Application Note

Analysis of axoCells™ Atrial and Ventricular Cardiomyocytes Electrophysiology Using CorePlate™ Enabled HD-MEA System

Last update: 02 Jun 25
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Abstract

A 7-day thaw-to-data protocol comparing axoCells Atrial (ax2518) and Ventricular (ax2508) iPSC-derived Cardiomyocytes using 3Brain's CorePlate™ enabled HD-MEA system.

Key findings:

- HD-MEA allows visualization of activity propagation across the plate.
- BrainWave software accurately calculates key electrophysiological parameters such as field potential duration (FPD) and inter-beat interval.
- Atrial cardiomyocytes have a shorter FPD than ventricular cardiomyocytes.
- Atrial cardiomyocytes give the expected pharmacological response to isoprotenerol including increased frequency and shorter QT interval.

Introduction

Understanding the functional differences between atrial and ventricular cardiomyocytes is crucial for advancing cardiovascular research, particularly in the context of arrhythmias such as atrial fibrillation (AF). Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) provide an invaluable tool for modelling human heart tissue, enabling detailed studies of electrophysiological properties across different cardiac regions. This application note highlights the use of high-density multielectrode arrays (HD-MEA) for characterizing atrial and ventricular iPSC-derived cardiomyocytes. HD-MEA offers exceptional sensitivity for capturing real-time electrophysiological data, making it ideal for studying re-entrant arrhythmias and the propagation of electrical waveforms. By leveraging this technology, researchers can better understand the distinct behaviours of atrial and ventricular cardiomyocytes, creating a powerful platform for investigating the mechanisms of AF and other arrhythmias *in vitro*.

Materials and Methods

Cell plating

The CorePlate™ microelectrode array was coated with 1 µg/ml human fibronectin overnight as per manufacturer's instructions. iPSC-derived cardiomyocytes were thawed in Cardiomyocyte Maintenance Medium supplemented with 10% FBS and 10 µM Y-27632 and seeded at a density of 200,000 cells/cm^2. The following day media was replaced with fresh Cardiomyocyte Maintenance Medium. Cells were subsequently fed every 2 days.

HD-MEA parameters

The dataset was recorded using 3Brain's BioCAM DupleX system with a CorePlate™ 1W 38/60 microelectrode array. The CorePlate™ 1W 38/60 features 4,096 recording electrodes arranged within a 3.8 ×3.8 mm² area. Each electrode is bidirectional, allowing both for signal recordings and for electrically pacing the sample. Each microelectrode measures 21 ×21 µm² with a 60 µm pitch. Data were sampled at

