submitted to the National University of Ireland as fulfilment of the requirements for the Degree of Doctor of Philosophy

Mechanical and Biomedical Engineering

College of Engineering and Informatics

National University of Ireland, Galway

2012

Supervisor of Research: Dr. J. P. McGarry

Head of Discipline: Prof S. B. Leen
Abstract

Numerous in-vitro studies have established that cells possess the ability to sense and react to their physical environment. However, the mechanisms underlying such mechanotransduction are poorly understood. Previous cell models neglect the active biomechanical cell processes that allow the cell to interact with the physical environment; therefore, ad-hoc adjustment of passive material properties is required.

The main objective of this thesis is to develop and implement a computational framework that incorporates stress fibre (SF) contractility and remodelling and focal adhesion (FA) formation and evolution in a fully 3D environment to better understand the biophysical processes underlying the mechanical behaviour of cells. SF formation in this fully predictive implementation is allowed to occur in any direction at every point in the cell cytoplasm. A thermodynamic FA formulation is expanded to consider both specific and non-specific adhesion dynamics under mixed mode conditions. The FA implementation is also entirely predictive; FA formation is driven by SF contractility and remodelling.

Compression of single cells is simulated for round and spread cells, and for fully 3D elongated and irregularly shaped cells. The effect of cell shape and contractility on the compression response of cells is examined, revealing that tension in dominant SF bundles acts to restrict the deformation of the cell, increasing the resistance to compression. The 3D framework successfully parses the contributions of SF contractility, the nucleus, and the cytoplasm to the mechanical behaviour of osteoblasts.

The mixed mode FA interaction model is used with the 3D SF framework to examine the effect of substrate stiffness on SF and FA formation. Results reveal that SF contractility plays a critical role in the substrate-dependent response of cells. Compliant substrates do not provide sufficient tension for stress fibre persistence, causing dissociation of stress fibres and lower focal adhesion formation. The simulations elucidate the link between substrate stiffness, SF formation, and nucleus stress, providing insight into the relationship between substrate stiffness and regulation of stem cell differentiation observed experimentally.

The predictions of this mutually dependent material-interface framework are strongly supported by experimental observations of cells adhered to elastic substrates and of cells subjected to whole cell compression.
Acknowledgements

I would like to sincerely thank my supervisor, Pat, who lured me into cell mechanics for an undergraduate research project many years ago. Pat has provided an unrivalled level of support, time, and knowledge over the years that it took to prepare this thesis.

I would like to thank Prof. Bob McMeeking for giving me the opportunity to work and study with him in UCSB. His immense knowledge of mechanics and modelling (not to mention his location in California) have been invaluable. I would also like to thank Dr. Vikram Deshpande, who has provided insightful analysis and suggestions about the work in this thesis. I would like to thank everyone I have had the pleasure of co-authoring journal articles with for all their input.

I would like to thank all the academic, technical, and administrative staff in the department formerly known as the Department of Mechanical and Biomedical Engineering; in particular: Prof. Peter McHugh, Prof. Sean Leen, and Dr. Mark Bruzzi for their advice and encouragement throughout my time in NUI Galway.

I would like to thank all the people I’ve shared offices with, had lunch with, and gone for tea with over the years, particularly those who joined me in exile out of Nuns’ Island and all in the McGarry group.

I would like to thank the following funding sources: the Irish Research Council for Science and Engineering Technology, through the EMBARK Initiative Scholarships; the SFI/HEA Irish Centre for High-End Computing (ICHEC) for the provision of computational facilities and support; the Science Foundation Ireland Research Frontiers Programme; the Science Foundation Ireland Short Term Travel Fellowship; the NUI Galway International Affairs Office; the University of California Education Abroad Program; and South Tipperary County Council through the Higher Education Grants Scheme. I would also like to thank the ASME Summer Bioengineering Conference for the opportunity to compete in the Student Ph.D. Paper competition in 2010 and 2011.

Finally, and most importantly, I would like to thank my mother, Ann, for all her support (emotional, nutritional, and financial) and for her selfless efforts to provide myself and my sister (not a real Dr) Sarah with the opportunities we have had.
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## Abbreviations and nomenclature

### Abbreviations

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<th>Description</th>
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<tr>
<td>1D, 2D, 3D</td>
<td>One, two, three dimensions (dimensional)</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>CH</td>
<td>Chondrocyte</td>
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<tr>
<td>Cyto</td>
<td>Cytoplasm</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>Eqn</td>
<td>Equation</td>
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<tr>
<td>FA</td>
<td>Focal adhesion</td>
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<tr>
<td>FB</td>
<td>Fibroblast</td>
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<tr>
<td>FE</td>
<td>Finite element</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal stem cell</td>
</tr>
<tr>
<td>Nuc</td>
<td>Nucleus</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary differential equation</td>
</tr>
<tr>
<td>PVW</td>
<td>Principle of virtual work</td>
</tr>
<tr>
<td>RVE</td>
<td>Representative volume element</td>
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<tr>
<td>SF</td>
<td>Stress fibre</td>
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<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
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<tr>
<td>Subs</td>
<td>Substrate</td>
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<td>VM</td>
<td>von Mises</td>
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### Nomenclature -

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<th>Symbol</th>
<th>Description</th>
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<tr>
<td>$C_{10}, D_1$</td>
<td>Hyperalstic material constants</td>
</tr>
<tr>
<td>$\delta_{ij}$</td>
<td>Kronecker delta</td>
</tr>
<tr>
<td>$E$</td>
<td>Young’s modulus</td>
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<td>$J$</td>
<td>Jacobian</td>
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<td>$K$</td>
<td>Bulk modulus</td>
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<td>Green’s strain</td>
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<td>$F$</td>
<td>Deformation gradient</td>
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<tr>
<td>$P$</td>
<td>First Piola-Kirchoff stress</td>
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<tr>
<td>$R$</td>
<td>Rotation tensor</td>
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<tr>
<td>$S$</td>
<td>Deviatoric stress</td>
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$U$ Right Cauchy stretch tensor 

$V$ Left Cauch stretch tensor 

$n$ Surface normal 

$\mathcal{U}$ Strain energy potential 

$\mu$ Shear modulus 

$\nu$ Poisson’s ratio 

$\sigma$ Cauchy stress 

$\tau$ Second Piola-Kirchoff stress 

$\tilde{k}_v$ Slope of tri linear contractility model 

$k_f, k_b$ Kinetic equation constants (forwards and backwards) 

$\eta$ Stress fibre activation level 

$\bar{\eta}$ Average SF activation level 

$\sigma_0$ Istometric fibre tension 

$\sigma_{\text{max}}$ Maximum possible SF tension 

$\Pi$ SF variance 

$C$ Signal magnitude 

$\theta$ Decay constant 

$\omega, \phi$ Angles/ spherical coordinates
1 Introduction

Mechanotransduction is an essential component of cellular functions such as cell motility (Lo et al. 2000); and the physical environment around the cell has been shown to direct stem cell lineage specification (Engler et al. 2006; Discher et al. 2005). Previous experimental studies have established that cells can sense the stiffness of underlying substrates and that cellular tractions depend on substrate stiffness (Tee et al. 2011; Discher et al. 2005). The contractile response of cells to ECM stiffness has been shown to be an important factor in wound healing (Danjo and Gipson 1998), atherosclerosis (Isenberg et al. 2009), and cancer progression (Paszek et al. 2005; Levental et al. 2009). Furthermore, static and dynamic compression of chondrocytes has been shown to effect the regulation of type II collagen and aggrecan gene expression (Shieh and Athanasiou 2007). Previous studies have also shown that compressive loading induces mineralization of osteoblastic cells (Gabbay et al. 2006) and is an effective up-regulator of osteogenesis (Rath et al. 2008). Clearly, these studies show that the physical environment plays a critical role in regulation of cell behaviour.

Despite such extensive experimental evidence, the underlying biomechanisms by which cells respond to the mechanical stimuli are poorly understood. Cell mechanics modelling has been used in an effort to provide insightful interpretation of experimental results. In particular, single cell experimentation has demonstrated that the cytoskeleton plays a significant role in the mechanical behaviour of cells (Dowling et al. 2012; Guilak 1995), also provides a mechanical link from the nucleus to the extracellular matrix (Buxboim et al. 2010). However, despite such experimental evidence of the importance of the cytoskeleton, previous computational investigations have overlooked the key active biomechanical features of the cytoskeleton, with many studies simply modelling the cytoplasm as a passive continuum (Caille et al. 2002; Mohrdieck et al. 2005). This approach gives limited insight into cell behaviour as it is necessary to artificially alter the material properties to account for remodelling of the cytoskeleton in different geometries or loading conditions (Caille et al. 2002; McGarry 2009; McGarry and McHugh 2008).
Recent computational investigations have included stress fibre (SF) and focal adhesion (FA) dynamics (Deshpande et al. 2008; Vernerey and Farsad 2011); however, these formulations have not been implemented in a framework that considers SF and FA formation in realistic cell geometries in 3D that include the nucleus and substrate interactions.

The recent studies of Deshpande et al. propose a novel computational model of contractile cytoskeleton behaviour based on the biochemistry of SF formation (Deshpande et al. 2007) and an FA formulation based on the thermodynamics of integrin interactions (Deshpande et al. 2008). These models are entirely predictive: the SF distributions and contractility are dynamically governed by cellular signalling and tension dependent dissociation; FAs form in response to tension in the cell. Previously, the SF material model has been used successfully to accurately predict the scaling of active cell tractions with cellular contractility and with substrate stiffness for cells adhered to arrays of microposts (McGarry et al. 2009). The SF and FA models have also been used to simulate SF distributions in cells on patterned substrates (Pathak et al. 2008). However, these studies have used a 2D implementation that doesn’t consider realistic cell geometries, therefore limiting the investigations to cases where the cell can be approximated as a thin planar structure. In the current study, these material and adhesion models are, for the first time, developed into a fully 3D framework. SFs are permitted to form in all possible directions in 3D space, and FAs are considered in the context of a cell adhering to and spreading on a substrate. This 3D framework allows for novel investigations of the response of cells to a variety of loading conditions and substrate characteristics.

Simulations presented in this thesis focus on the use of cell mechanics modelling to elucidate the role of the cytoskeleton in the mechanical behaviour of cells. This work is motivated by a number of key observations in the literature:

- Previous numerical investigation of compression of spread and round cells have artificially increased the apparent stiffness of spread cells to capture experimentally observed results (Caille et al. 2002; Darling et al. 2008). A
modelling framework that captures the behaviour of cells should not require such an ad-hoc modification of the material properties of the cell.

- Substrate stiffness has been shown to direct stem cell lineage specification (Engler et al. 2006), and also cause significant changes in SF and FA formation (Discher et al. 2005), as shown in Figure 1.1. The mechanisms underlying such mechanotransduction are poorly understood.

- The studies of Fu et al. (2010) and Han et al. (2012) show a clear relationship between FA area, cell tractions and substrate stiffness for cells seeded on micropost arrays (Figure 1.2,1.3). Cells on micropost arrays subjected to locally applied strains show a change in FA area only on actuated posts with no significant changes in FA area on un-actuated posts (Sniadecki et al. 2007), as shown in Figure 1.4. Computational investigation that considers tension dependent SF and FA remodelling is necessary to understand the role of the cytoskeleton in these phenomena.

1.1 Aim and objectives
The aim of the thesis is to develop and implement a computational framework that incorporates SF contractility and remodelling and FA formation and evolution in a fully 3D environment to better understand the biophysical processes underlying the mechanical behaviour of cells.

The specific objectives of the thesis are:

- To develop and implement a material model that considers SF formation in 3D space based on the kinetic formulation of Deshpande et al. (2007)
- To develop and implement a FA model that incorporates the specific and non-specific adhesion forces during mixed mode deformation of FAs.
- Analyse the role of SF contractility in the compressive resistance of spread and round cells.
- Present a methodology for validation and evaluation of the active and passive material parameters in a 3D loading scenario.
- Investigate the effect of substrate stiffness and substrate actuation for cells adhered to continuous substrates and to micropost arrays.
1.2 Thesis structure

In Chapter 2 an overview of the relevant mechanics theory, the finite element method, and of the published literature relating to cell mechanics is provided. A concise description of the SF and FA formulations developed by Deshpande et al. (2007; 2008) is given in the first half of Chapter 3. The details of the implementation of these formulations in a fully 3D environment, which was conducted by the author, is provided in the second half of Chapter 3.

In Chapter 4 the 3D stress fibre material model is utilised to investigate differences in SF evolution in a range of cell types with varying contractility. Simulations are performed for axisymmetric round and spread cells, and for a fully 3D elongated/polarised cell. The effect of cell shape and contractility on the compression response of cells is examined. The predictions of the active 3D framework are compared to published experimental data to illustrate the predictive capabilities of the model.

In Chapter 5 experimental observations of the compression of untreated cells and cells treated with cytochalasin-D are used to determine the active and passive material properties of osteoblasts. In order to determine material parameters, model predictions of active and passive cells are compared to the following experimental measurements: 1) the compression resistance of treated and untreated cells; 2) the change in cell morphology due to the removal of the contractile cytoskeleton. Simulations reveal that experimental cell compression, in tandem with an active computational framework, can be used to parse the contributions of the contractile cytoskeleton, the passive cytoplasm, and the nucleus.

In Chapter 6, a mixed-mode interface model that captures the evolution of focal adhesions (FAs) in parallel with a passive normal interface model is employed to investigate the behaviour of contractile cells on elastic substrates. The active mixed-mode interface model is implemented in tandem with a fully 3D constitutive material formulation that simulates the active remodelling of the actin cytoskeleton due to changes in cytoskeletal tension. The effect of substrate stiffness on (SF)
evolution in the cell cytoplasm and FA distribution at the cell-substrate interface is investigated. Additionally, results are presented for active cell spreading on rigid substrates.

In Chapter 7, 2D implementations of the SF and FA models are used to consider both SF and FA formation in cells adhered to static and dynamically loaded micropost arrays. The simulations investigate four experimentally observed phenomena: 1) cell behaviour on different sized arrays with a range of post stiffness; 2) SF and FA formation in irregularly shaped cells; 3) the response of cells to deformations applied locally to individual posts; 4) the response of cells to equibiaxial stretch.

Finally, a discussion of thesis as a complete body of work is presented in Chapter 8, outlining the novel contributions to the broad field of cell mechanics.

A number of appendices provide further details of the 3D material and interface models, including the FORTRAN subroutines developed, which were excluded from the main chapters for clarity. The reader should note that figures and a bibliography are provided at the end of each chapter.
1.3 Figures

Figure 1.1 A – Experimental images of FAs (vinculin) for cells seeded on substrates with different stiffness. B – corresponding images of actin SFs for each substrate. Note the nucleus is shown in blue in all images. Reproduced from Engler et al. (2006).

Figure 1.2 Experimentally observed relationship between total FA area and spread area for cells adhered to micropost arrays. Micropost arrays contain posts with either high, medium, or low stiffness. Reproduced from Fu et al. (2010).
Figure 1.3 Experimentally observed relationship between traction force and FA area for cells adhered to micropost arrays. Plots are shown for micropost arrays with a post stiffness of either 18, 11, or 4 nN μm⁻¹. Reproduced from Fu et al. (2010).

Figure 1.4 FA areas for actuated and un-actuated (magnetic) posts for cells with displacements applied to an individual post. Results are shown for a single actuation (left) and multiple actuations of the same post (right). Reproduced from Sniadecki et al. (2007).
1.4 References


1 – Introduction


2 Theory and background

2.1 Introduction

The present chapter presents an overview of continuum mechanics theory, the finite element method, the structure of the cell, and the state of the art of cell mechanics modelling. Relevant continuum mechanics theory is introduced (Section 2.2) as a precursor to the development of stress fibre and focal adhesion models. The specific equations underlying the cell material formulation and the focal adhesion interface formulation are presented later in Chapter 3. An overview of the finite element method is provided (Section 2.3) with a particular focus on non-linear material behaviour using the implicit method. A brief overview of the cell structure and key cellular components is provided in Section 2.4. Finally, different approaches to numerical modelling of cell mechanics in the published literature are presented in Section 2.5 with particular emphasis on recent developments that consider the cytoskeleton. In addition to the background literature presented in the current chapter, it should be noted by the reader that Chapters 4-7 contain detailed reviews of the literature that is directly relevant to each chapter.

2.2 Continuum Mechanics

2.2.1 Notation and nomenclature

The notation used in the current work is such that vectors, tensors, and matrix quantities are indicated with a bold face font and scalar quantities are indicated with italics. Vector and tensor components are indicated in a subscript font; quantities in two dimensions are indicated with Greek subscripts and quantities in three dimensions are indicated with Latin subscripts. Einstein summation convention is used for brevity in vector and tensor operations such that a dot product between two vectors is represented:

\[ \mathbf{u} \cdot \mathbf{v} = u_1 v_1 + u_2 v_2 + u_3 v_3 = \sum_{i=1}^{3} u_i v_i = u_i v_i \]  

(2.1)
A full list of abbreviations and nomenclature used in the current work is provided at the beginning of this manuscript.

### 2.2.2 Deformation and strain

Finite deformation kinematics describes the deformation of a body with respect to an earlier reference configuration, as shown in Figure 2.1. A point in the undeformed volume $V_0$ has a position vector $\mathbf{x}$ relative to a fixed system of axes $X_i$. Following deformation, the new location is given by the vector $\mathbf{y}$. The displacement vector $\mathbf{u}$ as a function of time can be then defined by:

$$u_i(x, t) = y_i(x, t) - x_i$$  \hspace{1cm} (2.2)

The velocity is then defined as the time derivative of $\mathbf{u}$:

$$\dot{u}_i = v_i = \frac{du_i}{dt}$$  \hspace{1cm} (2.3)

In order to further consider the deformation mapping from the undeformed reference configuration to the deformed current configuration, the deformation gradient $\mathbf{F}$ is defined as:

$$\mathbf{F} = \nabla \mathbf{y}; \quad F_{ij} = y_{i,j}$$  \hspace{1cm} (2.4)

which gives the change in position of a particle relative to its original location. The Jacobian of the deformation is given by the determinant of the deformation gradient $\mathbf{F}$ and is always positive for well defined deformations. The Jacobian also gives the volume change for the deformation:

$$\frac{V}{V_0} = J = \det(\mathbf{F})$$  \hspace{1cm} (2.5)

As $\mathbf{F}$ is a well defined second-order tensor ($\det(\mathbf{F}) > 0$), $\mathbf{F}$ can be decomposed such that:

$$F_{ij} = R_{ik}U_{kj} = V_{ik}R_{kj}$$  \hspace{1cm} (2.6)

where $\mathbf{U}$ and $\mathbf{V}$ are symmetric tensors and $\mathbf{R}$ is orthogonal. Therefore, the deformation can be considered as a rotation ($\mathbf{R}$) followed by a stretch ($\mathbf{U}$, the right stretch tensor) or a stretch ($\mathbf{V}$, the left stretch tensor) followed by a rotation ($\mathbf{R}$).
In order to describe the deformation of the vector \( dx_i \) to \( dy_j \) as shown in Figure 2.1, the difference in the squares of the lengths of the vectors can be written as:

\[
(dy)^2 - (dx)^2 = dy_k dx_k - dx_i dx_i
\]

\[
(dy)^2 - (dx)^2 = F_{ki} dx_i F_{kj} dx_j - dx_i \delta_{ij} dx_j
\]

\[
(dy)^2 - (dx)^2 = (F_{ki} F_{kj} - \delta_{ij}) dx_i dx_j
\]

Therefore, \( F_{ki} F_{kj} - \delta_{ij} \) can be considered to be a measure of stretch. The first term can also be written as the right Cauchy-Green deformation tensor:

\[
C_{ij} = F_{ki} F_{kj}
\]

The Green strain tensor is then defined as:

\[
E_{ij} = \frac{1}{2} (F_{ki} F_{kj} - \delta_{ij})
\]

The displacement gradient is defined as:

\[
B_{ij} = u_{i,j}
\]

\[
u_i = y_i - x_i
\]

\[
B_{ij} = y_{i,j} - x_{i,j}
\]

\[
B_{ij} = F_{ij} - \delta_{ij}
\]

Therefore, the Green strain can be rewrite as:

\[
E_{ij} = \frac{1}{2} \left( (B_{ki} + \delta_{ki})(B_{kj} + \delta_{kj}) - \delta_{ij} \right)
\]

\[
E_{ij} = \frac{1}{2} \left( (B_{ij} + B_{ji}) + (B_{ki} B_{kj}) \right)
\]

Therefore for small deformations, if \( |B|^2 \ll |B| \) and \( |B| \ll 1 \), the small strain tensor can be defined as:

\[
\varepsilon_{ij} = \frac{1}{2} (B_{ij} + B_{ji})
\]

\[
\varepsilon_{ij} = \frac{1}{2} (u_{i,j} + u_{j,i})
\]

The logarithmic strain can also be described:

\[
LE_{ij} = \ln(V_{ij})
\]

It is also useful to consider the stretch as a ratio:
This gives the stretch in a direction $n$. The principal stretches and associated
directions can be found by considering the eigenvalue problem:

$$(F^T \cdot F - \lambda I) \cdot n = 0 \quad (2.15)$$

It can also be shown that the left stretch tensor $V$ can be written in terms of the
principal stretches as the sum of three dyadic products:

$$V = \lambda_i n_i n_i^T + \lambda_{ii} n_{ii} n_{ii}^T + \lambda_{iii} n_{iii} n_{iii}^T \quad (2.16)$$

### 2.2.3 Trawions, stresses and invariants

A volume $V$ shown in Figure 2.2 is subjected to a surface force $F^S$ and is in
equilibrium. The vector $t$ is defined as the traction, or force per unit area, over the
internal surface $\beta$. Considering the equilibrium of forces gives:

$$F_i^S = \int_{\beta} t_i dA \quad (2.17)$$

The traction vector is related to the normal at a point of the surface by Cauchy’s
theorem:

$$t_i = \sigma_{ij} n_j \quad (2.18)$$

where $\sigma$ is a tensor, known as the stress tensor. It can also be shown that the
Cauchy stress tensor is symmetric. The Cauchy stress represents the force per unit
area in the deformed, or current, configuration. Other representations of the stress
state can also be considered. The first Piola-Kirchoff stress tensor measures the
force per unit reference area:

$$P = J\sigma \cdot F^{-T} \quad (2.19)$$
The nominal stress \( N = P^T \) is also given as the transpose of the first Piola-Kirchoff stress. However, \( P \) is not generally symmetric; therefore the second Piola-Kirchoff stress \( \tau \) is defined as:

\[
\tau = F^{-1} \cdot P = J F^{-1} \cdot \sigma \cdot F^{-T}
\]

(2.20)

\[
\sigma = \frac{1}{J} F \cdot \tau \cdot F^T
\]

(2.21)

By considering the energy balance in a material and looking at the rate of work in a reference volume, pairs of conjugate stress and deformation tensors can be identified. The nominal stress \( N \) and the deformation gradient \( F \) form a conjugate pair, however the second Piola-Kirchoff stress \( \tau \) and the Green strain \( E \) provide a more useful conjugate pair.

In order to readily compare computed stress states in real geometries, it is useful to consider stress measures that are independent of the orientation of the coordinate system. The stress tensor is unique, but the components depend on the coordinate system. It is possible to show that a plane exists such that the traction vector is in the direction of the plane normal. Such a plane is not subjected to any tangential stress. Three such planes exist which satisfy this condition and these planes are orthogonal to each other. These planes are the principal planes and the traction on that plane is the principal stress. The principal stresses can be determined from the solution to the eigenvalue equation:

\[
(\sigma - \sigma I) \cdot n = 0
\]

(2.22)

The principle stresses \( \sigma \) can be found if the equation has a non-trivial solution; \( n \) (which gives the normal of the principal planes) must be non-zero and \( (\sigma - \sigma I) \) must not have an inverse. Therefore:

\[
\det(\sigma - \sigma I) = 0
\]

(2.23)

The characteristic equation of this problem may then be written as:

\[
\sigma^3 - I_1 \sigma^2 - I_2 \sigma - I_3 = 0
\]

(2.24)

The three coefficients \( I_1, I_2, \) and \( I_3 \) are the stress invariants and are given as:
These invariants may also be written in terms of the roots of the characteristic equation, i.e. the principal stresses:

\[ I_1 = \sigma_1 + \sigma_2 + \sigma_3 \]  
(2.28)  
\[ I_2 = -(\sigma_1\sigma_2 + \sigma_2\sigma_3 + \sigma_3\sigma_1) \]  
(2.29)  
\[ I_3 = \sigma_1\sigma_2\sigma_3 \]  
(2.30)

The stress can also be split into the hydrostatic stress, which causes volume changes, and the deviatoric stress, which causes changes in shape. The mean or hydrostatic stress, which is a scalar quantity, is defined as:

\[ \sigma_m = \frac{\sigma_{kk}}{3} = \frac{I_1}{3} \]  
(2.31)

The deviatoric stress \( S \) is then obtained:

\[ \sigma_{ij} = \sigma_m \delta_{ij} + S_{ij} \]  
(2.32)  
\[ S_{ij} = \sigma_{ij} - \sigma_m \delta_{ij} \]  
(2.33)

Finally, it is useful to consider a tensile equivalent stress \( \sigma_e \), which is also called the von Mises stress. It should be noted that calculating the first invariant of the deviatoric stress \( I_1(S) = 0 \). The equivalent stress can then be written as:

\[ \sigma_e = \sigma_{vm} = \sqrt{3I_2(S)} = \sqrt{\frac{3}{2}S_{ij}S_{ij}} \]  
(2.34)

### 2.2.4 Linear elasticity and hyperelasticity

A linear elastic model for finite deformation relates the second Piola-Kirchoff stress to the Green strain simply as:

\[ \tau = C : E \]  
(2.35)
This is known as the *Saint-Venant Kirchoff* model and it contains only geometric non-linearities. The stress is obtained from the energy function:

\[
S = \frac{\delta U(E)}{\delta E} = \frac{\lambda}{2} (\text{tr}(E))^2 + \mu E : E
\]  

(2.36)

where \(\mu\) and \(\lambda\) are the Lamè constants. It should be noted that the *Saint-Venant Kirchoff* model is not suitable for pure compression as it predicts unreliable results for large compressive strains (Colli et al. 2009).

