Biomimetic Loading Promotes Maturation and Suppresses Pathological Progression of Chick Embryonic Cardiomyocytes in Engineered Heart Tissues

CNF Project Number: 2619-17
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Primary Source(s) of Research Funding: Additional Ventures Single Ventricle Research Fund; National Institutes of Health R01 HL160028

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Primary CNF Tools Used: Objet30 pro 3D printer

Abstract:

Mechanical stimulation of engineered cardiac tissue was shown to improve cardiomyocyte maturation by emulating the mechanical loadings cardiomyocyte experienced during cardiac cycle, namely resistance to contraction (Afterload) and cyclic stretching (Preload). Nevertheless, many existing platforms for cyclic stretching pose the risk of inducing human cardiac pathology. In this study, we developed a novel bioreactor system to more accurately recapitulate the in vivo loading condition, allowing cyclic stretching with active contractile work production and duty cycling incorporated in between each stretch. Our focus was to investigate the functionalities, maturation, and pathological progression of embryonic chicken engineered heart tissues (EHTs) subjected to three distinct mechanical stimulation regimens, including (i) static control, (ii) afterload no duty cycle (Afterload NoDC), and (iii) afterload duty cycle (Afterload DC). We showed that Afterload DC improved tissue functionalities, maintaining higher contractile force and frequency. This was achieved by prohibiting early tissue compaction during stimulation. Tissues exposed to Afterload DC demonstrated enhanced cardiomyocyte maturation evidenced by improved sarcomeric organization.

Moreover, Afterload DC suppressed the transcriptional expressions of pathological hypertrophy and fibrosis markers, including TGF.2, COL5A2, and POSTN. Collectively, Afterload DC significantly promoted the functionality of EHTs by enhancing cardiomyocyte maturation and suppressing cardiac pathology. This study highlighted the remarkable potential of biomimetic loadings in facilitating tissue maturation.

Summary of Research:

To determine if utilizing a mechanical stimulation regimen that can provide cyclic stretching while allowing contractile work production improves the tissue functionalities, contractile forces and frequencies of EHTs during auxotonic contraction were measured over seven days of stimulation (Figure 1). A decline in contractile force was observed from D5 to D11 in all conditions (Figure 1A). However, both Afterload DC and Afterload NoDC groups had a general maintenance of the contractile force across culture time with a less degree of a decline. When comparing each condition, the afterload DC conditioned tissues showed a significantly greater force compared to the other conditions observed as early as the fifth day of stimulation. In terms of contractile frequency, Afterload NoDC and Afterload DC led to an increase in contractile frequency to a level significantly higher than that of the static control starting from D7 (Figure 1B). This incline in contractile frequency was shown to be followed by a mild decrease until D11 while most of the tissues in static control gradually lost their contraction.

To examine the effect of different mechanical stimulation regimens on tissue compaction, brightfield images of EHTs were taken over the culture period (Figure 2A). EHTs from all regimens exhibited spontaneous compaction in volume from D4 to D5 prior to the application of mechanical stimulation (Figure 2B). From D5, both Afterload NoDC and Afterload DC interrupted the compaction process, leading to significantly higher tissue volume of EHTs on D8 relative to those from the static control. This prohibiting effect was sustained until D11 only in Afterload NoDC, whereas EHTs subjected to Afterload DC showed a tissue volume similar to that of the static control. Our results showed that the percentage change of tissue volume from D5 to D8 was significantly and positively correlated with the percentage change of contractile force, in which afterload NoDC and afterload DC promote higher tissue volume and therefore higher contractile force (Figure 2C).

We investigated whether the improved tissue functionalities of EHTs following Afterload NoDC and Afterload DC conditioning is associated with enhanced cardiomyocyte maturation [1]. To do so, EHTs were stained with .-Actinin to visualize the sarcomeric organization of cardiomyocytes (Figure 3A). As expected, tissues exposed to Afterload

NoDC and Afterload DC conditioning demonstrated more aligned sarcomere structure compared to those in the static control group. More importantly, both mechanical stimulation regimens led to sarcomere and z-band lengths resembling that of the mature chick cardiomyocytes.

Increasing afterload was shown to promote pathological hypertrophy and fibrosis despite improved tissue functionalities of EHTs. Here, EHTs subjected to Afterload NoDC condition were stretched while being prohibited from contractile work production by providing a consistently high afterload. To access whether pathological phenotypes were manifested in the tissues under Afterload NoDC and whether Afterload DC can mitigate the pathological progression, smFISH staining was performed on a panel of gene markers associated with pathological hypertrophy and cardiac fibrosis (Figure 4). Aligned with previous study, tissues in the Afterload NoDC group showed significantly higher expression of pathological hypertrophy markers, including POSTN, TGF.2, and COL5A2, compared to the static control. Surprisingly, despite being exposed to mechanical loading, EHTs in the Afterload DC condition showed similar marker expression to the static control. No significant difference among conditions was detected in FN1 expression.