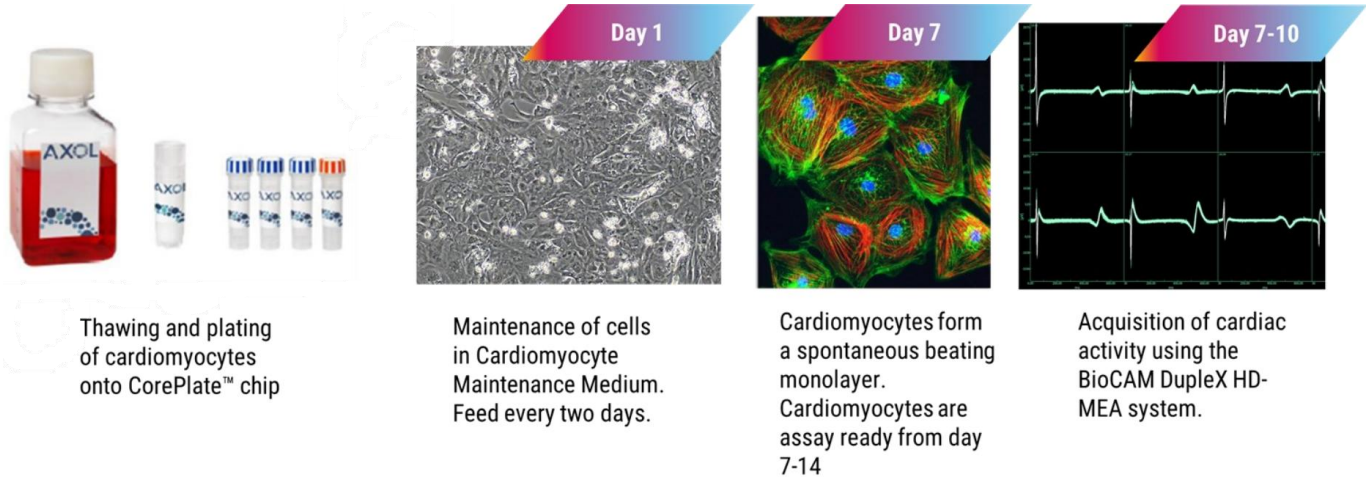

Figure 1. Experimental timeline

20 kHz and acquired using a 5 Hz high-pass filter.

Results

At Day 7 post-thawing, both atrial and ventricular hiPSC-derived cardiomyocytes exhibited robust beating activity when cultured on 3Brain's HD-MEA platform (Fig. 2a). Atrial cardiomyocytes displayed a higher beating frequency compared to their ventricular counterparts, consistent with their physiological roles *in vivo*. This increased activity was qualitatively evident when examining the raw traces and quantitatively confirmed by the beating calculations performed by BrainWave5 software (Fig. 2c). This behaviour aligns with their physiological roles, as atrial cells typically operate at a higher intrinsic pacing rate.

Table 1.Equipment List

Item	Vendor	Catalogue Number
CorePlate™	3Brain	CorePlate™ 24W 16/50 CorePlate™ 6W 38/60 CorePlate™ 1W 38/60
3Brain MEA Platform	3Brain	HyperCAM Delta HyperCAM Alpha BioCAM Duplex
Pipette Kit, 0.2µL – 1mL	Multiple Vendors	

Table 2.Software List

Item	Vendor	Catalogue Number
BrainWave Software	3Brain	

Table 3.Consumables List

Item	Vendor	Catalogue Number
axoCells™ Atrial Cardiomyocytes	Axol Bioscience	ax2518
axoCells™ Ventricular Cardiomyocytes	Axol Bioscience	ax2508
Cardiomyocyte Maintenance Medium	Axol Bioscience	ax2530
Human Fibronectin	Axol Bioscience	ax0050

Interestingly, we observed a distinct difference in the T-peak amplitude, which was higher in ventricular cardiomyocytes (Fig. 2c). This suggests early divergence in repolarization dynamics, potentially reflecting cell-type-specific electrophysiological properties even at this stage.

Effect of Isoproterenol on Atrial Cardiomyocytes at DIV 9

To investigate the responsiveness of atrial cardiomyocytes to adrenergic stimulation, we applied 10 µM isoproterenol to the cells at DIV 9. This well-known β-adrenergic agonist is commonly used to modulate the electrophysiological properties of cardiac cells, and its effect on atrial cardiomyocytes provides insights into their functional maturation.

The application of isoproterenol elicited significant changes in the electrophysiological activity of the atrial cells. Analysis of the waveforms using BrainWave5 software revealed that the T-peak became noticeably closer to the QRS complex in the presence of isoproterenol. This shift in timing is indicative of a faster repolarization process, reflecting the influence of β-adrenergic stimulation on the electrical dynamics of atrial cardiomyocytes (Fig. 3).

Consistent with this observation, the cardiac field potential rate increased markedly, accompanied by a decrease in the inter-cardiac field potential interval, suggesting a more rapid beating rate. Notably, both the R–S peak and T-peak amplitudes increased, further highlighting the augmented electrical activity within the atrial cells (Fig. 4 a-e).