In order to capture the nonlinear behaviour of compressible isotropic materials, a hyperelastic formulation is used. Hyperelastic materials are described in terms of a strain energy potential \(U(\varepsilon)\), which gives the strain energy stored per unit volume (i.e. the reference volume in the original configuration) as a function of the strain. There are numerous forms of strain energy potential and a more rigorous description and presentation of these is given in (Belytschko et al. 2000). The neoHookean form of the strain energy potential is used here; which is given as:

\[
\mathcal{U} = C_{10}(\mathcal{I}_1 - 3) + \frac{1}{D_1} (J - 1)^2
\]  

(2.37)

where \(C_{10}\) and \(D_1\) are material constants and \(J\) is the volume ratio or material Jacobian. The first deviatoric strain invariant is described as:

\[
\mathcal{I}_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2
\]  

(2.38)

\[
\lambda_i = J^{-\frac{1}{3}} \lambda_i
\]  

(2.39)

where \(\lambda_i\) are the principle stretches. The Cauchy stress can also be written:

\[
\sigma_{ij} = \frac{2}{J} C_{10} \left( \bar{B}_{ij} - \frac{1}{3} \bar{B}_{kk} \delta_{ij} \right) + \frac{2}{D_1} (J - 1) \delta_{ij}
\]  

(2.40)

where the deviatoric left Cauchy-Green tensor \(\bar{B}\) is determined from the deformation gradient \(F\):

\[
\bar{B}_{ij} = \frac{B_{ij}}{J^{2/3}} = \frac{F_{ik} F_{jk}}{(\epsilon_{mn} F_{1i} F_{2m} F_{3n})^{2/3}}
\]  

(2.41)

The elasticity constants are given in terms of Young’s modulus, \(E\), and Poisson’s ratio, \(\nu\), or in terms of the shear modulus, \(\mu\), and bulk modulus, \(K\), as:
2.2.5 Viscoelasticity

In viscoelastic materials, the stress is a function of strain and time. Mathematical models of viscoelasticity typically consist of spring and dashpot elements to represent the elastic and viscous responses of the material. Two common models, which are used in the current study, are the Maxwell model and the Standard Linear Solid (SLS), shown in Figure 2.3. The governing equation of the Maxwell model can be determined:

\[
\dot{\varepsilon} = \frac{1}{k} \dot{\sigma} + \frac{1}{c} \sigma \tag{2.43}
\]

The relaxation can then be expressed as:

\[
\sigma(t) = \sigma_0 e^{-t/\tau_r}; \quad \tau_r = \frac{c}{k} \tag{2.44}
\]

where \(c\) and \(k\) are the dashpot and spring constants. The governing equation of the SLS model can be written as:

\[
c_3 \dot{\sigma} + k_1 \sigma = c_3 (k_1 + k_2) \dot{\varepsilon} + k_1 k_2 \varepsilon \tag{2.45}
\]

where \(c\), \(k_1\) and \(k_2\) are the dashpot and spring constants, as shown in . The relaxation can then be expressed:

\[
\sigma(t) = \frac{\sigma_0}{k_1 + k_2} \left( k_2 + k_1 e^{\frac{k_1 t}{c_3}} \right) \tag{2.46}
\]

2.3 The finite element method and numerical solutions

The finite element method is used to solve the boundary value problems in the current work. The commercially available software Abaqus (Dassault Systemes, RI, USA) is used as it allows the user to define material behaviour not already included in the program library via user defined subroutines. Finite element programs can broadly be split into implicit and explicit solution methods. Explicit methods obtain values for dynamic quantities using only information available at the start of the increment, however, it should be noted that the central difference operator used by
Abaqus is only conditionally stable. The equations of motion in Abaqus\ Explicit are integrated as follows:

\[ \dot{\mathbf{u}}^{(i+\frac{1}{2})} = \dot{\mathbf{u}}^{(i \frac{1}{2})} + \frac{\Delta t^{(i+1)} + \Delta t^{(i)}}{2} \ddot{\mathbf{u}}^{(i)} \] (2.47)

\[ \mathbf{u}^{(i+1)} = \mathbf{u}^{(i)} + \Delta t^{(i+1)} \dot{\mathbf{u}}^{(i+\frac{1}{2})} \]

The accelerations at the beginning of the increment are calculated:

\[ \ddot{\mathbf{u}}^{(i)} = \mathbf{M}^{-1} \cdot (\mathbf{F}^{(i)} - \mathbf{I}^{(i)}) \] (2.48)

where \( \mathbf{M} \) is the diagonal lumped mass matrix, \( \mathbf{F} \) is the applied load vector, and \( \mathbf{I} \) is the internal force vector. As the lumped mass matrix is diagonal, it is trivial to invert; this provides the computational efficiency associated with the explicit method. In the current work, the implicit code Abaqus\ Standard is used and an overview is provided below.

2.3.1 Implicit solutions

The principle of virtual work provides the fundamental equation of the finite element method:

\[ \int_{V} \delta \mathbf{e}^T \sigma dV = \int_{S} \delta \mathbf{u}^T \mathbf{t} dS \] (2.49)

where the equilibrium is enforced on a reference volume, \( V \), which is bounded by a surface, \( S \), \( \sigma \) and \( \mathbf{t} \) are the stress and surface traction vectors respectively, while \( \delta \mathbf{e} \) and \( \delta \mathbf{u} \) are the virtual strain and virtual displacement vectors. The finite element approximation can now be introduced over the entire mesh and rewrite the displacements and strains:

\[ \delta \mathbf{u} = \mathbf{N} \delta \mathbf{u}_e \] (2.50)

\[ \delta \mathbf{e} = \mathbf{B} \delta \mathbf{u}_e \]

where \( \mathbf{N} \) is the global shape function vector and \( \mathbf{B} \) is the global shape function vector. The PVW then becomes:

\[ \int_{V} \delta \mathbf{u}_e^T \mathbf{B}^T \sigma dV = \int_{S} \delta \mathbf{u}_e^T \mathbf{N}^T \mathbf{t} dS \] (2.51)
The virtual displacement $\delta \mathbf{u}_e$ is arbitrary, therefore it can be stated:

$$\delta \mathbf{u}_e^T \int_V \tilde{B}^T \sigma dV = \delta \mathbf{u}_e^T \int_S \tilde{N}^T t dS$$

$$\delta \mathbf{u}_e^T \left( \int_V \tilde{B}^T \sigma dV - \int_S \tilde{N}^T t dS \right) = 0$$

The second term on the left of the equality is the external force vector and may also be written:

$$\int_S \tilde{N}^T t dS = \mathbf{F}$$  \hspace{1cm} (2.52)

If linear elasticity is introduced, it can be seen that:

$$\sigma_{ij} = C_{ijkl} \varepsilon_{kl}; \sigma = D \varepsilon = D \tilde{B} \mathbf{u}_e$$  \hspace{1cm} (2.54)

where $C_{ijkl}$ is a fourth order elasticity tensor and $D$ is a 6x6 matrix with $\sigma$ and $\varepsilon$ presenting the stress tensor in vector form, i.e Voight notation. For the case of linear elasticity the PVW can be rewritten as:

$$\int_V \tilde{B}^T D \tilde{B} \mathbf{u}_e dV - \mathbf{F} = 0$$

$$\left( \int_V \tilde{B}^T D \tilde{B} dV \right) \mathbf{u}_e - \mathbf{F} = 0$$

$$\mathbf{K} \mathbf{u}_e = \mathbf{F}$$  \hspace{1cm} (2.55)

which is the familiar form of the global finite element characteristic/stiffness equation. Returning to the PVW as shown in Eqn (2.52), it can be shown that:

$$\int_V \tilde{B}^T (\mathbf{u}_e) \sigma(\mathbf{u}_e) dV - \mathbf{F} = \mathbf{G}(\mathbf{u}_e) = 0$$  \hspace{1cm} (2.56)

where $\mathbf{G}(\mathbf{u}_e)$ gives us the residual forces vector. Therefore, a solution must be obtained for the non-linear set of equations:

$$\mathbf{G}(\mathbf{u}_e) = 0$$  \hspace{1cm} (2.57)
In Abaqus Standard, these equations are solved using an implicit solution scheme by applying loads in increments $\Delta t$ over the simulation time. Typically, an algorithm, such as the Newton Raphson method (or a modification of this), is used to step from time $t$ to time $t + \Delta t$ by taking an initial guess and iterating until the solution converges.

The Newton Raphson method is an iterative process where a better approximation to the solution is found by taking a tangent to the function and using this to find a closer solution. For an equation $f(x)$, each new iterated solution is found:

$$x_{n+1} = x_n - \frac{f(x_n)}{f'(x_n)} \quad (2.58)$$

Applying this method to the system of non-linear equations in Eqn (2.57) gives:

$$G(u^{t+\Delta t}) = 0 \quad (2.59)$$

For the $i^{th}$ iteration:

$$u_{i+1}^{t+\Delta t} = u_i^{t+\Delta t} - \left[ \frac{\partial G(u_i^{t+\Delta t})}{\partial u} \right]^{-1} G(u_i^{t+\Delta t}) \quad (2.60)$$

Considering the change in the nodal displacements $\partial u_{i+1}$ gives:

$$\partial u_{i+1} = u_{i+1}^{t+\Delta t} - u_i^{t+\Delta t} = - \left[ \frac{\partial G(u_i^{t+\Delta t})}{\partial u} \right]^{-1} G(u_i^{t+\Delta t}) \quad (2.61)$$

This can be rewritten in terms of the tangent stiffness matrix $K$:

$$K(u_i^{t+\Delta t}) = \left[ \frac{\partial G(u_i^{t+\Delta t})}{\partial u} \right]$$

$$K(u_i^{t+\Delta t})\partial u_{i+1} = G(u_i^{t+\Delta t}) \quad (2.62)$$

This gives the finite element equation that is solved during each iteration of the implicit method. It should be noted that, when comparing this form of the finite element equations to that given in Eqn (2.55), the solution variable here is the incremental displacement. Finally, the tangent stiffness matrix can be expressed:
where \( D^{tan} \) is called the consistent tangent matrix and \( \frac{\partial \sigma(\varepsilon)}{\partial \varepsilon} \) is the Jacobian of the constitutive law. It can be seen that for each iteration of each increment it is necessary to calculate and invert \( K \), which is computationally expensive. Two common modifications to the Newton Raphson method are: (i) the initial stress method, where \( K \) is calculated once at the beginning and used for each subsequent iteration in each increment; (ii) the constant \( K \) method, from the where \( K \) is calculated only at the start of each increment and the same matrix is used for each subsequent iteration. Both these methods may offer increased efficiency by reducing the cost of calculating \( K \), however, each increment may be slower to converge.

### 2.3.2 User defined subroutines in Abaqus

Abaqus offers the user the ability to define material behaviours not already included in the program library by means of user defined subroutines or a UMAT. During each equilibrium iteration, the subroutine is called by the main program for each integration point in the model. The deformation at the start of the increment and the increment of deformation during the increment is passed into the subroutine and the stress at the end of the increment, and the material Jacobian must be updated. The material Jacobian gives the change in the stress due to an infinitesimal perturbation of the strain increment. In Abaqus the material Jacobian is frequently written as \( \text{DDSDDE} \) and is defined as:
A full description of the variables that are passed into and updated with the *UMAT* subroutine is provided in Appendix A.

Abaqus also allows the user to define the contact or interaction behaviour between two surfaces (a master and slave surface) by means of a user defined interface subroutine or *UINTER*. The subroutine is called for each iteration for every point on a slave surface. The relative displacement between the two surfaces is passed into the routine and the interface stresses (or tractions) between the surfaces must be updated. The interface stiffness matrix $\text{DDSDDE}$ must also be updated and is defined as the change in the interface stresses due to an infinitesimal perturbation of the relative displacements.

### 2.3.3 Ordinary Differential equations – Runge Kutta method

An ordinary differential equation where an analytical solution is not readily available, such as:

$$
\frac{\mathrm{d}y}{\mathrm{d}x} = f(x, y)
$$

may be solved numerically. The Euler method advances the solution incrementally from $x_n$ to $x_{n+1} \equiv x_n + h$ using the formula:

$$
y_{n+1} = y_n + hf(x_n, y_n)
$$

This method, however, is not very accurate and can easily become unstable. The Euler method uses information about the derivative only at the beginning of the interval to step forward. The Runge-Kutta method takes a trial step to midpoint of the interval and uses the improved information to step across the entire interval. Using a single midpoint step is called the *second-order Runge-Kutta* method. A *fourth-order Runge-Kutta* method is commonly used to provide a reasonably accurate and fast method of numerically solving ordinary differential equations. The method is summarised as:
This method may be improved via implementation of an adaptive step-size control so that both accuracy and efficiency can be improved with fewer steps. Full details of this adaptive Runge-Kutta-Fehlberg method is given by Press (1996).

2.4 Cell structure

Eukaryotic cells consist of a number of organelles within a membrane. Organelles such as the nucleus, cytoskeleton, and cytosol, are important structural components of the cell and contribute significantly to the mechanical properties. Cells may also interact with a suitable substrate or extra-cellular matrix (ECM) through focal adhesions. A simplified schematic of a cell is shown in Figure 2.4 and a brief description of each component is given below.

2.4.1 Cytoskeleton

The cytoskeleton provides the main structural component of the cell and additionally provide a network for the transport of vesicles throughout the cell. The cytoskeleton can be broken down into three classes of protein filaments: actin filaments, intermediate filaments, and microtubules. These filaments remodel and interact to support and generate tension, and thus mechanically regulate the cell function and morphology.

Actin monomers form long helical filaments, which in turn are bound by other proteins to form a cross-linked gel, when bound by filamin, or to form bundles when bound by α-actinin and fimbrin (Alberts 2008). Actin, loosely packed by α-actinin, has sufficient space to interact with myosin II. Myosin II, through phosphorylation via light-chain kinase, assumes an activated state where the myosin tail is extended. The phosphorylated myosin spontaneously self-assembles

$$k_1 = h(x_n, y_n)$$
$$k_1 = h\left(x_n + \frac{h}{2}, y_n + \frac{k_1}{2}\right)$$
$$k_2 = h\left(x_n + \frac{h}{2}, y_n + \frac{k_2}{2}\right)$$
$$k_3 = h(x_n + h, y_n + k_3)$$
$$y_{n+1} = y_n + \frac{k_1}{6} + \frac{k_2}{3} + \frac{k_3}{3} + \frac{k_4}{6}$$  (2.67)
in bi-polar filaments, as shown in Figure 2.5. The bipolar myosin filaments interact with the loosely bound actin to form actin-myosin stress fibres (SFs), as shown in Figure 2.6. The cross-bridge cycling of myosin II generates tension in the stress fibre bundle. An experimental image showing SF formation by staining for actin is presented in Figure 2.7.

Microtubules are 20-25nm diameter cylindrical tube of tubulin dimers. The microtubules provide structural support within the cell and have a flexural rigidity of $2.2 \times 10^{-8}$ nN $\mu$m$^2$, compared to $7.3 \times 10^{-11}$ nN $\mu$m$^2$ for actin filaments, and a persistence length on the order of mm (Gittes et al. 1993). The dense network of microtubules throughout the cell is shown in Figure 2.8. Intermediate filaments range in size from 8 -10 nm in diameter and are thought to anchor the nucleus within the cell (Dupin et al. 2011), although their full role is unclear in the absence of a consensus in the literature (Eriksson et al. 2009).

2.5 Cell mechanics modelling

Computational models have been widely used to interpret experimental observations of cells (Vaziri et al. 2007a). Specific experiments have been modelled using analytical models, particularly for micropipette aspiration (Hochmuth 2000). Early models take material formulations which have been established for traditional engineering materials and apply them to cell mechanics: e.g. linear elasticity (Haga et al. 1998), hyperelasticity (Akkaş 1980), and viscoelasticity (Bausch et al. 1998). Early models also treated the entire cell as a homogenous material. Recent reviews provide an overview of cell mechanics models that were developed by applying existing continuum models to the cellular structure (Lim et al. 2006) and a synopsis of development of continuum based models in conjunction with different experiments (Vaziri et al. 2007a; Chen et al. 2012). Vaiziri et al also categorise models based on the approach taken, as shown below in Figure 2.9. An overview of key developments is provided below.

2.5.1 Passive liquid cell models

Liquid-drop models were developed in order to analyse the behaviour of suspended cells in micropipette aspiration, where the cell is pulled into the pipette using a
suction pressure. Reasonable success has been achieved by considering the cell as a drop of fluid within a membrane; commonly used models include the Newtonian, compound drop, Maxwell, and shear thinning liquid drop models.

The Newtonian liquid drop model considers the interior of the cell as a homogeneous linearly viscous fluid inside a membrane, which represents the outer cell cortex (Yeung and Evans 1989). This method was useful for modelling aspiration; however, the speed of the initial entry into the pipette observed experimentally is not reproduced in the model.

Compound liquid drop model: Dong et al. (1991) found that the earlier models could be improved by considering the main body of the cell as two separate materials; one a strongly viscous Newtonian fluid for the nucleus of the cell and a less viscous liquid droplet for the cytoplasm. This allows for more accurate modelling of the entry of the cell into the pipette, as the less viscous cytoplasm is deformed first. However, the large number of parameters involved in both materials makes model calibration very difficult. Figure 2.10 below shows the recovery of shape when the cell is removed from the pipette.

Shear thinning model: for larger deformations the previous models were found to differ from experimental data, and it was found that the viscosity of the cytoplasm was dependent on the shear rate (Tsai et al. 1993), and could be expressed as a power law of the shear rate. This was incorporated in the compound model and was applied using a finite element model to predict micropipette aspiration (Drury and Dembo 1999, 2001).

2.5.2 Viscoelastic/Viscoplastic models

The compound model above and its derivatives have come closer to modelling the initial deformations, however, the Maxwell fluid drop model has been found to more accurately reproduce the elastic like behaviour exhibited over the small deformations (Dong et al. 1991). The time dependent response of this model provides a closer match with experimental observations, however, this approach is
limited by the inherent limitations of the Maxwell model itself; e.g. limited recovery and no long-term stiffness.

Mofrad (2009) provides a review of models based on the rheology of the cytoplasm. Fabry et al. (2003) consider the cytoskeleton behaves like a soft glassy material that can be modelled by the soft glassy rheology model (SGR) as it obeys the following general rules:

- The material is soft (i.e. the modulus is in the Pa or kPa range)
- The loss tangent \( \tan(\delta) = G''(\omega)/G'(\omega) \) is nearly constant for a wide range of frequencies
- The frequency dependencies of these moduli are weak power laws of the frequency of the applied load
- The material displays an aging behaviour under certain conditions

Fabry et al (2003) found that the frictional (viscous) and elastic stresses were a result of the same underlying mechanism and that cytoskeletal proteins control the mechanical behaviour of the cell by changing an effective temperature (for the SGR) of the cytoskeleton.

The storage and loss moduli of the SGR are given as (Vaziri et al. (2007b):

\[
G'(\omega) = G_0 \left( \frac{\omega}{\omega_0} \right)^{x-1} \cos \left( \frac{(x - 1)\pi}{2} \right) & 1 \leq x \leq 2 \\
G''(\omega) = G_0 \left( \frac{\omega}{\omega_0} \right)^{x-1} \sin \left( \frac{(x - 1)\pi}{2} \right)
\]

2.5.3 Passive solid models

While liquid based models above are useful for simulating micropipette aspiration, they are not suited for other loading scenarios, such as compression or AFM indentation. Linear elastic models provide the simplest solid models and have previously been used for compression (Ofek et al. 2009) and AFM indentation (Solon et al. 2007). Hyperelastic models have been used more successfully to capture large deformations of cells during compression (Caille et al. 2002) and AFM (Kang et al. 2008). However, hyperelastic models do not capture the time dependent behaviour of cells; under compression chondrocytes have been shown to display a time-dependent creep response (Leipzig and Athanasiou 2005).
In order to capture the time dependent response of cells to mechanical loading, viscoelastic material models have been used for cell compression (Peeters et al. 2005) and micropipette aspiration (Guilak et al. 2002). Different viscoelastic models have been employed including the Standard Linear Solid (SLS) (Guilak et al. 2002), Maxwell (Vaziri and Mofrad 2007), and Kelvin-Voigt (Ragsdale et al. 1997) models.

Poroelastic materials consist of two interpenetrating continua: a solid containing a network of pores or voids, and a fluid which saturates those voids. The strain field in the solid material affects the fluid pressure and the solid stress. Poroelasticity has been used to model micropipette aspiration, and cyto-indentation (Baaijens et al. 2005; Koay et al. 2003)

While viscoelastic models capture the time dependent response of cells, they do not capture the difference in stiffness caused by cell spreading; in order to capture the compression behaviour of round and spread adhered cells it is necessary to artificially increase the modulus of spread cells compared to round cells. Therefore, it is necessary to account for the significant cytoskeletal remodelling that the cell undergoes as it changes from a round to a spread configuration (McGarry 2009; McGarry and McHugh 2008).

2.5.4 Cytoskeleton and active cell models

Previous passive models have only examined the apparent material properties of the cell; however, these properties do not reflect the true behaviour of the cell. Instead, the active cellular processes determine the observed mechanical response. Significant factors such as the polymerisation of actin filaments into stress fibres and the formation of focal adhesions have not been considered in these passive models. Through the use of cytoskeletal disrupting agents, it can clearly be shown that the cytoskeleton significantly contributes to the active mechanical response of cells (Dowling et al. 2012; Guilak 1995; Petersen et al. 1982).

The tensegrity model is based on an architectural system known as tensional integrity (Ingber 1993). The model incorporates isolated rigid struts, representing microtubules, which are connected together by elastic threads, representing actin-myosin stress fibres that are under tension. The model acknowledges that the
tension in the cell before external loading may be significant (Wang et al. 1993) and attempts to address previous shortcomings by considering cytoskeletal components. However, the discrete placement of the fibres requires a priori knowledge of the SF distribution in the cell and a new fibre network to be generated for each cell considered (McGarry and Prendergast 2004). The tensegrity model also assumes that SF tension is exclusively supported by microtubules, however, experimental investigation has shown that the disruption of microtubules results in an increase in the traction force generated by cells (Kolodney and Elson 1995).

A number of models have addressed the role of focal adhesions in regulation of the mechanical force balance in cells (Shemesh et al. 2005; Nicolas and Safran 2006; Bruinsma 2005). Bruinsma (2005) considers the activation of an FA through conformational changes in adhesion integrin due to applied forces. Shemesh (2005) uses a thermodynamic basis to account for the process of focal adhesion formation. However, the model only considers FA assembly and does not consider interplay between the cytoskeleton and the adhesions. Very few models look at both the focal adhesion formation and the contractile behaviour of the cell together in an integrated or cooperative formulation: Novak et al. (2004) proposed a model which incorporates a feedback loop between adhesion formation and the assembly of actin fibres. The inclusion of this feedback loop replicates two key phenomena observed in cell mechanics; the arrangement of long stress fibres, and the tendency for adhesions to form at the cell periphery. However, the model of Novak et al. assumes that stress fibres form randomly at adhesion sites in proportion to the size of the adhesion and dissociate at a constant rate. Mohrdieck et al. (2005) has developed a model for the actin cytoskeleton as a discrete set of fibres linking adhesion sites. However, this model assumes that a fibre connects each adhesion site to every other site in the cell and does not allow for changes in the cytoskeleton based on the underlying cell processes.

More recently, Kaunas et al. (2009) have proposed a kinematic model of SF formation. Fibres are initially randomly distributed throughout the cell and disassemble based on the stretch magnitude the fibre experiences. Disassembled
fibres immediately reform in a random orientation, keeping the mass fraction of SFs constant. Initially, all fibres are given a 10% prestrain to simulate the equilibrium tension in the cell. This formulation captures the reorientation of fibres subjected to cyclic stretch. An improved sarcomeric model of SF tension due to cross-bridge cycling describes the dependence of fibre tension on fibre strain (Kaunas et al. 2011). Recently, Tondon et al. (2012) have modified this formulation such that the dissociation of fibres is dependent on the sliding velocity and the tension in the fibre to investigate the effect of cyclic stretch waveform on fibre reorientation.

Vereney and Farshad (Vernerey and Farsad 2011) present a multiphasic formulation that considers actin monomer transport, cytosol fluid pressure, mass exchange of cytoskeletal components, and fibre contraction. Fibre tension is a function of both strain and strain rate, and new fibres are predicted to form in the direction of the maximum contractile stress. Fibre distributions at each point are described by a von Mises distribution with a preferential direction. The rate of fibre formation is a function of fibre stress in the given direction.

The model of De and Safran (2008) investigates a contractile force dipole acting in an elongated cell adhered to deformable substrate. The formulation considers the free energy of a cell subjected to an applied force. The cell, when subjected to either a high or low frequency cyclic stretch, will reorient such that the orientation of the cell relative to the applied loading minimizes the stress in the substrate. The simulations reveal that if the frequency of the applied stress is greater than the characteristic time of SF remodelling the cell with reorient; this mechanism allows the formulation to capture the alignment of cells subjected to high frequency stretching. Friedrich and Safran (2012) adapt the model of De and Safran (2008) to consider a cell with a uniform distribution of contractile dipoles within an unsymmetric cell geometry. Cells simulated with an initially isotropic distribution of dipoles are shown to develop an anisotropic fibre distribution.

A recent model for the contractile behaviour of the cytoskeleton proposed by Deshpande et al. (2006) is based on the key biochemical processes that underlie the mechanical behaviour of cells. The three key processes considered are: (i) actin
polymerisation triggering by an activation signal, (ii) tension dependent assembly of actin stress fibres, (iii) cross-bridge cycling between actin and myosin filaments that generates tension. The model is developed for the formation of a single stress fibre, but is generalised for a two dimensional continuum models. A focal adhesion model by the same authors considers the thermodynamics of bond formation, accounting for the mixing entropy and enthalpy of low-affinity and high-affinity integrins involved in the process (Deshpande et al. 2008). This model has previously been used for idealised 1D and 2D cell applications (Pathak et al. 2008; McGarry et al. 2009).

2.6 Conclusions

This chapter provides an overview of continuum mechanics theory, the finite element method, the structure of the cell, and the state of the art of cell mechanics modelling. A brief synopsis of the cell and the cytoskeletal components considered in this thesis are provided above. Additionally, this chapter provides a review of the current literature in cell mechanics and modelling, and recent developments in cell biomechanics considering the cytoskeleton are described. Once again, it should be noted by the reader that Chapters 4-7 contain detailed reviews of the literature that is directly relevant to the specific work presented therein. The mechanics and finite element concepts introduced in Section 2.2 and 2.3 are used in Chapter 3 for the development and implementation of a 3D framework for SF and FA formation and remodelling, following from the work of Deshpande et al. (2006, 2008). The detailed equations underlying these models are presented in the following chapter.
2.7 Figures

Figure 2.1 Finite deformation of a body with volume $V_0$ in the reference configuration that is mapped to the volume $V$ in the current configuration.

Figure 2.2 Surface forces acting on a volume $V$ (shown sliced in two) with internal traction $t$ shown at a point with surface normal $n$ on the internal surface $B$. 
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Figure 2.3 Mathematical models of viscoelasticity: Maxwell model left and Standard Linear Solid (SLS) right.

