Simple Label-free Monitoring of Robust Human Pluripotent Stem Cell Differentiation to Cardiomyocytes with the Maestro MEA System and STEMdiff™ Cardiomyocyte Products

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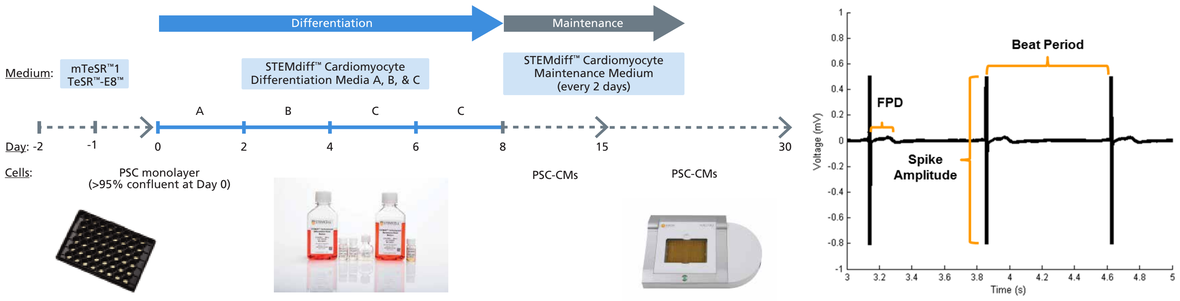
Introduction

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are an attractive model for studying heritable and drug-acquired arrhythmias. Although several studies have shown that independently produced populations of hPSC-CMs respond similarly to pro-arrhythmic drugs, variability has been observed. The ability to efficiently produce multiple patient-derived hPSC-CMs and easily track their excitability performance during differentiation is desirable. This would facilitate for example the determination of the effect of genetic diversity on drug-acquired arrhythmias between multiple different hPSC-CM lines. In pursuit of this objective we employed several novel technologies. The STEMdiff™ Cardiomyocyte Differentiation and Maintenance Media Kits were used to differentiate four different human pluripotent stem cell (hPSC) lines to cardiomyocytes and the transparent CytoView 48-well MEA plate and Maestro MEA system was used to measure their excitability.

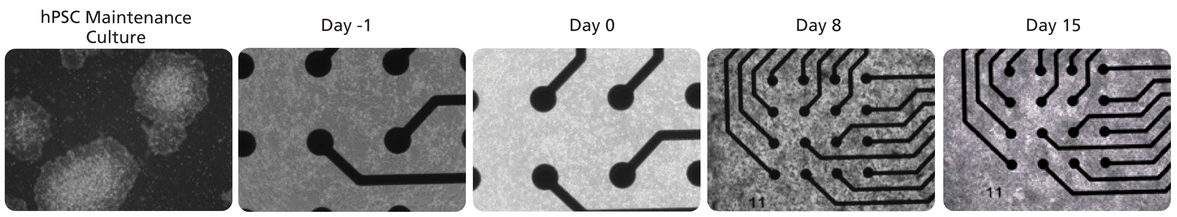
Methods



hPSCs (H1, H9, 1C, or F016) were seeded as single cells (~1x 10^5/well) with Y-27632 ROCK inhibitor in TeSR™-E8™ or mTeSR™1 medium on Corning® Matrigel®-coated wells of a 48-well CytoView™ plate (Day -2). The hPSCs were maintained with daily medium changes without Y-27632 (Day -1) until a confluent monolayer (>95%) was achieved (Day 0). The confluent monolayers of hPSCs were differentiated to cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit. At Day 0, the cells were treated with differentiation medium A. Day 2, medium A was removed and replaced with differentiation medium B. Day 4, medium B was removed and replaced with differentiation medium C. Day 6, medium C was removed and replaced with fresh differentiation medium C. Day 8, medium C was removed and replaced with maintenance medium. Maintenance medium was removed and replaced with fresh maintenance medium every 2 days until Day 15. At Day 15 cardiomyocytes were maintained with daily medium changes until Day 26 using the STEMdiff™ Cardiomyocyte Maintenance Kit. On Day 14, 5 minute MEA recordings were taken daily until Day 26 using the Maestro MEA system. On Day 26, 10 nM E-4031 was added for 10 minutes. MEA recordings were analyzed using the AxIS software (Axion BioSystems). The metrics analyzed were beat period, spike amplitude, and field potential duration (FPD).

