It's all in the timing: Modeling isovolumic contraction through development and disease with a dynamic dual electromechanical bioreactor system

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This commentary discusses the rationale behind our recently reported work entitled "Mimicking isovolumic contraction with combined electromechanical stimulation improves the development of engineered cardiac constructs," introduces new data supporting our hypothesis, and discusses future applications of our bioreactor system. The ability to stimulate engineered cardiac tissue in a bioreactor system that combines both electrical and mechanical stimulation offers a unique opportunity to simulate the appropriate dynamics between stretch and contraction and model isovolumic contraction in vitro. Our previous study demonstrated that combined electromechanical stimulation that simulated the timing of isovolumic contraction in healthy tissue improved force generation via increased contractile and calcium handling protein expression and improved hypertrophic pathway activation. In new data presented here, we further demonstrate that modification of the timing between electrical and mechanical stimulation to mimic a non-physiological process negatively impacts the functionality of the engineered constructs. We close by exploring the various disease states that have altered timing between the electrical and mechanical stimulation signals as potential future directions for the use of this system.

Introduction and Motivation

Our recently published work "Mimicking isovolumic contraction with combined electromechanical stimulation improves the development of engineered cardiac constructs" describes a bioreactor system that can precisely control the timing between combined electrical and mechanical stimulation of engineered cardiac tissue constructs, allowing us to study how synchronized vs. delayed electromechanical stimulation influences the development of function in engineered cardiac tissues.[1] The aim of this commentary is to provide additional insights into the rationale behind the simulation of isovolumic contractions in a dual stimulation bioreactor system, introduce new data that supports our claims that the timing of combined stimulation is important, and highlight potential uses of this technology in the study of various cardiac diseases.

Individually, both mechanical and electrical stimulation have been established as standard conditioning methods for engineered cardiac constructs and have been studied extensively previously. For instance, mechanical stimulation has been used in a wide variety of bioreactor systems,[2-4] and has been shown previously to activate FAK and RhoA, which initiates myotube
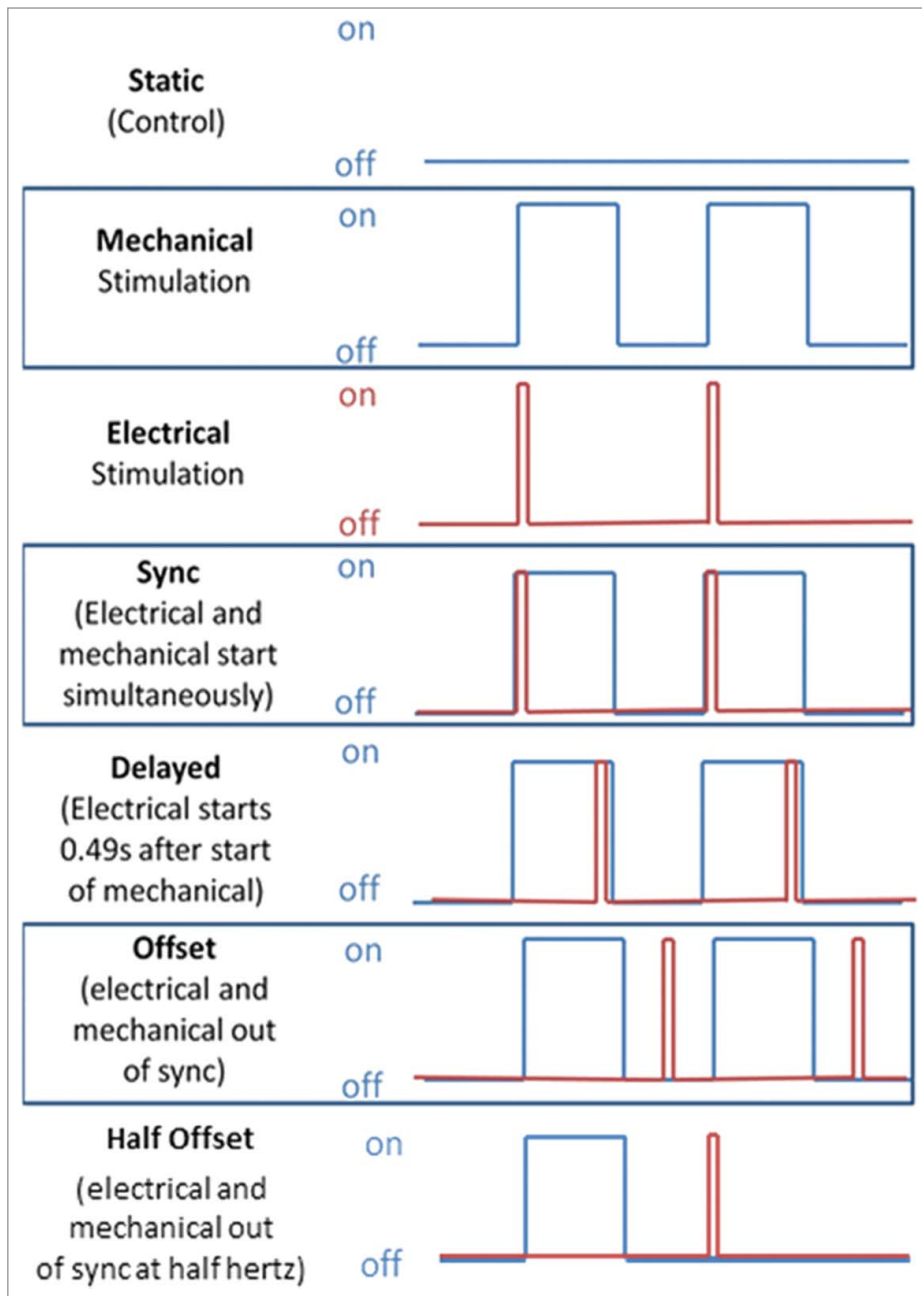
formation[5] and promotes extracellular matrix (ECM) formation,[6] improving the overall organization of the tissue and thus the contraction force.[2] Similarly, electrical stimulation has been used in other systems,[7-9] and has been shown to lead to synchronous contractions of the myocytes, by inducing alignment in the cardiomyocytes[10] and increasing the volume fraction and organization of sarcomeres.[11] Likewise, in our system, we observed that though electrical and mechanical stimulation both improve twitch forces over the static conditions, they influenced development in different ways. Mechanical stimulation had a statistically significant increase in the cell number in the constructs after 14 d in culture, while electrical stimulation appeared to have a slightly higher percentage of cardiomyocytes in the culture.

