Original Article

In silico stress fibre content affects peak strain in cytoplasm and nucleus but not in membrane for uniaxial substrate stretch

Tamer Abdalrahman[1,2], Neil H. Davies[3], Thomas Franz[1,4*]

[1]Division of Biomedical Engineering, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Observatory, South Africa
[2]Julius Wolff Institute for Biomechanics and Musculoskeletal Regeneration, Charité – Universitätsmedizin Berlin, Berlin, Germany
[3]Chris Barnard Division of Cardiothoracic Surgery, Faculty of Health Sciences, University of Cape Town, Observatory, South Africa
[4]Bioengineering Science Research Group, University of Southampton, Southampton, UK

*Corresponding author
Thomas Franz, PhD
Division of Biomedical Engineering
Department of Human Biology
Faculty of Health Sciences
University of Cape Town
Private Bag X3, Observatory 7935, South Africa
Tel.: +27 21 650 1795
Email: thomas.franz@uct.ac.za

Abstract

Existing in silico models for single cell mechanics feature limited representations of cytoskeletal structures that present inhomogeneities in the cytoplasm and contribute substantially to the mechanical behaviour of the cell. Considering these microstructural inhomogeneities is expected to provide more realistic predictions of cellular and subcellular mechanics. Here, we propose a micromechanical hierarchical approach to capture the contribution of actin stress fibres to the mechanical behaviour of a single cell when exposed to substrate stretch. For a cell-specific geometry of a fibroblast with membrane, cytoplasm and nucleus obtained from confocal micrographs, the Mori-Tanaka homogenization method was employed to describe cytoplasmic inhomogeneities and constitutive contribution of actin stress fibres. The homogenization was implemented in a finite element model of the fibroblast attached to a planar substrate through focal adhesions. With this model, the strain in cell membrane, cytoplasm and nucleus due to uniaxial substrate stretch was assessed for different stress fibre volume fractions in the cytoplasm of up to 20% and different elastic modulus of the substrate. A considerable decrease of the peak strain with increasing stress fibre content was observed in cytoplasm and nucleus but not the cell membrane, whereas peak strain increased in cytoplasm, nucleus and membrane for increasing elastic modulus of the substrate. With the potential for extension, the developed method and models can contribute to more realistic in silico models of cellular mechanics.

Keywords: cell mechanics; stress fibres; cytoskeleton; micromechanical homogenization; Mori-Tanaka scheme; finite element modelling

1. Introduction

The knowledge of how the cell structure deforms under different loads is crucial for a better understanding of physiological and pathological events [1, 2]. The cell comprises different components (e.g. cytosol, fibrous protein networks, nucleus, cell membrane) that are dispersed heterogeneously, display nonlinear behaviour [3,4], and can be highly anisotropic. The cytoskeletal components determine the mechanical properties of the cell. These properties can be quantified through experimental characterisation and theoretical formulations [5, 6] and have been found to vary even for the same type of cell [7].

Various techniques have been developed to obtain the mechanical properties of cells, including micropipette aspiration [8], use of optical tweezers [9], magnetometric examination [10] and atomic force microscopy (AFM) based strategies [11,12].

The cytoskeleton, and actin filaments in particular, affect the morphology and mechanical properties of the cell. For example, cytoskeletal changes associated with cellular remodelling may lead to substantial changes of the mechanical properties of a cell [14–17]. Park et al. [13] examined the localised cell stiffness and its correlation with the cytoskeleton. They reported that the local variation of cytoskeletal stiffness was related to regional prestress. This study comprehensively characterized the localized variations of intracellular mechanical properties that underlie localized cellular function.

Rotsch and Radmacher [18] found a significant reduction in the elastic modulus of fibroblasts when treated with actin disrupting chemicals, with similar findings reported by others [19, 20]. The variation in cellular stiffness has been linked to diseases [21], such as the decrease of the elastic modulus of cancerous cells by one order of magnitude compared to healthy cells [22]. With some exceptions [23-26], previous studies applied homogenization to the entire cell and did not explicitly consider the effect of inhomogeneities [27]. This resulted in non-physical relationships between assessed parameters and the mechanical properties of the cell.

In computational cell mechanics, an accurate description of the anisotropic, nonlinear

behaviour of the cytoskeleton is desired to account for cytoplasmic inhomogeneity. Finite element methods (FEM) have been utilized to study different aspects of cell mechanics [28-30]. The application of FEM to single cell and subcellular mechanics [31-33] is still limited because of the scarcity of data on material properties and shape of sub-cellular structures. The combination of image-based geometrical modelling and FEM have recently facilitated computational models with three-dimensional (3D) cellular morphology including cytoskeleton, cytoplasm, cell membrane, and nucleus [34-38]. However, including cytoskeletal stress fibres as discrete structural elements is one of the current challenges in computational models of cellular mechanics. Multiscale constitutive models may offer a way to overcome this challenge by capturing the mechanical properties of cellular structures at sub-cellular length scale and representing their mechanical contribution at the cellular length scale.

The current study aimed at the development of a multiscale computational model to predict the mechanical behaviour of single cells. Micromechanical homogenization facilitated the representation of the microstructural details of actin stress fibres in a FEM modelling framework. This improves the framework's capability to capture the mechanical contribution of sub-cellular micro-structures to the cellular mechanics at the macroscopic level.

2. Materials and methods

2.1 Geometrical modelling

The geometrical model of a human dermal fibroblast developed previously [30] was utilized in the current study. In brief, human dermal fibroblasts were seeded onto fibronectin-coated sterile cover slips, stained with Alexa Fluor 568 phalloidin (Invitrogen Molecular Probes, Eugene, Oregon, USA) and counterstained with Hoechst 33342 dye (Sigma Aldrich Chemie GmbH, Steinheim, Germany). Confocal images were acquired with a Zeiss 510 LSM Meta microscope at 40x magnification.

