

OPTOGENETICS FOR MATURATION OF hiPS-CM MICROTISSUES

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Introduction

The necessity for *in vitro* models of the human heart grows as the burden of cardiovascular disease continues to be the leading cause of patient mortality.¹ Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a promising tool due to their potential applications for disease modeling, drug testing, and regenerative medicine. However, hiPSC-CMs are less mature than their native counterparts. Electrical stimulation has been used to mature cardiomyocytes over the past decade; however, such stimulation regimes are associated with the presence of an electrical field as well as charge injection, the relative effects of which are not well understood.^{2,3} Cardiomyocytes expressing channelrhodopsin-2 (ChR2), a light-responsive ion channel first transduced into mammalian cells in 2005, would allow for optical pacing as a means for stimulation and maturation.⁴ Cells were seeded in collagen-fibrinogen gel and subjected to optical stimulation of progressive frequency increase. Here we show that optically stimulated cardiomyocytes exhibit enhanced Ca²⁺ handling and improved conduction velocity.

Generation of Optogenetic hiPSCs Lines

Optogenetics can be described as light-based actuation through genetic engineering. The classical example is light-gated membrane depolarization through the use of channelrhodopsin-2 (ChR2), a cation channel that opens in response to blue light. The precise spatiotemporal control enabled by optogenetics makes it promising for *in vitro* applications in tissue engineering. With that in mind, we have created induced human pluripotent stem cell (hiPSC) lines that express optogenetic proteins, from which we have created light-activated tissues.

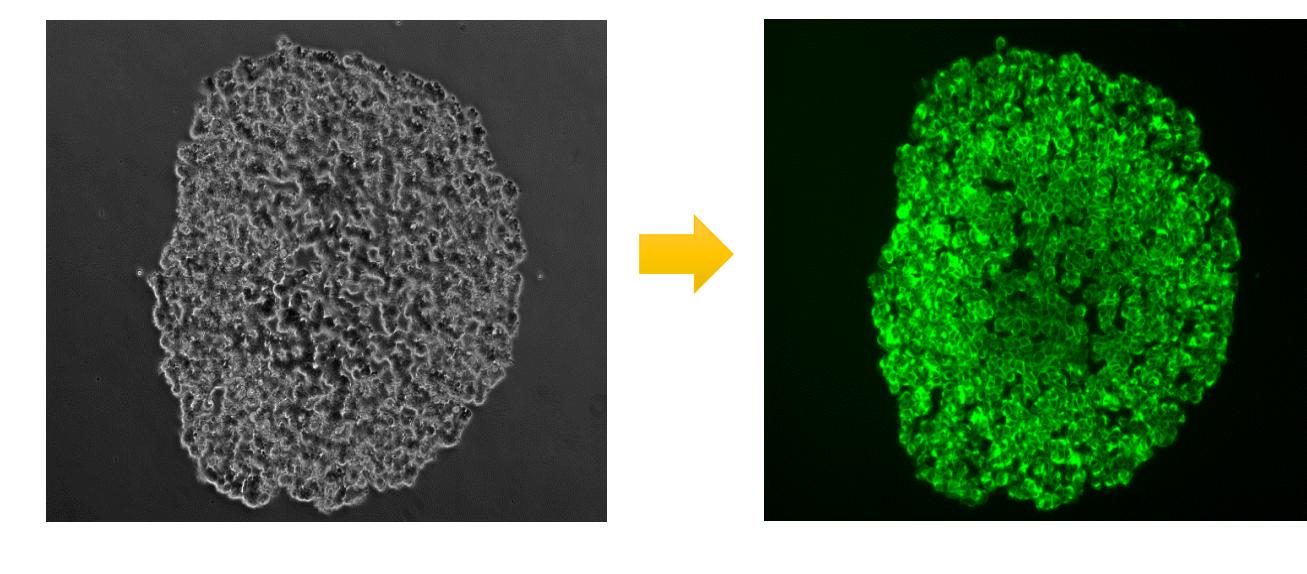


Figure 1: Generation of a ChR2-expressing hiPSC line by lentiviral infection. Lentiviral vectors were packaged and used to transfect C2A hiPSCs. Membrane localization of the fusion protein was demonstrated by fluorescent microscopy. The same method was used to generate the eNpHR line.