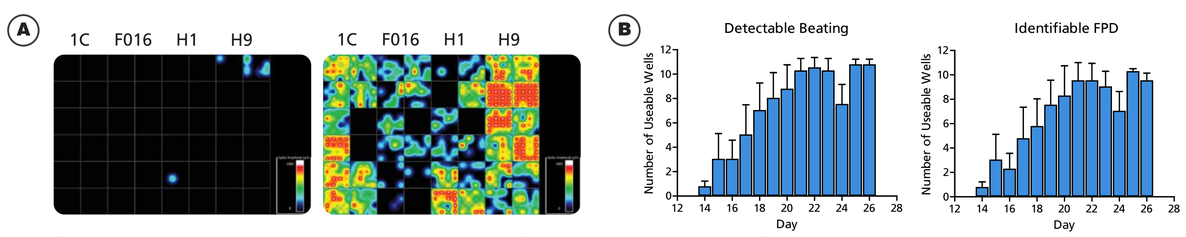
Results

Figure 1. Morphology Changes During hPSC Differentiation to Cardiomyocytes Using the STEMdiff™ Cardiomyocyte Differentiation Kit on the Cytoview™ MEA Plate



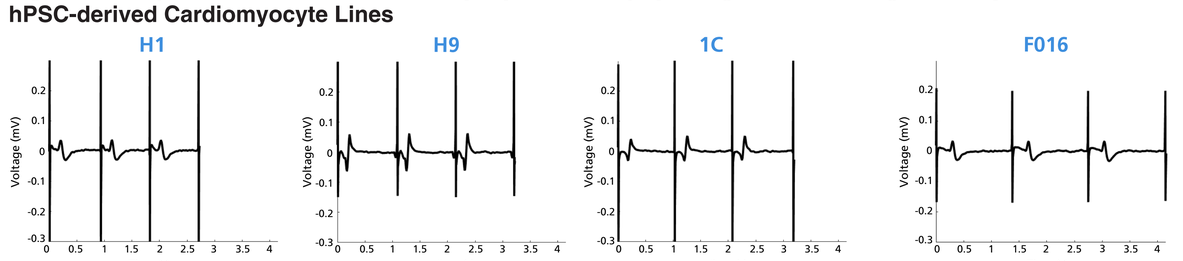
Morphology images of hPSCs in clumps, hPSC monolayer at day -1 and 0, and cardiomyocyte monolayers at day 8 and day 15.

Figure 2. Detection of Cardiomyocyte Excitable Parameters During hPSC Differentiation to Cardiomyocytes and Maintenance Using the STEMdiff™ Cardiomyocyte Maintenance Kit



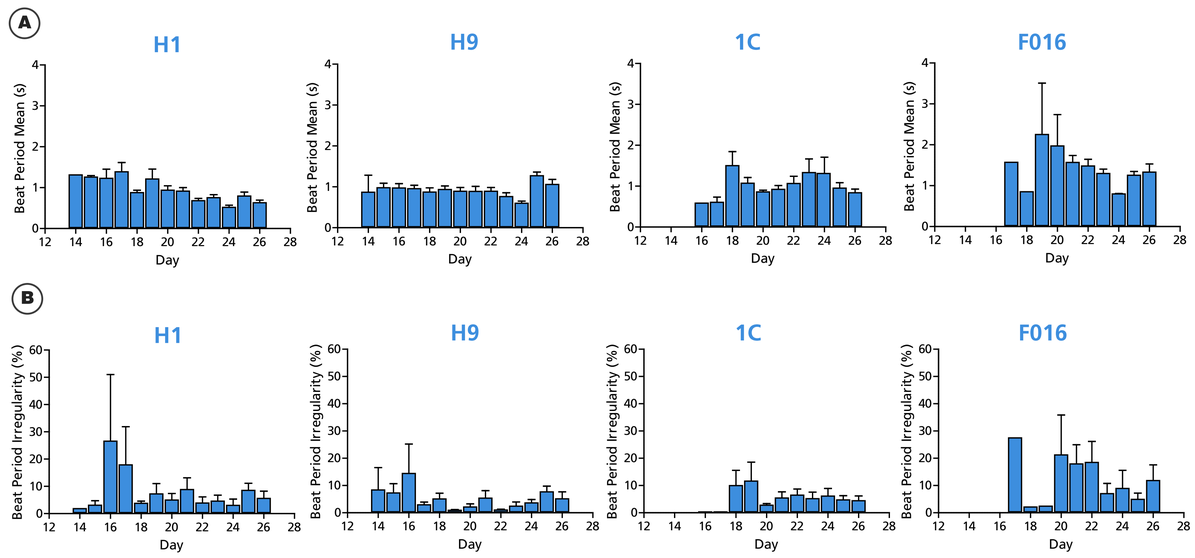
(A) Cardiomyocyte excitability heat map (48-well) derived from four hPSC lines measured at day 14 (minimal excitability) and day 25 (maximal excitability). **(B)** Bar graphs showing number of usable wells increases and plateaus at day 20 for both identifiable beating and FPD. Maximum number of useable wells is 12 in each condition. Plots show mean and SEM across four hPSC lines.