In modeling the dynamic electromechanical environment present in vitro, it is important to note that the mechanical stimulation simulates the ventricle filing with blood, while electrical stimulation mimics the induction of the ventricular contraction. While single-modality stimulation has its benefits, the environment that cells are subjected to in vivo involves both electrical and mechanical signaling, and the timing of these two events can be critical. The interaction of these two stimuli occurs during Isovolumic contraction, which is a critical period in the cardiac cycle

Figure 1. (**A**) A diagram demonstrating the stimulation regimens implemented. The blue line represents the mechanical stretch while the red line represents the electrical stimulation. Portions reprinted with permission from reference 1.

stimulation could be an important factor in promoting optimal construct function.

Though electrical and mechanical stimulation have been previously combined in other bioreactor systems,[16-19] the effect of delayed timing between electrical and mechanical stimulation had not been studied. Mimicking the timing present in vivo causes a switch in the contractile model of the muscle from an isometric mode when the valve is closed to an isotonic shortening, once the valve opens.[20] Previous studies have shown that muscles, on a tissue[21] and cellular level,[22] behave differently under isometric conditions vs. isotonic shortening. Under isometric conditions, mechanical stretch shortens the intracellular calcium transients,[23] and maximizes the forces generated by the tissue. Under isotonic shortening, the intracellular calcium transients are prolonged.[24] Thus, the switch in contractile modes that would be generated during a stimulation that mimics isovolumic contraction could allow for a higher force to be maintained during the shortening period, which would increase the network output,[25] and could modulate the calcium dependent action potential duration in our engineered constructs,[20] thus maximizing the forces generated.[26]

Our system allows for the study of changes in isovolumic contraction by changing the timing between the beginning of the mechanical stimulation and the beginning of electrical stimulation. To model the isovolumic contraction, the electrical stimulation was delayed until 10 ms before the end of the mechanical stimulation. While the ICT in the human heart is approximately 40 ms,[27] an ICT of 10 ms was chosen to model the ICT observed in the rat. This time was based on a derivation of the ICT

where the left ventricle is stimulated to contract, but it is maintained in a stretched position until enough pressure is built up in the ventricle to cause the aortic valve to open.[12] The alteration of Isovolumic contraction time has been established as a method of evaluating cardiac function[13] due to its direct relationship with myocardial contractility[14] and its sensitivity to alterations in both hemodynamic activity and contractile function.[15] Thus, when creating engineered cardiac tissue, in vitro methods of recapitulating the specific timing of combined electromechanical