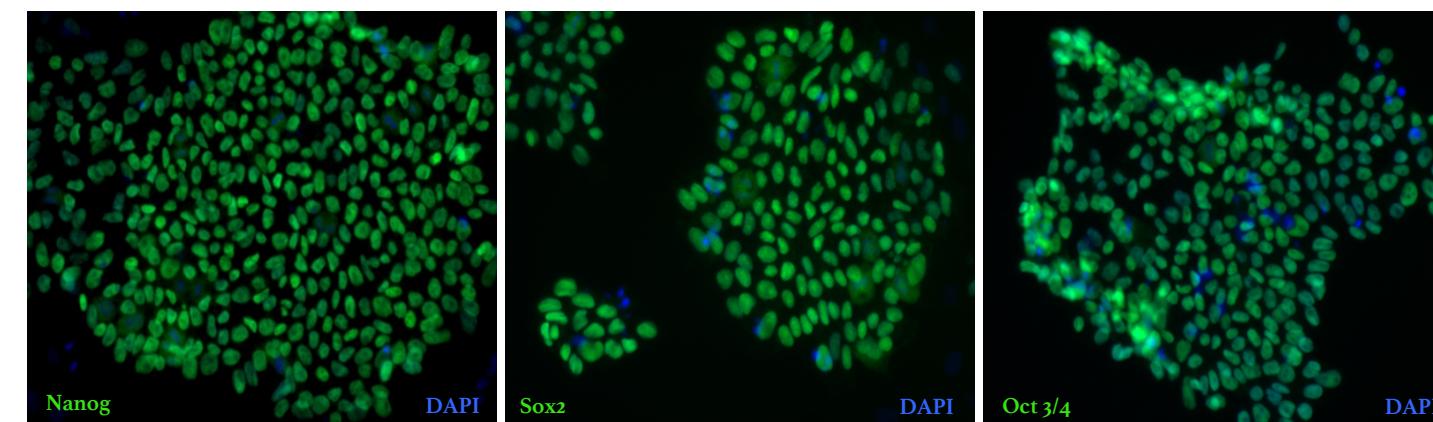


Figure 2: Pluripotency of transgenic hiPSC lines. A) Immunostaining shows nuclear expression of Nanog, Sox2 and Oct3/4. B) qPCR confirmed similar levels of Nanog and Sox2 expression compared to the parent line.

