Efficient Production and Processing of Cardiomyocytes from Human Pluripotent Stem Cells Using STEMdiff™ Cardiomyocyte Products

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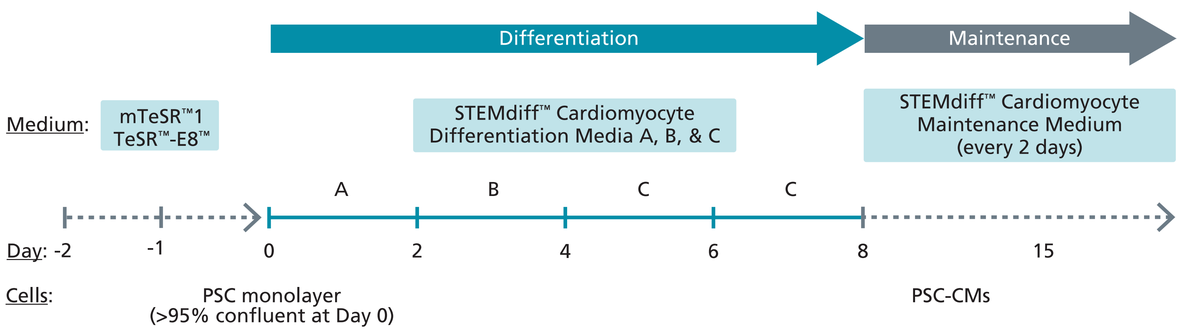
Introduction

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are used for disease modelling, drug discovery, and toxicology screening. However, the efficiency of producing hPSC-CMs is variable and, once established in culture, processing of hPSC-CMs for downstream assays is cumbersome. To overcome these limitations, we have developed a complete workflow comprising several novel products: 1) STEMdiff™ Cardiomyocyte Differentiation Kit, 2) STEMdiff™ Cardiomyocyte Maintenance Kit, 3) STEMdiff™ Cardiomyocyte Dissociation Kit, 4) STEMdiff™ Cardiomyocyte Freezing Medium, and 5) STEMdiff™ Cardiomyocyte Support Medium.

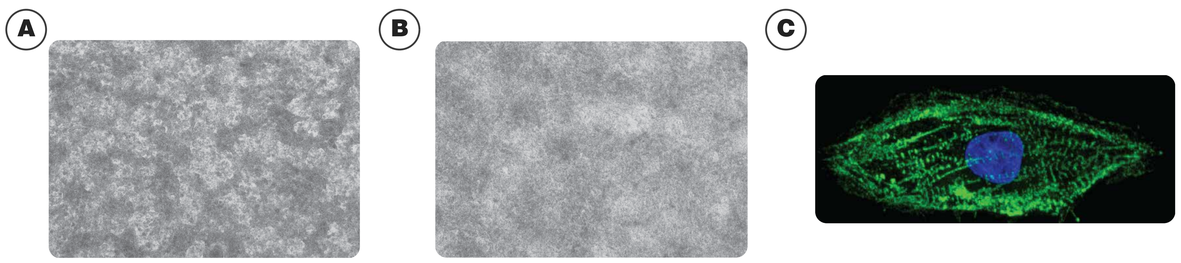
Methods



hPSCs were seeded as single cells (~3 x 10^5/well) with Y-27632 ROCK inhibitor in TeSR™-E8™ or mTeSR™1 medium on Matrigel®-coated 12-well plates and maintained with daily medium changes until a confluent monolayer (>95%) was achieved. 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 can be maintained up to a month or longer using the STEMdiff™ Cardiomyocyte Maintenance Kit or harvested with the STEMdiff™ Cardiomyocyte Dissociation Kit to replate for downstream applications (e.g, flow cytometry, immunocytochemistry, cryopreservation, electrophysiology, etc).

