

ELECTROPHYSIOLOGICAL SORTING OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN A MICROFLUIDIC PLATFORM

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One of the major challenges in translating stem cell biology into tissue replacement therapy is the establishment of effective separation methods which specifically isolate differentiated cells and exclude cells which may hamper graft performance or lead to teratoma formation.¹ Conventional separation techniques for stem cells require exogenous labeling or genetic modification, neither of which is ideal for clinical applications. However, many of the cell populations relevant for therapy are electrically-excitable (e.g. cardiomyocytes, neurons, skeletal muscle, and pancreatic beta cells), meaning they produce transmembrane ion currents in response to electrical stimulation. Microelectrode recordings of these signals can provide rich phenotypic information non-invasively and without labeling.^{2,3}

We propose a new cell sorting methodology based on electrophysiological response to stimulus. We have engineered a continuous-flow microfluidic platform which currently traps colonies of induced-pluripotent stem cell-derived cardiomyocytes, electrically stimulates them, and records their resulting extracellular field potential signals. Microelectrode field potential signals are notoriously weak and field stimulation produces dramatic artifacts in the recording which can obscure these signals. Our system addresses these problems in two ways. First, because the cells are confined in a microchannel, the ohmic voltage drop in the vicinity of the cells increases because the current is confined to the cross-section of the channel. Second, we differentially sense the field potential between a pair of electrodes which are equidistant between two large stimulus electrodes. This means that the stimulus artifact is common-mode and thus significantly suppressed. We also employ a blanking circuit to further eliminate stimulus artifact.

Our current efforts are aimed at using computational analysis of these field potential signals for ultimately providing a highly specific, label-free contrast mechanism for purification of individual cardiomyocytes at the single cell level. Our long-term goal is to apply this system toward cell purification for cardiac tissue engineering. We believe our label-free cell sorting method can substantially reduce the risk of teratoma formation and that electrophysiological homogeneity of implanted cardiomyocytes will lead to improved graft viability, improved electromechanical coupling within the host myocardium, and reduced incidence of arrhythmias when compared to other label-free sorting techniques.

Word Count: 342

REFERENCES:

1. Kirouac, D. & Zandstra, P. The Systematic Production of Cells for Cell Therapies. *Cell Stem Cell* **3**, 369-381 (2008).
2. Reppel, M. et al. The electrocardiogram of human embryonic stem cell-derived cardiomyocytes. *Journal of Electrocardiology* **38**, 166-170 (2005).
3. Banach, K., Halbach, M.D., Hu, P., Hescheler, J. & Egert, U. Development of electrical activity in cardiac myocyte aggregates derived from mouse embryonic stem cells. *Am J Physiol Heart Circ Physiol* **284**, H2114-2123 (2003).

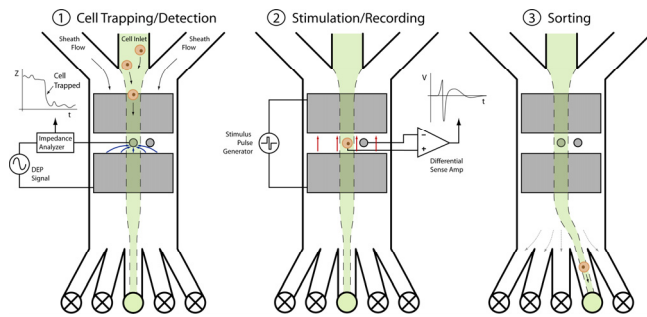


Figure 1. Conceptual diagram of microfluidic electrophysiological cell sorter. (1) Cells are hydrodynamically focused over detection electrodes and trapped there via dielectrophoresis. The presence of the cells is indicated by a drop in impedance. (2) Once trapped, cells are stimulated and the differential signal between the two detection electrodes is recorded. Because the detection electrodes are equidistant between the stimulus electrodes, the stimulus artifact is suppressed. (3) The field potential signal is analyzed and the cells are sorted accordingly.

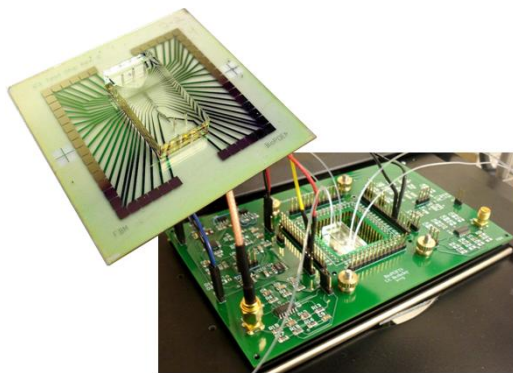


Figure 3. Top: Microfluidic device consisting of a PDMS microchannel on glass with Pt electrodes. **Bottom: Custom instrumentation amplifier PCB.** The amplifier is noise matched to the microelectrodes (noise factor = 1.02).