Embryoid Body Stimulation

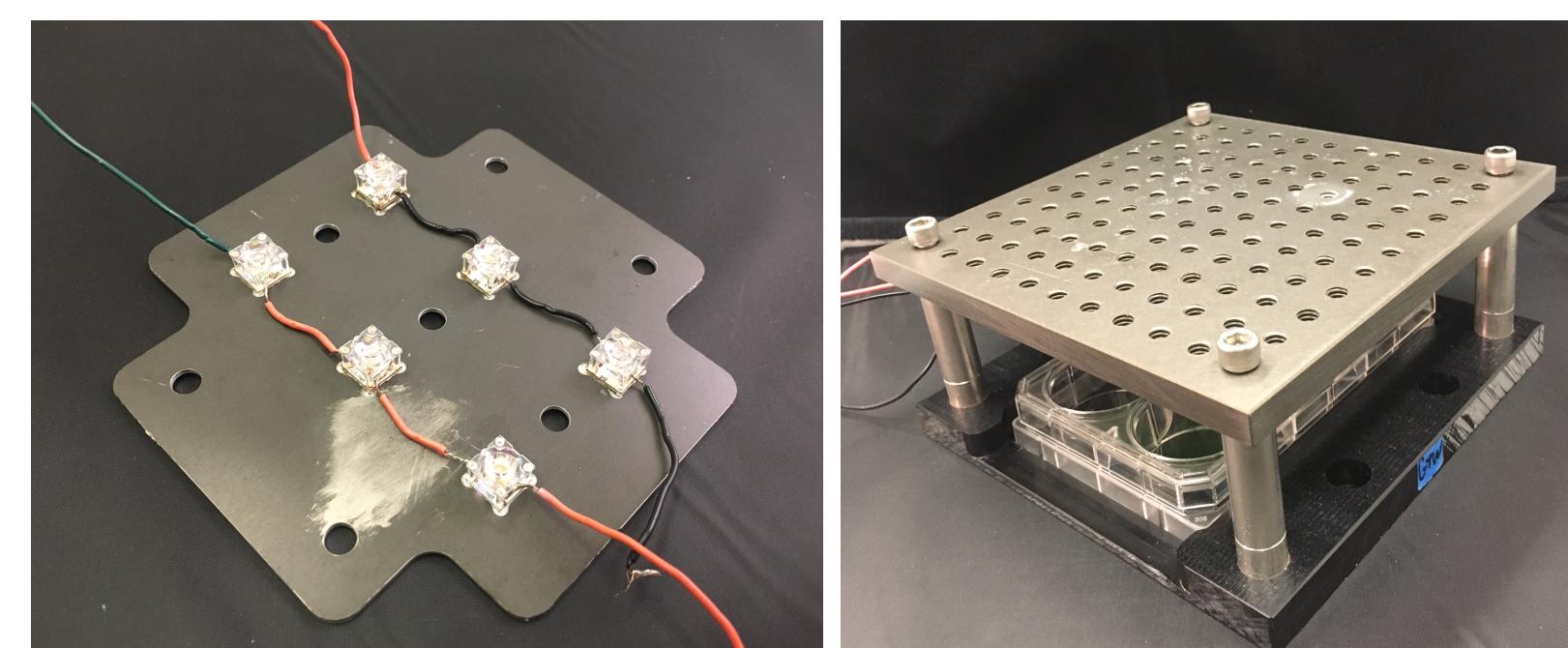


Figure 3: Experimental timeline and custom stimulation platform. ChR2-expressing hiPSCs were differentiated into cardiomyocytes following Burridge et al. Cardiomyocytes were then digested and seeded in AggreWell 400Ex plates. The newly formed embryonic bodies (EBs) were transferred into 6-well plates for optical stimulation using a custom stimulation platform.