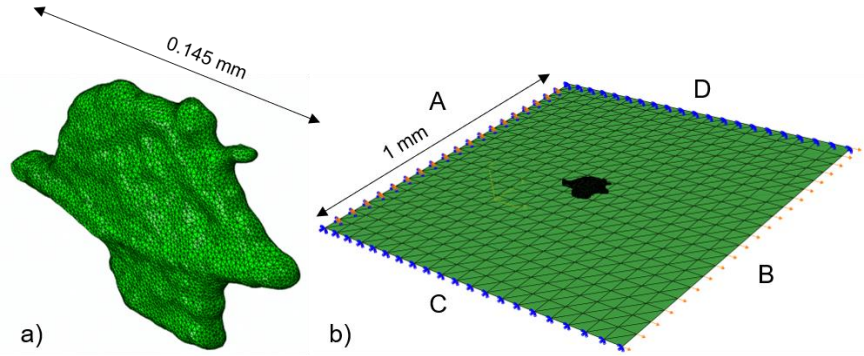

Figure 1. a) 3D reconstructed and meshed geometry of the fibroblast. b) Cell attached to substrate. Boundary conditions of the substrate: Edge (A) fixed in all directions; uniaxial quasi-static displacement applied normally to opposite edge B; edges C and D free in direction of displacement and fixed in the normal direction.

The 3D geometries of cytoplasm and nucleus were reconstructed from confocal images using threshold-based segmentation, meshed and converted to volumes (Simpleware ScanIP, Synopsys, Mountain View, CA, USA). The cell geometry has in-plane dimensions of 145 µm in the long axis and 92 µm in the short axis, and a thickness of 14 µm. The nucleus has an in-plane diameter of 19 µm and a maximum thickness of 3 µm. The reconstructed cell geometry was complemented with a 0.01 µm thick membrane enveloping the cytosol [39-41], see **Figure 1**(a).

2.2 Finite element modelling

2.2.1 Mesh generation

The cell membrane, cytosol and nucleus were meshed and identified as separate element sets. No-slip conditions were enforced at interfaces between the cellular components in Simpleware. The cell geometry was imported into Abaqus CAE (Dassault Systèmes, RI, USA) and assembled with a 1 x 1 mm flat substrate to simulate exposure of the cell to substrate stretch [30], see **Figure 1**(b).

For the cell-substrate attachment, 124 focal adhesions (FA) with a thickness of 1 µm [45-

48] and average contact area of 1 μm^2 [48] were randomly distributed across the basal cell surface [30] and meshed with cohesive elements [30, 42-44]. **Table 1** summarises element types and numbers for the various structures in the model.

Table 1. Types and number of elements for each component in the cell-substrate finite element model

Component	Type of element*	Number of elements
Nucleus	C3D4	1075
Cytosol	C3D4	47029
Plasma membrane	S3R	14832
Elastic substrate	S3	800
Focal adhesion	COH3D6	248

*C3D4 - four-node tetrahedral element, S3 - triangular three-node shell element, S3R - triangular three-node triangular shell element with reduced integration, COH3D6 - six-node three-dimensional cohesive element

2.2.2 Boundary conditions and loading

The cell was placed in the centre of the substrate with sufficient distance to the substrate boundaries to neglect edges effects (**Figure 1**b). One edge (A) of the substrate was fixed in all directions and a uniaxial quasi-static displacement was applied normally to the opposite edge (B). The other two edges (C and D) remained free in the direction of the displacement and fixed in the normal directions. The applied displacement generated a uniform deformation field in the substrate with a tensile strain up to 10% (i.e. stretch $\lambda = 1.1$).

2.2.3 Material Properties

The finite element simulations were limited to strain field. The cell membrane was represented as isotropic linear-elastic whereas nucleus and cytosol were assumed to be isotropic hyper-elastic compressible, and described with a Neo-Hookean strain energy function [51-53]:

$$\psi = \frac{\mu}{2}(\bar{I_1} - 1) + \frac{k}{2}(J - 1) \quad (1)$$

where ψ is the strain energy per unit of reference volume, μ is the shear modulus and k is the bulk modulus. $\bar{I_1}$ and J are the first and third invariant of the left Cauchy-Green deformation tensor, **P**, given by:

$$\mathbf{P} = \mathbf{F} \cdot \mathbf{F}^{\mathbf{T}} \quad (2)$$

where **F** is the deformation gradient tensor [54]. The substrate and focal adhesions were represented with an isotropic linear-elastic material model. All materials parameters are provided in **Table 2**.

Table 2. Mechanical properties of cell components, substrate, and focal adhesions.

Component	Constitutive law	Parameter
Nucleus	Hyperelastic	μ = 1.7 kPa, k = 16.4 kPa [55]
Cytosol	Hyperelastic	μ = 1 kPa, k = 9.7 kPa [51]
Stress fibre	Linear-elastic	E = 1.45 MPa, ν = 0.3 [32]
Membrane	Linear-elastic	E = 7 kPa, ν = 0.45 [56]
Focal adhesion	Linear-elastic	E = 10 kPa, ν = 0.3 [49, 50]
Elastic substrate	Linear-elastic	E_{sub} = 0.01, 0.14, 1, 10 MPa, ν = 0.45

2.3 Micromechanical homogenization of cytoplasm

The components of the cytoplasm include actin filament, intermediate filaments, and microtubules. The cell is able to organize the distribution of the cytoskeletal filaments according to its microenvironment, thus changing its mechanical properties. Stress fibres are contractile bundles of actin filaments [57] with diameter in the range of tenths of microns which. Experimental and theoretical studies have shown that cellular behaviour such as migration is dependent on the organization of stress fibres [58,59]. For simplicity, in this study we consider the stress fibres as randomly distributed in the cytoplasm, satisfying the continuum hypothesis.