Figure 2.4 Schematic diagram of cell structure including stress fibres (SFs), focal adhesions (FAs), and the nucleus.
Figure 2.5 Phosphorylation of myosin II and self-assembly into bi-polar filaments (Alberts 2008)

\[ \sigma = \sigma_0 \quad \sigma < \sigma_0 \]

Figure 2.6 Formation of actin-myosin stress fibres under isometric tension (left) and fibres dissociating due to a drop in tension (right).
Figure 2.7 Osteoblasts stained for actin (red), vinculin (green) and nuclei (blue). Section views were taken across the nucleus centre (dashed lines) in all cases. Scale bar = 20 µm. Adapted from Weafer, Ronan, et al. (2012)

Figure 2.8 Fluorescence microscopy imaging of microtubule network in cardiomyocytes. Scale bar: 20µm. Adapted from Devillard et al. (2008)
Figure 2.9 Categorisation of modelling approaches in cell mechanics. Reproduced from Vaiziri et al. (2007)

Figure 2.10 Micropipette aspiration and recovery of a cell using liquid drop model. Reproduced from Dong et al. (1991).
2.8 References


2 - Theory and background


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3 Model development

3.1 Introduction

The present chapter presents the stress fibre (SF) and focal adhesion (FA) models developed by Deshpande et al. (Deshpande et al. 2006; Deshpande et al. 2008) and the implementation of these models in a fully 3D framework, by the author. Sections 3.2 and 3.4 below provide a synopsis of the previously published formulations. Section 3.3 details the work performed in the current study; a 3D framework is developed and the material model of Deshpande et al (2006) is implemented in an environment that considers 3D fibre distributions at each point. Development of the passive components of the material model that represent non-contractile components is also described. Finally, in Section 3.5, the thermodynamic formulation for focal adhesion formation is modified such that mixed mode interface behaviour is considered, including the slipping of a focal adhesion on a substrate. The individual finite element models including cell geometries are presented in each results chapter.

3.2 Stress fibre material model

The following section provides details of the SF model, as described by Deshpande et al. (2006).

3.2.1 Overview of actin myosin and formation.

The biochemistry of stress fibre formation suggests that the process consists of three coupled phenomena

i. An activation signal triggers the formation of the stress fibres
ii. A formation rate dependent on the activation signal, coupled with a dissociation rate
iii. A contraction rate or contractility for the stress fibres that depends on the tension

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:

$$C = e^{\left(\frac{-t_1}{\theta}\right)} \quad (3.1)$$

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

The contractile actin-myosin cytoskeleton is formed via the phosphorylation of myosin (Figure 3.1B,C) and polymerization of actin filaments (Figure 3.1A). The myosin self assembles into bipolar filaments (Figure 3.1D) that interact with actin filaments that are loosely bound with $\alpha$-actinin to form contractile actin-myosin bundles (Figure 3.1E, Figure 3.2).

The activation level of the fibres is given by $\eta$ ($0 \leq \eta \leq 1$), where $\eta = 1$ corresponds to the maximum possible stress fibre activation level allowed by the biochemistry. The activation level $\eta$ is assumed to be governed by the first order equation:

$$\dot{\eta} = (1 - \eta) \frac{C k_f}{\theta} - \left(1 - \frac{\sigma}{\sigma_0}\right) \eta \frac{k_b}{\theta} \quad (3.2)$$

The overdot denotes the rate of formation with respect to time, measured from the application of the signal. The first part of the right hand of the equation is governed by the rate of formation of the stress fibres and is controlled by the dimensionless constant $k_f$. The latter part of formation gives the rate of dissociation and is governed by the dimensionless constant $k_b$, and is also dependent on the stress level $\sigma$, and the maximum fibre stress generated for that degree of fibre activation $\sigma_0$. This means that if the stress falls the rate of dissociation will increase and cause the overall activation level to fall. The isometric tension $\sigma_0$ is given:

$$\sigma_0 \equiv \eta \sigma_{max} \quad (3.3)$$

Therefore, for the maximum activation level of $\eta = 1$ the stress fibres will exert the maximum stress $\sigma_{max}$. The model is described firstly for a single stress fibre and then generalised by conducting a homogenisation analysis. The key assumptions made in generalising the model are
3 - Model development

a) The formation of stress fibres is not limited by the availability of actin and myosin
b) The cell can be modelled as a continuum; a representative volume element can be defined
c) Stress fibres can form in any direction

3.2.2 Contractile behaviour of fibres

Cytoskeletal tension is essential for sustaining SF bundles and a reduction below a defined isometric level leads to fibre dissociation (Franke et al., 1984; Kolega, 1986). The contractile behaviour of assembled SF bundles is similar to that of skeletal muscle. The tension in the SF bundle $\sigma_f$, which is generated by cross-bridge cycling of actin-myosin pairs (Warshaw et al., 1990), is related to the bundle contraction rate using the following Hill-like equation (i.e. similar to the contractile behaviour of muscle as observed by Hill (1938):

$$
\frac{\sigma_f}{\sigma_0} = \begin{cases}
0 & \frac{\dot{\varepsilon}}{\varepsilon_0} \leq -\frac{\eta}{k_v} \\
1 + \frac{k_v}{\eta} \left(\frac{\dot{\varepsilon}}{\varepsilon_0}\right) - \frac{\eta}{k_v} & -\frac{\eta}{k_v} \leq \frac{\dot{\varepsilon}}{\varepsilon_0} \leq 0 \\
1 & \frac{\dot{\varepsilon}}{\varepsilon_0} > 0
\end{cases}
$$

(3.4)

where $k_v$ is a non-dimensional constant that gives the fractional reduction in the fibre stress if the shortening rate $\dot{\varepsilon}$ is increased by a reference strain rate $\dot{\varepsilon}_0$. The reduction in stress is given as a fraction of $\sigma_0$. This relation can be explained graphically as shown in Figure 3.2. When the fibre is undergoing stretch, the stress $\sigma$ is equal to $\sigma_0$, and when the fibre is contracting, the stress drops in proportion with the strain rate. If the fibre is contracted very quickly such that $-\frac{\dot{\varepsilon}}{\varepsilon_0} \geq \frac{\eta}{k_v}$ the stress will be zero, and the fibre will completely dissociate in accordance with Eqn (3.2)

3.3 Development of a 3D SF model

The following section describes implementation of the material model in 3D by the author. The SF biomechanical model described in the previous section is expanded to consider 3D fibre formation and the challenges of integration in 3D are
3 - Model development

described. The material behaviour is implemented as a user defined material within
the finite element program Abaqus (Dassault Systemes, RI, USA).

3.3.1 Definition of an arbitrary fibre within an RVE

A representative volume element (RVE) is defined as a sphere with radius \( \rho \)
containing SFs that pass through the centre of the sphere. The orientation of an
arbitrary fibre within the RVE is defined using the unit vector
\[ m = \sin(\omega) \cos(\phi) \mathbf{x}_1 + \sin(\omega) \sin(\phi) \mathbf{x}_2 + \cos(\omega) \mathbf{x}_3, \]
where \( \mathbf{x}_i \) are the unit base vectors for a Cartesian basis in the current configuration as shown in Figure
3.3.

3.3.2 Rotations and deformation of a single fibre with an RVE

As the stress used in defining the material behaviour is the Cauchy stress, which is
in the current configuration, it is necessary to keep track of rigid rotations of fibre
orientations. A rigid body rotation can be determined from the deformations
gradient \( F \) by:

\[ R_{ij} = F_{im} U_{mj}^{-1} \tag{3.5} \]

where \( R \) is the rotation tensor, and \( U \) is the stretch tensor. Further details of this
relationship are given in Chapter 2. A rotation of a coordinate system in 3D may be
considered as a combination of 3 rotations

1. Rotation by an angle \( \beta \) about the \( X_3 \) axis to give \( X' \)
2. Rotation by an angle \( \alpha \) about the \( X_2' \) to give \( X'' \)
3. Rotation by an angle \( \gamma \) about the \( X_1'' \) to give \( X''' \)

The rotation tensor for the first rotation axis may be expressed:

\[ R_1 = \begin{pmatrix} \cos \beta & \sin \beta & 0 \\ -\sin \beta & \cos \beta & 0 \\ 0 & 0 & 1 \end{pmatrix} \tag{3.6} \]

Similar expressions can be determined for the subsequent rotations and the
complete rotation expressed as:

\[ R = \begin{pmatrix} \cos \beta \sin \alpha & -\cos \alpha \cos \beta \sin \gamma + \cos \gamma \sin \beta & \sin \alpha \sin \beta \\ -\cos \alpha \cos \beta \cos \gamma + \sin \beta \sin \gamma & \cos \beta \cos \gamma - \cos \alpha \sin \beta \sin \gamma & \cos \alpha \sin \gamma \\ \cos \alpha \sin \beta \cos \gamma + \sin \beta \sin \gamma & -\sin \alpha \sin \gamma \cos \beta + \cos \gamma \sin \beta & \cos \gamma \sin \alpha \end{pmatrix} \tag{3.7} \]
Therefore, rigid rotations of the unit vector \( \mathbf{m} \) can be found using the above matrix to relate the orientation of the current coordinate system to the reference orientation. The changes to the spherical coordinates describing \( \mathbf{m} \) are given by the following relations:

\[
\begin{align*}
\Delta \omega &= \Delta \alpha - \frac{\pi}{2} = \cos^{-1} R_{13} - \frac{\pi}{2} \\
\Delta \phi &= \Delta \beta = \frac{\sin^{-1} R_{12}}{\sin \Delta \alpha}
\end{align*}
\] (3.8)

The strain rate in a fibre in an arbitrary orientation may be determined by considering the material strain rate in a separate coordinate system where the fibre direction is aligned with the 1-direction of the system. Therefore, the first component in the rotated strain rate tensor will give the strain rate in the fibre direction. The fibre strain rate is then expressed in terms of the original material strain rate as:

\[
\dot{\varepsilon}_f = \dot{\varepsilon}_{ij} m_i m_j = \dot{\varepsilon}_{11} \sin^2 \omega \cos^2 \phi + \dot{\varepsilon}_{22} \sin^2 \omega \sin^2 \phi + \dot{\varepsilon}_{33} \cos^2 \omega + 2\dot{\varepsilon}_{12} \cos \phi \sin \phi \sin^2 \omega - 2\dot{\varepsilon}_{23} \sin \omega \cos \omega \sin \phi - 2\dot{\varepsilon}_{13} \sin \omega \cos \omega \cos \phi
\] (3.9)

### 3.3.3 Numerical integration and fibre orientations at a single point

A typical integral over the sphere may look like:

\[
I = \int_0^\pi \int_0^\pi f(\omega, \phi) \sin(\omega) d\omega d\phi
\] (3.10)

The \( \sin(\omega) \) term accounts for the large number of directions present at each pole. If \( f \) is integrated numerically using the trapezoidal rule:

\[
\int_a^b f(x) dx = \frac{(b - a)}{N} \left( \frac{f(x_0)}{2} + f(x_1) + \cdots + f(x_{n-1}) + \frac{f(x_n)}{2} \right)
\] (3.11)

For example, if \( \omega \) and \( \phi \) are defined at 18 intervals between 0 and \( \pi \), there would be 18 directions coincident on each pole.
However, the inclusion of the $sin(\omega)$ term means that at $\omega = 0$ the integral is forced to zero. Furthermore, the large number of fibres near the pole compared to the fewer fibres near the equator, as highlighted in Figure 3.4, introduce a bias into the integration. This bias also leads to computational inefficiencies, as the density of fibres required near the equator will lead to superfluous fibres near the pole. Therefore, in order to accurately and efficiently perform an integral over a sphere, it is necessary to consider evenly spaced fibres, or points on the sphere surface.

The idea of “evenly spaced” is simple to visualise for a 2D scenario. The distance from any point to its neighbour on either side must be the same. However, this does not easily expand for a 3D scenario. Different algorithms have been proposed to give different types of even distributions. Examples include considering each point as a charged particle and letting the repulsive forces move the points until equilibrium has been reached, or placing n points on a sphere so as to minimize the maximum distance of any point on the sphere from the closest one of the n points. Hardin and Sloane (1996) present an algorithm for determining N points on a sphere in order to perform a numerical integration using equal weights. These set of points are commonly called a spherical t-design and are more formally described: “A set of N points is called a spherical t-design if the integral of any polynomial of degree at most t over the sphere is equal to the average value of the polynomial over the set of N points”. In the current work, such a set containing 240 points is identified and used to perform the integration. As each fibre can be considered to pass through the centre of the sphere and onto the opposite surface, it is only necessary to consider points/fibres in one half of the sphere. Figure 3.5 shows 120 unit length fibres in a hemisphere. Therefore, the integral $I$ in Eqn (3.10) can be rewritten as the following sum:

$$I = \frac{2\pi^2}{nm} \sum_{k=1}^{m_k} \sum_{l=1}^{n_l} \sigma(\omega_k, \phi_l) m_l^* m_j^* sin(\omega_k)$$

(3.12)
where $\omega_k$ and $\varphi_k$ give the spherical coordinates of the point $k$ on the sphere surface.

The total active stress in the RVE can be calculated by integrating the contribution of each fibre over the volume of the RVE:

$$\sigma_{ij} = \frac{1}{V} \int_V \sigma_f(\omega, \phi) m_i m_j \, dV \quad (3.14)$$

Instead of evaluating this integral by integrating over both spherical coordinates, the numerical method described in Eqn (3.13) is employed. Using this method to sum the contribution of each fibre to calculate the total active stress gives:

$$\sigma_{ij}^A = \sum_{k=1}^{n} \sigma_f(\omega_k, \varphi_k) m(\omega_k, \varphi_k)_i m(\omega_k, \varphi_k)_j \quad (3.15)$$

A single element test where each node of the element is held rigid and SF formation is driven by an exponentially decaying signal is performed using this numerical integration and the double integration defined earlier. The results of these simulations are compared in Figure 3.6. Using the double integral over both spherical coordinates results considers 196 fibre directions (18 for each angle), however, there is a significant difference in the computed direct stresses (~1%), as shown in the magnified inset view in Figure 3.6. In contrast, the integration using the evenly distributed points considers 120 directions and a smaller error is computed (<<0.1%). In all further simulations in the current work, numerical integration of the active stress is performed using Eqn (3.15) with $n=120$.

### 3.3.4 Alternative forms of the tri linear SF contractility model

The tri-linear Hill-type model for SF contractility, described above in Eqn (3.4), is difficult to implement in a numerical formulation as the stress remains constant for a positive strain rate, i.e. when the strain is increasing, which leads to convergence problems. In order to implement this behaviour it is necessary to introduce a small strain rate dependence which modifies the equation such that:
The $\lambda$ term introduces a slope to horizontal areas of the curve shown in Figure 3.2, as shown in Figure 3.7.

However, these modifications could possibly lead to numerical errors at very high strain rates; the change in stress introduced to improve convergence may reach multiples of the maximum fibre stress. In order to reduce the possibility of such errors, alternative forms of the contractility model were developed.

The first approach to reducing the error due to the change in slope was to vary the slope introduced in Eqn (3.16) based on the strain state. This was achieved by modifying the $\lambda$ term in Eqn (3.16) such that:

$$\lambda = \lambda^* \exp \left| \frac{\varepsilon_f}{\varepsilon_0} \right|$$

(3.17)

where $\lambda^*$ and $\varepsilon_0$ are constants. This method allows for a larger slope to be specified at the beginning of the simulation, which is prone to convergence problems. As the material contracts and undergoes strain, the value of $\lambda$ increases such that the modification becomes less significant. However, this method is not suitable where an external load is applied later in the simulation. It is possible for the fibre strain to return back through zero, leading to artificially increased tension at zero strain.

The following modification modifies the contractility model in Eqn (3.4) such that the tri-linear relationship is replaces with a continuous function. An inverse tan function provides similar strain rate behaviour as the tri-linear model and the function is continuous and smooth. The contractile behaviour is defined:

$$\frac{\sigma_f}{\sigma_{max}} = \frac{\eta}{\alpha} \left[ \tan^{-1} \left( \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} + \eta \right) + 1 \right]$$

(3.18)

$$\alpha = \frac{\pi}{4} + 1$$
The constant $\alpha$ is introduced to ensure that $\frac{\sigma_f}{\sigma_{max}} = 1$ when $\dot{\varepsilon} = 0$. The resulting stress is shown in Figure 3.8 as a function of strain rate.

### 3.3.5 Constitutive Jacobian terms

The contribution of the active stresses to the constitutive Jacobian can be determined using a central difference operator:

$$f_{t+\Delta t} = \frac{\Delta f}{\Delta t}; f_{t+\Delta t/2} = f_t + \frac{\Delta f}{2} \quad (3.19)$$

Using the Voigt stress notation (with the ABAQUS numbering convention) where the stress tensor is represented as a vector, the active component of the Jacobian can be written using the chain rule as:

$$\frac{\partial \Delta \sigma_k}{\partial \Delta \varepsilon_j} = \sum_{k=1}^{n} \frac{\partial \Delta \sigma_k}{\partial \Delta \sigma} \left( \frac{\partial \Delta \sigma_f}{\partial \Delta \varepsilon_f} \right)_k \frac{\partial \Delta \varepsilon}{\partial \Delta \varepsilon_j} \quad (3.20)$$

where $(\frac{d\Delta \sigma_f}{d\Delta \varepsilon_f})_k$ is the derivative of the stress in the $k$th fibre with respect to the strain in that fibre. As the active stress depends on the tri-linear equation, as shown above (3.16), the material Jacobian must be determined for each of the three sections.

#### 3.3.5.1 Section 1

For large negative strain rates, the stress is given from Eqn 3.16 as:

$$\sigma = \eta \frac{\sigma_{max}}{\lambda} + \frac{\sigma_{max}}{\lambda} - \frac{k_v}{\varepsilon_0} \dot{\varepsilon}; \quad \frac{\dot{\varepsilon}}{\varepsilon_0} \leq -\frac{\eta}{k_v} \quad (3.21)$$

Applying the central difference operator:

$$\sigma_t + \frac{\Delta \sigma}{2} = \left( \eta_t + \frac{\Delta \eta}{2} \right) \frac{\sigma_{max}}{\lambda} + \frac{\sigma_{max}}{\lambda} - \frac{k_v}{\varepsilon_0} \Delta \varepsilon \quad (3.22)$$

Which gives the change in stress as:

$$\Delta \sigma = 2\sigma_t + \frac{2\sigma_{max}}{\lambda} \left( \eta_t + \frac{\Delta \eta}{2} \right) + \frac{2\sigma_{max}}{\lambda} k_v \frac{\Delta \varepsilon}{\varepsilon_0 \Delta t} \quad (3.23)$$

Taking the derivative with respect to $\Delta \varepsilon$ gives:
The derivative of $\Delta \eta$ is determined by first considering:

$$\frac{\partial \Delta n}{\partial \Delta \varepsilon} = \frac{\partial \Delta n}{\partial \Delta \sigma} \frac{d \Delta \sigma}{d \Delta \varepsilon}$$

$$\dot{\eta} = (1 - \eta_t) \frac{k_f c}{\theta} - \eta \frac{k_b}{\theta}$$

and applying the central difference operator:

$$\frac{\Delta \eta}{\Delta t} = \left(1 - \eta_t - \frac{\Delta \eta}{2}\right) \frac{k_f c}{\theta} \left(C_t + \Delta C \frac{2}{2}\right) - \left(\eta_t + \frac{\Delta \eta}{2}\right) \frac{k_b}{\theta}$$

which can be reorganized:

$$C^* = C_t + \frac{\Delta C}{2}$$

$$\Delta \eta = \left[1 + \frac{k_f c}{2\theta} + \frac{k_b}{2\theta}\right]^{-1} \left(1 - \eta_t \frac{k_f C^*}{\theta} - \eta \frac{k_b}{\theta}\right)$$

Therefore:

$$\frac{\partial \Delta \eta}{\partial \Delta \sigma} = 0$$

$$\frac{\partial \Delta \sigma}{\partial \Delta \varepsilon} = \frac{2\sigma_{max} k_v}{\lambda \dot{\varepsilon}_0 \Delta t}$$

### 3.3.5.2 Section 2

For small negative strain rates, the stress is given:

$$\sigma = \eta \sigma_{max} + \frac{k_v \sigma_{max}}{\dot{\varepsilon}_0} \dot{\varepsilon}; \quad -\frac{\eta}{k_v} \leq \frac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \leq 0$$

Applying the central difference operator:

$$\sigma_t + \frac{\Delta \sigma}{2} = \left(\eta_t + \frac{\Delta \eta}{2}\right) \sigma_{max} + \frac{k_v \sigma_{max} \Delta \varepsilon}{\dot{\varepsilon}_0 \Delta t}$$

Which gives the change in stress as:

$$\Delta \sigma = 2\sigma_t + \left(\eta_t + \frac{\Delta \eta}{2}\right) 2\sigma_{max}$$

Taking the derivative with respect to $\Delta \varepsilon$ gives:
The derivative of \( \Delta \eta \) is determined by first considering:

\[
\dot{\eta} = (1 - \eta) \frac{k_f C}{\theta} - \left(1 - \frac{\sigma}{\eta \sigma_{\text{max}}}\right) \eta \frac{k_b}{\theta}
\]  

(3.33)

and applying the central difference operator:

\[
\frac{\Delta \eta}{\Delta t} = \frac{k_f C^*}{0} - \eta_t \frac{k_f C^*}{\theta} \frac{\Delta \eta}{\Delta t} \frac{k_f k_f C^*}{2\theta} - \frac{k_b \eta_t}{\theta} - \frac{\Delta \eta}{\Delta t} \frac{k_b}{2\theta}
\]

\[
+ \sigma_t \frac{k_b}{\theta \sigma_{\text{max}}} + \Delta \sigma \frac{k_b}{2\theta \sigma_{\text{max}}}
\]

(3.34)

which can be reorganized:

\[
C^* = C_t + \frac{\Delta C}{2}
\]

(3.35)

Therefore:

\[
\frac{\partial \Delta \eta}{\partial \Delta \sigma} = \left[\frac{1}{\Delta t} + \frac{k_f C^*}{2\theta} + \frac{k_b}{2\theta}\right]^{-1} \frac{k_b}{2\theta \sigma_{\text{max}}}
\]

3.3.5.3 Section 3

For positive strain rates, the stress is given:

\[
\sigma = \eta \frac{\sigma_{\text{max}}}{\lambda} + \frac{\sigma_{\text{max}} k_v}{\dot{\varepsilon}_0} \dot{\varepsilon}; \quad \dot{\varepsilon} > 0
\]

(3.37)

Applying the central difference operator:

\[
\sigma_t + \frac{\Delta \sigma}{2} = \left(\eta_t + \frac{\Delta \eta}{\Delta t}\right) \frac{\sigma_{\text{max}} k_v \Delta \varepsilon}{\dot{\varepsilon}_0 \Delta t}
\]

(3.38)

Which gives the change in stress as:

\[
\Delta \sigma = 2\sigma_t + 2\eta_t \sigma_{\text{max}} + \Delta \eta \sigma_{\text{max}} + \frac{2\sigma_{\text{max}} k_v \Delta \varepsilon}{\dot{\varepsilon}_0 \Delta t}
\]

(3.39)

Taking the derivative with respect to \( \Delta \varepsilon \) gives:
The derivative of $\Delta \eta$ is determined by first considering:

$$\frac{\partial \Delta \sigma}{\partial \Delta \varepsilon} = \frac{\partial \Delta \eta}{\partial \Delta \varepsilon} \frac{\sigma_{\text{max}}}{\lambda \varepsilon_0 \Delta t} + 2k_v \sigma_{\text{max}}$$  \hspace{1cm} (3.40)

and applying the central difference operator:

$$\frac{\partial \Delta \sigma}{\partial \Delta \varepsilon} = \frac{\partial \Delta n}{\partial \Delta \sigma} \frac{d \Delta \sigma}{d \Delta \varepsilon}$$  \hspace{1cm} (3.41)

$$\hat{\eta} = \frac{k_f C}{\theta} (1 - \eta)$$

which can be reorganized:

$$\frac{\Delta \eta}{\Delta t} = \frac{k_f C^*}{\theta} - \left( \eta_t + \frac{\Delta \eta}{2} \right) \frac{k_f C^*}{\theta}$$  \hspace{1cm} (3.42)

Therefore:

$$C^* = C_t + \frac{\Delta C}{2}$$  \hspace{1cm} (3.43)

$$\frac{\partial \Delta \eta}{\partial \Delta \sigma} = 0$$

3.3.6 Output variables and interpretation of results

In order to visualise the resulting 3D SF distributions, two output variables are considered. Firstly, the average SF activation level $\bar{\eta}$ at each integration point is utilised, given as

$$\bar{\eta} = \frac{\sum_{k=1}^{n} \eta_k}{n}$$  \hspace{1cm} (3.45)

where $n$ is the total number (240) of discrete fibre orientations at each point. Secondly, in order to identify regions of the cell cytoplasm in which SFs are aligned in a dominant direction, a variance is defined to quantify the difference between the most highly activated fibre, $\eta_{\text{max}}$, and the average fibre activation, $\bar{\eta}$, at each integration point. The variance $\Pi$ is defined as:

$$\Pi = \eta_{\text{max}} - \bar{\eta}$$  \hspace{1cm} (3.46)
3.3.7 Cytoplasm and nucleus

The passive behaviour of the cytoplasm is simulated using an isotropic hyperelastic formulation, further details of which are given in Chapter 2. The passive cytoplasm is assumed to represent all other cytoplasm components except for the assembled SFs. The total Cauchy stress in the cytoplasm is then calculated by adding the passive and active stress.

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

(3.47)

It should be noted that the passive hyperelastic response is governed by two material properties: the Young’s modulus and the Poisson’s ratio. When relating to the cytoplasm, the subscript “cyto” is used with these properties.

The nucleus is modelled as a passive isotropic hyperelastic formulation, similar to the passive formulation used for the passive cytoplasm. The subscript “nuc” is used when referring to nucleus properties.

A passive viscoelastic cytoplasm component is also used in parallel with the active SF formulation. The passive stress component in Eqn (3.47) is instead determined using the formulation:

\[ \dot{\varepsilon}_{ij} = \frac{1}{E} \sigma_{ij} - \frac{\nu}{E} \sigma_{kk} \delta_{ij} + \frac{3}{2} \eta_s \varepsilon_{ij} + \frac{1}{\eta_v} \frac{\sigma_{kk}}{3} \delta_{ij} \]  

(3.48)

where \( \eta_s \) and \( \eta_v \) are the shear and volumetric viscous terms. The stress increment maybe determined by use of the central difference operator to first write an expression for \( \Delta \varepsilon_{ij} \). This expression is written for each component of the tensor. The components of the stress increment tensor are then determined by solving the system of equations \( \Delta \varepsilon_{ij} = \cdots \). The solution to this system of equations is provided in Appendix D.

3.3.8 Overview of UMAT

The full user material is provided in Appendix A and an overview of the UMAT structure is provided here. As shown in Figure 3.9, the UMAT can be broken down into a number of steps:
3 - Model development

- Initialize variables and define angles for fibre orientations
- Perform polar decomposition of $F$ to determine rotation tensor
- Calculate the passive cytoplasm stress using the hyperelastic formulation
- Determine the strain rate in each fibre direction
- Use the ODE integration scheme to determine $\eta$ in each direction at the end of the increment
- Calculate the contribution each fibre makes to the active stress and then calculate the total stress.
- Determine the contribution each fibre makes to the constitutive Jacobian.
- Calculate the output variables for interpretation of the results.

