A THERMODYNAMIC FRAMEWORK FOR SARCOMERE EVOLUTION IN CARDIOMYOCYTES SUBJECTED TO DYNAMIC LOADING

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Introduction

Development of in-vitro protocols to mature cardiomyocytes (CMs) with sarcomeric structures and aligned myofibrils is a key challenge in the field of cell and tissue engineering [1]. Physiologically sarcomeres consist of ordered actin-myosin (AM) bounded by Z-bands [2]. Similar to sarcomeres, stress fibres (SFs) actively generate tension through AM cross bridge cycling. However, SFs are unstructured and do not contain Z-bands or titin, and consequently generate lower levels of tension than sarcomeres [3,4]. The in-vitro study of *Dou et al.* [5] shows dynamic stretching increases sarcomere formation in comparison to static controls. The mechanism by which dynamic loading increases sarcomere formation and matures CMs is unknown. Understanding the role of mechanical loading in sarcomere formation would significantly contribute to the field of cardiac tissue engineering.

We propose a novel dynamic theoretical formulation for remodelling of sarcomeres and SFs. Our analyses uncover a thermodynamic basis for the relationship between dynamic loading and sarcomere formation, including the key energetic role of titin in this process.

Methods

In our thermodynamic framework, evolution and remodelling of sarcomeres and SF formation is driven by differences in chemical potential. Areal sarcomere evolution from unbound cytoskeletal proteins is derived:

$$\frac{\partial \hat{n}_{SU}}{\partial t} = \omega_S P \exp\left(\hat{n}_S \frac{\mu_U - \mu_Z}{k_B T}\right), \qquad (1)$$

where $\hat{n}_S$ is sarcomere concentration in series, μ_U is enthalpy of proteins in unbound states, and μ_Z is an activation barrier for sarcomere formation, P is a function of recruited bound AM proteins, where conservation of AM proteins is applied between unbound proteins $\hat{N}_U$, SFs $\hat{N}_{BF}$, and sarcomeres $\hat{N}_{BS}$. Areal sarcomere generation from bound SFs, is:

$$\frac{\partial \hat{n}_{SF}}{\partial t} = \omega_S P \left(\frac{\hat{\xi}_0}{\hat{N}_U} \frac{\hat{N}_{BF}}{\hat{\xi}_0 - \hat{N}_{BF}}\right)^{1/\nu_S} \exp\left(\hat{n}_S \frac{\mu_B - \mu_Z}{k_B T}\right), \qquad (2)$$

where μ_B is enthalpy of bound SF proteins. Sarcomere dissociation is controlled by the enthalpy of bound sarcomere proteins μ_H, which is a function of strain and titin elasticity. Total remodelling of sarcomeres is;

$$\frac{\partial \hat{n}_S}{\partial t} = \frac{\partial \hat{n}_{SU}}{\partial t} + \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \frac{\partial \hat{n}_{SF}}{\partial t} d\theta - 2\omega_S \hat{n}_S \, exp\left(\hat{n}_S \frac{\mu_H - \mu_Z}{k_B T}\right). \qquad (3)$$

Results

We simulate a CM spreading to a steady-state geometry over a two day period, which is then subjected to biaxial dynamic strain at 1Hz for a further eight day period. Evolution of SFs and sarcomeres in a cell subject to 20% loading, as well as static conditions shown in Fig. 1A. Dynamic loading leads to rearrangement of proteins in SFs, to form sarcomeres. Fig. 2B shows alignment and concentration of SFs and sarcomeres for static and dynamic cases. Fig. 2C shows model predictions of increased sarcomere concentration due to dynamic loading, in agreement with experimental values [5].

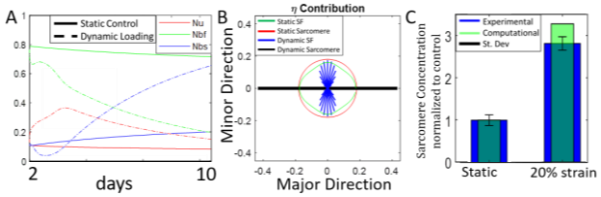

Fig 1: A) Dynamic evolution of protein concentration for static control (solid line) and biaxial dynamic loading (dashed line). B) SF and sarcomere orientation and concentrations following eight days of loading. C) Sarcomere concentration (normalised to static) following eight days of dynamic loading.

Discussion

The key finding that emerges from our novel thermodynamic framework is that titin stretch during applied dynamic loading of CMs results in a reduction in the standard enthalpy of sarcomeres, driving remodelling of cytoskeletal proteins from SFs to sarcomeres. Our model predicts that application of a dynamic biaxial stretch of 20% at 1 Hz results in a three-fold increase in sarcomere concentration, aligning with the in-vitro measurements of *Dou et al.* [5]. Our model suggests that titin knock-out experiments will lead to reduced sarcomere formation and increased SF formation under dynamic loading, as observed in-vitro [6]. The dynamic remodelling framework will also be extended to simulate hypertrophy due to altered mechanical loading of the myocardium [7].

References

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