The micromechanical homogenization allows to obtain the effective mechanical properties of the cytoplasm by assuming the cytoplasm as composite of cytosol and stress

fibres (see **Figure 2**). The homogenization is achieved by substituting the heterogenous microstructure with an equivalent homogenized structure. The micromechanical homogenization of the material properties is obtained considering a representative volume element (RVE). The RVE is a statistical representation of the material's microstructure and needs to provide sufficient details of the micro-fields to ensure an accurate sampling of the entire domain. The micro-length scale of the structure is represented by the RVE, which is small (l < L) compared to the macrostructure (i.e. the cell) and assumed to approximate the mechanical properties of the macrostructure. The RVE is, however, very large ($l >> \ell$) compared to the microstructural elements (i.e. the stress fibres) and not suited capture the microstructure in sufficient detail. The effective mechanical properties of the cytoplasm are then obtained based on the volume fraction of the stress fibres.

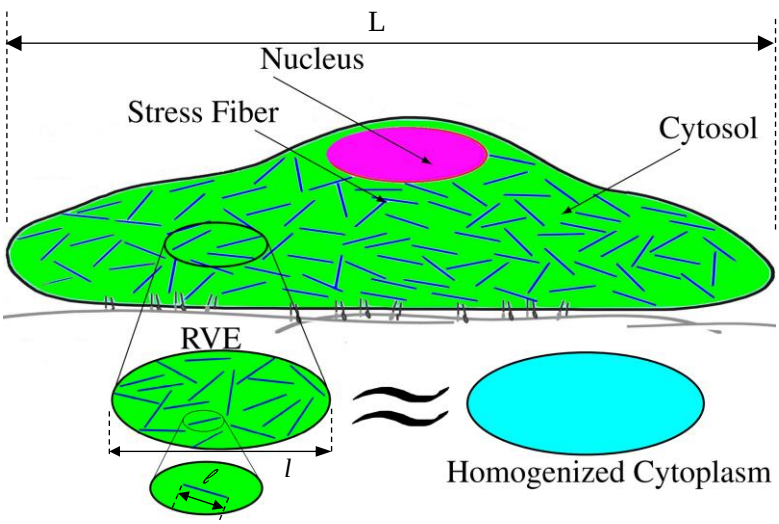

Figure 2. Schematic cross-section of the cell illustrating the random distribution of several actin stress fibres and the corresponding homogenized microstructure.

In microstructurally inhomogeneous materials, volume average stress and strain is obtained by integration of stress and strain over the RVE volume with respect to microscopic coordinates inside the RVE:

$$< \sigma >= \frac{1}{\mathrm{V}} \int_V (\sigma)\, d\mathrm{V} \tag{3}$$

$$< \varepsilon >= \frac{1}{\mathrm{V}} \int_V (\varepsilon)\, d\mathrm{V} \tag{4}$$

Here, σ(x) and ε(x) are the microscopic stress and strain, respectively, that are related to the average stress and strain by:

$$\sigma(\mathrm{x}) \;=\; \mathbf{A}(\mathrm{x}) < \sigma > \tag{5}$$

$$\varepsilon(\mathrm{x}) \;=\; \mathbf{B}(\mathrm{x}) < \varepsilon > \tag{6}$$

where **A** and **B** are the stress and strain concentration tensors, respectively.

Depending on complexity of the microstructure, the concentration tensors may be obtained by different approximation approaches. We consider a single ellipsoidal inclusion bonded to an infinite homogeneous elastic matrix that is subjected to a uniform strain and stress at infinity. For this problem, a suitable approximation approach is the mean field method, which is generally based on the Eshelby equivalent inclusion formulation [60]. The Mori-Tanaka (MT) homogenization model [61] is an effective field approximation based on Eshelby's elasticity solution, assuming that the strain concentration tensor **B** is equal to the strain concentration of the single inclusion problem. The Mori-Tanaka method is considered as an improvement over the Eshelby method, and the relationship for the effective strain is given as

$$< \varepsilon >^f \;=\; \mathbf{B}^{\mathrm{Eshelby}} < \varepsilon >^m. \tag{7}$$

The concentration tensor $\mathbf{B}^{\mathrm{Eshelby}}$ for Eshelby's equivalent inclusion is

$$\mathbf{B}^{\mathrm{Eshelby}} \;=\; \left[\mathbf{I} - \mathbf{S}\mathbf{M}^{\mathbf{m}}(\mathbf{E}^f - \mathbf{E}^m)\right]^{-1}, \tag{8}$$

where **I** is the identity tensor, **S** is the Eshelby tensor [61], **E** is the elastic tensor, **M** is the compliance tensor, and υ is the volume fraction. The superscripts *f* and *m* refer to the

fibre and matrix, respectively.

The Mori-Tanaka concentration tensor $\mathbf{B}^{\mathrm{MT}}$ is given as

$$\mathbf{B}^{\mathrm{MT}} = \mathbf{B}^{\mathrm{Eshelby}}\left[\left(1-\upsilon_f\right)\mathbf{I} + \upsilon_f \mathbf{B}^{\mathbf{Eshelby}}\right]^{-1} \tag{9}$$

The relationship between the macroscopic stress < σ > and strain < ε > can be obtained by:

$$< \sigma > = \mathbf{E}^{\mathrm{eff}} < \varepsilon > \tag{10}$$

where $\mathbf{E}^{\mathrm{eff}}$ is the effective elastic tensor of the homogeneous material that can be obtained as a function of the strain concentration tensor $\mathbf{B}^{\mathrm{MT}}$. To computationally model cellular mechanics, capturing the nonlinear deformation of the cytoplasm under large displacement is required. The Neo-Hookean material model has been used to describe the nonlinear stress-strain behaviour of the homogenized cytoplasm.