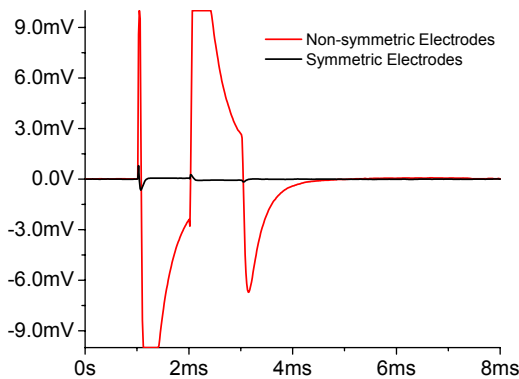


Figure 5. Symmetric differential electrode geometry suppresses stimulus artifact. Because the sensing electrodes are equidistant between the two stimulus electrodes, the stimulus artifact is common-mode and thus rejected by the instrumentation amplifier. Here, a 50mV stimulus pulse was applied between the stimulus electrodes and the response is measured between the two differential sensing electrodes (black) versus between one sensing electrode and a distant reference electrode (red).

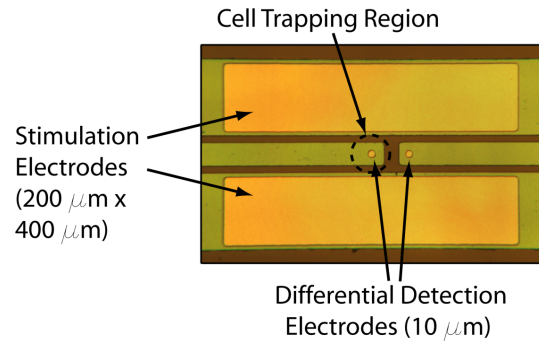


Figure 2. Stimulation and recording electrodes. Large stimulus electrodes allow high charge injection during stimulation while a pair small differential sensing electrodes suppress stimulus artifact while detecting the field potential of the cells. Electrodes are Pt with a Si_3N_4 passivation layer defining the electrode boundaries.

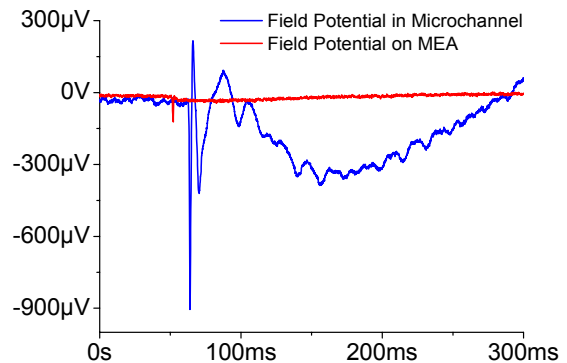


Figure 4. Microchannel confinement enhances field potential amplitude. Due to the spatial confinement of ionic current in the microchannel, cellular depolarization currents lead to substantially higher voltage drops as compared with conventional microelectrode arrays (MEAs). Here, we compare the spontaneous field potential from a cluster of iPSC-derived cardiomyocytes adhered on a commercial MEA (red) versus a non-adhered cluster in our device (blue).

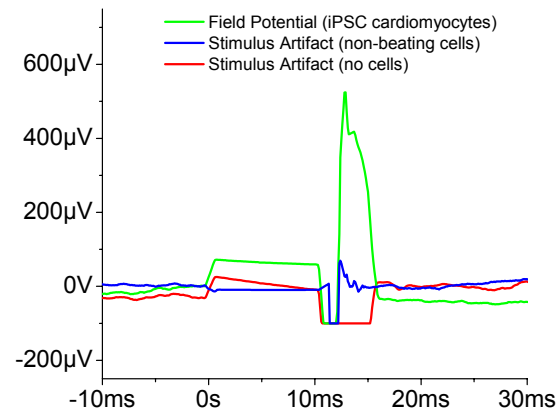


Figure 6. Field potential recorded from iPSC-derived cardiomyocyte cluster. The field potential signal from differentiated cardiomyocytes (green) can easily be distinguished from non-responsive cells (blue) and from the baseline stimulus artifact (red).