These observations are consistent with the expected physiological response to β-adrenergic stimulation, which typically

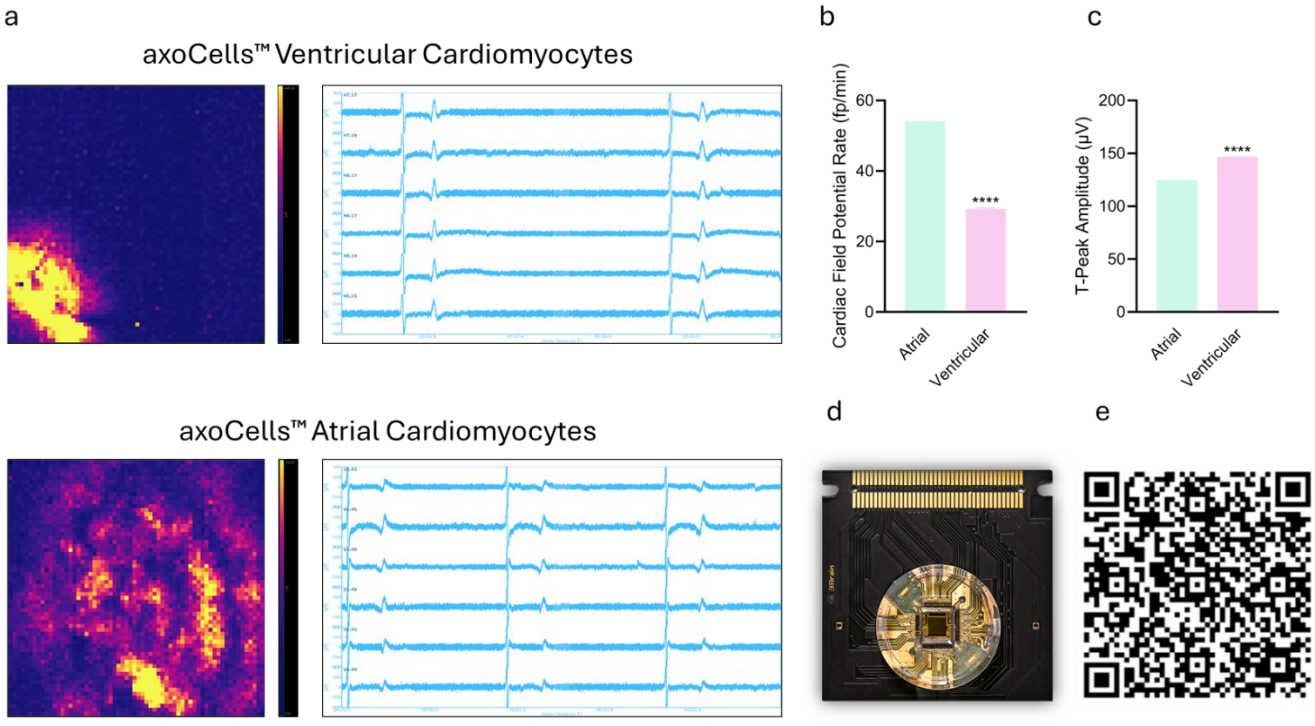

Figure 2. (a) Activity Map (left) and Raw Signal Map (right) of (top) axoCells Ventricular Cardiomyocytes & (bottom) axoCells Atrial Cardiomyocytes. (b) Beating Rate and (c) T-Peak amplitude comparison between Atrial and Ventricular Cardiomyocytes. (d) CorePlate™ 1W 38/60. (e) Scan the QR code to watch a video of the Ventricular Cardiomyocyte activity.

enhances the contractile and electrical activity of cardiac tissue *in vivo*.

HD-MEAs also allow for a detailed study of signal propagation. In this regard, treatment with isoproterenol resulted in changes to the activity propagation map, with the propagation duration increasing from approximately 40 ms under control conditions to 45 ms following treatment with 10 µM isoproterenol. This change was reflected in a corresponding increase in network burst duration. While this may seem counterintuitive, it is consistent with the electrophysiological profile of not-fully mature cardiomyocytes, where enhanced calcium influx and underdeveloped repolarization mechanisms can lead to prolonged depolarization (Fig. 5).