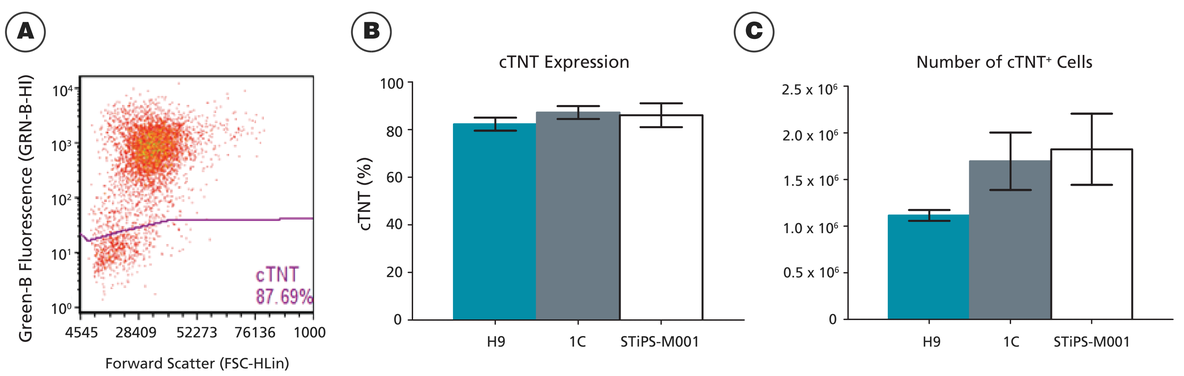
Results

FIGURE 1. hPSC-CMs generated with STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits



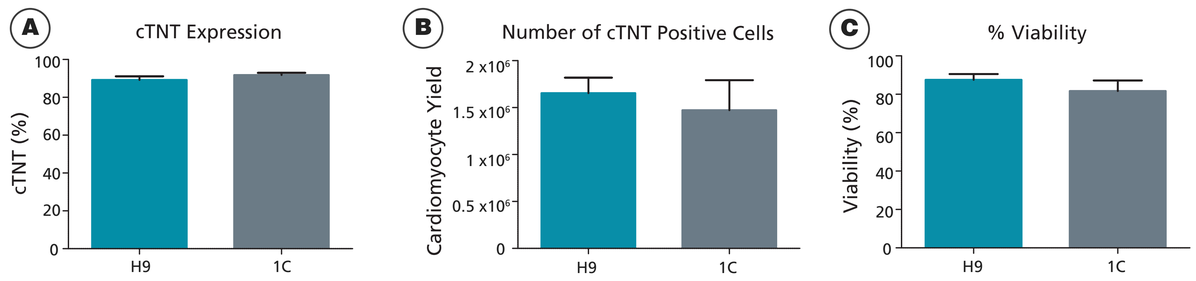
(A, B) Bright field images of hESC-CM and hiPSC-CM monolayers, respectively. **(C)** Confocal image of a single hiPSC-CM stained with cTNT (green) and Hoechst (blue).

FIGURE 2. STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits produce high percentage and yield of cTNT-positive cells from multiple human iPS and ES lines



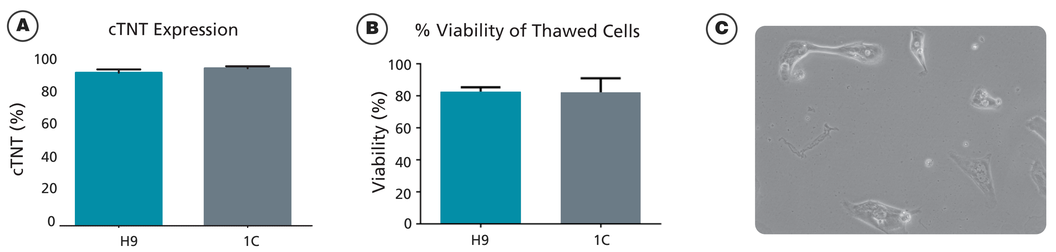
(A) Flow cytometry histogram of cTnT data for hPSC-CMs generated with the STiPs-MOO1 iPS line. **(B, C)** Bar graphs showing >80% cTNT-positive cells and >1 million cardiomyocytes are produced from hESC (H9) and hiPSC (1C, STIPS-MOO1) lines.