In order to ensure that the numerical integration of the kinetic ODE (described in Section 2.3.3) has temporally converged, a maximum time increment of 2 seconds was set for all analyses. A parameter study revealed that a maximum time increment of 10 seconds did not introduce a significant error; however, a 2 second limit was chosen as a conservative limit.

### 3.3.9 Adaptation to VUMAT

The above formulation maybe adapted for use with Abaqus\Explicit (a VUMAT in Abaqus terminology). As briefly discussed above, Explicit offers a faster solution scheme for non-linear analysis as it is not necessary to invert the tangent stiffness matrix. The stress calculated in a VUMAT is defined with respect to the corotational basis system. Therefore, the active and passive stresses should be calculated differently. For the case of the active stress, this simply involves neglecting to account for rigid rotations of the fibre vectors. For the passive hyperelastic stress, the form of the Cauchy stress presented in Chapter 2 is modified such that:

$$\sigma = R \left( \frac{2}{J} C_{10} \left( \bar{U}^2 - \frac{1}{3} tr(\bar{U}^2) I \right) + \frac{2}{D_1} (J - 1) I \right) R^T$$

(3.49)

$$\bar{U} = U / J^{\frac{1}{3}}$$

which can be express as:

$$\sigma = R \sigma^{\text{corotational}} R^T$$

(3.50)
3. Model development

Therefore:

\[ \sigma_{\text{corotational}} = \frac{2}{J} \bar{C}_{10} \left( \bar{D}^2 - \frac{1}{3} \text{tr}(\bar{D}^2)I \right) + \frac{2}{D_1} (J - 1) I \quad (3.51) \]

It is not necessary to calculate the material Jacobian in an explicit analysis. Despite the computational savings associated with the explicit method, large number of increments used, where each increment involves the numerical solution to the kinetic ODE, results in a prohibitive computational cost.

3.3.10 Axisymmetric considerations

The above implementation deals with a fully 3D implementation of the active SF formulation. In the case of an axially symmetric geometry, it is not sufficient to use a 2D plane strain formulation of the material model as out of plane SFs are possible in an axisymmetric geometry. Therefore, it is necessary to predict the formation and contractility of SFs in a fully 3D scenario. The resulting out of plane shear stresses should be zero, as the SF distribution will be symmetric about the revolved plane. In Abaqus Standard it is possible to employ an axisymmetric element that has three direct and three shear stress terms (CGAX4 in the Abaqus element library). This element is used to monitor and ensure that out of plane shear stresses are correctly predicted to be zero.

3.3.11 Comparison between 2D and 3D material models

In the current thesis, material parameters calibrated using previous 2D implementations of the material model are used in the 3D implementation. The 3D stress state may be written as:

\[ \sigma_{ij} = \frac{3}{4\pi} \int_0^{2\pi} \int_0^\pi \sigma(\omega, \phi) m_i^r m_j^r \sin(\omega_\nu) \, d\phi \, d\omega \quad (3.52) \]

This reduces to the 2-dimensional formulation by setting \( \omega = \pi/2 \) and only accounting for rotations about the \( x_3 \) axis:
The formation of active/specific adhesions of cells to a substrate is simulated using a recent thermodynamically motivated model (Deshpande et al. 2008). This model considers the formation of FAs via the bonding of integrins on the cell surface to suitable ligands on the ECM. Binding integrins on the cell surface exist in two conformational states: high affinity, or “straight”, integrins with a high reference chemical potential, $\mu_H$, and low affinity, or “bent”, integrins with lower reference chemical potential, $\mu_L$, as shown in Figure 3.10.

Only the high affinity integrins form bonds and low affinity integrins remain unbonded (Figure 3.10). Low affinity integrins with a concentration $\xi_L$ have chemical potential:

$$\chi_L = \mu_L + kT \ln \left( \frac{\xi_L}{\xi_0} \right)$$  \hspace{1cm} (3.54)

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

In contrast, high affinity integrins form bonds and undergo stretching; therefore, the potential energy stored in the bond is accounted for in the chemical potential via two additional terms:

$$\chi_H = \mu_H + kT \ln \left( \frac{\xi_H}{\xi_0} \right) + \Phi(\Delta_i) - F_i \Delta_i$$  \hspace{1cm} (3.55)
where $\Phi$ is the stretch energy and $F_l \Delta_l$ is the potential energy of the applied force $F_l$. The force $F_l$ is related to the stretch by:

$$F_l = \frac{\partial \Phi}{\partial \Delta_l} \quad (3.56)$$

The kinetics of bond formation and the diffusion of low affinity integrins along the cell membrane are considered fast compared with other time scales. Therefore, diffusive kinetics are neglected and the total concentration of integrins, i.e. the low plus the high affinity ones, is held fixed at $\xi_0$ everywhere on the membrane. Similarly, the kinetics of bond formation is so rapid that thermodynamic equilibrium, $\chi_H = \chi_L$, can be used to obtain the individual concentrations of high and low affinity integrins as:

$$\xi_H = \frac{\xi_0}{\exp\left[\frac{\mu_H - \mu_L + \Phi - F_l \Delta_l}{kT}\right] + 1} \quad (3.57)$$

$$\xi_L = \frac{\xi_0}{\exp\left[\frac{\mu_H - \mu_L + \Phi - F_l \Delta_l}{kT}\right] + 1} \quad (3.58)$$

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

$$\Phi = \begin{cases} 
\frac{\lambda_s \Delta_e^2}{2} & \Delta_e \leq \Delta_n \\
-\lambda_s \Delta_n^2 + 2\lambda_s \Delta_n \Delta_e - \frac{\lambda_s \Delta_e^2}{2} & \Delta_n < \Delta_e \leq 2\Delta_n \\
\lambda_s \Delta_n^2 & \Delta_e > 2\Delta_n 
\end{cases} \quad (3.59)$$

where $\kappa_s$ is the stiffness of the bond; $\Delta_e$ is the effective stretch and $\Delta_n$ is the peak bond length. The $\lambda_s \Delta_n^2$ term is the surface energy of the high affinity integrins and is found by $\Phi(\Delta_l \to \infty) = \lambda_s \Delta_n^2$, where $\lambda_s$ is the integrin stiffness, and $\Delta_e$ is the effective strain. The corresponding force for a single integrin follows as:

$$F = \begin{cases} 
\frac{\lambda_s \Delta_e}{2\lambda_s \Delta_n - \lambda_s \Delta_e} & |\Delta_e| \leq \Delta_n \\
0 & \Delta_n < |\Delta_e| \leq 2\Delta_n \\
& |\Delta_e| > 2\Delta_n 
\end{cases} \quad (3.60)$$

This piecewise linear function of $\Delta_e$ is shown in Figure 3.11, which also shows the variation of $\Phi$. 

55
3.5 Development of a mixed mode FA model

The current section describes the additions to the model of Deshpande et al. (2008) to include passive tractions and mixed mode deformation of FA bonds performed by the author.

3.5.1 Passive tractions

Cell-substrate interactions can be considered as active/specific interactions that involve binding proteins and passive/non-specific interactions such as electrostatic tractions, van der Waals interactions, hydrophobic forces, and steric repulsion (Bell 1978; Cheng et al. 2009). In the current study, passive forces are considered in the normal direction only and active forces are considered in both the normal and shear directions using a mixed mode formulation. Passive/non-specific forces are assumed to act on the portion of the membrane excluding the area covered by integrins. These passive forces are averaged over the entire membrane area giving the passive traction. Passive forces acting on integrins are neglected, as such forces are assumed to be significantly lower than active/specific forces on bound integrins.

The passive traction is defined in relation to the normal separation distance \( \Delta_1 \):

\[
T_1^p = \varphi_0^p \frac{\Delta_1}{\delta_p} e^{-\frac{\Delta_1}{\delta_p}} \tag{3.61}
\]

where the constant \( \delta_p \) is the passive characteristic distance, and the constant \( \varphi_0^p \) is the passive interaction potential.

3.5.2 Mixed mode bond stretching

The concentration of bound integrins, \( \xi_H \), and the stretch energy, \( \Phi_0 \), depend on the effective stretch of the integrin-ligand bond, \( \Delta_\nu \). The effective stretch is based on the movement between the cell and the substrate. However, a bond forms at a non-zero length \( \Delta_0 \) and it is possible for a bond to slip when an bound integrin jumps to a nearby ligand. A bond will slip if doing so would lower its chemical potential \( \chi_H \), i.e. if it is stretched past its peak length, \( \Delta_\nu \), and there is a suitable available ligand. The evolution of a single bond is shown below in Figure 3.12. The effective stretch is defined as
where $\Delta_1$ and $\Delta_2$ are the normal and tangential stretch components.

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:

$$T_1 = -\xi_H F_1 - T_1^p, \quad T_2 = -\xi_H F_2$$

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

$$\sigma_{ij} n_j = T_i$$

where $\sigma_{ij}$ is the Cauchy stress in the cell, and $n_j$ is the surface normal.

### 3.5.3 Overview of UINTER

As the UINTER does not require any numerical integration scheme, it is considerably shorter than the user defined material subroutine. A full copy of the UNITER is provided in Appendix B and brief overview is described below. The key steps in the implementation of the UINTER, as shown in Figure 3.13, are:

- Calculate relative positions of surfaces and determine if active bond has formed.
- Determine if bond is slipping and update effective stretch variables
- Calculate concentration of bound high affinity integrins
- Calculate passive normal traction
- Calculate shear and normal FA tractions
- Calculate the interface stiffness matrix $\mathbf{D\mathbf{S\mathbf{D}}\mathbf{D}}$

The interface stiffness matrix may be determined by first examining the FA stress:

$$T_i^A = -\xi_H F_i$$

Therefore, the interface stiffness $(\mathbf{D\mathbf{S\mathbf{D}}\mathbf{D}})$ is defined:

$$\frac{\delta T_i^A}{\delta u_j} = - \left[ \xi_H \frac{\delta F_i}{\delta u_j} + F_i \frac{\delta \xi_H}{\delta u_j} \right]$$
Therefore, it is necessary to evaluate the derivatives \( \delta F_i / \delta u_j \) and \( \delta \xi_H / \delta u_j \) based on the first two parts of the Eqns (3.59) and (3.60):

\[
\delta F_i \frac{\delta F_i}{\delta u_j} = \begin{cases} 
\frac{\lambda_s \delta_{ij}}{|u_j| \leq \Delta_n} \\
\frac{-\lambda_s \delta_{ij}}{\Delta_n < |u_j| \leq 2\Delta_n}
\end{cases} 
\]

\[
\frac{\delta \xi_H}{\delta u_j} = \frac{\xi_H \lambda_s u_j q^*}{kT(q^* + 1)^2}
\]

\[
q^* = \begin{cases} 
\exp\left(\frac{\mu_H - \mu_L - \frac{\lambda_s \Delta_e^2}{2}}{kT}\right) & |u_j| \leq \Delta_n \\
\exp\left(\frac{\mu_H - \mu_L + \frac{\lambda_s \Delta_e^2}{2} - \lambda_s \Delta_n^2}{kT}\right) & \Delta_n < |u_j| \leq 2\Delta_n
\end{cases}
\]

The passive contribution to the interface stiffness only occurs in the normal direction and is given:

\[
\frac{\delta T^p_{ij}}{\delta u_i} = \varphi \frac{\Delta_j}{\delta_p} \varphi \frac{\Delta_j}{\delta_p} \frac{\Delta_j}{\delta_p}
\]

### 3.6 Conclusions

This Chapter provides a synopsis of the material model of Deshpande et al. (Deshpande et al. 2006) (Section 3.2) and the original FA model (Deshpande et al. 2008) (Section 3.4). The material model is then expanded into a fully 3D framework by the author and the details of this implementation in Abaqus are provided in Section 3.3. The FA adhesion model is modified such that passive tractions are included and FA bonds slip and deform during the mixed mode displacement at the contact interface (Section 3.5). The reader should note that the subsequent Chapters 4 to 7 include details of the FE meshes specific to each application.
3.7 Figures

Figure 3.1 Overview of the formation of bipolar myosin filaments that interact with actin filaments to form SFs (Deshpande et al. 2007).

Figure 3.2 Left: Tri-linear contraction rate model used, which is an approximation of the Hill relation. Right: Stress fibre under isometric tension and fibre dissociation under reduced tension.
Figure 3.3 Angle convention used to define direction of an arbitrary fibre with a representative volume element (RVE).

Figure 3.4 Fibre directions considered using a numerical approximation of a double integration over 2 spherical coordinates, $\omega$ and $\phi$. 
Figure 3.5 Evenly distributed vectors in 3D space. Unit vectors are shown for a hemisphere.

Figure 3.6 Comparison of integration methods using an approximation of a double integral (left) or using evenly distributed points on the sphere (right). Stresses are shown for a rigidly held single element test where all fibres reach the maximum possible tension at equilibrium.
Figure 3.7 Modified SF contractility behaviour showing the slope introduced to the original trilinear relationship (dashed line). The modified relationship has a non-zero slope for all strain rates. Note that the slope of both green segments is identical. Changes in slope are not to scale.

Figure 3.8 Alternate form of cell contractility model using an arctan function to define a continuous smooth relationship between tension and strain rate.
Figure 3.9 Flowchart showing UMAT structure.
Figure 3.10 Left – The two conformational states of integrins. The low affinity state (left) and high affinity state are shown. Right – high affinity integrins bound to ligands on a substrate.

Image removed due to copyright

Figure 3.11 Left – schematic diagram showing direction of bond force relative to integrin movement. Top right – stretch energy of a bond as a function of effective stretch. Bottom right – bond force as a function of tangential displacement for a bond stretched in the tangential direction.
Figure 3.12 Evolution of a single FA integrin-ligand bond during mixed mode displacement of a cell relative to a static substrate. Bond forms (1) at a reference length (indicated by a dashed red line) and (2) is subjected to mixed mode stretch. Bond continues stretching until the bond reaches the peak length (3) and if possible the bond slips to maintain this stretch (4). Bond continues slipping (5) at the peak length until the bond is perpendicular to the substrate (6). Bond is stretched past its peak length, but keeps slipping to maintain the minimum possible bond length (7).
Figure 3.13 Flowchart showing overview of UINTER

1. Calculate relative positions of surfaces and determine if active bond has formed.
2. Determine if bond is slipping and update effective stretch variables.
3. Calculate concentration of bound high affinity integrins.
4. Calculate passive normal traction.
5. Calculate shear and normal FA tractions.
6. Calculate the interface stiffness matrix DDSDDR.
3.8 References

4 Compression of round and spread cells: a numerical investigation of the active role of the actin cytoskeleton in the compression resistance of cells

4.1 Introduction

Cell compression has been used to quantify differences in the mechanical response of different cell types and shapes. Previous studies have used finite element models in tandem with experimentally measured geometries and compression forces to show that spread cells have a higher apparent stiffness than round cells for a range of different cell types (Caille et al. 2002; Darling et al. 2008). However, these models only consider the cell as a passive entity and assume either elastic, viscoelastic, or biphasic material behaviour. For such passive cell models, a given, unchanged set of material parameters cannot be used to replicate experimentally observed compression forces for round and spread cells. The apparent stiffness of spread cells must be artificially increased to account for the significant cytoskeletal remodelling that the cell undergoes as it changes from a round to a spread configuration (McGarry 2009; McGarry and McHugh 2008). In order to gain insight into the mechanisms underlying cellular stiffening, it is necessary to employ an active material model that predicts the distribution and contractility of the actin cytoskeleton.

As discussed in Chapter 2, the cytoskeleton has previously been modelled using pre-positioned passive filaments and contractility has been included as a prescribed thermal strain (Mohrdieck et al. 2005; Storm et al. 2005). However, these attempts have not considered the cellular processes that drive cytoskeletal remodelling and contractility. A recent study has proposed a novel computational model of contractile stress fibre (SF) behaviour based on the biochemistry of SF formation (Deshpande et al. 2006). This model is entirely predictive; i.e., the SF distributions and contractility are dynamically governed by cellular signalling and tension dependent dissociation. Models predicting SF formation and contractility have also been proposed by Kaunas et al. (Kaunas and Hsu 2009), and Vernerey and Farsad
These models have been confined to 1D or 2D implementations; restricting SF formation to a single plane and restricting the ability to simulate in vitro experiments. The models of Kaunas et al. and Vernerey and Farsad differ from that of Deshpande et al. (2007): Vernerey and Farsad assume that the rate of SF formation is increased by fibre tension; Kaunas et al. assume that fibre dissociation occurs when a fibre has been stretched past a critical length. The Deshpande formulation has been used successfully to simulate SF distributions in cells on patterned substrates (Pathak et al. 2008). In a study by McGarry et al. (2009) this formulation is shown to accurately predict the scaling of active cell tractions with cellular contractility and with substrate stiffness for cells adhered to arrays of microposts.

In the current chapter, the fully 3D formulation described in Chapter 3 is used for the simulated compression of realistic round and spread cell geometries. This 3D implementation is used to investigate differences in SF evolution in a range of cell types with varying contractility. Simulations of SF formation and cell compression are performed for axisymmetric round and spread cells, and for a fully 3D elongated or polarised cell. The effect of cell shape and contractility on the compression response of cells is examined. The results of this study are compared to previous experimental data to illustrate the predictive capabilities of the model. Our findings highlight the importance of SF distribution and contractility in the mechanical response of a cell to applied compression.

4.2 Methods

4.2.1 Finite element models

Finite element meshes of round and spread cells were generated based on previously reported cell geometries of bovine aorta endothelial cells (Caille et al. 2002) and chick embryo fibroblasts (Thoumine et al. 1999) as shown in Figure 4.1. The initial dimensions of the cell used for each model are shown in Figure 4.1. Axisymmetry is assumed for the round and spread cells shown in Figure 4.1A and B. An elongated or polarised cell with an elliptical surface area is shown in Figure 4.1C; however, it is sufficient to model one quarter of the polarised cell as it contains two
symmetry planes. Thus a spread geometry is assumed for the cells in Figure 4.1 (A) and (C) prior to introduction of cellular contractility. The finite element model of each cell consists of two regions with different materials. The green regions in Figure 4.1 represent the cytoplasm and material consists of the active formulation in parallel with a passive hyperelastic material as described above. The blue regions represent the nucleus and the material is simulated using a hyperelastic material. The two regions are continuous and no slip is permitted between the nucleus and cytoplasm. A nominal element size of 0.075 \( \mu \text{m} \) was chosen for all meshes. A mesh sensitivity study was performed and negligible changes to the results were observed for smaller element sizes. The cells are attached to a rigid substrate using a previously published cohesive zone model that captures the tension dependent formation of focal adhesions (Deshpande et al. 2008). An overview of this model is given in Chapter 3. The cohesive zone model is implemented as a user defined interface subroutine in Abaqus.

In the first step of the simulation, fibre growth is driven by an exponentially decaying signal. The cell contracts on the rigid substrate due to the formation and remodelling of contractile SFs. Remodelling of the cytoskeleton continues until it reaches a steady state reached. Changes in cell height and nucleus shape also reach equilibrium during this step.

Following from this, in the second step of the simulation, the cell is compressed to 30\% of its steady state height, which was computed in step 1. A flat rigid indenter is brought into contact with the cell and moved downwards at a constant velocity of 0.02 \( \mu \text{m/s} \). The effect of friction between the indenter and the cell was investigated for smooth frictionless contact, contact with different friction coefficients, and for rough contact. Friction was found to have a negligible effect on compression forces (<1\%); therefore hard, frictionless contact is used to describe the interaction between the cell and the rigid indenter.

### 4.2.2 Model parameters and interpretation of results

Different levels of contractility are investigated in this study in order to represent different cell phenotypes. Specifically, \( \sigma_{\text{max}} \) values of 25kPa, 8kPa, and 3.5kPa are
used to represent smooth muscle cells (SMCs), mesenchymal stem cells (MSCs), and fibroblasts (FBs) respectively, based on previous calibrations for 2D simulations of cells on microposts (McGarry et al. 2009). A passive cytoplasm stiffness of $E_{\text{cell}} = 0.4 \, kPa$ is chosen for all cell types, and completely passive cells are represented by bare cytoplasm (i.e. $\sigma_{\text{max}} = 0$). For all cell types, the additional active parameters are set to $\theta = 70s$, $k_v = 7$, $k_f = 10$, $k_b = 1$, $\dot{\varepsilon}_0 = 0.003s^{-1}$. Previous experimental studies of isolated nuclei have estimated that the nucleus may be over 10 times stiffer than the cytoplasm (Thoumine et al. 1999). Therefore a passive nucleus stiffness of $E_{\text{nuc}} = 4.0kPa$ is assumed.

**Table 4.1 Cell properties**

<table>
<thead>
<tr>
<th></th>
<th>SMCs</th>
<th>MSCs</th>
<th>FBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_{\text{max}}$</td>
<td>25kPa</td>
<td>8kPa</td>
<td>3.5kPa</td>
</tr>
<tr>
<td>$E_{\text{cell}}$</td>
<td></td>
<td>0.4 kPa</td>
<td></td>
</tr>
<tr>
<td>$k_v$</td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>$k_f$ $k_b$</td>
<td></td>
<td>10,1</td>
<td></td>
</tr>
<tr>
<td>$\dot{\varepsilon}_0$</td>
<td></td>
<td></td>
<td>0.003s$^{-1}$</td>
</tr>
<tr>
<td>$\theta$</td>
<td></td>
<td></td>
<td>70s</td>
</tr>
</tbody>
</table>

In order to visualise the resulting 3D SF distributions, two output variables are considered. Firstly, we utilise the average SF activation level $\bar{\eta}$ at each integration point, given as

$$\bar{\eta} = \frac{\sum_{k=1}^{n} \eta_k}{n} \quad (3.70)$$

where $n$ is the total number (240) of discrete fibre orientations at each point. Secondly, in order to identify regions of the cell cytoplasm in which SFs are aligned in a dominant direction, a variance is defined to quantify the difference between the most highly activated fibre, $\eta_{\text{max}}$, and the average fibre activation, $\bar{\eta}$, at each integration point. The variance $\Pi$ is defined as:

$$\Pi = \eta_{\text{max}} - \bar{\eta} \quad (3.71)$$

Finally, vector plots are presented to illustrate the 3D orientation of dominant SFs throughout the cytoplasm.
4.3 Results

4.3.1 Stress fibre orientation and distribution

The distribution of the average SF activation level, $\bar{\eta}$, is presented in Figure 4.2 for round and spread SMCs. In both cases, a steady state distribution has been achieved following signal driven SF evolution. For both the round and spread cell, SFs are seen around the nucleus and along the base, where the cell is attached to a rigid substrate. It is important to note that the spread cell shows significantly higher levels of SF formation than the round cell, with a distinctive band of SFs leading from the cell periphery over the top of the nucleus. In contrast, large areas of the round cell cytoplasm contain no SF formation and there are no bands extending from the nucleus to the cell periphery. The stiff nucleus and the attachment to the rigid substrate support tension in the SFs and, in accordance with the SF kinetic equation, these fibres do not dissociate. The spread cell, which has a significantly larger adhesion area, provides more support to the SFs, preventing fibres from dissociating. In the round cell there is a smaller adhesion area and the cell radius is larger than the adhesion radius. Consequently, there is not enough support for fibre tension, leading to shortening of fibres and fibre dissociation.

In Figure 4.3, vectors indicating the direction and activation level of the most highly activated SF at each integration point are plotted, with the results corresponding to the state of the SMCs in Figure 4. The colour of the background and of the vectors corresponds to the variance $(\Pi)$ at each integration point. Long, red fibres indicate that the SFs have formed dominant bundles in a particular direction. Blue areas with very short or no fibres have very low SF formation in all directions. Magnified views of the round cell in Figure 4.3 (C, D) reveal that dominant SF bundles around the nucleus are oriented in the radial plane. These fibres have formed in a narrow band all around the nucleus. SFs at the base of the round cell are oriented at approximately 45° to the radial plane (Figure 4.3 (E)). In contrast, Figure 4.3 (B, G, H) show that large areas of the spread cell contain dominant bundles of SFs that are oriented circumferentially.
The angle of a fibre to the radial plane \((\pi/2 - \omega)\) is shown in Figure 4.4 (inset). The distribution of this angle for each integration point in SMCs corresponding to the condition depicted in Figure 4.2 & Figure 4.3 is presented in a histogram for both round and spread cells. For the spread cell, dominant fibres are oriented at 60° - 90° to the radial plane, i.e., circumferential (hoop) fibres. In contrast, the dominant fibres in the round cell are mostly radial ones that form an angle of 0°-15° to the radial plane. Additionally, it should be noted that there is a higher level of fibre activation in the spread cell.

### 4.3.2 Cells under compression

Following the simulation of SF evolution to a steady state in response to an exponentially decaying signal, parallel plate compression of cells to 30% of the steady state cell height is then simulated. Compression forces are presented in Figure 4.5 for round and spread SMCs. The round cell generates a peak force of \(~350\text{nN}\) at 70% compression strain (i.e. the cell height is 30% of the original value). However, significantly larger reaction forces are computed for the spread cell, with a peak force of \(~1317\text{nN}\). As the cell is compressed, the dominant fibres identified in Figure 4.3 undergo stretching. The majority of these dominant fibres are oriented in the circumferential direction and it should be noted that circumferential strain is higher at the cell periphery.

During compression of spread cells, these dominant contractile fibres are stretched and thus remain at a tension equal to the isometric level \((\sigma_0)\), providing resistance to cell compression. In these dominant fibres, the activation level is predicted to be close to its maximum value \((\eta \approx 1)\), therefore the computed isometric tension level is equal to the maximum possible value \((\sigma_0 \approx \sigma_{\text{max}})\). In contrast, fibres in the round cell which are stretched during compression are found to have a very low activation level \((\eta \ll 1)\). Furthermore, the majority of dominant fibres in the round cell do not undergo stretching during compression. The tension in these fibres drops and consequently, fibres are predicted to provide little resistance to compression.
4.3.3 Effect of cellular contractility

In addition to the previous simulations, where material properties were based on SMCs, the response of MSCs and FBs was also investigated by varying $\sigma_{max}$ as outlined in Section 4.2.2. Figure 4.6 shows the SF variance (\(\Pi\)) for cells following initial signal driven SF growth. FBs, which are the least contractile cells, have the lowest variance. The lower variance is due to a combination of higher average SF formation and a reduction in the activation level of the most highly activated fibre. The lower $\sigma_{max}$ causes less contraction of the cell, which in turn leads to less fibre shortening in all directions and therefore less dissociation of SFs. Consequently, highly aligned contractile bundles do not readily form.

The peak reaction forces are shown in Figure 4.7 for each cell type at 70% compression strain. Reaction forces for fully passive or non-contractile (i.e. with no SFs - $\sigma_{max} = 0$) cells are also included for comparison. The peak force for both round and spread cells decreases with decreasing contractility. The peak force for the spread cell is reduced from 1736nN for SMCs to 659nN and 335nN for MSCs and FBs respectively. This reduction is due to lower isometric tension in the dominant fibres in less contractile cells. The predicted isometric tension ($\sigma_0 = \eta \sigma_{max}$) is lower in contractile cells for two reasons: (i) the computed activation level $\eta$ in the dominant direction is found to be lower, as shown in Figure 4.6; (ii) the prescribed maximum fibre stress $\sigma_{max}$ is lower (8kPa and 3.5kPa for MSCs and FBs, respectively). As is also evident in Figure 4.7, the ratio of compression forces between spread and round cells increases with increasing cell contractility. A ratio of 2.9 is computed for SMCs compared to a ratio of 1.2 for passive non-contractile cells ($\sigma_{max} = 0$). Therefore, changes in the cell geometry do not cause the stiffer response predicted for spread cells; the computed increase in force is due to the presence and orientation of dominant fibres which are stretched during compression of highly contractile spread cells.