Discussion

The 7-day thaw-to-assay protocol for axoCells iPSC-derived cardiomyocytes enables rapid and reproducible maturation into functional atrial and ventricular cell populations. This simplicity makes it ideal for in vitro studies of cardiac electrophysiology. The CorePlate™ enabled HD-MEA systems provide real-time, high-resolution recordings, allowing for clear visualization of electrical activity propagation and the study of re-entrant arrhythmias. The ability to capture wavefront dynamics across

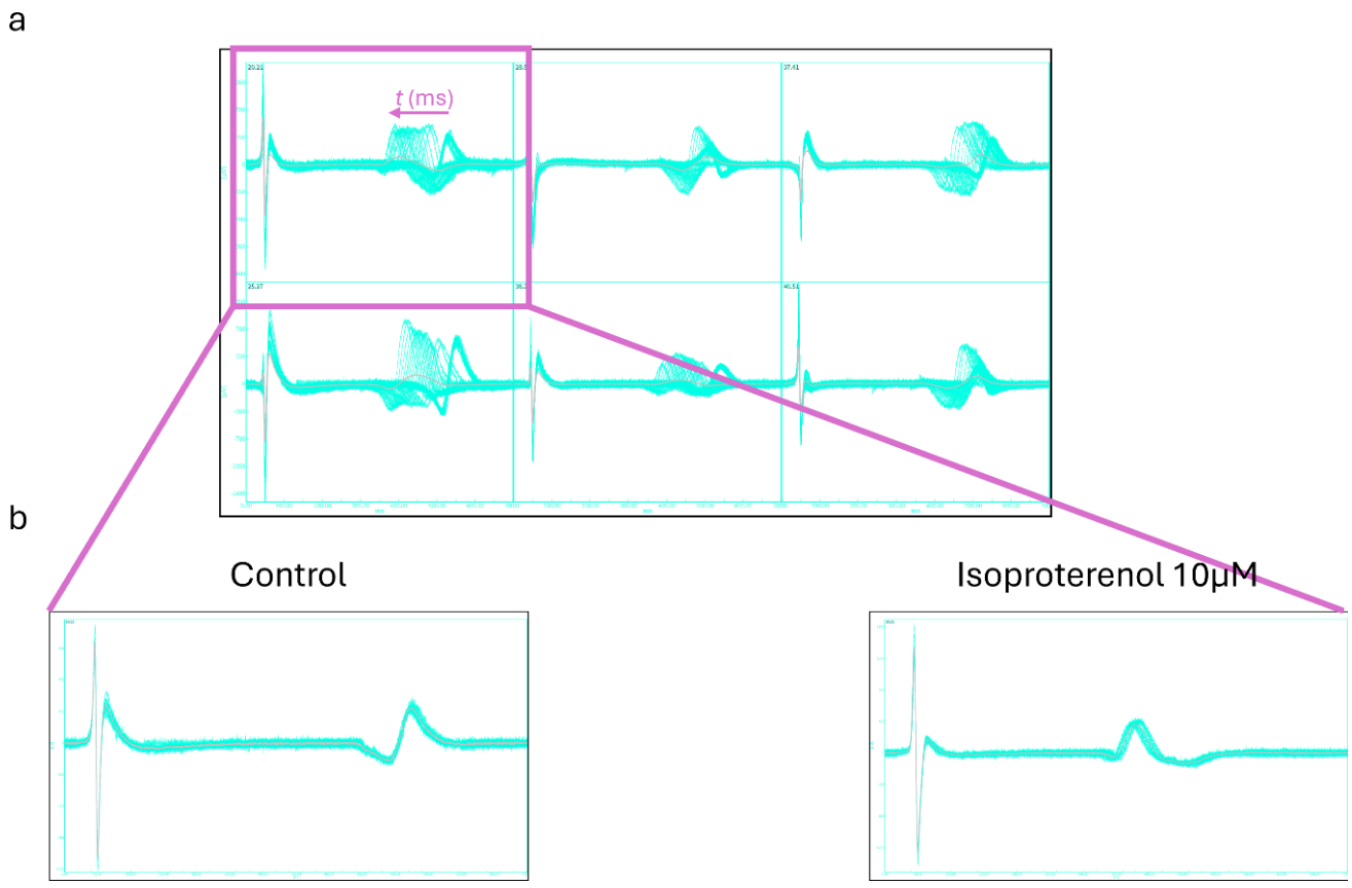

Figure 3. (a) Event waveforms recorded during a 3-minute exposure of atrial cardiomyocytes to 10 µM isoproterenol. The T-peak is visibly closer to the QRS complex following treatment. (b) Zoomed-in view of selected channels showing the average waveform calculated over 30 seconds before treatment and the final 30 seconds after treatment.

cell monolayers is particularly valuable for investigating arrhythmogenesis.

