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Photovoltaic Stimulation Induces Overdrive Suppression in Embryonic Chicken Cardiomyocytes

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Abstract: In this study, we employed calcium imaging to investigate the dynamics of intracellular calcium levels in embryonic chicken cardiomyocytes upon extracellular, optoelectronic stimulation. A photovoltaic layer of donor-acceptor pigments on a flexible PET substrate was used as a wireless stimulation electrode. Our findings revealed a distinct change in their spontaneous activity pattern in cardiac cells following asynchronous light stimulation. A short pause in cellular activity, indicative of overdrive suppression, was observed in recordings from several different cells. The pause in activity signifies a transient refractory period induced by stimulation of the photovoltaic device with red light. These findings suggest that photovoltaic electrodes can be used to effectively modulate the electrical activity of cardiac cells in a wireless, non-pharmacological manner. This opens new avenues for non-invasive and precise light-modulated control of cellular electrophysiology as well as potential therapeutic applications for cardiac rhythm disorders.

Keywords: Extracellular Stimulation, Arrhythmia, Optoelectronics, OEPC, Photocapacitor

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1 Introduction

Specialized cardiomyocytes, namely pacemaker cells, exhibit spontaneous electrical activity known as pacemaker activity. These spontaneous depolarizations and repolarizations coordinate contraction and relaxation of the cardiac muscle. Abnormalities in this pacemaker activity can lead to severe cardiac pathologies, such as arrhythmias and heart failure [1]. Therefore, a comprehensive understanding of the mechanisms underlying pacemaker activity is crucial for devising effective therapeutic strategies. Recent advances in electrical imaging and calcium imaging opened new possibilities to observe pacemaker activity and network formation *in vitro* while optoelectronic electrodes pave the way for effective wireless stimulation [2,3,4].

The objective of this study is to explore the manipulation of spontaneous activity in chicken embryo cardiomyocytes using optoelectronic stimulation, focusing specifically on photovoltaic extracellular stimulation techniques and the induction of overdrive suppression. Overdrive suppression refers to a transient pause in cellular activity observed after a burst of stimulation events. This mechanism helps to maintain the stability and synchronization of electrical signals in cardiac cells, contributing to the proper functionality of the heart muscle tissue [5].

By taking advantage of recent and ongoing advancements in the fabrication of versatile optoelectronic devices, we aim to optimize electrode geometries and their electrical properties to control the electrical activity of cardiomyocytes with high precision and minimal invasiveness. The intensity and duration of light stimulation can be modulated to explore the effects of different optoelectronic stimulation protocols on the frequency and timing of spontaneous activity. Additionally, we plan to investigate the mechanisms of photovoltaic extracellular stimulation and its impact on cellular signalling pathways in cardiomyocytes to improve the understanding of existing cell-electrode models [6,7].

2 Materials and Methods

2.1 Photovoltaic Electrode

The photovoltaic electrodes were fabricated as described in a previous study [8]. The photoconductor nanocrystalline (PN) layer, consisting of 13 mm diameter and 30 nm thickness each of H_2Pc as electron donor and PTCDI as electron acceptor, was vapour-deposited on polyterephthalate (PET) foils of 25 mm diameter, which were pre-coated with indium tin oxide (ITO) [8]. In contrast to the formerly described production, Poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) was spin-coated on the entire surface of the PN and ITO layers to improve the device capacitance. The photovoltaic electrodes are non-toxic and can generate a transient surface voltage of up to 330 mV when illuminated by a 10 W LED with an emission wavelength of 660 nm (Roschwege GmbH) making it a suitable device to test the effects of light-induced extracellular stimulation in a microscopy imaging setup. The devices were sterilized by rinsing with 70 % ethanol, followed by 15 min exposure to UV light [3,8].

2.2 Cell Isolation and Culture

Ventricular myocytes were isolated from the hearts of 7-day old chicken embryos as described previously [9]. The isolated cells were suspended in cell culture medium (M199 supplemented with 4% fetal calf serum, 2% horse serum, and 0.7 mM glutamine, pH 7.4) to yield a density of 5×10^5 cells/ml. The cells were seeded on the devices (Figure 1) and glass coverslips in 35-mm cell culture dishes and incubated at 37°C, 5% CO_2. Experiments were performed 24–36 h later.