The cytoplasm was considered as composition of randomly oriented stress fibres in the cytosol representing the matrix. The cytosol matrix was assumed to be hyperelastic with an elastic modulus of 1 kPa, see **Table 2**. For the stress fibres, mechanical properties obtained from stretch tests [33] were utilised. The effective linear-elastic bulk modulus K^{eff} and shear modulus μ^{eff} of the cytosol matrix with randomly oriented and distributed stress fibres are given as:

$$K^{eff} = \frac{\upsilon_m K_m + \upsilon_f K_f \mathrm{B}_K^{\mathrm{Eshelby}}}{\upsilon_m + \upsilon_f \mathrm{B}_K^{\mathrm{Eshelby}}} \tag{11}$$

$$\mu^{eff} = \frac{\upsilon_m \mu_m + \upsilon_f \mu_f \mathrm{B}_\mu^{\mathrm{Eshelby}}}{\upsilon_m + \upsilon_f \mathrm{B}_\mu^{\mathrm{Eshelby}}} \tag{12}$$

$$\nu^{eff} = \frac{3K^{eff} - 2\mu^{eff}}{2(3K^{eff} + \mu^{eff})} \tag{13}$$

where μ, K, ν, and υ are the shear modulus, bulk modulus, Poisson's ratio and volume fraction, respectively, of the fibre and matrix defined by subscripts f and m. The stress is obtained by:

$$\sigma_{11} = \frac{4C_1}{3J^{5/3}}\left(\lambda^2 - \frac{J}{\lambda}\right) + 2D_1(J-1) \tag{14}$$

where $C_1 = \mu/2$, $D_1 = K/2$, and λ is the principal stretch.

2.4 Parametric numerical study

Changes in the actin cytoskeletal structure have been reported to affect cell fate [19]. The mechanical properties of the cellular components including nucleus, cytoplasm, actin cortex and actin stress fibres contribute to the effective mechanical stiffness of the cell [40].

Parametric simulations were conducted to determine the effect of stress fibre content and substrate elasticity on the deformation of the cell. The volumetric fraction of filamentous F-actin in the cytoskeleton has been reported to be of the order of 1% [62]. Since we expect the stress fibre volume fraction in actin-rich intracellular regions (such as the cortex or actin bundles) to be higher than normal, we considered networks with stress fibre volume fractions of υ_f = 0, 1, 10, and 20%. Furthermore, we considered an elastic modulus of the substrate of E_S = 0.01, 0.14, 1 and 10 MPa. The reference mechanical properties of the cell are summarised in **Table 2**. The computed strain in plasma membrane, homogenised cytoplasm and nucleus of the cell exposed to uniaxial stretch as described above were assessed.

3. Results

3.1 Homogenization of cytoplasm

Figure 3 illustrates the relationship between the effective mechanical properties of the homogenised cytoplasm and the stress fibre volume fraction. The shear modulus μ^{eff}, bulk modulus K^{eff} and effective elastic modulus E^{eff} increase with increasing stress fibre volume fraction υ_f whereas the Poisson's ratio decreases for increasing stress fibre volume fraction.

The stress-stretch relationship for the macroscopic behaviour of the homogenized

cytoplasm is illustrated in **Figure 4**(a) for different stress fibre volume fractions. An increase in stress fibre volume fraction resulted in an increase in stress for a given stretch. For small stretch values of $\lambda \leq 1.1$, the stress-stretch relationship can be approximated as linear (see **Figure 4**b).

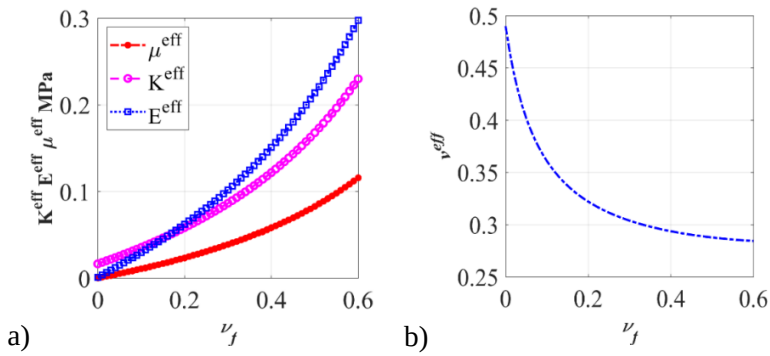

Figure 3. The effect of stress fibre volume fraction ν_f on (a) the effective elastic modulus $\mathbf{E}^{eff}$, shear modulus $\boldsymbol{\mu}^{eff}$ and bulk modulus $\mathbf{K}^{eff}$, and (b) the effective Poisson's ratio of the homogenised cytoplasm.

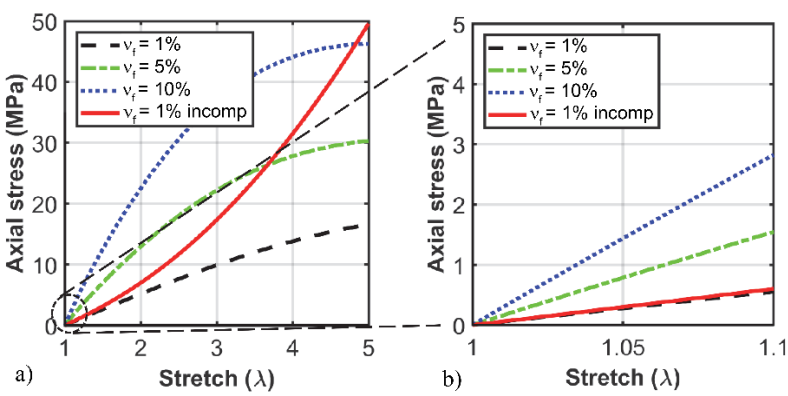

Figure 4. The axial stress as a function of uniaxial stretch for the homogenised cytoplasm predicted by hyperelastic constitutive laws of Neo-Hookean material model for (a) large stretch $\lambda = 1.0$ to 5.0 and (b) small stretch $\lambda = 1.0$ to 1.1 (incomp means incompressible).