These findings underscore the distinct phenotypic and functional properties of the two cell types and confirm the sensitivity and reliability of the HD-MEA technology in detecting and differentiating subtle electrophysiological signatures. Together, these results highlight the capability of Axol's hiPSC-derived cardiomyocytes to model chamber-specific characteristics and the value of 3Brain's HD-MEA system in capturing detailed and reproducible functional readouts at the single-cell and network level.

Notably, atrial cardiomyocytes exhibit shorter field potential durations (FPD) compared to ventricular cells, highlighting important chamber-specific differences. These distinctions

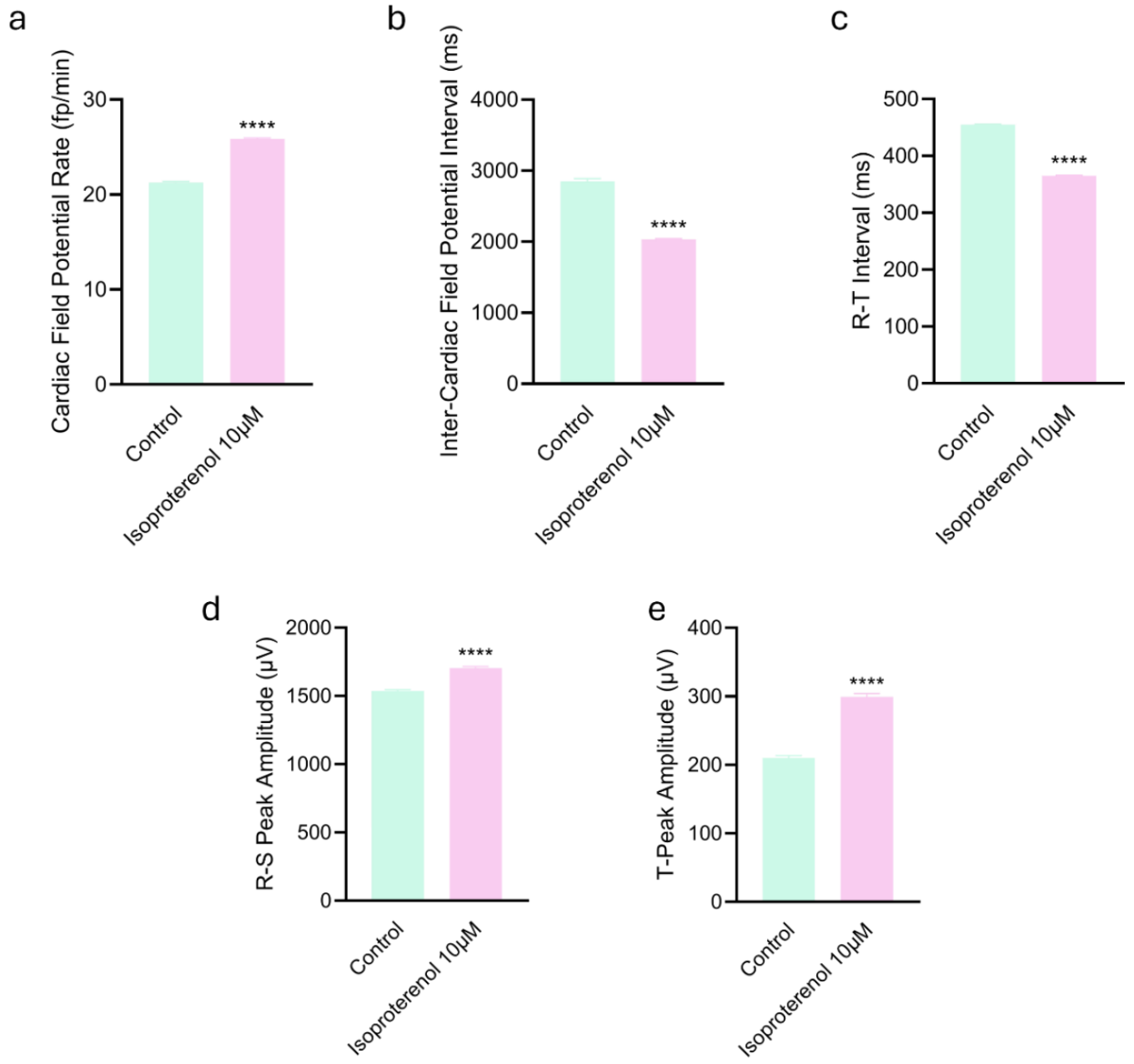