FIGURE 3. STEMdiff™ Cardiomyocyte Dissociation Kit harvests high yield of viable cardiomyocytes



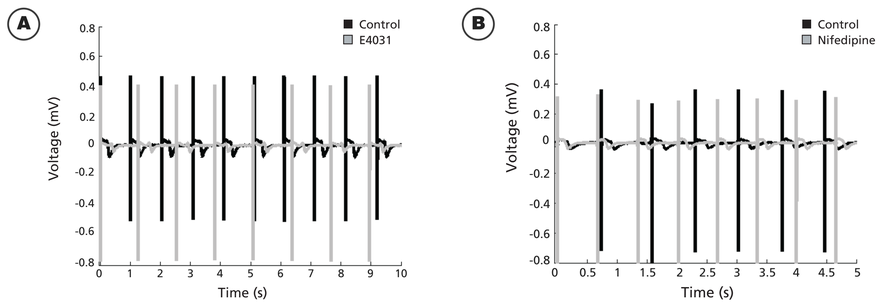
(A, B & C) hPSC-CMs have high purity (>85% cTnT-positive cells), yield (>1.5 million cardiomyocytes) and viability (>80% live cells) using the STEMdiff™ Cardiomyocyte Dissociation Kit. These values were determined from hPSC-CMs dissociated from a single well of a 12-well plate seeded initially with ES (H9) and iPS (1C) cells (n = 3).

FIGURE 4. STEMdiff™ Cardiomyocyte Freezing and Support Media used together support the cryopreservation, thawing and the replating of viable cardiomyocytes



(A, B) Cryopreserved hPSC-CMs have high purity (>85% cTnT-positive cells) and viability (>75% live cells) using the STEMdiff™ Cardiomyocyte Freezing Medium and after thawing with STEMdiff™ Cardiomyocyte Support Medium. These values were determined using 0.5 million cryopreserved hPSC-CMs at 1 and 3 months using ES (H9) and iPS (1C) cells (n = 2). **(C)** Bright field image of replated single hPSC-CMs at day 7 that were cryopreserved with STEMdiff™ Cardiomyocyte Support Medium. The cryopreserved hPSC-CMs were thawed and plated using the STEMdiff™Cardiomyocyte Support Medium for 24 hours. After 24 hours, the spent medium was replaced with STEMdiff™ Cardiomyocyte Maintenance Medium. The medium was then replaced every 2 days.

FIGURE 5. hPSC-CMs produced with STEMdiff™ Cardiomyocyte Products are sensitive to HERG Potassium channel and L-type Calcium channel blockers



(A, B) Microelectrode Array (Maestro MEA, Axion Biosystems) recordings of hPSC-CMs (Day 27) show characteristic electrical profiles and drug response to E4031 (10 nM) and Nifedipine (300 nM). E4031 prolonged (**A**, gray line) and Nifedipine (**B**, gray line) shortened the repolarization, respectively.

Conclusions

- The STEMdiff™ Cardiomyocyte Products provide a robust and standardized complete workflow to produce and process hPSC-derived Cardiomyocytes.
- Easily and efficiently produce and maintain hPSC-derived Cardiomyocytes with the STEMdiff™ Cardiomyocyte Differentiation and Maintenance Kits. Confluent beating monolayers observed by day 15 and can be maintained up to a month or longer.
- Robust Cardiomyocyte differentiation from multiple PSC lines resulting in >80% cTnT-positive cells with a yield of >1 million cardiomyocytes from one well of a 12-well plate.
- Easy and fast dissociation of confluent beating monolayers of hPSC-derived cardiomyocytes in just 15 minutes using the STEMdiff™ Cardiomyocyte Dissociation Kit. Achieve >80% viable cardiomyocytes that can used in downstream applications such as flow cytometry, cryopreservation, immunocytochemistry, or electrophysiology.
- Efficient cryopreservation and thawing of hPSC-derived Cardiomyocytes using the STEMdiff™ Freezing Cardiomyocyte Freezing Medium and STEMdiff™ Cardiomyocyte Support Medium.
- hPSC-derived Cardiomyocytes produced with the STEMdiff™ Cardiomyocyte Products show characteristic electrical waveforms and response to cardio-active drugs.