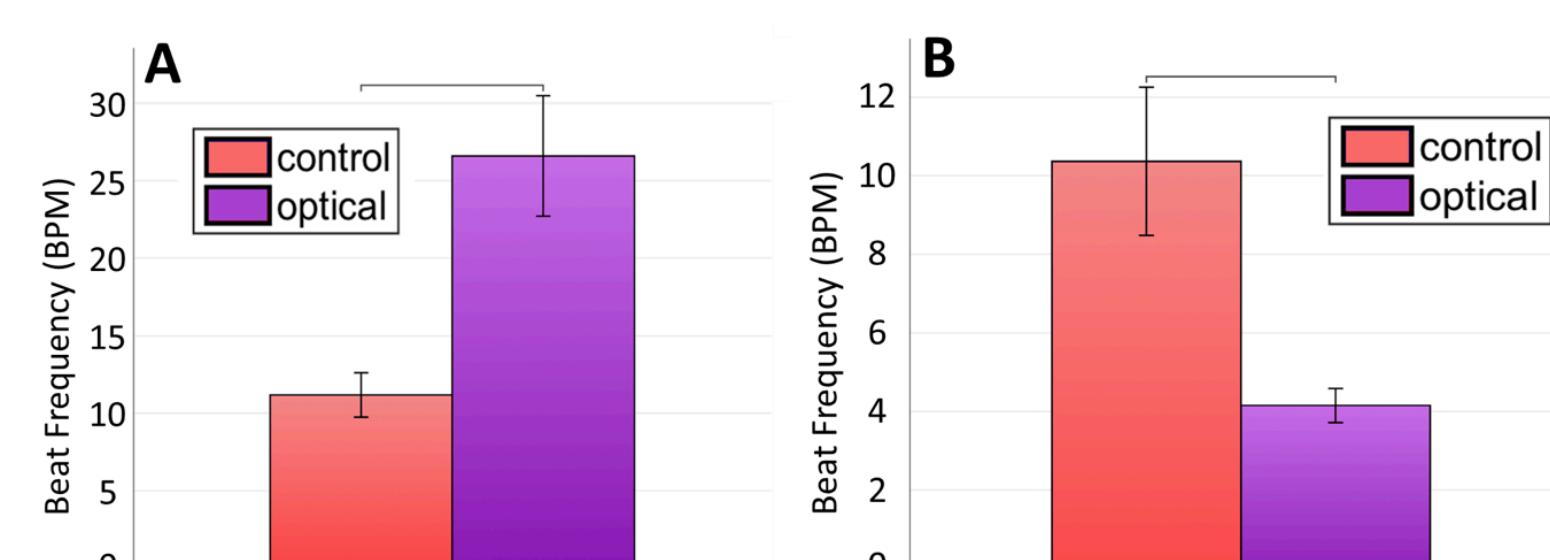
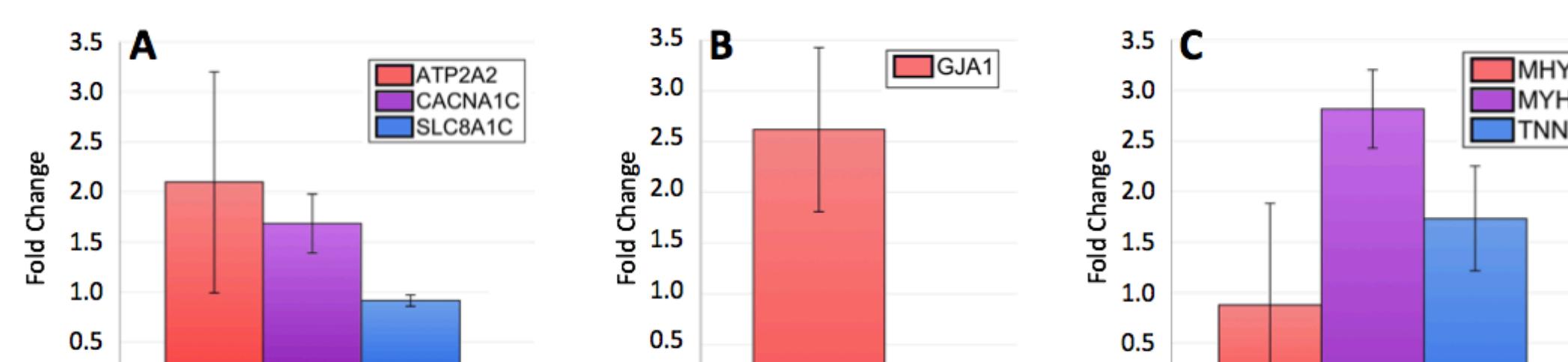


Figure 4: Beating frequency for control and stimulated EBs for optical pacing at (A) 0.50Hz and (B) 0.15Hz. Videos analysis of EBs after six days of optical stimulation in two separate experiments revealed the ability to entrain EBs to both (A) higher and (B) lower pacing frequencies respectively ($n=15$, unpaired two-sample t-test, $p < 0.05$). This finding is consistent with Eng et al, where electrical stimulation was used to entrain the spontaneous beating frequency of hiPSC-CMs.