3.2 Effect of stress fibre volume fraction and substrate modulus on cell deformation

The predicted distribution of the maximum principal strain in the mid plane of the cytoplasm and nucleus is presented in **Figure 5**. The results are shown in the mid plane of the cytoplasm to disregard potential numerical localization effects of the contact between the cell and the substrate. The intracellular strain distribution, i.e. spatial pattern, was found to be insensitive to the variation of the stress fibre volume fraction, whereas different strain magnitudes were apparent for the different cases. It was also noted that the peak strain in the cytosol and nucleus decreased considerably when stress fibres were included.

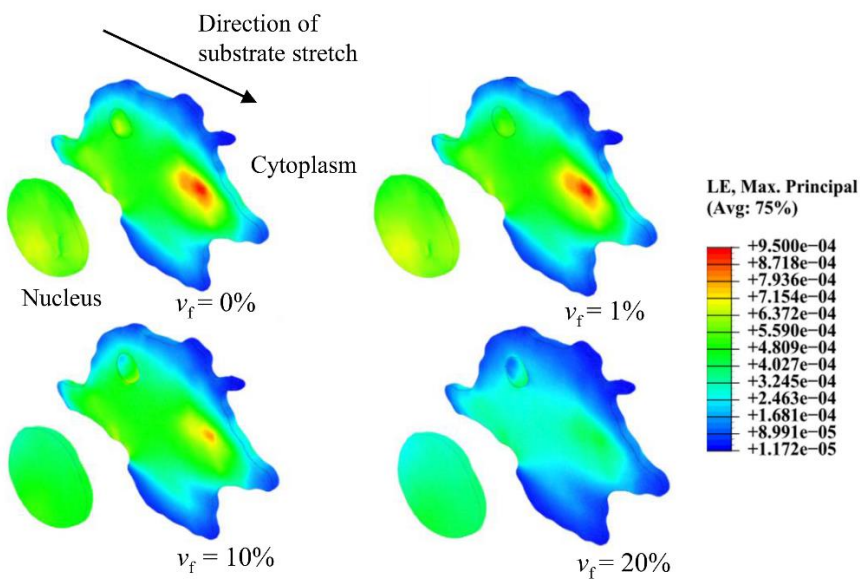

Figure 5. Distribution of maximum principal strain in the mid-section of cytoplasm and nucleus for E_{sub} = 0.01 MPa at different stress fibre volume fractions υ_f = 0, 1, 10 and 20%.

Figure 6 illustrates the peak maximum principal strain in the mid-section of the cell (as illustrated in **Figure 5**) versus stress fibre volume fraction and substrate elastic modulus for cytoplasm, nucleus and membrane. With increasing stress fibre volume fraction υ_f, the strain decreased in the cytoplasm and in the nucleus whereas it did not change

considerably in the cell membrane. A consistent response of cytoplasm and nucleus was also observed in the strain sensitivity to the change of υ_f. The change in stress fibre volume fraction had a negligible effect on the peak strain in the membrane, **Figure 6**c. The peak strain in all three cell components increased with increasing elastic modulus of the substrate, E_{sub} (**Figure 6**). The changes in peak strains in the cell were larger for changes in the lower range of E_{sub}, where it is of the same order of magnitude as the elastic modulus of the cell components, compared to the upper region of E_{sub} where the elastic modulus of the substrate is much higher than that of the cell components.

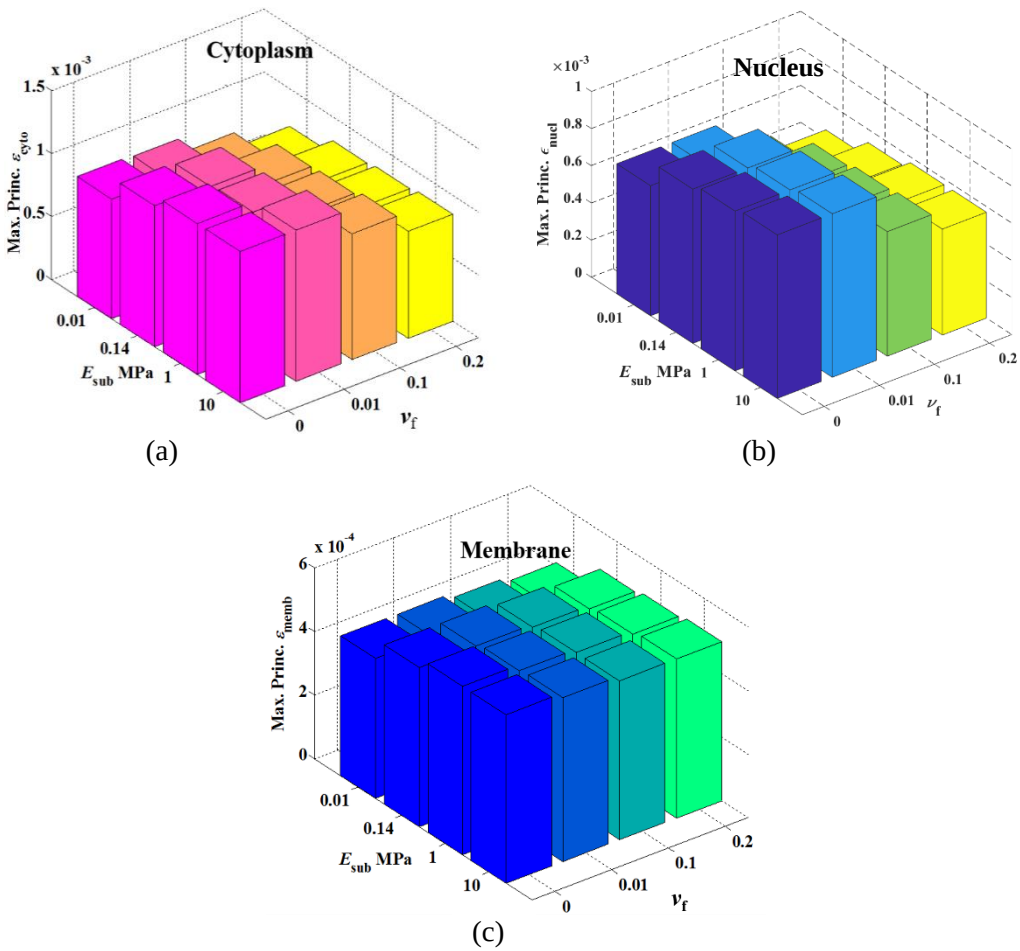

Figure 6. Peak maximum principal strain in the mid-section of cytoplasm (a), nucleus (b) and membrane (c) at a substrate stretch of $\lambda = 1.1$ for different ν_f and E_{sub}. (The values of the peak maximum principal strains in cytoplasm, nucleus and cell membrane are provided in the online supplement, **Table S1**).