4.3.4 Stresses in Cells

Figure 4.8 shows the tensile equivalent stress (also known as the von Mises stress) in axisymmetric cells before and after compression. Compression of round and
spread SMCs causes a ~2 fold increase in stress throughout the cytoplasm and more than a ~10 fold increase in the nucleus (Figure 4.8 (A-D)). In the spread FB, the stress in the cytoplasm before compression is ~5 times lower than the spread SMC. Figure 4.8 (G) shows that the stress in the nucleus is significantly lower in FBs than in SMCs (Figure 4.8 (C)) before compression. After compression, FB cytoplasm stress increase by a factor of ~2 (Figure 4.8 (F)). However, nucleus stresses increase by a factor of ~30. Finally, Figure 4.8 (H) shows the stress distribution in passive cells following compression. Stresses are highly localised above and below the nucleus. This distribution differs significantly from the predicted stress distributions for SMCs (Figure 4.8 (B, D)).

4.3.5 Stress fibre formation in polarised/elongated cells

Figure 4.9 shows SF orientation and distribution in a polarised SMC after SF formation has reached steady state in response to an exponentially decaying signal. A quarter cell showing fibre orientations on 3 orthogonal planes is shown in Figure 4.9(A), i.e., the two vertical planes of symmetry and a horizontal plane at the cell base. SFs are further illustrated in the horizontal basal plane in Figure 4.9(B) and in additional vertical planes in Figure 4.9(C). In order to further visualise the 3D SF distribution throughout the entire cytoplasm a 3D animation is presented in two videos in the online supplementary material (MovieS1, S2). Dominant fibre bundles are predicted to form parallel to the long axis of the cell. Due to the polarised shape of the cell, the cell is more deformable in the direction of the minor axis, which leads to fibre shortening and dissociation in this direction. In contrast, the cell is less deformable in the direction of the major axis, supporting fibre tension and preventing dissociation. Similar to axisymmetric cells, SF activation levels are highest at the base of the cell near the adhesion to the rigid substrate where fibre tension is supported, preventing fibres from dissociating.

4.3.6 Compression and release of polarised/elongated cells

As implemented for axisymmetric cells, polarised cells were compressed to 30% of the steady state height following the signal driven growth described above. The simulated compression forces are presented in Figure 4.10(A) for SMCs, MSCs, FBs,
and a passive cell with no contractility. Similar to axisymmetric cells, larger compression forces predicted for highly contractile cells; a peak reaction force (after compression to 30%) of 2340nN is computed for SMCs, 1270nN for MSCs, 945nN for FBs, and 700nN for passive cells. The increased compression resistance computed for highly contractile polarised cells is due to the stretching of dominant SF bundles. Figure 4.10(C) shows dominant fibre bundles that are stretched during compression for a cell that has been compressed to 50% of its steady state height, i.e., dominant fibres that are not undergoing stretch are not shown. These SFs have a high activation level and have a positive strain rate; therefore they exert an isometric tension close to the maximum possible value, \( \sigma_0 = \eta \sigma_{max} \approx \sigma_{max} \). Although the prediction of long parallel SFs for polarised cells differs from the circumferential SFs computed for axisymmetric cells, the dominant fibre bundles in both geometries are stretched during compression, and SF tension causes an increase in compression resistance. Increasing the Poisson’s ratio of the cell from 0.3 to 0.4 resulted in an \(~18\%\) increase in compression reaction forces for both passive and active cells (data not shown).

Following compression to 30% of the steady state height, the indenter is immediately withdrawn and the cell is allowed to return to an equilibrium configuration. The recovery of the cell height is shown in Figure 4.10(B) for each contractile cell type. In the 1200 seconds following the release both the MSC and FB recover to a steady state value, with the least contractile FB recovering fastest. As the cell relaxes, fibres which were stretched during compression are now shortening. The tension in these shortening fibres drops, which leads to fibre dissociation. The time-dependent response computed for contractile cells in Figure 4.10(B) is entirely due to SF dynamics, as no viscoelasticity has been included in the material formulation. It should be noted that all cells have recovered to a cell height greater than the steady state value prior to compression. Although the SMC has not yet reached a steady state by 1200 s, it has relaxed to a height greater than that prior to compression. The steady state heights of MSCs and FBs have increased by 20% and 30%. SF dissociation during release leads to an overall drop in tension throughout the cell and consequently to an increase in the steady state height.
4.4 Discussion

The simulations presented here demonstrate that a computational model based on the active remodelling and contractility of stress fibres (SFs) captures the compression response of adherent cells. By implementing this predictive bio-chemo-mechanical formulation in a fully 3D framework (as described in Chapter 3), it is shown that the orientation of dominant fibre bundles significantly influences the mechanical response of cells to compression. Specifically, if the dominant fibre bundles in the cell are stretched during compression, they significantly increase the computed compression force. In round cells, low SF formation is computed, with fibres occurring predominantly in the radial plane; hence, these fibres shorten during compression and therefore provide little resistance to compression. In contrast, high SF formation is computed for both axisymmetric and polarised spread cell geometries and, generally, the dominant fibres are stretched during compression. The resultant isometric fibre tension causes a significant increase in compression resistance. Simulated SF formation is significantly affected by cell shape, by the contractility level of the cell phenotype, and the presence of the nucleus. In axisymmetric spread cells the dominant fibres are predicted to align in the circumferential direction and in polarised cells they are predicted to align parallel to the long axis of the cell. In comparison to SMCs, decreased levels of cellular contractility associated with MSCs and FBs alter SF distributions and cause a reduction in the computed reaction forces. SF distributions and compression forces for both round and spread cells are simulated using a given, unchanged set of material parameters for each of three cell phenotypes.

4.4.1 Stress fibres resist compression

In the present study, SF distributions and compression forces for both round and spread cells are simulated using a given, unchanged set of material parameters for each of three cell phenotypes. Simulations predict that spread cells have significantly more dominant SF bundles than round cells. In axisymmetric spread cells, these are oriented in the circumferential direction and in polarised cells these are parallel to the major cell axis. In both axisymmetric and polarised spread cells, our model is in strong agreement with experimentally observed distributions. The
following experimental observations are reported for cells displaying an approximately axisymmetric geometry: Potter et al. (1998) show axisymmetric fibroblasts with clearly defined actin SFs oriented circumferentially near the cell periphery; Goffin et al. (2006) show that myofibroblasts on micropatterned substrates and on isotropic substrates fibroblasts acquire an axisymmetric shape with actin SFs throughout the cytoplasm; Schober et al. (2007) also show distinct bands of circumferential fibres for keratinocytes. Cells with polarised geometries are reported to have long parallel fibres: Peeters et al. (2004) show elongated myoblasts with distinctive long SFs parallel to the long edge of the cell; Goffin et al. (2006) also show elongated myofibroblasts with long parallel fibres oriented with the long axis of the cell; finally Engler et al. (2006) show polarised cells with long parallel fibres for MSCs seeded on glass substrates. In contrast, experimental images of rounded cells, such as unspread chondrocytes, show no distinct alignment of fibres, with actin staining having a smeared appearance throughout the cytoplasm (Ofek et al. 2009a). This is captured by the simulations of round cells, where low SF activation levels are predicted (\(\bar{\alpha}<<1\)), with no dominant bundling direction (\(\Pi<<1\)) in most of the cytoplasm. The present study also demonstrates that the nucleus plays a significant role in SF formation by providing a stiff structure around which a thin band of SFs form. As a stiff structure in the centre of the cell, the nucleus provides support for the tension that is essential for the persistence of SFs. Houben et al. (2007) give a comprehensive review of the importance of structural interactions between the nucleus and the cytoskeleton. Broers et al. (2004) also report a significant decrease in compression resistance for cells in which nuclear lamins have been removed. Broers et al. further show that the removal of nuclear lamins also causes disruption of the actin cytoskeleton and a decrease in cell stiffness. Khatau et al. (2009) also highlight the structural relationship between the nucleus and the actin cytoskeleton in spread cells.

Circumferential SFs have been described by Naumanen et al (2008) as transverse arcs. Such transverse arcs are generated from \(\alpha\)-actinin-decorated actin filaments which assemble endwise with myosin bundles to form contractile transverse arcs. The contractile nature of these transverse arcs is confirmed by Hotulainen &
Lappalainen (2006) where myosin ATPase activity is inhibited by treating cells with blebbistatin. This cell treatment resulted in complete dissociation of transverse arcs in the cell, and after 30 minutes focal adhesions disappeared. Furthermore, Oakes et al (2012) report that transverse arcs generate large amounts of tension that is relayed to FAs.

4.4.2 Compression of round and spread cells

The current study predicts a stiffer response for spread cells compared to round cells. This result is supported by the experimental data of Darling et al. (2008) which reports significant differences in apparent cell stiffness between round and spread osteocytes (7.5 fold increase) and MSCs (4.5 fold increase). The experimental findings of Caille et al. (2002) suggest that the force required to compress endothelial cells is six times higher for spread cells compared to round cells. Previous finite element simulations of cell compression have used passive material models, requiring an artificial increase in the passive stiffness of the cell material in order to capture the increased compression forces for spread cells (Caille et al. 2002; Thoumine et al. 1999). It is important to note that the active framework implemented in this study captures this trend while using a given, unchanged set of model parameters for each cell phenotype. The increased resistance of spread cells to compression results from the stretching of dominant contractile SF bundles. This mechanism is supported by the experimental observations of Peeters et al. (2004) for polarised cell geometries, where it is reported that long actin fibres restrict deformation of the cell and nucleus in the direction perpendicular to the dominant fibre orientation. This observation underlines the fully predictive nature of our modelling framework, which provides an insightful link between cell geometry, SF distribution and response to mechanical stimuli. In the current study, for both axisymmetric and polarised spread cells, a similar mechanism of increased compression resistance is predicted. In both cases the dominant fibre directions are oriented such that cell compression results in stretching of these fibres at isometric tension, leading to an increase in resistance to compression. In the case of axisymmetric cells, these dominant fibres occur in a circumferential direction whereas in a polarised cell these dominant fibres occur along the length of the cell.
4.4.3 Contractility of different phenotypes underlies viscous response

The link between cell contractility and resistance to compression is further underlined by our prediction that increased cell contractility leads to an increase in compression force. In spread cells, the dominant fibre bundle is stretched during compression and these bundles exhibit tension equal to the isometric level \( \sigma_0 = \eta \sigma_{max} \). Therefore, higher levels of cellular contractility \( \sigma_{max} \), which are associated with different cell phenotypes, result in higher tension in the dominant bundles and consequently a greater resistance to compression. The active modelling framework predicts that spread SMCs provide most resistance to compression (1400nN), followed by MSCs (600nN) and fibroblasts (400nN). These predictions are strongly supported by previous experimental studies: peak reaction forces of 2500nN have been reported for compression of highly spread myoblasts (Peeters et al. 2005). However, less contractile cells such as endothelial cells generate peak forces in the region of 500nN (Caille et al. 2002) and spread fibroblasts generate a reaction force of \(~360nN\) (Lulevich et al. 2006). Lower forces have been observed experimentally for round cells, with \(~100nN\) being measured for round endothelial cells and \(~80nN\) for round chondrocytes (Ofek et al. 2009b). Furthermore, in the study of Darling et al. (Darling et al. 2008), spread osteoblasts and MSCs were shown to be 4.5 and 2.3 times stiffer than spread chondrocytes, respectively. In contrast, round osteoblasts and MSCs were shown to be only 1.3 and 1.15 times stiffer than round chondrocytes.

The present study also suggests a link between contractility and creep recovery of a cell following compression. Upon removal of the indenter, the current study predicts a time-dependent recovery of the cell geometry to a steady state. The apparent relaxation time for FBs is similar to that reported by Thoumine et al (1997). Similarly shaped creep recovery curves have been reported by Ofek et al. (2009c) for chondrocytes, with a lower relaxation time of \(~5s\), suggesting a faster recovery time for less contractile phenotypes. Our simulations suggest that this characteristic time-dependent response is primarily due to the remodelling and shortening of the dominant SF bundles as SFs are observed to dissociate both fibres.
when the cell is released (the converse of the lengthening of these fibres during the compression phase and the recovery phase.). As fibres shorten, they obey a Hill-type tension-strain rate behaviour, with the fibre tension reducing from the isometric value. This tension reduction during release leads to partial fibre dissociation, further adding to the time dependent release behaviour. It is important to note that in the current study, unlike previous studies using simple standard viscoelastic material behaviour (Sato et al. 2006; Thoumine and Ott 1997), no phenomenological viscoelasticity was used to capture this time-dependent behaviour, suggesting that SF dynamics contribute to the creep recovery of cells. This highlights the capability of our model to provide mechanistic insight in addition to quantitative prediction.

4.4.4 Implications

The mechanical cellular environment is closely linked to cell differentiation (Engler et al. 2006) and, in particular, the cytoskeleton provides a mechanical link from the nucleus to the extracellular matrix (Buxboim et al. 2010). Nucleus stresses play an important role in stem cell differentiation and significant differences have been reported between stem cells and differentiated cells in terms of nucleus stiffness and nucleus stresses (Pajerowski et al. 2007). The accurate prediction of cell and nucleus stresses would represent a significant step in understanding and controlling cell mechanotransduction. In the current study, it is demonstrated that the stress distribution following compression in the cell and nucleus computed using a passive hyperelastic cytoplasm differs significantly to that computed using the active SF formulation. The passive formulation predicts highly localised stress concentrations at the centre of the cell and very little stress in the peripheral regions of the cytoplasm, in contrast to the active formulations, where significant stresses are computed throughout the cytoplasm for highly contractile cells. In the experimental study of Pajerowski et al. (2007), the differences in nucleus stiffness between stem cells and differentiated cell were determined using the micropipette technique in tandem with a simple passive analytical solution for the deformation of the aspirated cell. Therefore the change in apparent stiffness of the nuclei during aspiration may be due to the increased contractility of differentiated cells, rather
than a change in the stiffness of the nucleus material. Furthermore, the active formulation offers a prediction of cell and nucleus stresses in the absence of external stimuli.

In line with previous implementations of the material formulation (Pathak et al. 2008; McGarry et al. 2009) and experimental observations of chondrocytes and endothelial cells (Shieh et al. 2006; Ferko et al. 2007), a Poisson’s ratio of 0.3 is assumed for the majority of simulations in the current study. Other experimental studies have observed a Poisson’s ratio of 0.4 (Trickey et al. 2006; Jones et al. 1999; Freeman et al. 1994); however, Darling et al. (2008) found that varying the Poisson’s ratio between 0.3 and 0.5 only caused a ±20% change in the apparent elastic modulus of the cell. In the current study, increasing the Poisson’s ratio from 0.3 to 0.4 results in an ~18% increase in compression reaction forces for both passive and contractile cells; suggesting that the ratio of active to passive compression force is independent of the Poisson’s ratio. A previous investigation of the role of compressibility and Poisson’s ratio for passive cells (McGarry 2009) determined that assuming a fully incompressible cell (ν=0.5) leads to significant bulging of the cell during compression. Such bulging is not consistent with the experimental images of compressed endothelial cells reported by Caille et al. (2002).

4.4.5 Limitations and future directions

The model geometries in the current study are based on previous experimental observations of round and spread cells (Caille et al. 2002; Thoumine et al. 1999). However, these studies only investigated the mechanical response of actively contractile cells and did not isolate the role of the cytoskeleton through the use of SF inhibitors. The different levels of contractility associated with each cell type cause a reduction in cell height to a steady state value that is different for each cell type simulated. In order to overcome this limitation, all compression forces are presented as a function of change in cell height normalised by the steady state height. In a follow on study presented in Chapter 5, the effect of contractility on cell and nucleus height is analysed, presenting a framework to accurately parse the nucleus stiffness, cell contractility, and passive cytoplasm stiffness in spread cells.
Compression of round and spread cells

(Weafer et al. 2012). Additionally, all cell types were simulated in the current study using the same passive material properties. Previous investigations have calculated stiffness values by assuming the cytoplasm and cytoskeleton form a passive homogenous material. In contrast, the passive stiffness used in the current study is separated from the active contribution of the SFs. In the current study, non-contractile elements such as microtubules and intermediate filaments have been represented using a non-linear hyperelastic continuum. However, previous experimental studies have shown that microtubules undergo more complex deformation; for example, Brangwynne et al. (2006) show that in unloaded cells the microtubule network can buckle under cellular contractility.

4.4.6 3D implementation builds on previous studies

In the current study, each phenotype has been modelled by changing the cellular contractility, based on the calibration of cells on arrays of microposts and on patterned substrates (McGarry et al. 2009; Pathak et al. 2008). These previous implementations of the active SF formulation have been limited to 2D simplifications. In this thesis, this framework has been extended so that SF evolution is predicted in a fully 3D environment (Chapter 3). The alignment of dominant SF bundles which cause the increased resistance to compression in spread cells are not considered in a 2D formulation. Therefore, in order to accurately simulate experiments in which the cell deformation occurs in more than one plane, it is essential to consider SF formation in all directions. A 3D formulation also allows for the simulation of realistic polarised cell geometries and the inclusion of a cell nucleus. Cell compression has been used in a range of in vitro studies to quantify the mechanical properties of different cell types, including endothelial cells (Caille et al. 2002), fibroblasts (Thoumine and Ott, JCS), myoblasts, Peeters 2005, adipocytes (Or-Tzadikario and Gefen 2011) and MSCs, osteoblasts, chondrocytes, adipocytes (Darling et al. 2008). Previous computational investigations have assumed axisymmetric geometries for compression (Caille et al. 2002; Nguyen et al. 2010; Ofek et al. 2009b) and micropipette aspiration (Haider and Guilak 2002; Rowat et al. 2005) without investigating the effect of polarised cell geometries. The implementation of a fully 3D framework provides insight into the link between the
contractility and alignment of dominant SFs and cellular resistance to compression. It should also be noted that the model parameters calibrated for the pseudo-2D environments of cells attached to micro-posts (McGarry et al. 2009) and cells on fibronectin patches (Pathak et al. 2008) are shown to provide reasonable predictions of compression forces compared to published experimental values. This illustrates the robustness and applicability of the active SF formulation to a wide range of single cell experiments.

4.5 Conclusion

In the current study, the contractile cytoskeleton has been considered in a 3D, predictive framework with no predefined SF arrangement. This implementation elucidates the role of the cytoskeleton in the response of different cell types and shapes to compression between two rigid plates. The orientation and distribution of fibres significantly affects the forces generated by the cells. In both axisymmetric and polarised cell geometries dominant bundles of highly aligned SFs are computed: in the case of axisymmetric cells such dominant bundles occur in the circumferential direction whereas for polarised cells dominant bundles occur parallel to the long axis of the cell. In both cases the dominant SF bundles are stretched at isometric tension during the compression event, leading to an increased resistance to cell compression. This effect is most pronounced for highly contractile cells. In contrast, round cells provide a low resistance to compression as lower levels of SF formation are predicted, and SF tension does not significantly contribute to compression resistance. The framework employed here provides a powerful platform to consider the role of the cytoskeleton in different loading modes. In the following chapters, this 3D framework will be used to investigate the mutual dependence of SF formation and focal adhesion assembly during cell spreading, the effect of contractility on cell and nucleus height, and the effect of irregular cell geometries on SF distributions (Chapters 5&6).
4.6 Figures

Figure 4.1 Dimensioned diagram of spread (A), round (B), and polarised (C) cell geometries. The nucleus is shown in blue and the cytoplasm is shown in green. Due to axial symmetry it is only necessary to consider the half cell plane shown here for (A) and (B). The polarised cell contains two vertical symmetry planes and the cell is simulated using the quarter cell geometry shown.

Figure 4.2. Average stress fibre (SF) level $\bar{\eta}$ for round (A) and spread (B) smooth muscle cells. Note the bands of SFs in the spread cell leading from the top of the nucleus to the cell periphery. In contrast, much of the round cell contains low levels of SFs. The dashed lines depict the original cell size prior to the introduction of cellular contractility.
Figure 4.3. Orientation of the most highly activated fibre is shown for round (A) and spread (B) smooth muscle cells. Vector length and orientation describe the most highly activated fibre, where length is proportional to the activation level. The colour corresponds to the variance at each point. Long red fibres indicate that the stress fibres (SFs) have formed dominant bundles in a single orientation. Blue areas with short or no fibres have very low SF formation in all directions. Fibre orientation is shown in detail in for each cell: above the nucleus (C, F), in the cytoplasm (D, G), and at the base of the cell (E, H).
Figure 4.4. Distribution of angle \((\pi/2 - \omega)\) between radial plane and \(\eta_{\text{max}}\) orientation for all fibres for round (top) and spread (bottom) smooth muscle cells. An angle of 90 degrees means that fibres are in the hoop direction, and 0 degrees means the fibres are in the radial plane.
Figure 4.5. Compression reaction forces for round and spread smooth muscle cells when strained to as little as 30% of the original cell height. Note that the peak reaction force for the spread cell (1439nN) is ~2.2 times larger than for the round cell (635nN).

Figure 4.6. Stress fibre variance $[\Pi]\text{ for round (top) and spread (bottom) cells for fibroblasts (FBs)} (A, D), \text{mesenchymal stem cells(B,E), and smooth muscle cells (C, F). Note that the variance is lowest for the FBs, which are the least contractile cells. The dashed lines depict the original cell geometry prior to the introduction of cellular contractility.}$
Figure 4.7. Peak reaction forces at 70 % compression strain for round and spread cells. The peak force ratio for spread to round is 2.3, 1.8, 1.6, and 1.25 for smooth muscle cells (SMCs), mesenchymal stem cells (MSCs), fibroblasts (FBs) and passive cells respectively.
Figure 4.8. Tensile equivalent stress (also known as the von Mises stress) before and after compression for round smooth muscle cells (SMCs) (A,B), spread SMCs (C,D), and spread fibroblasts (FBs) (E,F). Von Mises stress is shown for the nucleus only for SMCs and FBs before compression (G). Stresses are also shown for passive cells after compression.
Figure 4.9 Stress fibre (SF) orientation is shown for an smooth muscle cells (SMC) with a polarised cell geometry. Vector length and orientation describe the most highly activated fibre, where length is proportional to the activation level and the colour corresponds to the variance at each point. SFs are plotted on 3 orthogonal planes (A), with 2 vertical symmetry planes and a horizontal plane near the base of the cell. SFs are further illustrated on the horizontal plane (B) and on two additional vertical planes (C). Note: SF distribution is computed throughout the cytoplasm and is further illustrated in Movie S1 in the online supplementary material.
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Figure 4.10 Compression reaction forces for smooth muscle cells (SMC), mesenchymal stem cells (MSC), fibroblasts (FB), and a passive cell with no stress fibres for cell compressed by 70% of their steady state height (A). Creep recovery curves for SMCs, MSCs and FBs following release after compression (B). Cell height was 30% of the original steady state height before release. Vector plot of dominant stress fibre bundles that undergo stretch during compression (C), i.e. dominant SF bundles that shorten during compression are not shown. The highlighted region represents a SF bundle running the length of the cell. These fibres remain at isometric tension, providing resistance to compression.
4.7 References


5 Compression based calibration of the active and passive material properties of osteoblasts

5.1 Introduction

An analysis of the role of the cytoskeleton in the compression resistance of cells is provided in Chapter 4. Following from this, the current chapter presents a methodology to parse the contributions of the contractile cytoskeleton, the passive cytoplasm, and the nucleus based on detailed experimental measurement of active and passive osteoblasts (Weafer, Ronan, et al. 2012b). As described in Chapter 2, previous computational interpretation of cell compression experiments have determined the properties cells using passive hyperelastic or viscoelastic material models (Thoumine et al. 1999; Ofek et al. 2009a). Cell compression has previously been used to fit such entirely passive material models for a wide range of cell phenotypes: chick embryo fibroblasts (Thoumine et al. 1999), bovine aorta endothelial cells (Caille et al. 2002), bovine articular chondrocytes (Ofek et al. 2009a), and mouse skeletal myoblasts (Peeters et al. 2005). Mechanical loading is necessary for the healthy function of osteoblasts. For instance, mechanical deformation is considered essential for bone remodelling (Frost 2004). Previous studies have shown that compressive loading induces mineralization of osteoblastic cells (Gabbay et al. 2006) and is an effective up-regulator of osteogenesis (Rath et al. 2008). The experimental images of Weafer, Ronan et al. (2012b) demonstrate that osteoblasts form highly aligned stress fibre (SF) bundles, suggesting that the acting cytoskeleton contributes significantly to the mechanical behaviour of this cell phenotype.

In the current study, experimental observations of the compression resistance and morphology of untreated cells and of cells treated with cytochalasin-D are used to determine the active and passive material properties of osteoblasts. Specifically, measured changes in cell morphology due to the removal of the contractile cytoskeleton, in tandem with experimental cell compression data, are used to parse the contributions of the active cytoskeleton, the passive cytoplasm, and nucleus to
osteoblast mechanical behaviour. Additionally, in this chapter, following evaluation of the material properties, the predicted SF distribution in an irregularly shaped 3D cell is compared to experimental data.

5.2 Experimental data and calibration methodology

5.2.1 Experimentally observed compression resistance and contractile behaviour

The experimental data (Weafer, Ronan, et al. 2012b) used for the calibration of the active and passive material properties of osteoblasts are presented in the current section. Briefly, whole cell compression was performed on untreated osteoblasts and osteoblasts treated with Cyto-D. Cell compression was performed using a modified AFM system, previously described by Weafer et al. (2012a). Three key experimental results are used in the current study: the compression resistance of untreated cells; the compression resistance of treated Cyto-D cells; and the change in cell height due to the suppression of the contractility of the actin cytoskeleton.