Figure 4. (a) Cardiac Field Potential Rate, (b) Inter-Cardiac Field Potential Interval, (c) R–T Interval, (d) R–S Peak Amplitude, and (e) T-Peak Amplitude of axoCells Atrial Cardiomyocytes in the absence and presence of 10 µM isoproterenol.

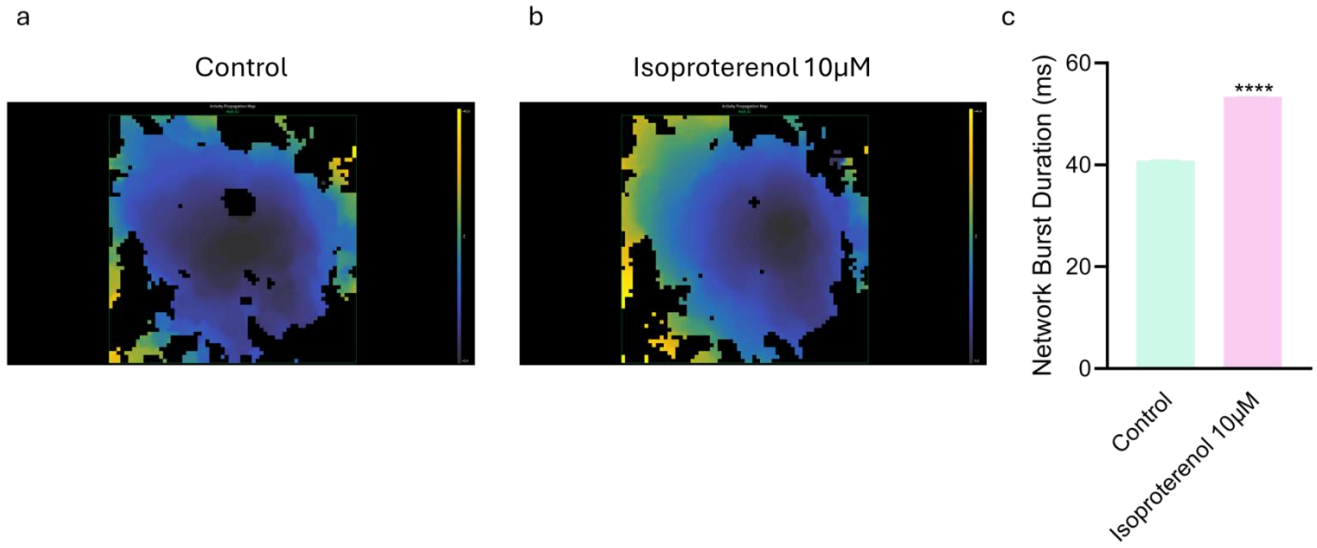

Figure 5. Propagation Map of axoCells Cardiomyocytes under (a) control conditions, and with (b) Isoproterenol 10 µM. (c) Network Burst Duration of axoCells Atrial Cardiomyocytes before and after the treatment with isoproterenol.

are crucial for drug screening and disease modelling, as therapeutic responses may vary between atrial and ventricular tissues. This platform offers significant potential for understanding the electrophysiological basis of arrhythmias, advancing both disease research and drug development.

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