Acta Biomaterialia 27 (2015) 251–263

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Single cell active force generation under dynamic loading – Part II: Active modelling insights

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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 14 August 2015
Accepted 6 September 2015
Available online 7 September 2015

Keywords:
Active cell model
Fading memory contractility
Dynamic loading
Stress fibre remodelling
Non-linear visco-hyperelasticity

ABSTRACT

In Part I of this two-part study a novel single cell AFM experimental investigation reveals a complex force–strain response of cells to cyclic loading. The biomechanisms underlying such complex behaviour cannot be fully understood without a detailed mechanistic analysis incorporating the key features of active stress generation and remodelling of the actin cytoskeleton. In order to simulate untreated contractile cells an active bio-chemo-mechanical model is developed, incorporating the key features of stress fibre (SF) remodelling and active tension generation. It is demonstrated that a fading memory SF contractility model accurately captures the transient response of cells to dynamic loading. Simulations reveal that high stretching forces during unloading half-cycles (probe retraction) occur due to tension actively generated by axially oriented SFs. On the other hand, hoop oriented SFs generate tension during loading half-cycles, providing a coherent explanation for the elevated compression resistance of contractile cells. Finally, it is also demonstrated that passive non-linear visco-hyperelastic material laws, traditionally used to simulate cell mechanical behaviour, are not appropriate for untreated contractile cells, and their use should be limited to the simulation of cells in which the active force generation machinery of the actin cytoskeleton has been chemically disrupted. In summary, our active modelling framework provides a coherent understanding of the biomechanisms underlying the complex patterns of experimentally observed single cell force generation presented in the first part of this study.

Statement of significance

A novel computational investigation into the active and passive response of cells to dynamic loading is performed. An active formulation that considers key features of actin cytoskeleton active contractility and remodelling throughout the cytoplasm is implemented. Simulations provide new insights into the sub-cellular biomechanical response, providing a coherent explanation for the complex patterns of cell force uncovered experimentally in the first part of this study. Our computational models also reveal that passive non-linear visco-hyperelastic material laws, traditionally used to simulate cell mechanical behaviour, are not appropriate for untreated contractile cells, and their use should be limited to the simulation of cells in which the active force generation machinery of the actin cytoskeleton has been chemically disrupted.

1. Introduction

In Part I of this two-part study a novel single cell AFM experimental investigation uncovers the complex force–strain response of cells to cyclic loading [1]. Using a bespoke AFM system [2], force generated by single cells during dynamic loading is measured. Cells in which the actin cytoskeleton is disrupted using cytochalasin-D (cytoD) are also tested. Experimental results reveal that the biomechanical behaviour of untreated contractile cells is fundamentally different to non-contractile cytoD treated cells. The following key findings are uncovered in Part I of this study:

- Untreated contractile cells are relatively insensitive to changes in applied strain magnitude. In contrast, cells treated with an actin cytoskeleton inhibitor, cytoD, are shown to be highly sensitive to changes in strain magnitude.

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http://dx.doi.org/10.1016/j.actbio.2015.09.004
1742-7061/

- Untreated contractile cells greatly resist the retraction of the AFM probe during unloading, pulling strongly on the probe. In contrast, cells treated with an actin cytoskeleton inhibitor provide little resistance to AFM probe retraction.
- Untreated contractile cells provide a higher resistance to compression than cells treated with an actin cytoskeleton inhibitor.

The above findings from our experimental study (Part I) provide new characterisation of the complexity of single cell response to dynamic loading. However, the biomechanisms underlying such complex behaviour cannot be fully understood without a detailed mechanistic analysis incorporating the key features of active stress generation and remodelling of the actin cytoskeleton. Importantly, the trends outlined above cannot be replicated or explained in terms of passive hyperelastic or viscoelastic material behaviour.

A simplified approach to modelling the behaviour of the actin cytoskeleton commonly entails the ad-hoc placement of pre-stressed beam elements in the cell cytoplasm [3,4]. This approach neglects intercellular processes governing active cytoskeletal contractility and remodelling, and does not allow for the simulation of the multi-axial evolution and stress-generation of the actin cytoskeleton throughout the cell. In contrast, the active model of stress fibre (SF) contractility and remodelling proposed by Deshpande et al. [5] incorporates the key features of SF formation, dissociation, and contractility. The extension of this active SF framework to a fully predictive 3D finite element (FE) setting [6] allowed for the simulation of complex multi-axial patterns of SF morphology and contractility [7], with complex experimental tests being accurately simulated under quasi-static conditions, e.g. non-linear force–deformation behaviour of chondrocytes under direct shear [8], and the response of spread adhered endothelial cells to micropipette aspiration [9].

This active 3D framework has not previously been used to simulate single cell dynamic loading experiments. Here we present a computational investigation of the experimental results reported in Part I [1]. Simulations reveal that different families of SFs generate tension during probe pulling and probe pushing, resulting in elevated forces during both unloading and loading half-cycles, respectively. Additionally, it is shown that a "fading memory" strain rate contractility model is required to replicate the transient behaviour of cells under dynamic loading. Furthermore, results highlight that passive visco-hyperelastic material models cannot accurately simulate the dynamic behaviour of contractile cells.