Conclusion and Future Steps:

Our study highlights the promising potential of Afterload DC in promoting EHTs functionality by promoting cardiomyocyte maturation while preventing pathological phenotypes. To expand, assessing the effect of biomimetic loading on human induced pluripotent stem cell-derived cardiomyocyte will greatly facilitate the clinical translation of this novel mechanical stimulation regimen. Meanwhile, our bioreactor system could also serve as a platform for drug screening and disease modeling.

References:

[1] Leonard, Andrea, et al. "Afterload promotes maturation of human induced pluripotent stem cell derived cardiomyocytes in engineered heart tissues." Journal of molecular and cellular cardiology 118 (2018): 147-158.

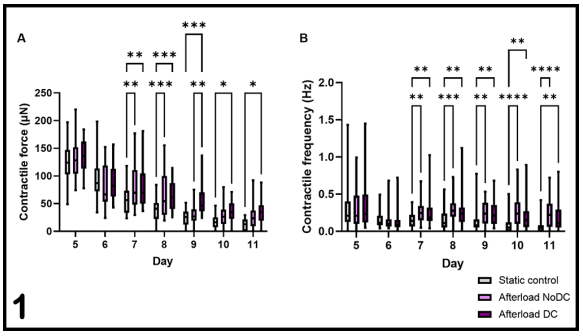

Figure 1: Auxotonic contractile capacity of EHTs on post constructs across stimulation period. Contractile force (A) and frequency (B) across culture time for all three stimulation conditions.

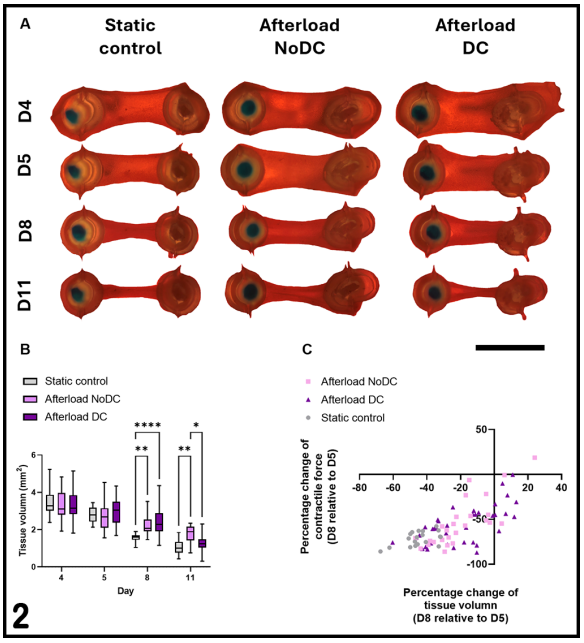

Figure 2: Compaction of EHTs on post construct across stimulation period. (A) Bright field images of EHTs across culture time for all three stimulation conditions. (B) Quantitative measurement of tissue volume of EHTs across culture time. (C) Correlation between percentage change of contractile force and percentage change of tissue volume from D5 to D8 and D8 to D11.

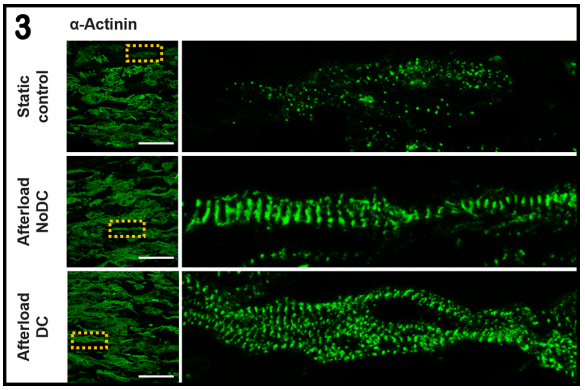

Figure 3: Cardiomyocyte maturation in EHTs on Day 11. (A) Representative immunofluorescent images of .-actinin. Quantitative measurement of the sarcomere length (B) and the z-band length

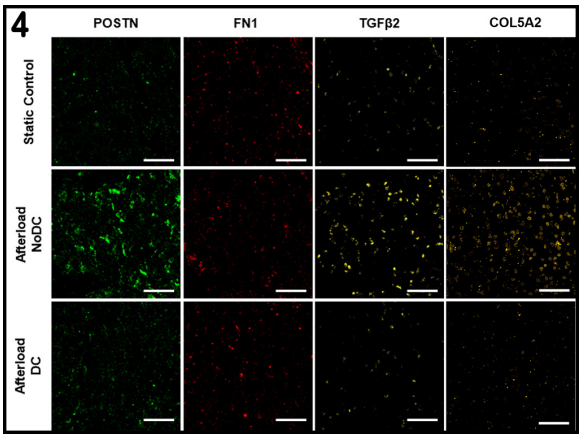

Figure 4: Expression of gene markers associated with fibrosis and pathological hypertrophy in EHTs on Day 11.