Figure 5: qRT-PCR. qRT-PCR showed transcriptional upregulation of multiple genes associated with (A) calcium handling, (B) connexins, and (C) sarcomeric structure (average \pm s.e.m. of fold change relative to control, $n = 4$) in optically stimulated embryoid bodies



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Microtissue Stimulation



Figure 6: Tissue platform. Fibrin-collagen hydrogel suspensions of ChR2 hiPSC-CMs were seeded into custom tissue platforms for auxotonic stress and stimulated with a ramp stimulation regime for two weeks.

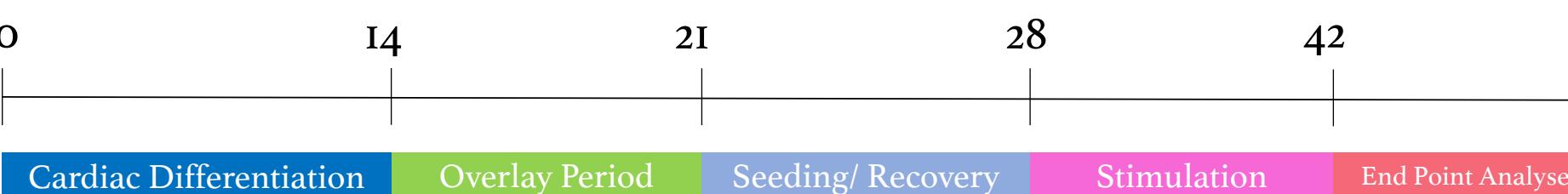


Figure 7: Conduction velocity measured by a custom projector system. Simultaneous optical mapping and patterned illumination of ChR2-expressing tissue for paced conduction measurements