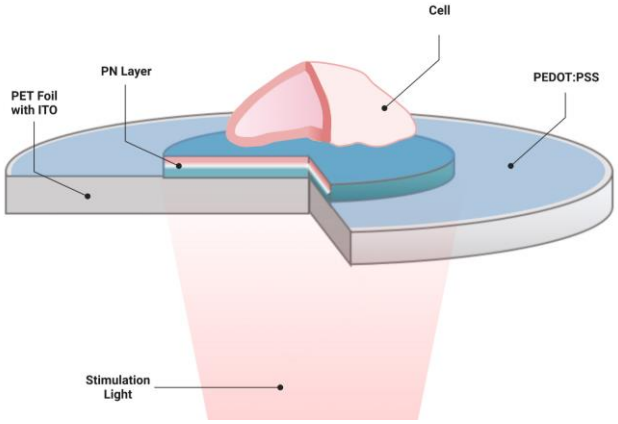

Figure 1: Schematic of the structure and individual components of a photovoltaic electrode with an enlarged cell on its surface [10].

2.3 Intracellular Calcium Imaging

Changes in intracellular Ca^{2+} were monitored using Fura-2 ratiometric imaging with slight modifications to a previously used protocol [11]. The cells were loaded with 1 µM Fura-2 AM dye and 0.02 % Pluronic F-127 (Invitrogen) in Tyrode's solution at 20-22 °C for 40 min in a dark environment, washed twice with fresh Tyrode's solution and kept for 15 min to allow de-esterification of Fura-2 AM. The device with Fura-2-loaded cells was mounted on an upright microscope (Olympus BX51WI) with a 20x/0.5 water objective. The cells were excited by high-power LEDs of an Omicron LEDHUB (Omicron-Laserage Laserprodukte GmbH), with wavelengths of 340 nm and 380 nm every 10 ms. The fluorescent images were captured in VisiView v5.0 software (Visitron Systems) using a 510/80 nm emission filter (Chroma) with an ORCA-Flash 4.0 v2 CMOS camera (Hamamatsu). The 340/380 fluorescence ratio following background subtraction was used as an index of intracellular Ca^{2+} levels.

2.4 Stimulation Protocol

The photovoltaic electrode was activated using a 10 W high-power LED pulse with a beam angle of 120° and a wavelength of 660 nm at 6.2 mm distance, illuminating the active layer fully from below the microscope stage, resulting in a power density of approximately 10 mW/mm². The device was illuminated by light pulses with a length of 100 ms, every 2 s. The resulting stimulation frequency of 0.5 Hz was higher than the frequency of spontaneous activity of about 0.3 Hz.

3 Results

Cells on either side of the PN-ITO boundary were selected for monitoring spontaneous changes in fluorescence intensity. Selected cells for the recordings are highlighted in red in Figure 2. Controls on glass showed no activation on illumination, excluding the possibility of stimulation of light-triggered ion channels. The intensity ratio curve was filtered with a moving average filter of 10 data points to attenuate signal spikes caused by the light stimulation (Figure 3).

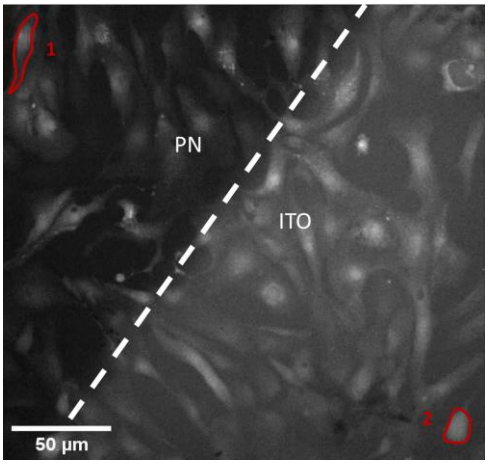

Figure 2: Representative 340 nm fluorescent image of Fura-2-loaded embryonic chicken cardiomyocytes on a photovoltaic electrode. The two selected cells are highlighted in red and the boundary between the PN and ITO layers is shown as a white dashed line.