Figure 3. Representative MEA Cardiomyocyte Electrophysiological Recordings at Day 25 From Four hPSC-derived Cardiomyocyte Lines



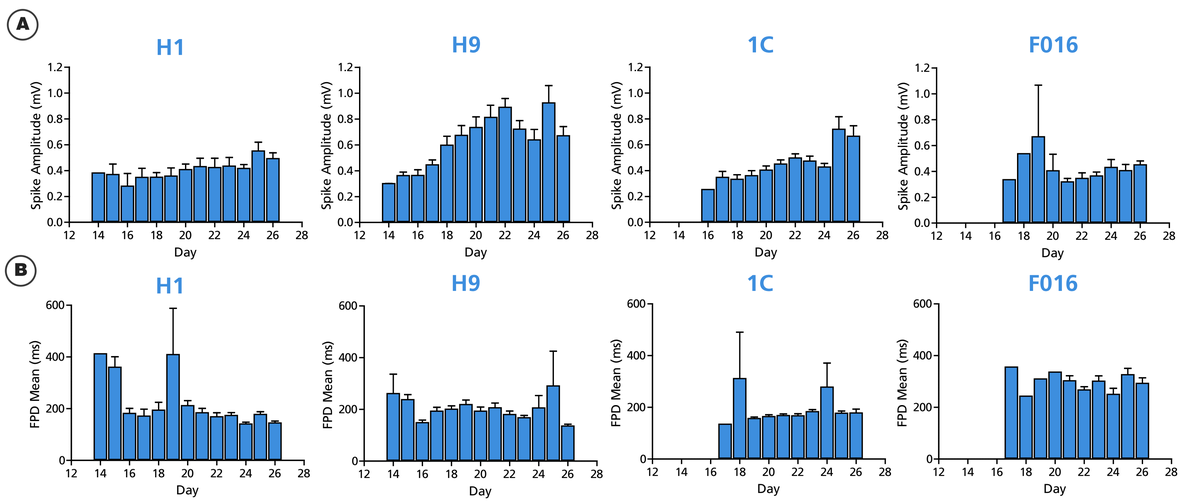
Cardiomyocytes derived from the four hPSC lines have a characteristic MEA electrophysiology profile. Large spike amplitude, small repolarization waveform, and stable beating frequency were measured.

Figure 4. Beating Metrics Are Comparable and Stable Across the Four PSC-Derived Cardiomyocyte Lines From Day 14 to 26



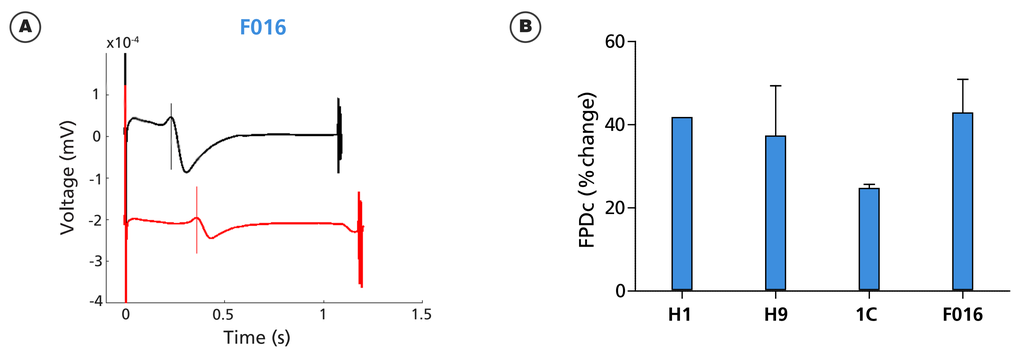
(A, B) Bar graphs showing that beat period mean is comparable and relatively stable and beat period irregularity is less than 10% after Day 20.

Figure 5. Large Spike Amplitudes and Reproducible Field Potential Duration (FPD) Means Are Observed Across the Four PSC-Derived Cardiomyocyte Lines From Day 14 to 26



(A) Bar graphs show that the spike amplitude remains large and discernable (> 0.3 mV) from Day 14 to Day 26. **(B)** bar graphs of FPD are relatively stable and comparable after Day 20.

Figure 6. Comparable FPD Prolongation With hERG Blocker (E-4031) Across the Four PSC-Derived Cardiomyocyte Lines



(A) Representative MEA electrophysiological recording before (black) and after (red) applying 10 nM E-4031 (10 min) to hiPSC-F016-derived cardiomyocytes on Day 26. The FPD is prolonged with 10 nM E-4031. **(B)** Bar graph showing that the FPDc is 20 - 40% longer with 10 nM E-4031 compared to before E-4031 in all four hPSC-derived cardiomyocyte lines.

Conclusions

Features of the STEMdiff™ Cardiomyocyte Kits:

- All four hPSC lines reliably differentiated to form beating monolayers of cardiomyocytes by day 15 with stable excitability profiles observed by day 21
- 83 - 100% of the wells contain regular beating cardiomyocytes with very consistent excitability profiles between replicate wells by day 25
- Variability across hPSC-CM lines was small with beat periods of 0.795-1.27 sec, FPDs of 177-326 ms, and less than 10% beat period irregularity
- Cardiomyocytes have a comparable response to a hERG potassium channel blocker, E-4031
- STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits combined the Maestro MEA System support medium to high throughput drug discovery and toxicology screening with multiple genetically diverse hPSC-derived cardiomyocytes in a label-free manner