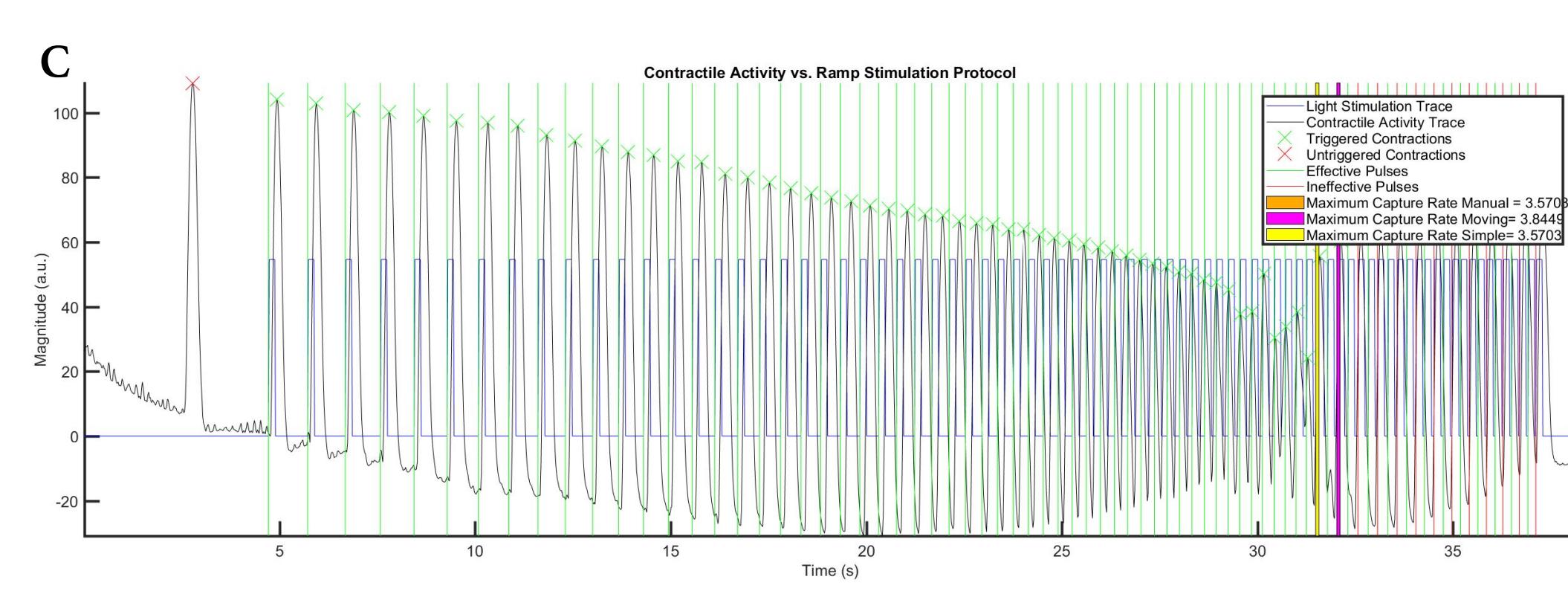
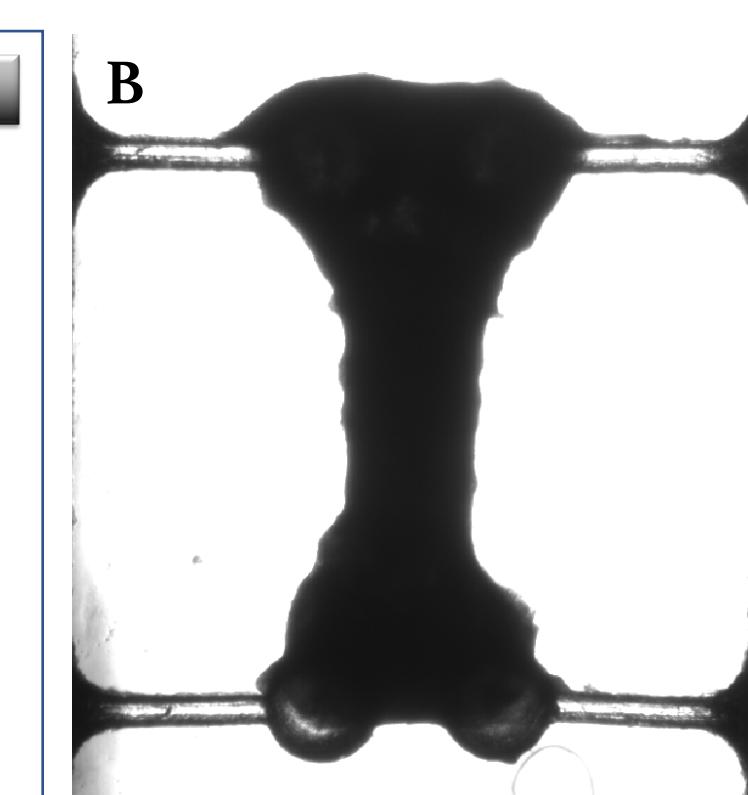
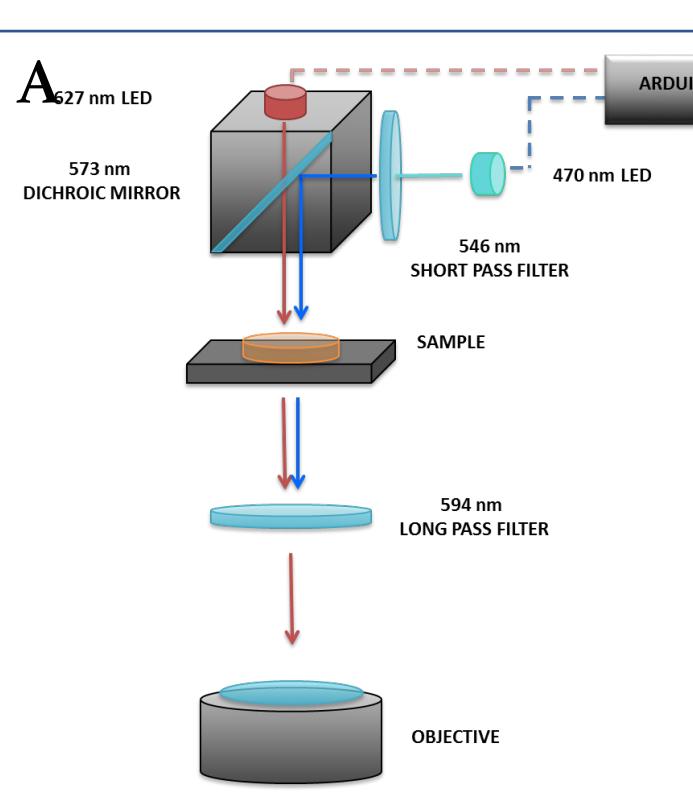