Figure 7 and **Figure 8** provide graphs of overall peak maximum principal logarithmic strain in the cytoplasm $\varepsilon_{1,Peak,Cyt}$ and the nucleus $\varepsilon_{1,Peak,Nuc}$ versus the peak maximum principal strain in the substrate $\varepsilon_{1,Peak,Sub}$ for different stress fibre volume fractions and substrate elastic moduli. (Here, 'overall strain' refers to the strain in the entire cytoplasm and nucleus in contrast to the strain in the mid-section presented in **Figure 5** and **Figure 6**.) The overall peak strain in the cytoplasm increased linearly with increasing strain in the substrate. The overall peak strain in the nucleus also increased linearly with increasing substrate strain.

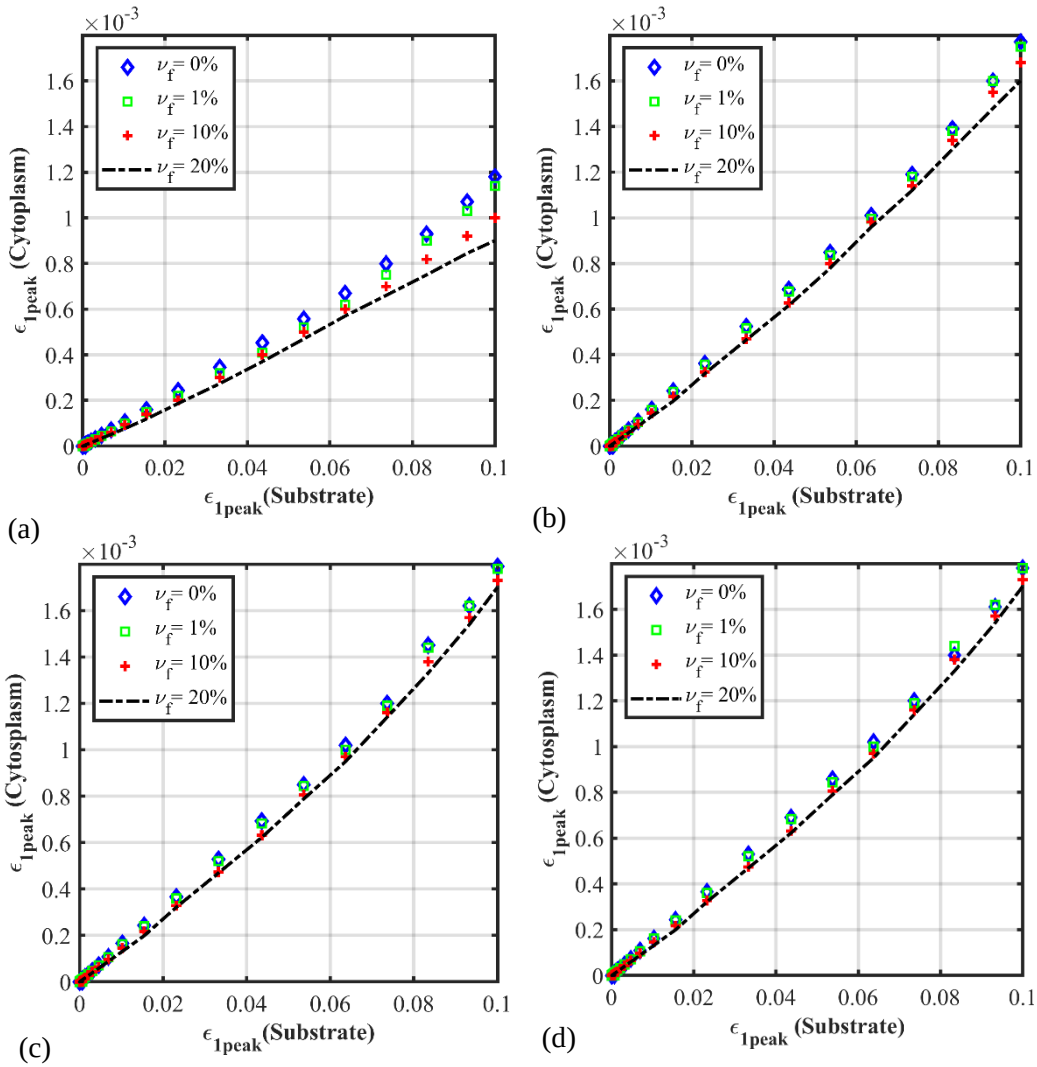

Figure 7. Overall peak maximum principal strain in cytoplasm versus same strain in substrate for different stress fibre volume fractions of 0, 1, 10 and 20% for the substrate elastic modulus E_{sub} of 0.01 MPa (a), 0.14 MPa (b), 1 MPa (c) and 10 MPa (d).