2. Materials and methods

2.1. Modelling the bio-chemo-mechanical behaviour of the actin cytoskeleton

2.1.1. Fibre activation level and tension

A bio-chemo-mechanical model is used to simulate signal induced SF formation, active SF contractility, and tension dependant SF dissociation. Originally proposed by Deshpande et al. [5] and implemented in a predictive 3D framework by Ronan et al. [6], a brief description of the key features of the model is provided here. Formation and dissociation of SFs are described using a first order kinetic equation:

$$\frac{d\eta(\phi,\omega)}{dt} = [1-\eta(\phi,\omega)]Ck_f - \left[1-\frac{\sigma_f(\phi,\omega)}{\sigma_0(\phi,\omega)}\right]\eta(\phi,\omega)k_b \tag{1}$$

where η is the non-dimensional activation level of a fibre ($0 \leqslant \eta \leqslant 1$) and k_f and k_b are forward and backward reaction rate constants, respectively. θ is the decay constant. The first term in the first order kinetic equation captures fibre formation via a spatially uniform signal in the cytoplasm, C, that is typically represented as an exponent ($C = \exp(-t_s/\theta)$) governed by the decay constant, θ, and the time since the most recent signal, t_s (Fig. 1A). The second term describes SF dissociation when the fibre tension is lower than the isometric tension, $\sigma_f < \sigma_0$ (Fig. 1B). The fibre isometric tension is proportional to the fibre activation level, such that $\sigma_0 = \eta\sigma_{MAX}$, where the model parameter, σ_{MAX}, is the isometric tension of a fully activated fibre ($\eta = 1$).

Fibre tension, σ_f, is related to the fibre axial strain rate, $\dot{\varepsilon}_f$, via a linearised approximation of the Hill tension–velocity relationship [5]:

$$\frac{\sigma_f}{\sigma_0} = 1 + \frac{\bar{k}_v}{\eta}\frac{\dot{\varepsilon}_f}{\dot{\varepsilon}_0}; \quad -\frac{\eta}{\bar{k}_v} \leqslant \frac{\dot{\varepsilon}_f}{\dot{\varepsilon}_0} \leqslant 0, \tag{2}$$

where $\bar{k}_v$ is the reduction in stress upon increasing the shortening strain rate, $\dot{\varepsilon}_f$, by $\dot{\varepsilon}_0$. As described by Eq. (2) (and Fig. 1C), during fibre shortening (negative strain rate) fibre tension decreases linearly from the isometric tension (at zero strain rate) to zero tension at a strain rate of $-\eta\dot{\varepsilon}_0/\bar{k}_v$. Fibre tension remains at zero for strain rates less than $-\eta\dot{\varepsilon}_0/\bar{k}_v$. Finally, when subjected to positive (lengthening) strain rates, fibres yield at the isometric tension, σ_0.

2.1.2. Fading memory of fibre strain rate

The SF model outlined in Section 2.1.1 has recently been shown to accurately predict the response of contractile cells to externally applied shear deformation [8,10], parallel-plate compression [6,11], micropipette aspiration [9], and spreading on 2D elastic substrates [7]. All of these aforementioned studies investigate the cell response under static conditions or during single monotonic applied load (such loading is commonly referred to as "static loading"). In contrast, the current study considers dynamic cyclic loading applied at a frequency of 1 Hz for a duration of two hours. In order to provide enhanced predictions of actin–myosin contractility under dynamic conditions, here we modify SF contractility model presented in Section 2.1.1 to incorporate dynamic effects. Based on empirical observations for cardiac muscle [12] under dynamic conditions, we define a history dependant fibre strain rate:

$$h_f = \sum \int_{-\infty}^{t} Ae^{-\alpha(t-\tau)}\dot{\varepsilon}_m(\tau)d\tau \quad \dot{\varepsilon}_m = \begin{cases} \dot{\varepsilon}_f & \dot{\varepsilon}_f \leqslant 0 \\ 0 & \dot{\varepsilon}_f > 0 \end{cases} \tag{3}$$

where $\dot{\varepsilon}_f(\tau)$ is the instantaneous fibre strain rate and A and α are material parameters. To include fading memory effects in the active SF model, the Hill contractility equation is modified so that now:

$$\frac{\sigma_f}{\sigma_0} = 1 + \frac{\bar{k}_v}{\eta}\frac{h_f}{\dot{\varepsilon}_0}; \quad -\frac{\eta}{\bar{k}_v} \leqslant \frac{h_f}{\dot{\varepsilon}_0} \leqslant 0, \tag{4}$$

An example of fibre strain rate, h_f, calculated from the actual strain rate, $\dot{\varepsilon}_f$, is graphically illustrated in Fig. 2A. A motivation for the incorporation of this fading memory contractility law will be presented in Section 3.3.2.

2.2. Numerical implementation

2.2.1. Fibre remodelling and contractility

In order to predict the distribution of SFs throughout the cell, the fibre remodelling and contractility equations are solved in 240 discrete directions at every integration point in the cytoplasm. At each integration point the fibre activation level in any one of these 240 directions depends on the local stress state and the signal intensity. This provides a fully predictive framework to determine the inhomogeneous three dimensional SF distribution throughout the cytoplasm. The representative volume element (RVE) is defined as a sphere containing fibres that are equally distributed in 3D space (Fig. 1D). The strain rate experienced by each

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