Figure 8: Optical control and evaluation of cardiac microtissue. (A) Schematic of custom optical stimulation platform. (B) A cardiac microtissue. (C) Trace of cardiac microtissue contractions (black line) with respect to light stimulation (blue square trace/green line). First instance of non-capture is denoted with by the solid yellow block.

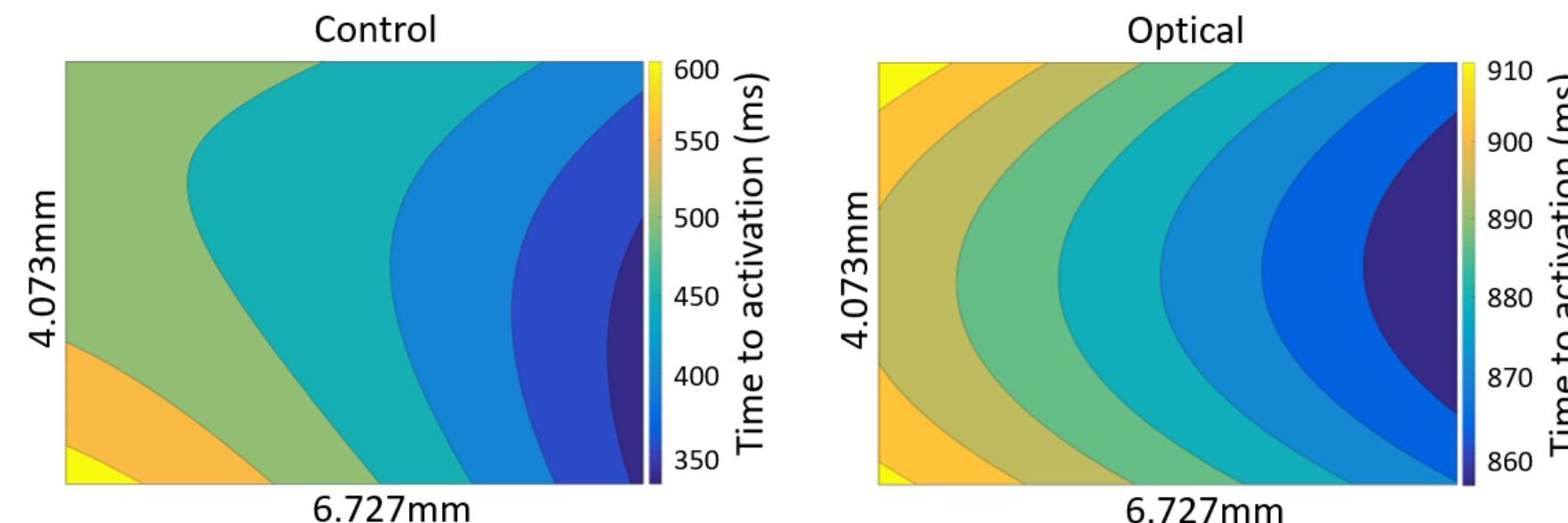


Figure 9: Activation Map. Example activation map of cardiac microtissue. These can be analyzed to determine the conduction velocity of microtissues, which can be correlated to the maturation of gap junction proteins