The main geometrical characteristics of untreated and Cyto-D cells recorded from cell compression tests are reproduced in Table 5.1. The experimental data also reveals that, prior to compression, cell height increases by 71 % in response to Cyto-D treatment. Furthermore, analysis of stained cells shows that the nucleus height increases by 57 % and nucleus diameter decreases by 41 % in response to Cyto-D treatment. Also, the cytoplasm thickness above and below the nucleus increases by over 200 %. Figure 5.1(A,B) shows long, linear bundles of polymerized actin that traverse the cytoplasm of the cell surrounding the nucleus (blue) for untreated cells (Weafer, Ronan, et al. 2012b). Focal adhesions (vinculin) were observed as intense spots (green) at the terminal ends of actin filaments Figure 5.1 (A,B). This arrangement changes drastically due to the addition of Cyto-D: actin depolymerizes to its monomer form resulting in the disassembly of SFs and focal adhesions. The mean experimental force versus compressive strain for untreated (blue) and Cyto-D (red) cells are reproduced in Figure 5.4. At 0.6 compressive strain, the mean maximum force recorded for the untreated cells is 936 ± 149 nN versus 511 ± 191 nN for the Cyto-D cells.
Table 5.1 – Cell geometry characteristics for untreated and Cyto-D cells (mean ± standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Cyto-D</th>
<th>% Change of Mean Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell height (µm)</td>
<td>6.3 ± 2.49</td>
<td>10.8 ± 2.78</td>
<td>71.40%</td>
</tr>
<tr>
<td>Cell base area (µm²)</td>
<td>3898 ± 1776</td>
<td>2776 ± 1824</td>
<td>-28.80%</td>
</tr>
<tr>
<td>Nucleus Height (µm)</td>
<td>4.6 ± 0.8</td>
<td>7.2 ± 3.0</td>
<td>56.50%</td>
</tr>
<tr>
<td>Nucleus Diameter (µm)</td>
<td>25.6 ± 4.9</td>
<td>15.1 ± 2.4</td>
<td>-41.00%</td>
</tr>
<tr>
<td>Cytoplasm above nucleus (µm)</td>
<td>0.7 ± 0.5</td>
<td>2.7 ± 2.0</td>
<td>285.70%</td>
</tr>
<tr>
<td>Cytoplasm below nucleus (µm)</td>
<td>0.4 ± 0.3</td>
<td>1.3 ± 1.0</td>
<td>225.00%</td>
</tr>
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5.2.2 Simulating Cyto-D (treated) cells

Cells treated with cytochalasin-D are not contractile and are modelled as a passive hyperelastic material. Previous experimental observations suggest that the nucleus is 10 times stiffer than the cytoplasm (Caille et al. 2002); however, different values have been reported in other studies (Ofek et al. 2009b; Guilak and Mow 2000). Three different ratios of nucleus stiffness to passive cell stiffness (5:1, 10:1, and 20:1) are considered in the present study. Cell geometries are assumed to be axisymmetric with cell and nucleus height and diameter being based on experimental measurements. Simulations of the passive elastic treated cells consist of a single load step during which the cell is compressed to 60% of its original height. This corresponds to Part A of the material calibration, as shown in Figure 5.3.

5.2.3 Simulating untreated cells.

The active constitutive formulation is added to the cytoplasm material model used for passive cells. The nucleus is included as a separate hyperelastic material and all passive parameters ($E_c$, $E_n$, $\nu_c$, $\nu_n$) are kept the same as for passive cells. Cellular contractility is adjusted by varying $\sigma_{max}$ and $k_v$. All other parameters for the active formulation are based on previous applications (Ronan et al. 2012). Simulations of untreated cells consist of two steps: in the first step SF growth is driven by an exponentially decaying signal. This leads to a change in the cell geometry whereby active contractility of the SFs reduce the height of the cell until an equilibrium is reached. This is shown as Part B in Figure 5.3. In the second step of the simulation...
Compression of osteoblasts

the cell is compressed to 60 % of its equilibrium height, which is shown as Part C in Figure 5.3.

5.3 Results

5.3.1 Simulated compression of passive (Cyto-D) cells (Calibration Part A)

Figure 5.5 (A) shows the simulated reaction forces for a passive cell subjected to compression to 60 %. Reaction forces are shown for three different ratios of nucleus stiffness to cell stiffness: 5:1, 10:1, and 20:1. The nucleus stiffness was chosen such that reaction force at 60 % compression was equal to that obtained experimentally for cells treated with Cyto-D (~500 nN). This corresponds to a nucleus stiffness of 3.15 kPa, 4.5 kPa, and 6.3 kPa for \( E_n:E_c = 5:1; 10:1; \) and 20:1 respectively. It should be noted that the different ratios of \( E_n:E_c \) did not significantly alter the form of the force-strain curve.

The von Mises stress at a compressive strain of 0.6 is shown in Figure 5.5(B) for the different \( E_n:E_c \) ratios. Increasing the ratio \( E_n:E_c \) causes an increase in the cytoplasm stress directly above and below the nucleus; however the stress in the rest of the cytoplasm is significantly lower for all simulations. The stress in the nucleus is significantly larger than that in the cytoplasm, and increases with increasing \( E_n:E_c \).

5.3.2 Cellular contractility (Calibration Part B)

SF distributions are shown in Figure 5.6 for different values of \( \sigma_{max}, k_v, \) and \( E_n:E_c \) at the end of the signal driven SF growth during the first step. Increasing the cellular contractility (higher \( \sigma_{max} \)) causes a higher SF variance (I) throughout the cytoplasm. Increasing the slope of the Hill-curve (lower \( k_v \)) also leads to dominant bundles of SFs. Lowering \( k_v \) leads to a greater tension drop and therefore fibres that are shortening will dissociate faster. The lowest values of \( k_v \) and the highest value of \( \sigma_{max} \) causes the highest variance and, consequently, the greatest reduction of cytoplasm and nucleus height as a result of active SF contractility. Increasing the ratio of the nucleus to cytoplasm stiffness \( (E_n:E_c) \) leads to the formation of highly aligned dominant SFs and a larger reduction of the cell height. The ratio of 20:1 has
the lowest cytoplasm stiffness (0.315 kPa) which provides less resistance to fibre contractility and hence the highest levels of SF dissociation as a result of tension reduction in the cytoplasm. The lower cytoplasm stiffness provides little support for SF tension, leading to a bigger drop in tension, and therefore more SF dissociation in certain directions. The SFs in the dominant direction will still have a high activation level, hence a higher SF variance is computed. In Figure 5.7, the effect of SF contractility on computed cell height at the end of the first step is shown. The height for a range of simulated cells with different values of $\sigma_{max}$, $k_v$, and $E_n:E_c$ are presented. The starting cell height corresponds to the mean cell height for treated Cyto-D experimental cells and the target height corresponds to the mean cell height for active or untreated experimental cells (also indicated in Figure 5.7). Computed cell height is lowered by either decreasing $\sigma_{max}$, or decreasing $k_v$. Increasing $E_n:E_c$ causes a small decrease in cell height. For each value of $k_v$ and $E_n:E_c$, the value of $\sigma_{max}$ that results in a reduction in cell height to the experimental target is identified in Table II. In summary, three pairings ($\sigma_{max}$, $k_v$) which correctly simulate the change in cell height due to SF contractility are identified for each $E_n:E_c$ ratio.

Table II – Calibrated values of $\sigma_{max}$ for three different values of $k_v$ and three different passive stiffness ratios $E_n:E_c$

<table>
<thead>
<tr>
<th>$E_n:E_c$</th>
<th>$k_v = 2$</th>
<th>$k_v = 5$</th>
<th>$k_v = 7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_n:E_c =$5</td>
<td>60 kPa</td>
<td>150 kPa</td>
<td>300 kPa</td>
</tr>
<tr>
<td>10</td>
<td>30 kPa</td>
<td>150 kPa</td>
<td>300 kPa</td>
</tr>
<tr>
<td>20</td>
<td>30 kPa</td>
<td>150 kPa</td>
<td>300 kPa</td>
</tr>
</tbody>
</table>

5.3.3 Compression of contractile cells (Calibration Part C)

Using the nine possible ($\sigma_{max}$, $k_v$) pairings identified in Part B of the calibration above, cell compression is now simulated and results are compared to experimentally observed compression forces for untreated contractile cells.
Computed compression forces at a compressive strain of 0.6 are shown in Figure 5.8. The reaction forces generated for $k_v = 5$ and $k_v = 7$ are ~5 and ~15 times larger than the experimentally observed value. The reaction forces for $k_v = 2$ are closer to the experimentally observed target. Furthermore, using a passive stiffness ratio of 5:1 and $k_v = 2$ gives the peak reaction force which is closest to the experimental value. This corresponds to a $\sigma_{max}$ of 60 kPa and the passive stiffness of the cytoplasm and nucleus are 0.63 kPa and 3.15 kPa respectively.

In summary, in the first step of the computational investigation, as shown in Part A of Figure 5.3, three different ratios of $E_n:E_c$ were initially simulated. In order to determine the correct level of nucleus stiffness for each ratio, these simulations were compared to the experimental compression forces observed for treated cells. Using these three selected values of passive cytoplasm and nucleus stiffness, in addition to the active parameters $\sigma_{max}$ and $k_v$, the change in cell height between treated and untreated cells was simulated, as shown in Part B in Figure 5.3. For each value of nucleus stiffness identified in Part A of the calibration, 3 pairings of $\sigma_{max}$ and $k_v$ were obtained, giving 9 separate parameter sets. In the final step of the calibration, Part C in Figure 5.3, compression of untreated cells was performed using these 9 parameter sets. The computed compression results were then compared to the experimentally observed results to give the parameter set that provided the closest match. Thus, the parameter set that gives the best prediction of passive compression of treated cells and active compression and height change in untreated cells was selected.

Finally, the full compression force-strain curves using these parameters for the passive and active simulations are compared with experimental curves in Figure 5.9. The shapes of the computational and experimental force-strain curves are in good agreement. The predicted passive compression force is within 5% of the experimental force at high strains; however, the error is larger (up to 40%) for higher strains. The experimental active force is higher than the computed value at high applied strains by 30% to 50%.

5.3.4 Fibre orientation in irregularly shaped cells
Following model calibration using an idealised axisymmetric cell geometry, a finite element model of an irregular cell geometry was constructed based on experimental confocal microscopy images (Figure 5.3(B)) in order to directly compare predicted patterns of SF formation and orientation to experimental observations. The predicted orientation of dominant SFs throughout the cytoplasm are shown by the vectors plotted in Figure 5.10. High levels of SF formation are predicted near the periphery of the cell, particularly in areas of high curvature, in strong agreement with the experimental image in Figure 5.1(B).

5.4 Discussion

The present study, for the first time, presents a detailed evaluation of the passive properties of the cytoplasm and nucleus and the active properties of the actin cytoskeleton for osteoblasts. This evaluation is performed using experimental compression data for both untreated contractile osteoblasts and Cyto-D treated non-contractile osteoblasts (Weafer, Ronan, et al. 2012b). In order to determine the passive elastic components of the cytoplasm, compression data for cells in which active contractility has been removed is used. Following from this, observed changes in cell and nucleus height following disruption of cell contractility are used to uncover the contribution of SF contractility. Finally, compression data for untreated contractile cells are also used for the calibration in order to provide a unique set of active and passive properties for osteoblasts. As the calibration involves three interlinked steps, and exact match is only achieved for the first two steps, i.e., the compression of treated cells and the change in height between treated and untreated cells due SF contractility. The predicted compression forces for untreated cells, i.e. the third step of the calibration, are slightly higher than those observed experimentally; however, the model provides a good approximation of the increase in compression resistance due to the presence of contractile SFs. The work presented in the current chapter demonstrates the capability of the active bio-chemo-mechanical framework for SF remodelling and contractility, as described in Chapter 3. This approach to the interpretation of measured cell compression forces represents a considerable advance on previous studies, in which the cell cytoplasm was treated as a passive homogeneous hyperelastic or viscoelastic
material (Caille et al. 2002; Ofek et al. 2009b; Peeters et al. 2005). These simulations presented in this chapter demonstrate that active contractility in osteoblasts contributes significantly to compression resistance.

### 5.4.1 Osteoblast contractility and compression resistance

The present study directly investigates the role of the actin cytoskeleton in the compression resistance of osteoblasts. Experimental compression of spread cells vary widely with cell type. Peak reaction forces at 70% compression of 2500 nN are reported for highly contractile myoblasts (Peeters et al. 2005), while lower forces are reported for less contractile cells: 500 nN for endothelial cells (Caille et al. 2002) and 360 nN for fibroblasts (Deng et al. 2010). This trend suggests that highly contractile cells provide a greater resistance to applied compression. As shown in Chapter 4, the stretching of dominant fibre bundles during compression leads to a significant increase in the compression resistance of spread cells compared to round cells; this trend was observed for a number of different cell types and the increase in compression resistance was strongly dependent on the level cell contractility (Ronan et al. 2012). The compression force measured for osteoblasts in the experimental work reported in Weafer, Ronan, et al. (2012b) (~900 nN at 60% compression) is significantly higher than the aforementioned reported compression forces for endothelial cells and fibroblasts, suggesting that osteoblasts are highly contractile. The value of $\sigma_{\text{max}} = 60$ kPa determined for the active model used in the current study suggests that osteoblasts are highly contractile, and the largest change in cell height between treated and untreated cells was computed for this value of cell contractility. The higher level of contractility results in increased fibre tension leading to a lower steady state height being computed for untreated cells. In contrast, a recent study found that less contractile chondrocytes ($\sigma_{\text{max}} = 0.85$ kPa) show little change in cell height upon the addition of Cyto-D (Dowling et al. 2012). In comparison to the value of $\sigma_{\text{max}}$ computed in the current study, a computational-experimental investigation of cells adhered to an array of microposts reports values of $\sigma_{\text{max}} = 25$ kPa, 12 kPa and 3.5 kPa for smooth muscle cells, MSCs and fibroblasts, respectively (McGarry et al. 2009). This investigation of cells adhered to microposts was performed using a previous 2-dimensional
implementation of the constitutive formulation used in the current study, demonstrating the predictive capabilities of the formulation under different boundary conditions. While different boundary conditions will lead to different stress states and SF distributions in the cell, the formulation used in the current study has been shown to accurately simulate cells in compression (Chapter 4) (Ronan et al. 2012), cells under shear (Dowling et al. 2012), cells adhered to micropatterned islands (Pathak et al. 2008), and cells adhered to continuous elastic substrates (Chapter 6), using the same material parameters for each cell phenotype. The modelling framework used in the current study is entirely predictive and the material properties are not influenced by different boundary conditions.

5.4.2 Nucleus stiffness

The finding that the passive nucleus stiffness is five times higher than the passive cytoplasm properties differs from the predictions of Ofek et al. (2009a) for chondrocytes. However, it should be noted that Ofek et al. ignored the contribution of active SF contractility in the cytoplasm. The present study suggests that this omission would lead to an overestimation of the passive cytoplasm Young’s modulus. The nucleus stiffness calibrated in the present study (~3 kPa) is similar to the stiffness of ~5 kPa reported by Caille et al. (2002) for isolated endothelial cell nuclei. However, it is possible that removal of nuclei from the cell in the experiments of Caille et al. (2002) may alter their mechanical behaviour. Interactions between the nucleus and cytoskeleton have been shown to significantly contribute to the mechanical regulation of the cell (Houben et al. 2007). Furthermore, the removal of nuclear lamins leads to a significant decrease in the compressive strength of cells (Broers et al. 2004) and disruption of the actin cytoskeleton (Khatau et al. 2009). Knockdown of the nuclear protein nesprin-1 has also been shown to alter the cytoskeleton and inhibit cell reorientation under applied cyclic strain in endothelial cells (Chancellor et al. 2010).

Significant nucleus deformation due to SF contractility is observed in the present study. Similarly, in work of Avalos et al. (2011), nuclei in contractile cells are shown to be significantly flatter than spherical nuclei observed in treated Cyto-D cells. In
contrast to the present study, Avalos et al. did not implement force measurements in order to uncover the mechanical properties of the nucleus. Furthermore, the present study, for the first time, investigates the level of SF contractility required to generate correct nucleus deformed geometries in contractile spread cells. Results reveal that a ratio of maximum SF contractility to nucleus stiffness of ~20 predicts the correct change in nucleus height. In the current study, the nucleus has been simulated as a homogenous hyperelastic material. However, previous studies have shown that isolated nuclei exhibit a viscoelastic response (Guilak et al. 2000) and that the nucleus is not a homogenous body (Rowat et al. 2005).

5.4.3 SF contractility leads to changes in cell morphology

In the current study, simulations of treated and untreated cells to determine the change in cell height and compression resistance of cells were performed using axisymmetric finite element meshes. Axisymmetry has been used previously to simulate the compression (Caille et al. 2002; Ofek et al. 2009b) and micropipette aspiration of cells (Haider and Guilak 2002; Rowat et al. 2005). As shown previously in Chapter 4, SF distributions in both axisymmetric and polarised cell geometries contain SFs that are stretched during compression, providing increased resistance to compression (Ronan et al. 2012). Previous studies which have considered realistic cell geometries have neglected the role of SF contractility (Slomka and Gefen 2010). As shown here and in Chapter 4, the active cellular biomechanical processes are the most important factor influencing the compression resistance of cells. (Ronan et al. 2011). In Chapter 6, starting with an unadhered cell, it is demonstrated that interlinked SF and FA remodelling drive the evolution of the cell shape during spreading, resulting in the formation of fibre bundles leading from the cell periphery over the nucleus.

5.4.4 Evaluation of osteoblast properties using cell compression

In the current study, the active and passive material parameters of osteoblasts are successfully determined based on experimental observations of cell compression. In order to determine the passive material properties through comparison with experimental data, it is necessary to identify possible values of the dimensionless
ratio $E_c:E_n$. Initial estimates of this ratio are based on previous experimental observations (Caille et al. 2002; Maniotis et al. 1997), however, further investigation is required to ensure that the initial estimates do not affect the final calibrated values. Micropipette aspiration has previously been used to evaluate cell properties using passive homogenous models (Baaijens et al. 2005; Tan et al. 2008; White et al. 1984). In order to demonstrate the ability of the SF contractility model to simulate micropipette aspiration, some initial simulations are presented in Appendix C. Previous experimental studies have also demonstrated that treatment with CytoD can reduce the apparent cell stiffness by ~85% (Trickey et al. 2006; Tan et al. 2008); however such studies have not parsed the individual contributions of the nucleus, passive cytoplasm, and SFs.

5.5 Conclusion

In summary, this chapter presents a detailed evaluation of the passive mechanical properties in parallel with the active SF contractility parameters for osteoblasts. Simulations also reveal that osteoblasts are highly contractile and that significant changes to the cell and nucleus geometries occur when SF contractility is removed. It is also shown that the active 3D modelling framework accurately predicts the contribution of the actin cytoskeleton to osteoblast compression resistance, and furthermore, accurate predictions of SF distributions in an irregular shaped cell are provided. Future investigations should include the seeding of osteoblasts on arrays of microposts in order to validate the high levels of contractility predicted by the present simulations.
5.6 Figures

Figure 5.1 Stained cell images of untreated (A, B) and Cyto-D (C, D) cells. Cells were stained for actin (red), vinculin (green) and nuclei (blue). Section views were taken across the nucleus centre (dashed lines) in all cases. Scale bar = 20 µm. Reproduced from Weafer, Ronan, et al. (2012b)
Figure 5.2 Schematic diagram of an axisymmetric cell, showing the axisymmetric plane in detail. The exploded view shows the 3D orientation of fibre directions (n=240) at every point. The nucleus is modelled as a hyperelastic material. The cytoplasm is modelled as a hyperelastic material in parallel with SF contractility.

Figure 5.3 Flowchart showing simulations performed to calibrate the active and passive material models.
5 - Compression of osteoblasts

Figure 5.4 Experimentally observed reaction force versus compressive strain curves (mean ± SD) obtained experimentally for untreated cells (red) and Cyto-D (blue) cells. Reproduced from Weafer, Ronan, et al. (2012b)

Figure 5.5 (A) Simulated compression reaction forces (nN) in passive cells for three different ratios of nucleus stiffness to cytoplasm stiffness ($E_n:E_c$). (B) Von Mises stress ($\sigma_{VM}$) in the cell for each ratio $E_n:E_c$. 
5 - Compression of osteoblasts

Figure 5.6 Computed stress fibre variance ($\Pi$) in each contractile cell shows where fibres have aligned in a dominant orientation to form a large stress fibre bundle. The variance is shown for 3 different values of $k_v$ and $E_n:E_c$, and for both a high and low value of $\sigma_{max}$. 
Figure 5.7 Simulated cell height after the addition of cellular contractility. Heights are shown for different values of $k_v$ and $\sigma_{max}$ and for different ratios of nucleus to cell stiffness (A-C). The starting height of the cell (dotted line) is the experimentally observed mean height for treated cells. The target cell height (dashed line) is the experimentally observed mean height for untreated cells.
Figure 5.8 Simulated reaction forces at 60% compression for different values of $k_v$ and $E_n:E_c$. For each simulation, $\sigma_{max}$ is chosen to give the correct reduction in cell height after the addition of contractility. The experimentally observed mean reaction force at 60% compression is shown as a dotted line.

Figure 5.9 Simulated (dashed lines) reaction forces superimposed over experimental (solid lines) reaction forces for active/untreated (red) and passive/treated (blue) cells. Experimental curves show the mean ± standard deviation and reproduced from Weafer, Ronan, et al. (2012b)
Figure 5.10 Predicted SF (stress fibre) orientation in an irregularly shaped cell. The computed orientation and activation level of the dominant fibre at each point is shown by the vector direction and length. The SF variance $\Pi$ is shown via the colour of the vectors.
5.7 References


6 Cellular contractility and substrate elasticity: a numerical investigation of the actin cytoskeleton and cell adhesion

6.1 Introduction

Previous experimental studies have established that cells can sense the stiffness of underlying substrates, and that cellular tractions depend on substrate stiffness (Discher et al., 2005; Tee et al., 2011). Substrate stiffness has also been shown to direct stem cell lineage specification (Engler et al., 2006) and affect cell motility (Lo et al., 2000). The contractile response of cells to ECM stiffness has been shown to be an important factor in wound healing (Danjo and Gipson, 1998), atherosclerosis (Isenberg et al., 2009), and cancer progression (Levental et al., 2009; Paszek et al., 2005).

Changes in substrate stiffness have been shown to significantly alter the actin cytoskeleton and focal adhesions (FAs) of a range of cell types, including fibroblasts, mesenchymal stem cells, endothelial cells, and chondrocytes (Byfield et al., 2009; Engler et al., 2006; Goffin et al., 2006; Schuh et al., 2010). Different ranges of substrate stiffness that influence cytoskeletal remodelling have been reported for different cell phenotypes; particularly, more contractile cells, such as myoblasts, are most sensitive up to ~400 kPa (Ren et al., 2008), but less contractile cells, such as fibroblasts, are sensitive up to up to ~20 kPa (Yeung et al., 2005). Previous in-vitro studies have quantified the effect of different substrate stiffness on stress fibre (SF) formation (Solon et al., 2007), FA area (Goffin et al., 2006), cell traction (Califano and Reinhart-King, 2010), and cell shape (Yeung et al., 2005). However, the cellular mechanisms underlying these phenomena are poorly understood. Experimental observations of cells adhered to micropatterned surfaces have previously been combined with numerical analyses to investigate this substrate sensing phenomena (McGarry et al., 2009); however, these investigations were not performed for continuous elastic substrates. In order to provide insight into the role of the contractile cytoskeleton and cellular adhesions, it is necessary to employ an active
constitutive formulation for them that considers the remodelling and contractility of the cytoskeleton in tandem with the formation of FAs in a fully three-dimensional framework.

FAs provide a mechanical link between the cytoskeleton and a substrate or extracellular matrix and also play an important role in cellular signalling (Schwartz et al., 1995; Wang, 2000). FA formation is modulated by traction forces on the adhesion and the size of the adhesion increases with force (Balaban et al., 2001; Tan et al., 2003). FAs are typically observed experimentally at the end of SF bundles (Burridge et al., 1988) and disrupting SF contractility has been shown to cause FAs to disappear (Oakes et al., 2012). Previous attempts to simulate FA dynamics have not included the role of cellular contractility (Bruinsma, 2005; Shemesh et al., 2005) and offer limited insight into the role of FAs in mechanotransduction. The study by Deshpande et al. (2008) presents a coupled thermodynamic and mechanical framework that incorporates SF formation kinetics, SF contractility and the force sensitivity of FA formation and growth.

In the current chapter, the mixed mode FA interaction model, which was expanded (Chapter 3) to include both specific and non-specific adhesion dynamics under mixed mode conditions, is used to examine the effect of substrate stiffness on SF and FA formation. Changes in cell shape, nucleus stress, and cell tractions with substrate stiffness are also shown. The effect of different levels of contractility (i.e. the internally generated traction forces exerted by the cell on the surrounding matrix or substrate) associated with a range of cell phenotypes when seeded on elastic substrates is investigated. Finally, the role of SF and FA formation in the spreading of cells by considering a cell that is not initially in contact with a substrate is examined.

6.2 Materials and methods

6.2.1 Finite element simulations

A finite element mesh was generated for a spread cell in contact with an elastic substrate, and for a cell that is initially not in contact with a rigid substrate. In both
cases, axisymmetric geometries are assumed, as shown in Figure 6.1. Each finite element model consists of 3 sections with different material models: the substrate is modelled as an elastic material; the cell nucleus is modelled as hyperelastic material; and the cytoplasm is modelled using the active formulation defined above. The cytoplasm and nucleus are continuous and no slip is permitted between the nucleus and cytoplasm. The contact interaction between cell and the substrate is simulated using the mixed-mode FA formulation for integrin stretching (i.e., both tangential and normal movement between the cell and substrate) defined above.

The material and interaction formulations are implemented in the finite element software Abaqus (Dassault Systemes, RI) as a user-defined-interface (UINTER) and user-defined-material (UMAT), respectively. A mesh sensitivity study was performed and no changes to the results were observed for smaller element sizes. The simulations consist of a single analysis step: for the cell adhered to the elastic substrate, SF formation is driven by an exponentially decaying signal; for the cell spreading on the rigid substrate, SF formation and the spreading process are driven by a constant signal.