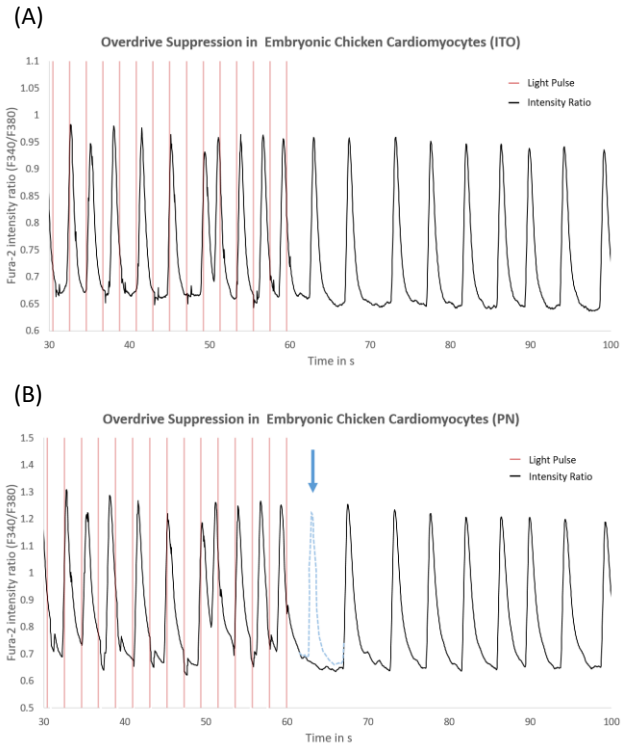

Figure 3: Traces of intracellular Ca^{2+}-sensitive Fura-2 intensity ratio, representing spontaneous Ca^{2+}-transients in the selected cell 2 on the ITO (A) and cell 1 on the PN (B) (highlighted in Figure 2), during and after light stimulation. Stimulation events are marked as red lines. The expected activity that is missing in cell 2 (B) after stimulation, is highlighted in blue.

4 Discussion and Conclusion

Our findings from this study on cardiomyocytes cultured on a photovoltaic electrode suggest that it is possible to stimulate cell layers externally and effectively suppress their spontaneous activity through overdrive suppression. In Figure 3, a distinct pause in spontaneous activity after stimulation can be observed in cell 1 (B) but no pause in cell 2 (A). A slight change of spontaneous activity frequency after stimulation is observed in both exemplary cells. The presence of synchronous behaviour suggests the emergence of conduction pathways in the early stages, prior to the formation of a complete monolayer. However, the fact that the pause occurred only on the active layer indicates that the cathodic stimulation is more effective at the PN layer than the anodic stimulation on the ITO back electrode. Thus, the change in activity of the ion channels is more prominent and exceeds a certain threshold to induce overdrive suppression.

One of the biggest challenges is the estimation of the capacitive stimulation voltage on both sides due to its transient behaviour, which highly depends on the distance between cardiomyocytes and electrode surface. A two domain model of the cell-electrode coupling utilized in previous studies provides a valuable platform for investigating the effects of light stimulation on cardiac cells, offering insights into the broader field of extracellular capacitive stimulation and optoelectronic electrodes as implants [6,7,8]. The distance between the cells and the surface of the electrode after 24 hours was not examined in this study. Additional experiments are required to precisely characterize the signal transduction at the cell-electrode interface and get a comprehensive understanding of the limitations of this process.

However, the findings of this observation could help in understanding the effects of local overdrive suppression in cardiac monolayers and allow precise spatial manipulation of spontaneous activity in single cells. Future research directions may investigate the specific signalling pathways involved in the observed overdrive suppression response and optimize the parameters of photovoltaic device stimulation to achieve more efficient stimulation. For example, a laser could be used to generate spatially precise charges to stimulate single cells.

In conclusion, this study demonstrates that photovoltaic devices can effectively induce overdrive suppression in a layer of adhered embryonic chicken cardiomyocytes through light stimulation with protocols previously applied in literature [5]. A short pause observed following a burst of light stimuli signifies the modulation of cellular electrical activity, providing a foundation for further investigations into the

potential therapeutic applications of light-based interventions in cardiac electrophysiology.

Author Statement

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