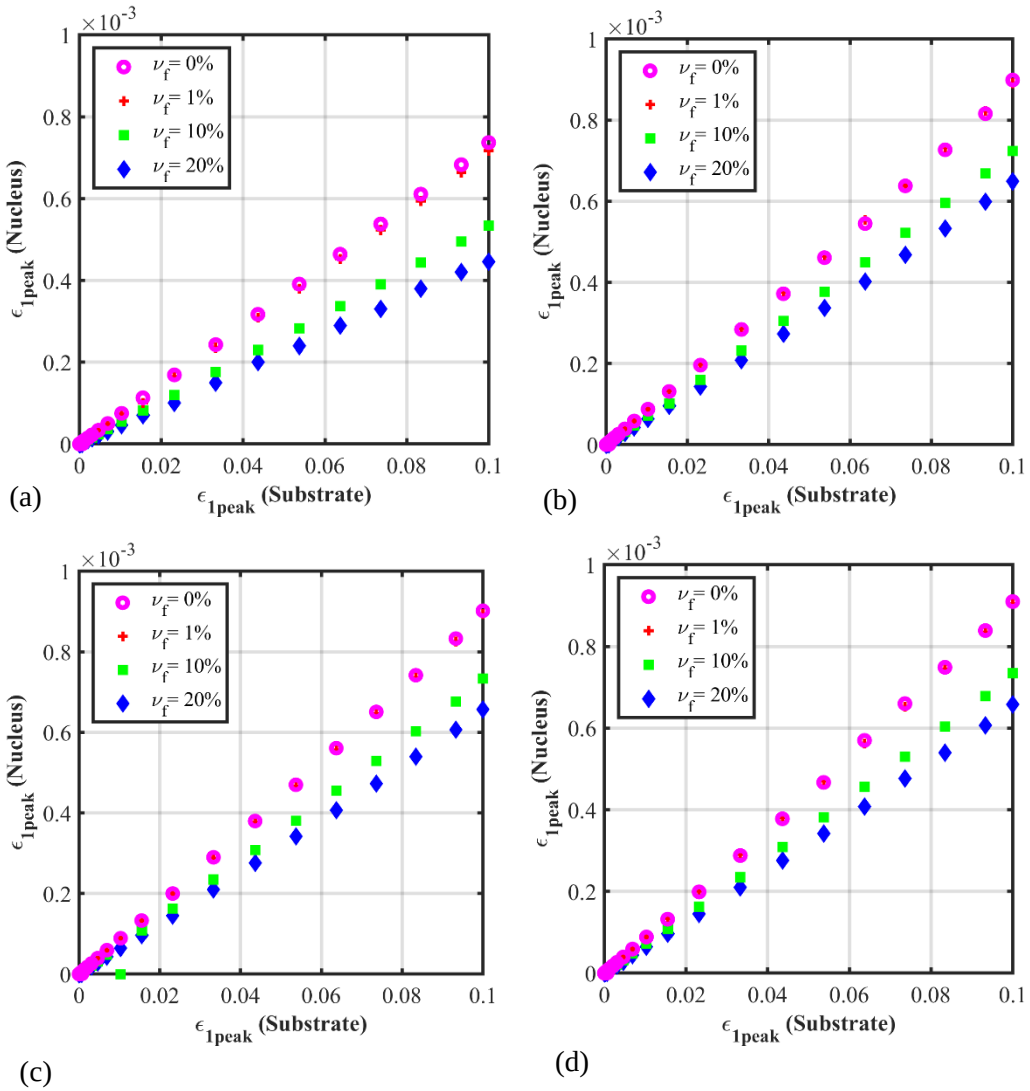

Figure 8. Overall peak maximum principal strain in nucleus versus the same strain in the substrate for different stress fibre volume fractions of 0, 1, 10 and 20% and substrate elastic modulus E_{sub} of 0.01 MPa (a), 0.14 MPa (b), 1 MPa (c) and 10 MPa (d).

The relationship between the overall peak strain in the cytosol and nucleus, respectively, and the overall peak strain in the substrate can be approximated with linear functions for the cytosol,

$$\epsilon_{1,Peak,Cyt} = \alpha_{Cyt} \cdot \epsilon_{1,Peak,Sub} + \beta_{Cyt} \tag{15}$$

and the nucleus

$$\epsilon_{1,Peak,Nuc} = \alpha_{Nuc} \cdot \epsilon_{1,Peak,Sub} + \beta_{Nuc} \,. \tag{16}$$

The values for parameters α and β for nucleus and cytoplasm for different values of the substrate elastic modulus and the stress fibre volume fraction are provided in **Table 3**.

Table 3. Parameter values for estimation of overall peak strain in cytoplasm and nucleus (see Eqs. 15 and 16).

E_{sub} (MPa)		Cytoplasm υ_f 0%	1%	10%	20%		Nucleus υ_f 0%	1%	10%	20%
0.01	α_{Cyt}	0.0112	0.0107	0.0096	0.009	α_{Nuc}	0.0073	0.0071	0.0053	0.0045
	β_{Cyt}	$-5e^{-6}$	$-6e^{-6}$	$-4e^{-6}$	$-4e^{-6}$	β_{Nuc}	$-5e^{-7}$	$-8e^{-7}$	$-5e^{-7}$	$-2e^{-7}$
0.14	α_{Cyt}	0.0168	0.0167	0.016	0.0156	α_{Nuc}	0.0088	0.0087	0.0072	0.0064
	β_{Cyt}	$-8e^{-6}$	$-8e^{-6}$	$-1e^{-5}$	$-1e^{-5}$	β_{Nuc}	$-1e^{-7}$	$-1e^{-7}$	$-1e^{-7}$	$-1e^{-7}$
1	α_{Cyt}	0.017	0.17	0.0164	0.0161	α_{Nuc}	0.0089	0.0088	0.0073	0.0065
	β_{Cyt}	$-8e^{-6}$	$-9e^{-6}$	$-1e^{-5}$	$-1e^{-5}$	β_{Nuc}	$-2e^{-7}$	$-1e^{-7}$	$-4e^{-7}$	$-1e^{-7}$
10	α_{Cyt}	0.169	0.169	0.0163	0.0156	α_{Nuc}	0.009	0.0089	0.0073	0.0065
	β_{Cyt}	$-7e^{-6}$	$-8e^{-6}$	$-1e^{-5}$	$-1e^{-5}$	β_{Nuc}	$-3e^{-7}$	$-2e^{-7}$	$-1e^{-7}$	$-1e^{-7}$

4. Discussion

Previous studies of eukaryotic cells have shown that the cytoskeletal structures largely determine the distinct mechanical properties of the cytoplasm. In some computational studies of single cell mechanics, the assumption of homogenous mechanical properties for the entire cell has been a simplification particularly for cells with focal adhesions where stress fibre presents critical inhomogeneity. Hybrid computational cell models [37, 38, 39] with a restricted number of tensegrity elements to represent the mechanical contribution of stress fibres have remained limited in their capabilities to capture real cellular behaviour. One of the main shortcomings of continuum-based models is the lack of representation of the functional contribution of cytoskeletal fibres [63].

