Velocity of shortening tunes myosin heavy chain isoform expression in human engineered heart tissues

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Non-standard Abbreviations and Acronyms

MHC: Myosin Heavy Chain

iPSC-CMs: Induced pluripotent stem cell derived cardiomyocytes

EHT: Engineered Heart Tissue

Cardiac myosin heavy chain (MHC) is expressed as one of two isoforms in the mammalian heart, α or β. Despite near sequence homology, α-MHC has a higher ATPase rate and is capable of greater shortening velocities and power output[1]. Relative abundances of each isoform are known to shift in response to aging, hormonal changes, and altered hemodynamic load.

Human embryonic stem cell derived cardiomyocytes express high levels of α-MHC under traditional culture conditions, but near-complete β-MHC isoform expression can be achieved by extended culture on stiff substrates[2]. Induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) display shifts in MHC isoform expression in response to mechanical loading[3]. Although such studies point toward regulation of MHC isoform by mechanical factors, further elucidation of a mechanosensitive mechanism is limited by the fact that in previous model systems, load and shortening are inextricably linked.

In order to investigate relationships between muscle loading, shortening, and MHC isoform ratio in a precisely controlled environment, we previously developed a bioreactor which allows engineered heart tissues (EHTs) to be cultured under a prescribed loading regime with coordinated electrical pacing (Fig 1A)[4]. This allows for independent modulation of load and shortening. Here, we report measurements of relative MHC abundance made on tissues dynamically cultured as part of that previous study[4]. We present evidence for the first time that the α/β-MHC expression ratio is highly sensitive to the velocity of cardiomyocyte shortening. By contrast, MHC isoform expression was not altered by isolated changes in mechanical load.

EHTs were subjected to one week of culture under one of seven length transients which differed in their amount of shortening, velocity of shortening, and applied afterload (Fig 1B). Protein was isolated from EHTs and MHC isoforms were separated on 7% acrylamide gels (1:100 Bis) at a constant current of 5 mA

for 24 hours at 4°C. Gels were stained using Silver Stain Plus (Biorad) and relative amounts of α and β MHC isoforms were determined by densitometry analysis (Fig 1C). Linear regressions with post-hoc Bonferroni correction were performed to identify significant correlates of MHC isoform expression. Partial least squares regression (PLSR) was conducted to determine the relative importance of each factor.

MHC isoform ratio was strongly correlated with the velocity of shortening administered to tissues in culture ($R^2 = 0.54$, $p < 0.0001$). Tissues exposed to more rapid shortening velocities responded by shifting protein expression to a higher proportion of α-MHC compared to β-MHC. Total amount shortened ($R^2 = 0.37$, $p < 0.0001$) and contractile work ($R^2 = 0.19$, $p < 0.01$) were also significantly correlated with MHC isoform ratio (Fig 1D). Increased amounts of shortening or contractile work both provoked an increase in the relative abundance of α-MHC. Surprisingly, MHC relative abundance was not sensitive to either the peak force attained by the tissue during twitch (afterload) or the normalized force integrated over the cycle length. This lack of force-dependent MHC isoform regulation was made apparent by the mavacamten-treated group, in which peak force and force-time integral were reduced while holding muscle length constant. One week of mavacamten application (0.25 μM) decreased peak force by 89% and normalized force-time integral by 92%, while the β-MHC percentage did not significantly change. Variable importance projection (calculated from PLSR analysis) ranked shortening velocity as the only predictor of MHC isoform ratio that exceeded the importance threshold (Fig 1E).

While previous investigations have shown that MHC isoform expression determines maximum shortening velocity, this work is the first to demonstrate the reverse, namely that a prescribed shortening velocity can shape isoform selection. This work underscores the importance of controlled shortening for attaining adult-like levels of β-MHC in human iPSC-CMs. As many disease-linked

mutations are located in the β-MHC gene, understanding in detail how to obtain correct stoichiometric expression of MHC is essential for accurate *in vitro* disease modeling.

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Disclosure:

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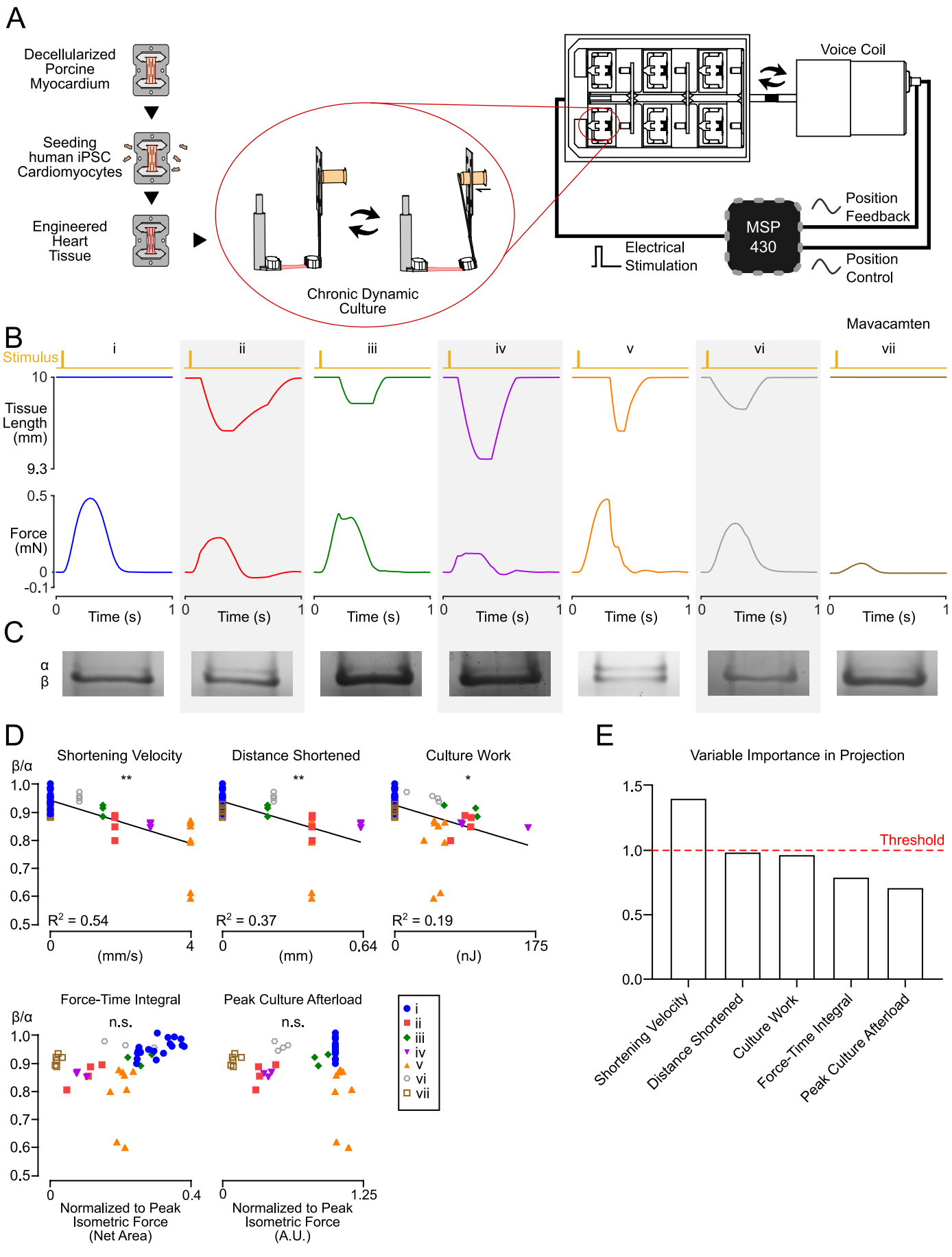
Figure 1: β-Myosin Heavy Chain (MHC) abundance is most closely correlated with the shortening velocity experienced during chronic culture. (A) Schematic showing construction of engineered heart tissues (EHT) from induced-pluripotent stem cell derived cardiomyocytes. EHTs were loaded into a bioreactor and cultured for one week under coordinated electrical and mechanical stimulation. **(B)** Representative shortening protocols of seven unique culture conditions with corresponding force records (From left, n = 18, 4, 3, 3, 8, 4, 6). One group of EHTs was cultured under isometric conditions with 0.25 μM mavacamten added to culture media to decrease normalized force-time integral and peak afterload without affecting shortening (right-most graph). **(C)** Representative 7% SDS-PAGE gels showing MHC composition of EHTs cultured under each shortening regime. Relative amounts of each isoform were calculated using GelBandFitter densitometry analysis. **(D)** Proportion of β/α MHC isoform plotted against different shortening and loading parameters resulting from varied shortening protocols. Color and marker of data points correspond to culture conditions. Linear regression with post-hoc Bonferroni correction revealed a significant decrease of β-MHC expression correlated with increased shortening velocity, distance shortened, and contractile work performed during culture (**$p < 0.0001$; * $p < 0.01$). β-MHC expression was not significantly correlated with force-time integral or peak culture afterload ($p = 0.0137$, 0.9091 respectively; regression lines omitted) **(E)** Variable Importance in Projection scores were calculated from partial least squares regression. Only shortening velocity exceeded the threshold (VIP = 1) to provide an increase in the predictive power of the PLSR model.


A
Decellularized Porcine Myocardium
Seeding human iPSC Cardiomyocytes
Engineered Heart Tissue
Chronic Dynamic Culture
Voice Coil
MSP 430
Position Feedback
Position Control
Electrical Stimulation
B
Mavacamten
Stimulus
i
ii
iii
iv
v
vi
vii
Tissue Length (mm)
10
9.3
Force (mN)
0.5
0
-0.1
Time (s)
C
α
β
D
Shortening Velocity
Distance Shortened
Culture Work
β/α
R² = 0.54
R² = 0.37
R² = 0.19
(mm/s)
(mm)
(nJ)
Force-Time Integral
Peak Culture Afterload
n.s.
Normalized to Peak Isometric Force (Net Area)
Normalized to Peak Isometric Force (A.U.)
E
Variable Importance in Projection
Threshold
Shortening Velocity
Distance Shortened
Culture Work
Force-Time Integral
Peak Culture Afterload