A THERMODYNAMIC FRAMEWORK FOR SARCOMERE FORMATION IN CARDIOMYOCYTES SPREAD ON MICRO-PATTERNED SUBSTRATES

Ryan J. Coleman (1), Vikram S. Deshpande (2), Patrick McGarry (1)

1. *Department of Biomedical Engineering, University of Galway, Galway, Ireland;*
2. *Department of Engineering, University of Cambridge, Cambridge, UK*

Introduction

Identification of in-vitro protocols to develop mature cardiomyocytes (CMs) with ordered sarcomeric structures and aligned myofibrils is a challenge in cellular and tissue engineering [1]. An experimental study by *Ribeiro et al.* [2] reports human pluripotent stem cells (hPSCs) on high aspect ratio (AR) rectangular ligand patches develop aligned sarcomeres, however cells on square patches develop unaligned stress fibers (SFs). This mechanism of differentiation of hPSCs to mature CMs as a function of cell AR is not understood. We propose a novel thermodynamically based theoretical model for sarcomere and SF formation within a cell. We simulate the spreading of cells on square and rectangular adhesive patches and quantitatively compare our model predictions to experimentally measured levels of sarcomere formation.

Methods

We consider that cytoskeletal proteins can exist in three states within a CM: (i) bound as part of a SF ($\widehat{N}_{BF}$), (ii) bound as part of a structured sarcomere ($\widehat{N}_{BS}$), or (iii) unbound within the cytoplasm ($\widehat{N}_U$). At steady-state conditions the chemical potentials of these three states are in equilibrium, which derives the areal density of sarcomeres at any point within the cell, given as

$$\hat{\eta}_S = \frac{\frac{1}{\hat{n}_S}(\hat{\xi}_1 - \widehat{N}_{BF})(\widehat{N}_U + \widehat{N}_{BF})\exp(\hat{n}_S \Delta\hat{\mu})}{\hat{\xi}_1 + (\widehat{N}_U + \widehat{N}_{BF})\exp(\hat{n}_S \Delta\hat{\mu})}. \qquad (1)$$

We develop a statistical mechanics framework for CM spreading, analysing over two million cell spreads with MCMC walks [3]. The Gibbs free energy for a potential cell state is accepted if the energetic competition between sarcomere recruitment and elastic deformation reduces free energy relative to a reference state. Simulations are performed on micro-patterned ligand patches for comparison with in-vitro experiments [2].

Results

A sample of computed spread cells are shown in Fig. 1 for CMs on rectangular ligand patches. Highly aligned dense sarcomere structures are predicted for cells on 7:1 rectangular patches. In contrast, for a cell on a square patch our model predicts a low-density of sarcomeres. Computed sarcomere formation increases with AR, shown in Fig. 2B, in agreement with experimental measurements. Fig. 2C shows the computed distribution of sarcomere orientations as a function of patch AR. Again, strong agreement with experiments is observed, with elongated CMs exhibiting higher levels of alignment. Experimental images of *Ribeiro et al.* [2] for comparison, with similar trends to our model prediction. Our model uncovers the following mechanism: Gibbs free energy decreases with increased sarcomere density. Sarcomere areal density exponentially increases with in series units, driving an increase in sarcomere length. Such an increase in length is facilitated by elongated cells and opposed by isotropic cells of similar area.

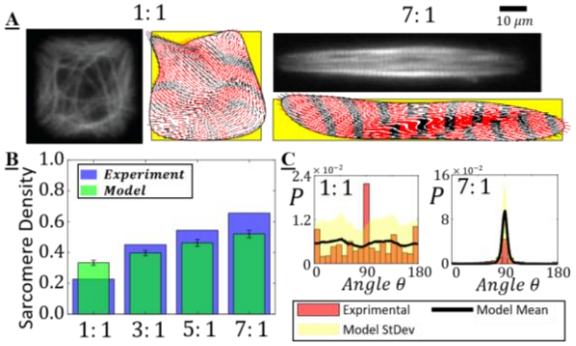

Fig. 1. (A) Predicted CM states on ligand patches, sarcomere orientations red quivers (thickness scales with sarcomere density). Ligand-patch AR on (B) sarcomere density, (C) sarcomere alignment. Experimental results (Ribeiro et al. [2]) for comparison.

Discussion

We present the first thermodynamically based theoretical model for sarcomere formation in CMs, and implement this model in a novel statistical mechanics framework to simulate cells spreading on micro-patterned ligand patches. Decreased Gibbs free energy is driven by sarcomere formation as cells spread into highly elongated states. Our model predictions are in strong agreement with the experimental observations of *Ribeiro et al.* [2]. The mechanism of sarcomere formation uncovered by our model may guide in-vitro strategies to generate mature contractile CMs. Optimal dynamic loading regimes promoting sarcomere formation can be identified using our model [4]. Our framework can be implemented into finite element models of a heart predicting the influence of a range of pathologies on remodelling of sarcomeres in-vivo [5].

References

1. Nunes, S et al., Nat. Methods, 781-787, 10(8), 2013.
2. Ribeiro, A et al., PNAS, 112(41), 12705–12710, 2015.
3. Shishvan, S et al., BMMB, 17, 1631–1662, 2018.
4. Dou, W et al., Biosens. Bioelectron. 175, 112875, 2021.
5. McEvoy, E et al., J Mech Behav Biomed Mater, 113:104074, 2021.

Acknowledgements

Science Foundation Ireland (grant 18/ERCD/5481);