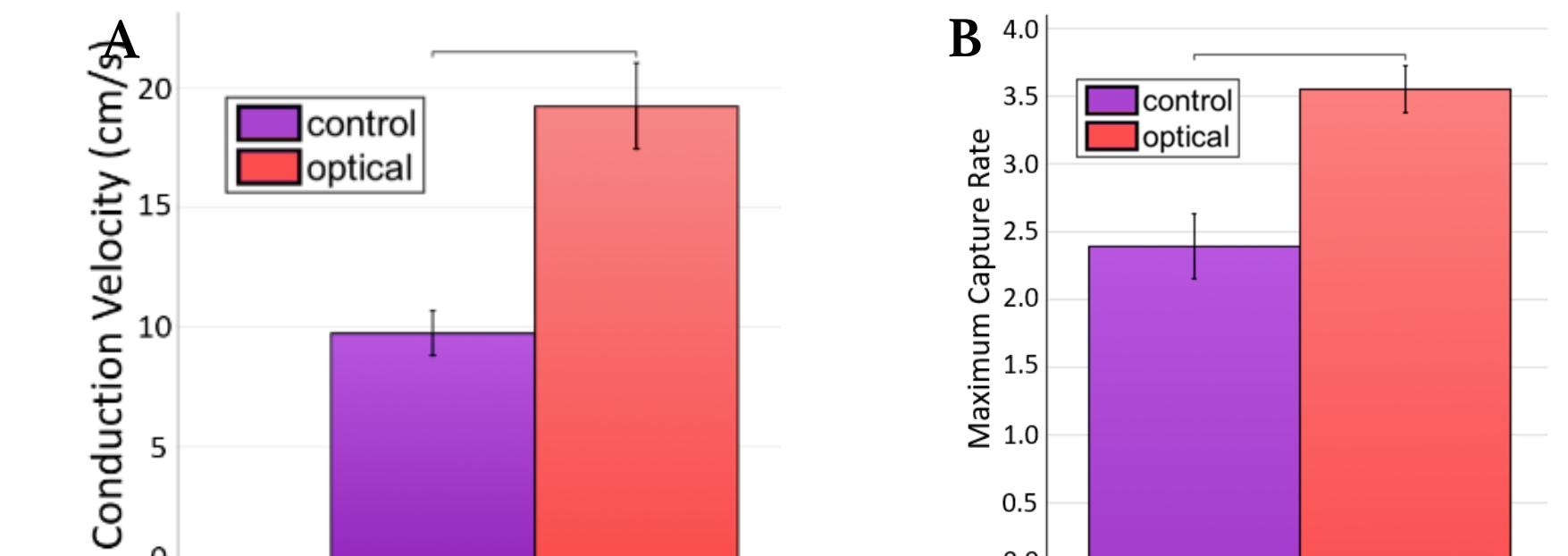


Figure 10: Maximum Capture Rate (A) Conduction velocity was increased in optically stimulated tissues, as revealed by optical mapping during localized pacing. (average \pm 95% CI, $n>8$, unpaired two-sample t-test, $p < 0.05$). (B) MCR was increased in the optically stimulated tissues

Discussion and Future Directions

In vitro optogenetics offers a novel approach to stem cell and tissue engineering research. Our results show that optical pacing of transgenic hiPSC-CMs can be used to produce functional changes linked to cardiac maturation. An increase in conduction velocity, along with an increase in maximum capture rate are both promising and convincing pointers to more mature cardiac microtissues through better Ca²⁺ handling. Immunochemistry staining as well as qRT-PCR have not yet been conducted on these tissues and are currently being analyzed for ultrastructural and transcriptional changes.

In comparison to electrical pacing, membrane depolarization using optical pacing is neither associated with the presence of electrical fields, nor with direct charge injection. Further studies will directly compare these two stimulation methods to better flesh out potential differences in gene transcription, protein expression, sarcomeric alignment, and cardiomyocyte function. Additional assays to analyze the metabolic activity of these tissues will be conducted.

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