from previously published data on the Myocardial Performance Index(MPI)[28] and literature values of ejection time and isovolumic relaxation time.[29,30] With the delayed condition, we observed an increase in the twitch force as compared with a simultaneous electromechanical stimulation. The increase in function was likely due to the increases in the protein expression of Troponin T, the thin-filament regulatory protein necessary for coordinated force generation[31] and cardiac sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), the

protein responsible for pumping calcium back into the sarco-plasmic reticulum during diastole.[32] The increase in Tropo-nin T and SERCA2a indicates that modeling the isovolumic contraction improves calcium sensitivity and handling, which is necessary for cardiomyocyte maturation. Observed increases in Akt expression further suggested that this maturation could be via physiological hypertrophic pathway.[33,34]

Offsetting Electrical and Mechanical Stimulation

While the data from our previous paper[1] indicate that the tim-ing between electrical and mechanical stimulation can be utilized to enhance the function of engineered cardiac tissues, we only investigated variations in the timing where the two stimuli were overlapping. To further build the case on the importance of tim-ing in a combined electromechanical bioreactor system, here we present new data investigating the effects of offsetting the electri-cal and mechanical stimulation so that they were completely out of phase. As shown in **Figure 1**, two additional conditions beyond those studied in the original paper were added: an Offset (O) condition where the electrical stimulation was completely out of phase with the mechanical stimulation but the rate of mechanical stimulation was maintained (1Hz), and a Half Offset (H) condition in which the electrical and mechanical stimulation alternated at 1 Hz stimulation. The offset condition represents a condition that is physiologically irrelevant, given that electrical and mechanical stimulation does not occur in isolation in the native heart. In this case, though the alternating electrical and mechanical stimulation could potentially combat the cellular adaptation to stimuli, the electrical stimulation would cause the construct to contract against no resistance, likely reducing its effect. Thus, we hypothesized that the offset conditions would have less functionality than the delayed electromechanical stimulation.

It is important to note that all experimental groups, including both the new and previously published data, were created from the same cell isolation and cultured at the same time in the biore-actor system. Twitch force results (**Fig. 2**) indicate that the static condition (2.08 ± 0.77 mN) had a statistically significantly lower contraction force than all other conditions but the half offset con-dition (2.03 ± 1.13 mN) ($P < 0.05$). The offset condition exhib-ited a similar twitch force (3.33 ± 1.02 mN) to the delayed condition (3.29 ± 1.09 mN). The force generation time (**Fig. 3A**) and the force relaxation time (**Fig. 3C**) were statistically greater in the half offset condition than in the static, mechanical only, electrical only and synchronized conditions. The force gen-eration and relaxation rates were also statistically less in the static and half offset conditions (**Fig. 3B and D**, respectively). Contrary to our initial hypothesis, the offset condition maintained a simi-lar twitch force to the delayed conditions, indicating that an addi-tional factor could be influencing the development of the constructs and offsetting the potential negative effects of mis-timed stimulation. One potential explanation was that by offset-ting the electrical and mechanical stimulation in the manner that we did, we increased the effective rate of stimulation from 1 Hz
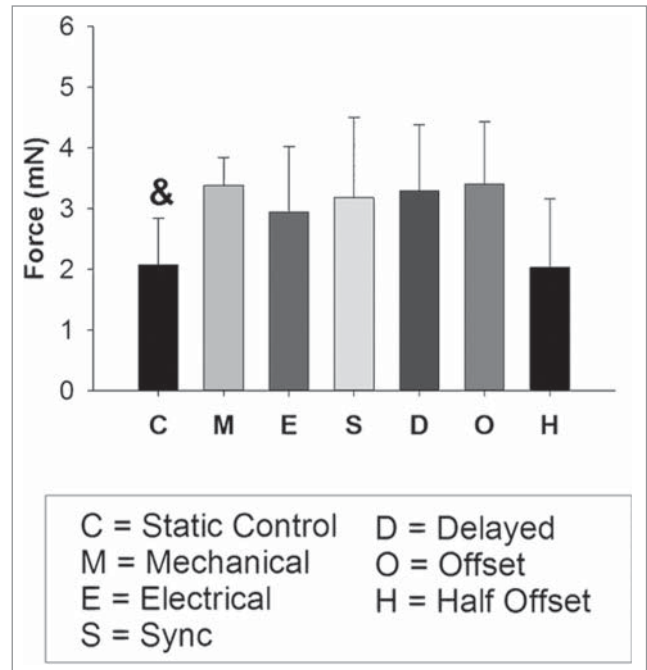
Figure 2. Observed twitch forces for all groups. and denotes statistically significantly different than all other conditions but the half offset (O) ($P < 0.05$). Portions reprinted with permission from reference 1.