6.2.2 Model parameters and interpretation of results

The material parameters used in the current study for the different phenotypes considered are based on previous implementations of the current formulation which were used to simulate cells adhered to microposts (McGarry et al., 2009) and cells under direct shear (Dowling et al., 2012). In order to investigate the role of contractility, three representative cell types are selected from those used by McGarry et al. (2009) and Dowling et al. (2012): smooth muscle cells (SMCs), fibroblasts (FBs), and chondrocytes (CH). The contractility of these cells is captured by setting the value of $\sigma_{\text{max}}$ to 25 kPa, 3.5 kPa, and 0.85 kPa for SMCs, FBs, and CHs respectively; as was previously defined in Table 4.1. A passive cytoplasm stiffness of $E_{\text{cell}} = 0.4$ kPa and a nucleus stiffness of 4.0 kPa are chosen for all cell types, and the additional active parameters are set to $\theta = 70$ s, $k_v = 7$, $k_f = 10$, $k_b = 1$, $\dot{\varepsilon} = 0.003$ s$^{-1}$. The parameters for the FA model are chosen based on previous calibrations of this model (Deshpande et al., 2008; Pathak et al., 2008) as: $(\mu_H - \mu_L)$
= 2.14 \times 10^{-20} \text{ J}; \xi_0 = 200 \mu m^2; \kappa_s = 0.15 \text{ nN } \mu m^{-1}; \Delta_n = 0.13 \mu m. The parameters of the passive component of the interface model are: \delta_p = 0.13 \mu m; \varphi_0^p = 50 \text{ fJ.}

In order to visualise the resulting 3D SF distributions, two output variables are considered, as described in Chapter 3. Firstly, the average SF activation level \( \bar{\eta} \) at each integration point is utilised, given as

\[
\bar{\eta} = \frac{1}{n} \sum_{k=1}^{n} \eta_k
\]

(6.1)

where \( n \) is the total number (240) of discrete fibre orientations at each point. Secondly, in order to identify regions of the cell cytoplasm in which SFs are aligned in a dominant direction, a variance is defined to quantify the difference between the most highly activated fibre, \( \eta_{\text{max}} \), and the average fibre activation, \( \bar{\eta} \), at each integration point. The variance \( \Pi \) is defined as:

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

(6.2)

In order to quantify the results on a whole-cell level, volume averaged and area averaged quantities are calculated by summing the quantity over the cell volume and area respectively:

\[
X^* = \frac{1}{V} \int_V X \, dV \quad \quad \quad \quad \quad \quad X^+ = \frac{1}{A} \int_A X \, dA
\]

(6.3)

where \( X \) is the quantity in question and \( V \) and \( A \) are the total volume of the relevant volume or area, where area is defined as the segment of the cell membrane adhering to the substrate.
6.3 Results

6.3.1 Increasing substrate stiffness increases SF and FA formation

The predicted distribution of SFs in cells adhered to elastic substrates is presented in Figure 6.2(A). The contour plots show the location of the dominant SFs, i.e. the SF variance ($\Pi$), on substrates with moduli of 0.2 kPa – 20000 kPa. The results show the steady state distribution following 800s of signal driven SF growth. The variance is computed to be highest in cells simulated on the stiffest substrate. SFs are predicted to form near the base of the cell at the adhesion to the substrate. In order to quantify the differences in SF formation, volume average values of SF formation and SF variance are calculated for the entire cytoplasm, as shown in Figure 6.2(B,C). Both the average SF and variance increase between 0.1 kPa and 100 kPa, at which point a plateau is reached and no further increase in SF formation is computed.

The stiffer substrates provide more support for SF tension, which leads to less fibre dissociation, as predicted by the kinetic SF equation. The activation level of the most highly activated fibre at a given point in the cell, $\eta_{max}$, is higher, causing increased SF formation. It should be noted that although the average SF formation, $\overline{\eta}$, is generally higher on stiffer substrates, the maximum fibre activation shows a greater increase, and therefore a higher variance ($\Pi = \eta_{max} - \overline{\eta}$) is computed on stiffer substrates.

The concentration of FA binding integrins on the cell surface is shown in Figure 6.3(A) for cells adhered to a range of different substrates. The dimensionless concentration of bound, high affinity integrins ($\xi_H/\xi_0$) is shown as a function of the dimensionless radial coordinate. The area averaged FA concentration is computed for each cell and presented as a function of substrate stiffness in Figure 6.3(B). Higher FA concentrations are computed for cells adhered to stiffer substrates, as expected due to tension from the higher levels of SF formation described above. FA concentrations are higher near the cell periphery for all substrates and on the stiffer substrates (>200 kPa), a small area of FAs are predicted to form beneath the nucleus.
6.3.2 Substrate stiffness alters cell shape, nucleus stresses, and cellular tractions

In Figure 6.4(A), the effect of substrate stiffness on steady state cell height is shown. On compliant substrates (<2 kPa), the cell height is 6.7 μm and on stiffer substrates (>100 kPa), the height is 5.3 μm. The height of the cell before the introduction of cellular contractility is shown as a dashed line (7.5 μm). The average stress in the nucleus is computed to increase from 70 Pa on compliant substrates to 600 Pa on stiffer substrates, as shown in Figure 6.4(B). The stress in the nucleus increases between 1 kPa and 100 kPa; however, outside of this range substrate stiffness does not significantly alter the stress. The increased SF formation seen on stiffer substrates generates more tension in the cell, which causes the computed increase in nucleus stress and decrease in cell height. The computed shear traction exerted on the substrate by the cell is shown in Figure 6.4(C) as a function of the dimensionless radial coordinate. As expected, higher shear tractions are predicted for stiffer substrates, and, for each cell, the tractions are highest near the cell periphery. Traction in the normal direction are shown in Figure 6.4(D), with positive tractions only at the cell periphery. The negative normal tractions indicate that the cell is compressed into the substrate in the central region.

6.3.3 Cellular contractility and substrate stiffness

The effect of cellular contractility on the substrate-dependent response of cells is shown in Figure 6.5. The SF variance for each cell is shown for contractility levels representing smooth muscle cells, fibroblasts, and chondrocytes. Figure 6.5(A) shows that the SF variance is decreased on more compliant substrates for all cell types. On very stiff substrates, the variance increases with increasing contractility, however, on very compliant substrates, the variance decreases with increasing contractility. The SF variance, normalized by the maximum variance for each cell type, is shown in Figure 6.5(B). This normalized plot shows that the relative change in variance is greatest for more contractile cells. Lower substrate stiffness causes a decrease in both the average and maximum SF formation; however, a smaller decrease of average SF formation is predicted for less contractile cells causing the trends in Figure 6.5(A,B). It should also be noted that in Figure 6.5(B), the range of
substrate stiffness that causes the greatest change in the variance is lower for less contractile cells. Chondrocytes are predicted to be most sensitive on substrates more compliant than 2 kPa. In contrast, smooth muscle cells are predicted to be most sensitive in the range 1-100 kPa and fibroblasts in the range 0.1-10 kPa.

The nucleus stress computed for different cell types seeded on elastic substrates is shown in Figure 6.5(C). For all elastic substrates, decreasing contractility reduced the average nucleus stress. As was observed for the SF variance, two plateau regions were computed for very stiff and very compliant substrates in which the nucleus stress did not change significantly. Each cell type underwent the same relative change in nucleus stress and highly contractile cells were more sensitive to stiffer substrates, as shown in the normalised plot in Figure 6.5(D).

### 6.3.4 Cells spreading on rigid substrates

Figure 6.6(A-E) shows the evolution of the cytoskeleton during spreading in a cell that is initially detached from a rigid substrate. SF formation and cell spreading is driven by a constant signal over 1400s. The mixed mode interface model pulls the cell towards the surface and the formation of FAs prevents lateral shortening of the cell. As the cell spreads, SFs are predicted to form at the periphery of the cell-substrate adhesion where FAs have formed. Further spreading leads to SF formation in a band extending from the edge of the cell-substrate contact over the top of the nucleus. The combined active and passive interface model provides support for fibre tension, causing the distinct bands to emerge as the contact area between the cell and the substrate grows radially. Figure 6.6(F) shows the location of the FAs near the cell periphery at the end of the dominant band of fibres.

In Figure 6.7, SF formation in a cell subjected to only passive adhesion forces acting in the normal direction is compared to SFs in a cell spreading with the previously described active FA formation. SFs are shown after 60 seconds and after 550 seconds of spreading for each cell (note: only half of the cell cross section is shown). Due to the absence of FAs providing support for fibre tension, SFs parallel to the substrate shorten and hence dissociate near the base of the cell. Consequently, no SFs are predicted at the cell base and the band of SFs extending from the periphery
to the top of the nucleus is diminished in comparison to that computed with the active FA formation. Additionally, when SF tension is not supported by FAs, the cell contracts laterally and a significantly smaller contact area is computed.

### 6.4 Discussion

The current study presents a computational investigation of mutually dependent stress fibre (SF) and focal adhesion (FA) formation on elastic substrates in a 3D framework. Tension dependent SF remodelling results in changes in the interface traction between the cell and substrate, which consequently lead to traction dependent FA formation. Simulations reveal that SF contractility plays a critical role in the substrate-dependent response of cells. Compliant substrates do not provide enough tension for SF persistence, causing dissociation of SFs and lower FA formation. In contrast, cells on stiffer substrates are predicted to contain large amounts of dominant SFs, with FAs forming near the cell periphery at the end of the SF bundles. A transitional range of substrate stiffness is identified over which substrate stiffness effected the most significant changes in SF and FA formation, nucleus stress, and cell height. Outside of this transitional range substrate elasticity has a less significant effect on cell behaviour. Furthermore, different levels of cellular contractility representative of different cell phenotypes are found to alter this stiffness range. It should be noted that, for a given cell type, the substrate-dependent response is investigated using an unchanged parameter set. Finally, the spreading of a cell initially not in contact with a substrate is simulated. SF and FA formation evolves as the cell spreads and leads to the formation of bands of SFs leading from the cell periphery over the nucleus. Inhibiting the formation of FAs during cell spreading is found to limit SF formation.

#### 6.4.1 Stiff substrates lead to more SF and FA formation

In the current study, more SFs and FAs are predicted to form in cells adhered to stiffer substrates. Simulations reveal that SFs form near the base of the cells, particularly at the cell periphery. Higher levels of bound FA integrins are computed in cells with a large amount of SFs. The inter-dependence of FAs and SFs is further shown on compliant substrates where low levels of SFs are accompanied by
significantly smaller FAs. This trend has been widely observed experimentally for a range of cell types including endothelial (Byfield et al., 2009), mesenchymal stem cells (Engler et al., 2006), fibroblasts (Solon et al., 2007), and chondrocytes (Schuh et al., 2010). Experimental studies have attempted to quantify the changes in SF formation for cells seeded on elastic substrates: Byfield et al. (2009) report a doubling of the average SF fluorescence in endothelial cells on a 9 kPa substrate compared to 1.7 kPa. Solon et al. (2007) use densitometric quantification of Western blots to report a ~50 fold increase in sedimented actin for fibroblasts seeded on a 15.2 kPa substrate compared to 0.7 kPa, as shown in Figure 6.8. Engler et al. (2006) show a linear increase in non-muscle myosin fluorescence in mesenchymal stem cells on elastic substrates ranging from 0.1 to 10 kPa, as shown in Figure 6.9. Furthermore SFs are reported to form distinct bundles on stiffer substrates, in contrast to more compliant substrates where stains for actin appear smeared (Engler et al., 2006; Yeung et al., 2005). Increased levels of FA formation are also reported for cells on stiffer substrates (Engler et al., 2006). These changes have been quantified experimentally by counting the number of distinct adhesions (Ren et al., 2008) or by calculating the total area of FAs on the cell surface (Goffin et al., 2006). Elineni and Gallant (2011) measured the intensity of FA integrins for cells adhered to circular adhesive patches, observing a significant peak of high affinity integrins at the cell periphery and a uniformly low level in the interior of the adhered area, which is in strong agreement with the distributions predicted here. For comparison, the results of Figure 6.3 are superimposed upon the experimental data of Elineni and Gallant (2011) for axisymmetric fibroblasts adhered to circular micropatterned islands, as shown in Figure 6.10.

### 6.4.2 Role of cell contractility

The role of contractility was investigated in the current study by considering three cell phenotypes representing a spectrum of cellular contractility: smooth muscle cells, fibroblasts, and chondrocytes (in order of decreasing contractility). The parameters required to simulate the active contractility of these cells were identified in previous implementations of the material formulation employed here (Dowling et al., 2012; McGarry et al., 2009). The range of substrate stiffness that has
the greatest effect on the substrate-dependent response of each cell type is identified by considering the level of SF formation and average nucleus stress. Simulations reveal that highly contractile SMCs were most sensitive over the range 1-100 kPa. In contrast, less contractile fibroblasts are sensitive over 0.1-10 kPa, and chondrocytes are most sensitive on substrates with a modulus less than 2 kPa. This link between substrate elasticity and the cellular contractility is supported by experimental observations. Ren et al. (2008) report that highly contractile myoblasts form well defined FAs and numerous highly organised SF bundles on substrates stiffer than 300 kPa and also note significant changes in cell morphology over the range 100 – 300 kPa. In contrast, less contractile cells, such as chondrocytes, are reported to be sensitive to much more compliant ranges: substrates stiffer than 4 kPa are reported to lead to flattened morphologies, while less stiff substrates lead to rounded chondrocyte morphologies (Schuh et al., 2010; Subramanian and Lin, 2005). The predictions presented in the current study, which are strongly aligned with these experimental observations, suggest a strong link between the contractility of cells and the range of substrate stiffness over which they exhibit the greatest changes. The coupled SF and FA framework offers a unique insight into the role of SF contractility and tension dependent remodelling in the ability of cells to actively respond to substrate stiffness.

6.4.3 Nucleus stress as a result of SF contractility and substrate stiffness

The current study predicts that the level of stress in the nucleus is affected by both cellular contractility and substrate stiffness. Previous experimental studies have established the mechanical link between the cytoskeleton and the nucleus (Buxboim et al., 2010), and, in particular, that the removal of nuclear lamins also leads to disruption of the cytoskeleton (Broers et al., 2004). Significant differences have been reported in nuclear stresses and apparent stiffness levels between stem cells and differentiated phenotypes (Pajerowski et al., 2007). Furthermore, it is well established that substrate stiffness directs stem cell lineage (Discher et al., 2005). Compliant substrates are reported to be neurogenic, while stiffer substrates are found to be myogenic, and comparatively rigid substrates are osteogenic (Engler et
al., 2006). The current study predicts substrate-dependent changes in nucleus stress for a given cell type using an unchanged parameter set. The ability to accurately simulate nucleus stress offers a unique insight into the mechanical regulation of stem cell differentiation and will potentially provide a powerful predictive tool for tissue engineering applications.

### 6.4.4 Cell tractions

The current study predicts the traction stress exerted by a cell on an elastic substrate. Simulations predict that shear tractions are highest at the cell periphery and highly dependent on substrate elasticity. On compliant substrates, shear tractions are computed to be ~100 Pa in the interior of a smooth muscle cell, rising to 250 Pa at the cell periphery, whereas on stiffer substrates, tractions in the interior range up to 800 Pa rising to over 1.5 kPa at the periphery. Previous experimental studies have measured the surface shear tractions exerted by a cell using microbeads embedded in elastic gels (traction force microscopy) to calculate the deformation of the gel and hence determine the traction field (Wang et al., 2002). Traction near the interior of the cell are typically 4-5 times lower than peak tractions at the cell periphery, with peaks of over 900 kPa reported for smooth muscle cells, dropping to 400 Pa halfway from the periphery to 200 Pa at the interior (Wang et al., 2002). Lower tractions are reported for less contractile epithelial cells with ~50 Pa internally and 150-200 Pa at the cell periphery (Gavara et al., 2006; Roca-Cusachs et al., 2008). The tractions predicted in the current study are strongly aligned with such experimental data. Furthermore, experimental studies have also reported that substrate stiffness influences cellular tractions; tractions for endothelial cells seeded on a 1 kPa substrate are reported to be ~200 Pa, whereas significantly higher tractions of ~1000 Pa are measured for endothelial cells on 5 kPa or 10 kPa substrates (Califano and Reinhart-King, 2010). A similar dependence of shear tractions on substrate stiffness is predicted by the modelling framework used in the current study. It should be noted that, in the present study, significant tractions in the normal direction are computed for cells on stiffer substrates. Experimental techniques, such as traction force microscopy, typically measure only the shear component. However, both normal and shear tractions can
lead to stretching of FA bonds. Considering only the shear component of the contact tractions may lead to underestimation of the forces exerted on FA integrins at the periphery of cells on very stiff substrates.

### 6.4.5 Cell spreading

The latter part of the current study presents simulations of a cell attaching to and spreading on a rigid substrate. The mixed mode FA model promotes the spreading and flattening of the cell, while resisting the lateral contraction of the cell due to SF contractility. During cell spreading, the actin cytoskeleton evolves as the size of the adhered surface grows. FAs are predicted to form at the cell periphery and support fibre tension leading to the formation of a band of SFs from the cell periphery over the nucleus. Such long SFs that extend from the cell periphery over the nucleus have been observed experimentally (Elineni and Gallant, 2011; Goffin et al., 2006). Removal of the active FA component of our computational framework at the cell-substrate interface inhibits the formation of such dominant SF bands. This highlights the importance of considering both SF and FA formation in a mutually dependent framework.

While the current study represents a step forward in the computational investigation of SF and FA behaviour of cells spread on elastic substrates, a number of limitations exist that should be considered in future studies. Experimental studies have shown that protrusion forces exerted by filopodia and lamellipodia during spreading can be ~5pN and ~20 pN (Cojoc et al., 2007). These phenomena have not been considered in the current study; however, future computational studies of cell spreading should consider these processes. Previous experimental studies have also shown that microtubules undergo complex deformations that merit investigation in future studies (Brangwynne et al., 2006); however, microtubules and other components of the cell have all been included in the current formulation as part of a passive hyperelastic component. Axially symmetric finite element models used in the current study are based on experimental observations of spread cell morphologies (Caille et al., 2002; Thoumine et al., 1999). Axial symmetry has been successfully used in previous computational studies to minimize computational cost.
(Caille et al., 2002; Haider and Guilak, 2002; Nguyen et al., 2010; Ofek et al., 2009; Rowat et al., 2005). Previously, in Chapter 4, the investigation of the role of SF contractility in the compression resistance of cells reported similar trends for axisymmetric and for elongated cell geometries (Ronan et al., 2012).

6.5 Conclusion

In the current study the substrate dependent response of contractile cells is predicted with no predefined SF or FA arrangement. Each cell phenotype is simulated using an unchanged set of parameters, predicting increased levels of SF and FA formation on stiffer substrates. The predictions of this mutually dependent material-interface framework are strongly supported by experimental observations of cells adhered to elastic substrates. SF contractility is found to strongly influence the substrate-dependent response of cells, including changes in nuclear stress and cell tractions. This framework is also used to simulate cell spreading where an unadhered cell geometry is initially assumed. Again, spreading simulations highlight the importance of inter-dependent SF and FA formation.
6.6 Figures

Figure 6.1. Schematic diagram of a 3D cell showing detail of the radial plane with stress fibres (SFs) shown in red and the nucleus shown in blue. Insets shows 240 fibre orientations and unit vector m and angles (ω,φ). A mixed mode focal adhesion formulation is used with a passive or non-specific formulation to simulate the interaction between the cell (yellow) and substrate (grey).

Figure 6.2. Contour plot of average stress fibre (SF) formation for cells seeded on elastic substrates (A-E). The average SF activation level is summed over the volume of the cytoplasm (F) and variance of SF formation (max – average) at each point is summed of the volume of the cytoplasm (G) as a function of substrate stiffness.
Image removed due to copyright

Figure 6.3. Predicted concentration of focal adhesion (FA) integrins as a function of distance from cell centre for cells seeded on elastic substrates (A). Surface averaged FA concentration for each cell as a function of substrate stiffness (B).

Image removed due to copyright

Figure 6.4. Cell height (A) and averaged nucleus stress (B) as a function of substrate stiffness. Surface shear tractions (C) and normal tractions (D) as a function of distance from cell centre for cells on elastic substrates.
Figure 6.5. Stress fibre (SF) variance averaged over the cytoplasm volume (A) and volume averaged nucleus stress (C) as a function of substrate stiffness. The plots A and C are reproduced in B and D, normalized by the maximum value on the stiffest substrate to show relative changes on softer substrates.

Figure 6.6. Stress fibres evolution during cell spreading at 50s, 150s, 300s, 400s, and 550s for a cell which is initially not in contact with a rigid substrate (A-E). The concentration of focal adhesion integrins is also shown (F).
Figure 6.7. Stress fibre (SF) formation for a cell simulated with a passive interface model that acts only in the normal direction (A) and for a cell simulated with an active mixed mode formulation. SFs are shown after 60 seconds (left) and 550 seconds (right). Note: only half of the spread cell is shown due to a vertical symmetry axis at the left of the cell as presented here.

Figure 6.8 Effect of substrate stiffness of F-actin organization quantified using densitometric quantification of Western blots. The relative increase in sedimented actin is shown for fibroblasts plated on polyacrylamide gels of 0.7 kPa, 4.5 kPa, 15.2 kPa, or tissue culture plastic. Adapted from Solon et al. (2007)
Figure 6.9 Immunofluorescence of myosin (non-muscle myosin IIb) in cells seeded on substrates with different stiffness. Note that the last data points on the right are for cells seeded on glass and the x-axis is broken at this point. Adapted from Engler et al. (2006).

Figure 6.10. Comparison of experimental observations of focal adhesion (FA) proteins for fibroblasts cells seeded on circular adhesive patches (shown as solid black lines) and computational predictions of cells seeded on elastic substrates (shown as dashed lines for 200 kPa (blue) 20 kPa (green) and 2kPa (magenta). Experimental data adapted from (Elineni and Gallant, 2011)
6.7 REFERENCES


Cells on microposts: the response of stress fibres and focal adhesions to changes in micropost stiffness and to applied micropost deformations

7.1 Introduction

Mechanotransduction is an essential component of cellular functions such as cell motility (Lo et al. 2000); and the physical environment around the cell has been shown to direct stem cell lineage specification (Engler et al. 2006; Discher et al. 2005). The contractile response of cells to ECM stiffness has been shown to be an important factor in wound healing (Danjo and Gipson 1998), atherosclerosis (Isenberg et al. 2009), and cancer progression (Paszek et al. 2005; Levental et al. 2009). In order to elucidate further mechanotransduction, previous experimental studies have employed arrays of microposts, or micropillars (i.e. a surface patterned with vertical cylindrical features with dimensions on the order of microns), to quantify the traction generated by a cell and provide a tuneable mechanical environment (Tan et al. 2003; Chen et al. 2004). These micropost arrays have been used to study the adhesion and contractility of static cells (Fu et al. 2010; Tan et al. 2003), cells subjected to local (Sniadecki et al. 2007) and global applied deformation (Mann et al. 2012), cell migration (Du Roure et al. 2005; Sochol et al. 2011a), and cell-cell adhesion (Liu et al. 2010). 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 a comprehensive modelling formulation that considers the key biochemical processes, i.e. the tension dependent formation of focal adhesions and the remodelling and contractility of the actin-myosin cytoskeleton (i.e. stress fibres).
FAs provide a mechanical link between the SF contractility and the physical environment around the cell, in this case the microposts. Previous studies of cells on microposts have established a relationship between cell tractions and FA area (Fu et al. 2010; Tan et al. 2003) and also the changes in SFs and FAs on arrays with different post stiffness and geometry (Han et al. 2012; McGarry et al. 2009). Post spacing and stiffness has also been spatially varied with an array to investigate the migration of cells over gradients in substrate stiffness (Sochol et al. 2011b; Sochol et al. 2011a). Micropost arrays have 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 (Liu et al. 2010). Arrays of microposts have also been employed to apply deformations to a cell: Sniadecki et al. (2007) 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. (2012) 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.

In the current study, the thermodynamically motivated adhesion model is used in tandem with the active SF contractility model (as described in Chapter 3) to investigate the response of cells adhered to micropost arrays. In contrast to previous studies which studied cells on patterned substrates (Pathak et al. 2008) or only considered SF formation in cells on microposts (without FAs) (McGarry et al. 2009), the formulation in the current study considers both SF and FA formation in cells adhered to static and dynamically loaded micropost arrays. The simulations are presented in four sections and relevant experimental results are discussed in each section:

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 applied locally to individual posts is predicted;

4) Cells are simulated on micropost arrays subjected to equibiaxial stretch.

7.2 Methods

7.2.1 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:

$$F_p = \left(\frac{3E_p I}{L^3}\right) \delta_p \quad (7.1)$$

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.

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 7.1. 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, representing 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 (McGarry et al. 2009) and based on simulations in Chapters 4 and 5 and are identified in Table 7.1. 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. (2010); Sniadecki et al. (2007); and (Tan et
al. 2003) and Mann et al. (2012), respectively. The contractility of each cell type is represented by altering the maximum stress in a fully activated fibre ($\sigma_{\text{max}}$).

Table 7.1 Material and interface parameters for SMCs, MCSs, and FBs.

<table>
<thead>
<tr>
<th>Material parameters</th>
<th>Interface parameters</th>
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<tr>
<td>$\sigma_{\text{max}}$(kPa)</td>
<td>$E_{\text{cell}}$(kPa)</td>
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<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>SMC</td>
<td>25</td>
</tr>
<tr>
<td>MSC</td>
<td>8</td>
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<tr>
<td>FB</td>
<td>3.5</td>
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7.2.2 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, 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:

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

(7.2)

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

$$\Pi = \eta_{\text{max}} - \bar{\eta}$$

(7.3)

Finally, vector plots are presented to illustrate the direction in $\eta_{\text{max}}$ occurs at each point.

7.3 Results and discussion
7.3.1 Simulation of fibroblasts adhered to static micropost arrays.

Figure 7.2 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 µm² and ~2920 µm² 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 Figure 7.3.

The effect of post stiffness on predicted SF and FA formation is shown in Figure 7.4. Simulations are performed and for a high post stiffness of 1556 nN/µm (Figure 7.4 (A,B)) for a low post stiffness of 1.9 nN/µm (Figure 7.4(A,B)) for a cell adhered to a 13x13 micropost array (note that the cell in Figure 7.4(A,B) 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; 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 7.4E 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 7.5(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 7.5(B), 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. (2012) 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. (2012) 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. (2003) for smooth muscle cells spread on arrays of 2 – 25 posts and was also captured in the study of McGarry et al. (2009). The post arrangement of these two experimental studies have different post spacing, and the study of Han et al. (2012) 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. (2012).

The total FA area for each cell was calculated by summing the areas of the cell membrane where the concentration of high affinity integrins (ξh/ξ0) above a threshold level. Figure 7.6A 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. (2010) 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 7.6C. The changes in FA area with post stiffness in Figure 7.4 show that cells on stiffer posts contain a small number of large FA in contrast to cells on compliant posts, which 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 7.6B. For each micropost stiffness, total force increases approximately linearly with FA area and total force also increases 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 7.6D as a function of post stiffness. This slope has previously been referred to as the “FA Stress” (Fu et al. 2010). Similar to the experimental observations of Fu et al. (2010), this slope is predicted to double over the range 1.9 nN/μm to 18.16 nN/μm, as shown in Figure 7.6E.

### 7.3.2 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. (2003). 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 600 s of signal driven formation in Figure 7.7A. Dominant bundles are predicted to form near the cell periphery, particularly 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 7.7B. Such long fibres parallel to the concave edges have been observed experimentally (McGarry et al. 2009; Tan et al. 2003). The computed FA distribution for the same cell is shown in Figure 7.8A. Adhesions are predicted to form distinctive “horseshoe” type shapes, similar to those observed by Tan et al. (2003), (reproduced in Figure 7.8C for comparison). The predicted reaction forces exerted by the cell on each post are superimposed over the FA plot and are in good agreement with the experimental observations.
Magnified views of two regions are presented in Figure 7.8C to further illustrate 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 7.8(D). This prediction is in very good agreement with the experimental observations of Tan et al. (2003) (reproduced in Figure 7.8(E)).

### 7.3.3 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 Sniatecki et al. (2007), 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 7.9B: 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 7.9A. 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 are 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 Figure 7.10) 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. (2007), where an increase in area was observed only on the moved post for single post actuation. The computed results presented in Figure 8 highlights the importance of considering both the location and direction of any applied deformation in single post actuation experiments.