In our current study, we developed a finite element method and models for single cell

mechanics with the micromechanical homogenization of actin stress fibres as primary cytoskeletal elements in the cytoplasm. The method and models were used to investigate the influence of stress fibre content on the deformation of cytoplasm, nucleus and cell membrane of a fibroblast when subjected to uniaxial strain of up to 10% on substrates of different stiffness.

This study demonstrates the importance of the representation of cytoskeletal components in computational models for single cell mechanics since the predicted intracellular strains varied substantial for different stress fibre contents.

The results for stress fibre volume fractions of 0% and 20% indicated that an increase in stress fibre volume fraction led to a decrease in the peak strain in cytoplasm and nucleus but did not affect the strain in cell membrane to the same degree (**Figure 6**). As shown in **Figure 5**, the spatial distribution of strain is similar for the four cases since distribution of the focal adhesions is the same for all cases. We have investigated the effect of focal adhesions on intracellular strain distribution in previous studies [30, 64, 65].

We observed that the mid-section peak strain in the cytoplasm and nucleus decreased more for the change of stress fibre volume fraction from 0% to 10% than for the change from 10% to 20%. Our results support the hypothesis that the cytoskeleton plays a significant role in transmitting extracellular mechanical forces to the nucleus, possibly mediating mechanotransduction [66]. The negligible change of the strain in the cell membrane indicates that the membrane is less sensitive to a change in stress fibre content than the cytosol and nucleus.

Our findings indicate that the omission of stress fibres in computational models may overestimate intracellular strain. Due to the challenges in discretely reconstructing the stress fibres, numerous computational studies utilized beam elements to represent stress fibres [8, 9, 35, 37, 67]. However, this is not sufficient to represent the global effect of stress fibres for high volume fractions [68] and random orientations [69]. Our approach utilising micromechanical homogenization offers a new method to address this limitation

in single cell computational mechanics.

A substantial variation of the effective Poisson's ratio of the micromechanically homogenised cytoplasm was predicted for the change in stress fibre content (**Figure 3**). This is of interest in the context of the wide range of values reported in literature, from nearly incompressible hyperelastic with 0.49 [70] to 0.3 [71, 72].

Numerical relationships were identified between overall peak strain in the cytosol and the nucleus, respectively, and the strain in the substrate (**Figure 7** and **Figure 8**) for varying stress fibre volume fraction and elastic modulus of the substrate. Once validated, such relationships can provide simple tools to estimate the maximum deformation in cellular components for a given stiffness of the extracellular environment, and consequently a design tool for cellular microenvironments to guide cellular deformation in therapeutic applications.

The hyperelastic response of the cytoplasm was captured by a Neo-Hookean strain energy density function based on isotropic elastic shear modulus and bulk modulus that were derived with by micromechanical homogenization. Based on the spread morphology of the fibroblast with a multitude of stress fibre orientations observed microscopically, the homogenized cytoplasm was treated as mechanically isotropic with randomly aligned stress fibres.

There are some simplifications in the finite element model presented here. Focal adhesions were assumed to be arbitrarily distributed over the entire contact area between cell and substrate. We have investigated the impact of focal adhesions in more detail in our previous studies [30,64,65]. The cell-specific morphology of the model was intended to resemble to some degree the in vivo interactions of the cell with the substrate. This was deemed appropriate for the comparison with experiments that investigated cellular stretching with adherent monolayers [73,74,75]. In the context of the primary goal of this study, namely to investigate the role of stress fibres on cellular mechanics with changing substrate stiffness, the complexity of the model was appropriate.

Shortcomings of the current study include the lack of quantitative information on the distribution of stress fibres within the cell and the omission of microtubules and intermediate filaments in the micromechanical homogenization of cytoskeletal structures. This was based on the technical challenge to identify these structures in the microscopic images. A further limitation is the lack of validation of the numerical results. However, at present, there is a scarcity of experimental data of cellular and subcellular physical parameters (e.g. mechanical properties of FA, structure and mechanical properties of cytoskeletal components) and on localized intracellular deformation such as provided by the FE models in this study (**Figure 5**).

Future research will aim at describing computationally the cytoskeletal dynamics including the nonlinear constitutive behaviour of the cell in terms of stress-strain relationships shown experimentally [76,77]. Experimental studies have also demonstrated that the functions of microtubules, i.e. to maintain viscosity [78] and to define the time of recovery to equilibrium of a cell after external stimuli [79,80]. The inclusion of microtubules would serve as a good basis for the future extension of our studies.

5. Conclusion

This study demonstrated that the representation of stress fibres should be considered in computational models for cell mechanics since predicted cytoplasm elastic modulus values changed substantially with stress fibres content. The results also show that the stress fibres content influences the deformation of cytoplasm and nucleus but not of the membrane, at least for uniaxial substrate stretch. These developed methods and models offer potential for refinement and extension, for example to capture the regional variability of cytoskeletal content and mechanical anisotropy of the cytoplasm. This study can as such contribute to the development of more realistic and accurate computational models of cell mechanics based on continuum approaches that will provide a better understanding of mechanotransduction in living cells.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Data

Abaqus input files of the finite element models used in this study are available on are available on ZivaHUB (http://doi.org/10.25375/uct.9782798).

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