to 2 Hz. Increases in the frequency of both mechanical[3] and elec-trical[35] stimulation have been shown to lead to increased force development. To assess what effect this was having in our system, we added a half offset condition in which the stimulation occurred at half of the frequency of the other groups (total stimu-lation frequency of 1 Hz, individual stimulation was at 0.5 Hz) to determine if the offset electromechanical stimulation or the increased frequency was the cause of the observed functionality. Though twitch force testing occurred at the same rates for all groups, unlike the standard offset condition, the half offset con-dition demonstrated a reduced twitch force and decreased rate of force generation and relaxation, similar to the static conditions. Twitch force generation rate signifies the ability of the tissue to contract simultaneously, indicating high levels of cell-cell cou-pling, which allows for the quick propagation of the calcium sig-nal and a faster rate of force generation.[36] Previous contraction kinetics experiments in skinned fibers, skinned myocytes, and subcellular cardiac myofibrils have also indicated that elevated Ca^{2+} increases both the forces generated and the rate of force generation.[37] Furthermore, alterations in relaxation rate are likely due to changes in the expression, function, and interactions of Ca^{2+} handling proteins, such as SERCA2a.[38,39] Our results indi-cate that increased stimulation rate was likely the cause of the similar levels of contractile function observed in the standard off-set condition as compared with the other conditions, analogous to previously published results indicating that increased fre-quency of mechanical stimulation improved contractile func-tion.[3] In the case of the half offset we see the true effect of a 1 Hz
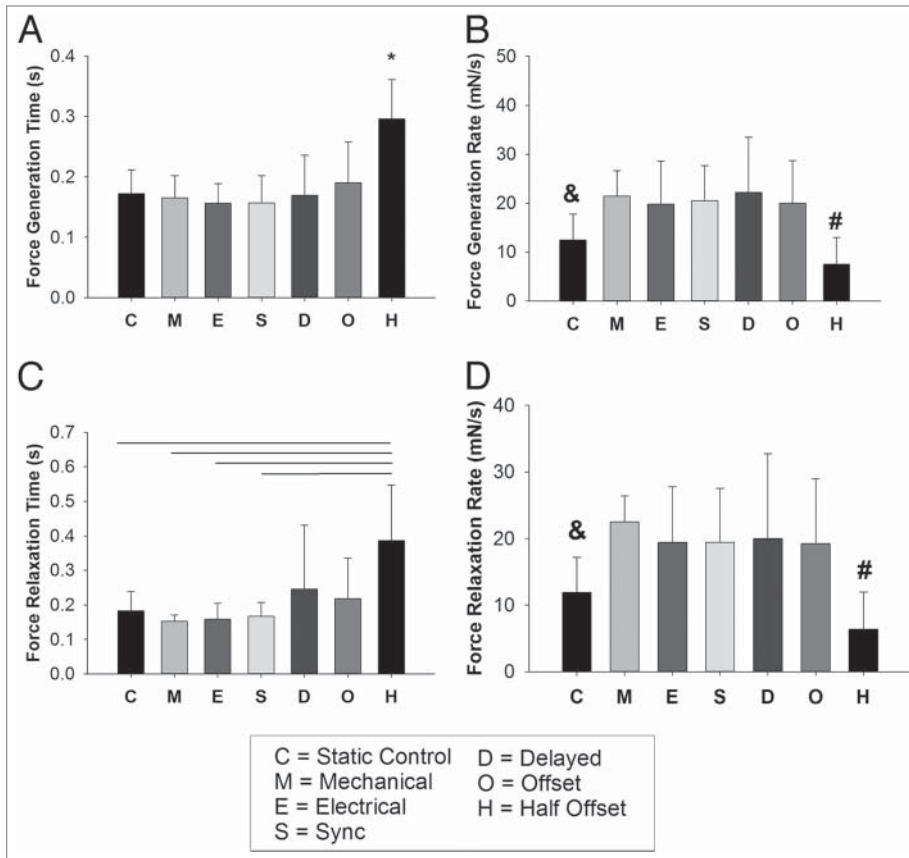
Figure 3. (**A**) Force generation time for all conditions. (**B**) Force generation rate for all conditions (mN/s). (**C**) Force relaxation time and (**D**) force relaxation rate for all conditions (mN/s). For all figures A–D, * denotes statistically different than all other conditions ($P < 0.05$), and denotes statistically significantly different than all other conditions but the half offset (O) ($P < 0.05$). # denotes statistically significantly different than all conditions but the static control (C) ($P < 0.05$). Portions reprinted with permission from reference 1.