7.3.4 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 Section 7.3.1 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 7.1. Simulations consist of two steps: an equilibrium step lasting 600 s, where SF formation is driven by an exponentially decaying signal; and a loading step of 3600 s, 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 7.11(A). In order to determine the effect of cellular signal during stretch, three stretch activated signalling regimes were considered during the second step of the analysis: (i) a constant, fully activated signal; (ii) an exponentially decaying signal; and (iii) no signal.

The post force normalized by the force at the end of the equilibrium step is shown in Figure 7.11(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 which 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. (2012) 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. (2012), 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. (2012), 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 modulus. Figure 7.11 (C) shows the post force before stretching, the peak immediately following stretch, and the force 3600 s following stretch. The equilibrium force and peak force are not affected by the addition of the viscous component. The post force 3600 s 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 7.11(D) using the active model in parallel with the passive Maxwell cytoplasm. Peak force increases linearly with increasing strain and the force computed 3600 s after stretching decreases with increasing applied strain. In the case of the smallest applied strain (1.5%), the post force after 3600 s is ~15% above the equilibrium value. The larger applied strains of 7% and 15% lead to a reduction in force after 3600 s 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 3600 s after stretch is shown in Figure 7.12 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. (2012) observes a larger drop in post force 3600 s after loading with increased applied strain, as shown reproduced in Figure 7.11(D). However, Mann et al. (2012) 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.

### 7.4 Concluding remarks

In the current study, a thermodynamically based focal adhesion model, combined with an active stress fibre contractility model (as described in Chapter 3), was 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. (2010) and Han et al. (2012), is successfully captured. In particular, the distinctive shape of FAs and the direction and magnitude of post tractions observed by Tan et al. (2003) 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 (Sniadecki et al. 2007) 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. (2012), is successfully simulated using the active formulation with a passive viscoelastic cytoplasm component.

Micropost arrays are novel tools that provide quantifiable data relating to the contractile response of cells, i.e. post defections, from which the post forces can be determined. However, interpreting this data is not trivial. In-vitro studies to date have quantified average post force magnitude and total post force magnitude as a measure of cell contractility. These quantities are influenced by the number and
7 - Cells on microposts

arrangement of posts, in addition to post geometry and stiffness. Post spacing varies from 4 to 10 μm in most cases in either square or triangular arrangements (Sniadecki et al. 2007; Cesa et al. 2007), however, some arrays use very large post spacing of 20 – 75 μm (Baker et al. 2011). The stiffness of an individual post is then varied by adjusting the post radius and length, e.g. for a post diameter of 2 μm, lengths between 0.97 and 12.9 μm will give post stiffness between 1556 nN/μm and 1.90 nN/μm (Fu et al. 2010). Yang et al. (2007) compared the response of cells adhered to microposts with a diameter of 0.67 – 0.83 μm and spacing of 1.75– 4 μm with stiffness of 8 – 37 nN/μm and found no significant difference in the total strain energy of the posts or in the spread area of the cell. However, this study was conducted over a limited range of stiffness and did not compare SF intensity or FA area. In contrast, a computational investigation of SF formation in cells adhered to micropost arrays found that post radius and spacing resulted in significant differences in the average post force (McGarry et al. 2009). In the current study, post stiffness is found to have a significant effect on average cell force and on the distributions of SFs across the cell. Furthermore, the current study elucidates the relationship between cell size, FA area, traction force, and post stiffness.

In the current study, the dynamic responses of cells to local and global deformations were successfully simulated without the introduction of a signalling cascade that accompanied the actuation. In both the simulations of the current study and the experimental observations of Sniadecki et al. (2007), the movement of a single post did not cause a change in FA area on unactuated posts; this suggests that this method of loading did not trigger global signal in the cytoplasm that would have resulted in further SF formation and FA remote from the point of actuation. Furthermore, for cells subjected to biaxial stretch, the addition of a signal upon application of the stretch led to incorrect post forces whereas simulations without a deformation activated signal provide a closer agreement with the observations of Mann et al. (2012). A recent mechano-sensitive intracellular signalling formulation incorporates a feedback loop between SF contractility, FA tractions, and signalling pathways (Pathak et al. 2011). In order to further investigate the role of signalling in
the results of Sniadecki et al. (2007) and Mann et al. (2012), such a formulation should be included in future computational studies.

In the current study, the cell is simulated using an active material formulation that predicts the distribution and contractility of stress fibres. The passive non-contractile elements of the cytoplasm are initially included as a hyperelastic formulation. In the latter part of the study this hyperelastic formulation is replaced with a viscoelastic component in order to capture the long term reduction in the post force after the application of biaxial stretch observed by Mann et al. (2012). The viscoelasticity of the cytoplasm allows for further shortening of stress fibres following the applied stretch, with the resulting dissociation of the cytoskeleton leading to a drop in the equilibrium post force. Furthermore, the viscoelastic type response for cells treated with cytochalasin, i.e. with no contractility, observed by Thoumine and Ott (1997) suggest that the passive cytoplasm does contain a viscous component. Previous models have also treated the cytoskeleton as a soft glassy material and found that under cyclic loading the elastic and viscous stresses displayed similar frequency dependent responses, suggesting that the both stresses are caused by the same cellular components (Fabry et al., 2003; Vaziri et al., 2007). The current SF and FA model did not capture the lower peak force observed for a larger applied strain observed by Mann et al. (2012). This lower peak force maybe as a result of the breaking and reforming of FAs due to the instantaneous stretch. A kinetic model of FA formation may capture this phenomenon.

The current study identifies a number of future directions for both experimental and computational studies. The response of FAs throughout a cell subjected to a locally applied deformation, as observed by Sniadecki et al. (2007), is successfully captured by the modelling framework; however, simulations reveal the sensitivity of this response to the relative location and direction of the applied displacement. Future studies should further investigate this phenomenon by either patterning the microposts to restrict cell spreading to a predetermined shape, or by considering the direction and location in the interpretation of experimental results. This method of locally applied deformation also allows for the investigation of the rupturing of FAs at different strain rates and amplitudes. Such an experimental investigation
would also provide a robust validation method for the further development and calibration of a FA formulation that includes formation and rupture kinetics. Finally, Han et al. (2012) observed an initial increase, and subsequent decrease in average force with increasing cell size for cells seeded on compliant microposts. However, this trend was not observed for all micropost stiffness, as was seen in the previous study of Tan et al. (2003). Future studies of cells adhered to micropost arrays should consider a wider range of cell sizes and post stiffness to investigate if this phenomenon is reproduced on different micropost array geometries.
7.5 Figures and Captions

Figure 7.1 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.

Figure 7.2 Predicted stress fibre formation (A,C) and focal adhesion formation (B,D) in cells adhered to 5x5 and 13x13 arrays of microposts. Microposts have a 1µm 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 600 s of signal driven formation.
7 - Cells on microposts

Figure 7.3 Predicted stress fibre (SF) formation (A) and focal adhesion (FA) formation (B) in a cell adhered to a 25x25 array of microposts. SFs and FAs are shown have reached a steady state following 600 s of signal driven formation.

Figure 7.4 Predicted stress fibre formation (A,C) and focal adhesion formation (B,D) in cells adhered to 13x13 arrays of microposts. Microposts have a 1µm 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 600 s of signal driven formation. Individual post forces are shown for different levels of post stiffness as a function of distance from cell centre (E).
Figure 7.5 Average force per post (A) for and total force per cell (B) for cells adhered to micropost arrays of different stiffness as a function of both cell area (top axis) and number of posts (bottom axis). Each data point represents an individual cell. Similar experimental observations by Han et al. (2012) of average post force and total cell force for endothelial cells (C,D).

Figure 7.6 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. (2010) 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. (2010) are presented for comparison (E); note that the experimentally observed FA stress is computed in the same way.
Figure 7.7 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 (B). The length of each vector corresponds to the SF activation level in the dominant direction.

Figure 7.8 Comparison of computed FA formation (A) and experimental observations of focal adhesion formation (B). Predictions show the dimensionless concentration of high affinity integrins ($\xi_h$). Experimental images are reproduced from Tan et al. (2003). 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 red together with the corresponding experimental forces and FA areas observed by Tan et al. (2003) (D).
Cells on microposts

Figure 7.9 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 1 μm, as shown in (B). For comparison, the experimental results of Sniadecki et al. (2007) are reproduced with permission (C).

Figure 7.10. Change in stress fibre formation in cells subjected to locally applied deformations. The change is calculated by subtracting the average level of SF formation at two time points: immediately prior to the actuation and 40 seconds after the actuation. Note that the contours are plotted on the undeformed cell shape. Changes in SF formation are shown for four separate loading scenarios: A – a corner post moved towards the cell centre; B – a corner post moved away from the cell centre; C – an edge post moved towards the cell centre; D – an edge post moved away from the cell centre.
Figure 7.11 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 (Mann et al. 2012).

Figure 7.12 Change in stress fibre formation in cells subjected to biaxial stretch. The change is calculated by subtracting the average level of SF formation at two time points: 3600 seconds after the stretch and immediately prior to the stretch. Note that the contours are plotted on the undeformed cell shape. Changes in SF formation are shown for three different stretch magnitudes: A –1.5%; B –7%; C –15%.
7.6 References


8 Discussion and conclusions

8.1 Introduction

This chapter provides an overview of the work presented in this thesis in the broad context of cell biomechanics. It should be noted that each chapter also contains a focused discussion section that provides a more in depth analysis of the individual results. The novelty and challenges of implementing a stress fibre (SF) and focal adhesion (FA) model in a fully 3D environment are summarised below. The following key findings are also discussed:

- Simulations reveal that polarised and axisymmetric spread cells contain dominant SF bundles that are stretched during compression, causing an increase in computed compression forces compared to round cells (Chapter 4).
- The 3D computational framework predicts changes in cell morphology due to the addition of cytoskeletal disrupting agents and simulated compression of treated and untreated cells allows for the evaluation of the active and passive material parameters for osteoblasts (Chapter 5).
- Compliant substrates are found to provide insufficient tension for SF persistence in adhered cells, causing dissociation of SFs and lower focal adhesion formation. Different levels of cellular contractility, which are representative of different cell phenotypes, are found to alter the range of substrate stiffness that causes the most significant changes in SF and FA formation (Chapter 6).
- The computational framework quantitatively predicts changes in FA sizes and tractions for cells adhered to micropost arrays with different post stiffness and with externally applied post deformations (Chapter 7).

Future perspectives building on the work presented in this thesis are also discussed. Finally, some concluding remarks summarise the findings and implications of the thesis.
8.2 Discussion

The current work, for the first time, presents the development of a computational framework that incorporates stress fibre contractility and remodelling, in addition to focal adhesion formation and evolution, in a fully 3D environment. Previous computational models of stress fibre formation assume that the cell has a planar geometry, or that SF formation is limited to a basal plane (Hsu et al. 2009; Kaunas and Hsu 2009; Vernerey and Farsad 2011; De and Safran 2008). This 2D assumption limits the applicability of the model and provides an incomplete representation of the role of the cytoskeleton. Such an assumption is appropriate only in the cases where the height of the cell is sufficiently small compared to the other dimensions, e.g. for cells adhered to micropost arrays, which are used to quantify cellular tractions that are acting in the horizontal plane only. However, experimental observations suggest that fibre formation is not generally limited to a 2D basal plane; experimental images of actin filaments show that SFs may exist over the top of the nucleus (Weafer, Ronan, et al. 2012; Peeters et al. 2004) and SFs have been shown to interact with and attach to the nucleus (Wang et al. 2009). Moreover, disrupting the contractility of the cytoskeleton in spread cells has been shown to cause the nucleus and cell to change height significantly, suggesting that SF tension is responsible for the flattened nuclear morphology observed experimentally (Avalos et al. 2011). Clearly, SF formation is not generally limited to a single plane and it is essential to consider fibre formation in all possible directions. Furthermore, a planar representation of cell geometries precludes the inclusion of the nucleus as a separate domain in simulations. By considering realistic cell geometries in 3D, simulations in this thesis explicitly include the nucleus and also consider a range of cell shapes. Predicted 3D SF distributions for irregularly shaped cells presented in Chapter 5 are in strong agreement with experimentally observed distributions (Weafer et al. 2012). Additionally, the simulations presented in Chapter 7 demonstrate the importance of fibre formation and contractility in 3D during cell spreading.

Simulations of compression of round and spread cells reveal that SF tension in dominant fibre bundles that are stretched during compression causes an increase in
the compression resistance of spread cells compared to round cells. In the case of elongated spread cells, dominant SF bundles are predicted to form parallel to the long axis of the cell. Similar to the observations of Peeters et al. (2004), the tension in these long fibres acts to restrict the deformation of the cell, increasing the compression resistance. In contrast, a lower level of SF formation is predicted for round cells and, furthermore, dominant fibres are not stretched during compression of round cells, leading to significantly lower compression resistance. The prediction of lower SF formation and reduced compression resistance for round cells is supported by the experimental observations of Caille et al. (2002) and Huang et al (2003). Previous attempts to capture the compression behaviour of round and spread cells have employed isotropic homogenous material formulations such as hyperelasticity and viscoelasticity (Caille et al. 2002; Ofek et al. 2009; McGarry 2009). Such passive models cannot capture the experimentally observed data without artificially increasing the apparent stiffness of spread cells. Clearly, SF contractility and remodelling must be considered in computational models of cell deformation. Additionally, in both the simulations presented in the current work, and in the experimental work of Peeters et al.(2004), SFs are also shown to restrict the deformation of the nucleus, as SFs are not limited to the basal plane. This further highlights the importance of considering fibre orientations in 3D and simulating realistic cell shapes that include the cell nucleus.

The simulations presented in the current study have been extensively discussed in the context of the existing experimental literature. The simulated compressive forces presented in Chapter 4 are quantitatively in agreement with experimentally observed forces for highly contractile myoblasts (Peeters et al. 2005) and less contractile fibroblasts (Deng et al. 2010). Furthermore, the predicted SF distributions are in good agreement with previous studies (Oakes et al. 2012; Peeters et al. 2004). Additionally, the predicted SF distributions and mechanical behaviour of osteoblasts are in strong agreement with the experimental observations of Weafer, Ronan, et al. (2012). Furthermore the SF distributions of an irregularly shaped 3D cell are accurately predicted (Chapter 5). The evolution of the 3D cytoskeleton is shown to play a key role in the active spreading of a contractile
cell on a substrate. Mixed mode FA interactions are shown to regulate SF tension during spreading (Chapter 6). Such quantitative validation of the predicted link between SF contractility and compression resistance demonstrates the potential of this 3D modelling framework to elucidate the important role of the cytoskeleton in the mechanical behaviour of cells and provides a powerful tool to interpret experimental data. In addition to the simulations in this thesis, this 3D framework was also used by Dowling et al. (2012) to simulate the direct shear of untreated chondrocytes and of chondrocytes treated with cytoskeletal disrupting agents; SF contractility was found to significantly contribute to the mechanical behaviour of chondrocytes.

Substrate stiffness has been shown previously to direct stem cell lineage specification (Engler et al. 2006), and also cause significant changes in SF and FA formation (Discher et al. 2005). Experimental studies have established the mechanical link between the cytoskeleton and the nucleus (Buxboim et al. 2010), and, in particular, that the removal of nuclear lamins also leads to disruption of the cytoskeleton (Broers et al. 2004). Clearly, SF contractility plays a vital role in substrate regulation of cell differentiation; however, the mechanisms underlying such mechanotransduction are poorly understood. The present study, for the first time, predicts the stress in the nucleus due to SF contractility in 3D cell geometries for a range of cell phenotypes adhered to elastic substrates. Substrate elasticity was found to significantly alter SF distributions and, hence, nucleus stress. Furthermore, a range of substrate stiffness was identified for each cell phenotype over which the stress changes significantly. Outside of this transitional stiffness range, nucleus stresses do not change significantly. Additionally, experimental studies have linked nucleus deformations with gene expression (Roca-Cusachs et al. 2008; Thomas et al. 2002). Engler et al. have also established a clear link between substrate stiffness and MSC differentiation; with compliant substrates leading to a neurogenic response and stiffer substrates leading to osteogenesis, as shown in Figure 8.1. The simulations provided in the current thesis demonstrate a link between substrate stiffness, SF formation, and nucleus stress. Importantly, the transitional stiffness ranges predicted in the current study are supported by quantifications of SF activity.
in a range of different cell types (Solon et al. 2007; Schuh et al. 2010; Engler et al. 2006). The ability to accurately simulate SF formation and nucleus stress for cells in different mechanical environments provides a powerful predictive tool for the functional design of ECM stiffness in tissue engineering applications.

In the current work, simulations are performed on cells with different levels of SF contractility, representing different cell phenotypes: smooth muscle cells, mesenchymal stem cells, fibroblasts, and chondrocytes. The material parameters used to represent the contractility of each cell type have been determined in previous simulations of cells adhered to micropost arrays (McGarry et al. 2009). In the current work, these values are used to simulate SF and FA formation in a range of different boundary conditions and cell geometries. In contrast, previous computational predictions of passive material properties for osteoblasts are inconsistent: AFM indentation suggests the stiffness is 17 kPa (Takai et al. 2005) while whole cell compression suggest 6.5 kPa (Darling et al. 2008). In order to provide meaningful insight and ensure confidence in predicted results, material formulations should not predict different material properties for different boundary conditions.

Further quantitative validation of the constitutive formulation for SF and FA formation is provided in Chapter 7 through the simulation of cells on micropost arrays. Predicted FA areas and traction forces are in particularly good agreement with the observations of Fu et al (2010) and Han et al. (2012). Changes in FA area surrounding a single actuated micropost are in good agreement with the experimental observations of Sniadecki et al. (2007) and the simulations also suggest that the actuation does not trigger a significant cell-wide signalling cascade. Simulations of cells subjected to biaxial stretching are performed using a number of different signalling cascades and also introducing passive cytoplasm viscoelasticity. The predicted response to biaxial stretch, when compared to the results of Mann et al. (2012), suggest that cytoplasm viscoelasticity plays a role in the remodelling of the cytoskeleton following stretch.
8.3 Future directions

In the current work, for the first time, SF and FA formation are considered in a fully 3D environment to simulate the behaviour of cells under mechanical loading and in different physical environments. This computational framework will provide a powerful predictive tool to further investigate the active contractility of cells in different loading environments and will also provide a foundation for further model development. A number of common cell mechanics experiments have not been analysed using a comprehensive 3D SF and FA framework; in particular, micropipette aspiration would provide further validation of the computational framework presented in the current work while also providing valuable insight into the role of the cytoskeleton and nucleus deformation during aspiration. Micropipette aspiration would also provide a platform to investigate FA rupture as cells are pulled away from a substrate; such experiments, in tandem with the mixed mode FA interface presented here, would provide quantitative insight into the kinetics of bond dissociation and rupture.

The current computational framework represents a significant step forward compared to existing models as it considers the remodelling and contractility of the cytoskeleton and adhesions in realistic 3D cell geometries; however, further model development will continue to provide insight into cell mechanics. In particular, cellular signalling in the current framework is based on the assumption of a spatially uniform decaying signal that drives SF formation. A recent study by Pathak et al. (2011) presents a one-dimensional implementation of a signalling feedback loop between FA traction and SF formation based on the release of IP$_3$ molecules and diffusion of a Ca$^{2+}$ signal. Incorporating such a feedback loop into the 3D framework presented here would provide further insight into the mutually dependent relationship between SF remodelling and FA formation.

The current study deals exclusively with the behaviour of single cells, however, the constitutive framework presented here could also be adapted to provide insight into tissue mechanics. The proliferation and migration of cells into 3D porous scaffolds is dependent on the physical properties of the scaffold; however, it is unclear how individual cells experience the mechanical environment due to the
complex shapes of the scaffold and the varied arrangements of cells in relation to struts and other scaffold features. Additionally, an investigation of the role of contractile smooth muscle cells in the medial layer of an artery, particularly in a stented artery, would provide new insight into tissue injury in stented vessels. SF remodelling has also been linked with matrix anisotropy in fibrous tissues (Foolen et al. 2012); however, the mechanisms underlying the interdependent remodelling of SFs and collagen fibres is poorly understood. Further development of the current formulation in tandem with a collagen remodelling formulation should be performed to investigate these mechanisms.
8.4 Conclusions

The computational framework presented in this thesis incorporates the contractility and remodelling of SFs, in addition to the formation and evolution of FAs, in a fully 3D environment in order to provide unique insight into the biophysical processes underlying the mechanical behaviour of cells.

- The simulations elucidate the role of the cytoskeleton in the compression resistance of cells; tension in dominant SF bundles acts to restrict the deformation of the cell, increasing the resistance to compression.
- The 3D framework, in tandem with experimental observations (Weafer, Ronan, et al. 2012), successfully parses the contributions of SF contractility, the nucleus, and the cytoplasm to the mechanical behaviour of osteoblasts and, hence, allows for the accurate determination of the active and passive material parameters.
- Simulations presented in the thesis use a consistent, unchanged, parameter set to successfully capture the mechanical behaviour of a specific cell phenotype for a diverse range of boundary conditions. This further demonstrates that it is essential that computational models of cell biomechanics consider the underlying biomechanical cellular processes. Passive cell models neglect the active response of cells to the physical environment and, hence, require ad-hoc adjustment of material properties.
- The mutually dependent responses of SFs and FAs, effected by changes in micropost stiffness and by applied micropost deformations, are successfully simulated and predictions are in strong agreement with experimental data (Sniadecki et al. 2007; Han et al. 2012; Fu et al. 2010; Mann et al. 2012).
- The simulations provided in the current thesis elucidate the link between substrate stiffness, SF formation, and nucleus stress. These predictions provide insight into the relationship between substrate stiffness and regulation of MSC differentiation observed experimentally (Engler et al. 2006).

The 3D SF and FA formulation developed in the current thesis has elucidated the role of the cytoskeleton in a number of experimentally observed phenomena. This formulation can serve as a powerful predictive tool for cell biomechanics and tissue engineering.
8.5 Figures

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Figure 8.1. Fluorescent intensity of differentiation markers for neurogenesis, myogenisis, and osteogenesis versus substrate elasticity reveals a preferential range of substrate stiffness for each cell type. Adapted from Engler et al. (2006).
8.6 References


Doi 10.1371/Journal.Pone.0004853


A. FORTRAN user material (UMAT)

Image removed due to copyright
B. FORTRAN user interface (UINTER)

Image removed due to copyright
C. Micropipette aspirations

Adhered and suspended cell models are created including analytically rigid micropipettes (Figure D.1). Simulations consist of two steps, an initial equilibrium stage where the cell responds to a signal cascade and a second stage where an aspiration pressure is applied instantaneously and held at a constant value. In the first step the cytoskeleton is formed and in the second step the aspiration pressure causes the cell to move upwards into the pipette, and the amount of cell deformation into the pipette is defined as the aspiration length.

Figure D.1 Schematic diagram of axisymmetric micropipette aspiration models

Stress fibre concentration levels in the cell cytoplasm are plotted in Figure 2. The dominant stress fibre bundles are examined by subtracting the average activation level over all directions, $\bar{\eta}$, from the maximum activation level in any direction, $\eta_{\text{max}}$.

The adhered spread cell shows large concentrations of dominant stress fibres in the region surrounding the adhesion along the base of the cell at the end of the first step (Figure 2A). Micropipette aspiration causes significant cytoskeletal remodelling locally at the aspiration site (Figure 2A), and this trend was also
observed for the suspended cell (Figure 2B) and the adhered rounded cell (Figure 2C).

The predicted aspiration lengths are shown in Figure 2D for suspended and adhered round and spread cells. Higher SF concentrations in the spread adhered cell lead to a stiffer response, decreasing the aspiration length. In contrast, the low SF levels in the round adhered cell result in a larger aspiration length. Very low SF concentration levels are computed for an un-adhered suspended cell resulting in high aspiration lengths.
Figure D.2 SF concentrations following micropipette aspiration for spread (A) and round (B) adhered cells and suspended cells (C). Aspiration lengths as a function of time for an applied step pressure of 500Pa (D).
Figure D.3 Aspiration lengths as a function of time for aspiration for spread, suspended, and adhered cells. Reproduced from Thoumine et al [1].

These results strongly agree with experimentally observed phenomena, in which cell spreading and actin distributions affect micropipette aspiration dynamics. Thoumine et al highlighted the effect of spreading on material properties through micropipette aspiration and computation modelling using a biphasic viscoelastic cell model[1]. However, this viscoelastic model used different material constants for each scenario to best fit the data. Our stress fibre model uses the same material properties for each scenario, and the effect of cell spreading on growth and remodelling of the cytoskeleton explains the different aspiration lengths observed.

These results demonstrate the ability of our computational model to simulate cytoskeletal remodelling and predict differences in experimentally observed responses to mechanical stimuli for suspended and adhered cells.
Figure D.4. Aspiration lengths for suspended, adhered and spread cells.

D. Viscoelastic passive cytoplasm UMAT

```
viscous model - direct
D1 = 1./3.etav + 1./etas
D2 = 1./3.etav - 1./2.etas
A = 1./6.etav + 1./2.etas + 1./dtime*emod
B = 1./6.etav - 1./4.etas - enu/(dtime*emod)

BBABAA = 2*B*B-A*B-A*A

C11 = D1 * PiolaK20(1,1) + D2 * (PiolaK20(2,2)+PiolaK20(3,3))
C22 = D1 * PiolaK20(2,2) + D2 * (PiolaK20(1,1)+PiolaK20(3,3))
C33 = D1 * PiolaK20(3,3) + D2 * (PiolaK20(1,1)+PiolaK20(2,2))

F11 = -C11 + dEgreen_11 / dtime
F22 = -C22 + dEgreen_22 / dtime
F33 = -C33 + dEgreen_33 / dtime

d_PiolaK2(1,1) = (B * (F33+F22) - (A+B) * F11) / BBABAA
d_PiolaK2(2,2) = (B * (F33+F11) - (A+B) * F22) / BBABAA
d_PiolaK2(3,3) = (B * (F22+F11) - (A+B) * F33) / BBABAA
```

viscous model - shear

```
A12 = (1+enu) / (emod*dtime) + 3.0/(2.0*etas)
D12 = 3.0 / (2.0 * etas)

d_PiolaK2(1,2) = (dEgreen_12/dtime - D12*PiolaK20(1,2))/A12
d_PiolaK2(1,3) = (dEgreen_13/dtime - D12*PiolaK20(1,3))/A12
d_PiolaK2(2,3) = (dEgreen_23/dtime - D12*PiolaK20(2,3))/A12

d_PiolaK2(2,1) = d_PiolaK2(1,2)
d_PiolaK2(3,1) = d_PiolaK2(1,3)
d_PiolaK2(3,2) = d_PiolaK2(2,3)
```

add increment to old 2nd PK stress

```
PiolaK21 = PiolaK20 + d_PiolaK2
```

convert 2nd pk stresses to Cauchy stresses

```
do i=1,3
  do j=1,3
    do m=1,3
      do n=1,3
        stress_cauchy1(i,j) = stress_cauchy1(i,j) + PiolaK21(m,n) * dfgrd1(i,m) * dfgrd1(j,n) / xj1
      enddo
    enddo
  enddo
enddo
```

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