contraction time (ICT) that occurs in normal development[40-42] or to model various myocardial diseases,[13,43-45] specifically those that affect the ICT. For example, in fetal heart development, four different distinct stages of development occur, each with its own contraction mechanics and conduction parameters.[41] Contractions coupled with action potential appear by the end of the 3-somite stage.[42] Early cardiac development is characterized by changes in the blood flow and heart function, which are necessary to maintain the circulatory needs of the developing embryo as the heart changes morphologically and physiologically.[46] Previous studies have shown that the ICT appears by 6 wk of gestation to be approximately 20% of the total cardiac cycle.[46] ICT as a proportion of the total cardiac cycle decreases in the first trimester, even though a steady fetal heart rate is maintained, indicating a decrease in the total ICT.[47] Through 18–31 wk of gestation, the ICT remains constant at approximately 43 ± 14 ms.[48] By modeling fetal ICT over time in our system, we can more closely recapitulate the mechanical and electrical signals observed and its changes, which is likely to be beneficial in engineered cardiac tissue development, particularly when the cells utilized are immature cardiomyocytes derived from stem cell sources.

offset stimulation and the reduction in force is accompanied by a reduction in both generation and relaxation rates indicating that altered calcium handling may be the culprit, but further testing is needed to verify this hypothesis. Additionally, these results in sum further implicate the critical importance of the timing of combined electromechanical stimulation in vitro. In summary, our experiments have demonstrated that varying the timing between electrical and mechanical stimulation in our bioreactor system can be an important factor to consider when conditioning engineered myocardium. When optimized, dual electromechanical stimulation can be a powerful tool, but mismatched stimulation can also hinder the conditioning process.

Future Directions in Modeling Development and Disease

One possible future direction of study for our system is utilizing its unique ability to vary the timings between electrical and mechanical stimulation to mimic the change of isovolumic

With respect to disease modeling, patients with aortic stenosis, a disease in which the aortic valve does not fully open, demonstrate a decrease in ICT[15] when they have preserved systolic left function, and an increase in ICT when depression of the systolic left ventricular function occurs.[43] A delay in the contraction occurs likely due to the prolonged effort of the left ventricle in ejecting the blood through the obstructed aortic valve,[49] which ultimately results in an increased afterload and slower contractions.[50] Aortic valve regurgitation can lead to ventricular pump failure due to a mismatch of afterload with preload.[51] When increases in afterload occur through reductions in the wall shortening and stroke volume, it leads to decreased performance, ultimately causing heart failure. Another potential example where the ability to alter the timing between electrical and mechanical stimulation could offer greater insight into the disease process involves patients with LV myocardial disease, where the ICT is increased significantly, from 35 ± 26 ms to 69 ± 30 ms,[44] likely due to decreased myocardial contractility.[52] In patients with heart failure, the widening of the QRS complex indicates an impaired or slowed propagation of the electrical input, which is associated with increased morbidity and

mortality.[53] This impairment of the electrical input causes ventricular dysynchrony. Biventricular devices used in cardiac resynchronization therapy have been established as a therapeutic treatment option for patients. However, studies have indicated that optimal pacing combinations change over time and high temporal individual variability of the activation pattern exists,[54] indicating electrical remodeling of the affected tissue.[53] In these cases, a system such as ours may be able to shed light on the role that the mismatch between mechanical and electrical signaling is playing in promoting or maintaining the diseased phenotype.

The creation of a dual electromechanical stimulation bioreactor system has demonstrated the importance of modeling the isovolumic contraction in the conditioning process of engineered cardiac tissues.[1] The ability to vary the timing between electrical and mechanical stimulation is a powerful tool that allows us to mimic both normal developmental processes that are likely important in cardiomyocyte and myocardial tissue maturation as well as diseased tissues, such as those that exist in aortic valve stenosis and heart failure. Future studies will aim to investigate the effects of elongated and shortened isovolumic contraction time and its effects on tissue development with the goal of simulating a disease model system in vitro for studying potential treatment options.

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No potential conflicts of interest were disclosed.

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