Robust and Efficient Generation of Functional Human Pluripotent Stem Cell-Derived Atrial Cardiomyocytes

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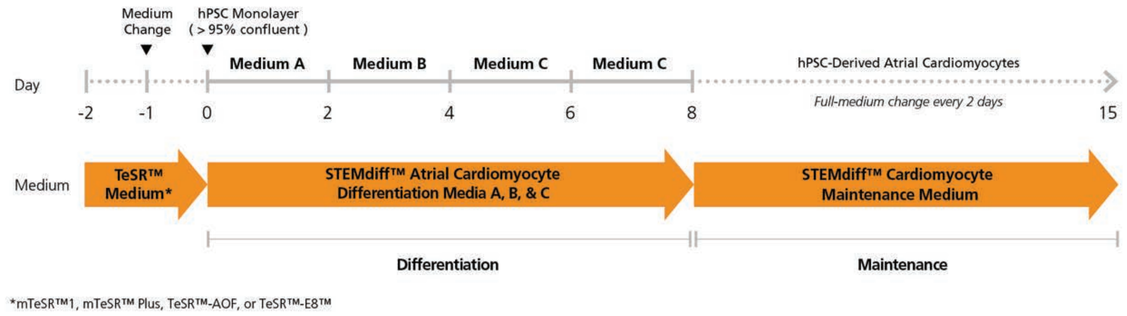
INTRODUCTION

The ability to generate and maintain high-quality human pluripotent stem cell (hPSC)-derived atrial cardiomyocytes is necessary to model atrial cardiomyocyte disease and for drug discovery and toxicology. Protocols and reagents to make hPSC-derived atrial cardiomyocytes are variable and not standardized. We have developed the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit, a defined, serum-free cell culture medium to differentiate hPSCs to atrial cardiomyocytes. This kit provides a standardized differentiation workflow to generate functional hPSC-derived atrial cardiomyocytes that are ready for use in downstream applications.

METHODS



*mTeSR™1, mTeSR™ Plus, TeSR™-AOF, or TeSR™-E8™

FIGURE 1. STEMdiff™ Atrial Cardiomyocyte Differentiation Kit Workflow

hPSCs were seeded as single cells (3.5 - 8 x 10^5 cells/well) onto Matrigel®-coated 12-well plates in TeSR™ medium containing Y-27632 and maintained for two days. On day 0, differentiation of hPSCs to atrial cardiomyocytes was initiated with a full-medium change using Differentiation Medium A. On days 2 and 4, the medium was replaced with Differentiation Medium B and C, respectively. On day 8, Differentiation Medium C was replaced with STEMdiff™ Cardiomyocyte Maintenance Medium. A full-medium change was completed every other day until day 15. On day 15, a confluent, beating monolayer was observed, and the hPSC-derived atrial cardiomyocytes were harvested for characterization using flow cytometry, qPCR, RNAseq, and electrophysiology.

RESULTS

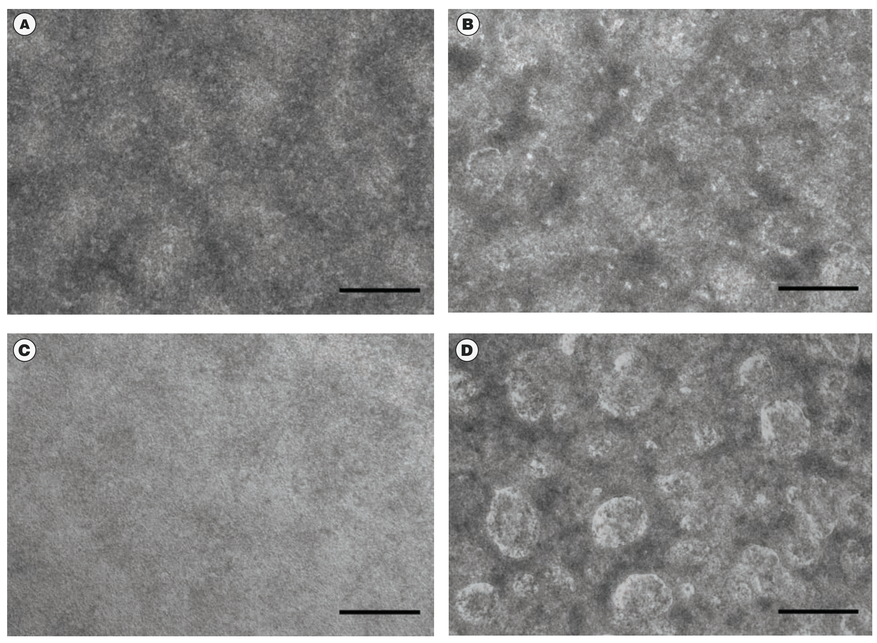


FIGURE 2. STEMdiff™ Atrial Cardiomyocyte Differentiation Kit Produced Robust, Uniform Monolayers of hPSC-derived Atrial Cardiomyocytes Across hPSC Lines

hPSC-derived atrial cardiomyocytes generated by the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit exhibited confluent, beating monolayers on day 15 of differentiation. Robust, uniform beating monolayers were observed in four hPSC lines, including 2 embryonic stem (ES) cell lines **(A)** H9 and **(B)** H7 and 2 induced pluripotent stem (iPS) cell lines **(C)** WLS-1C and **(D)** STiPS-R038. Scale bar = 500 µm.

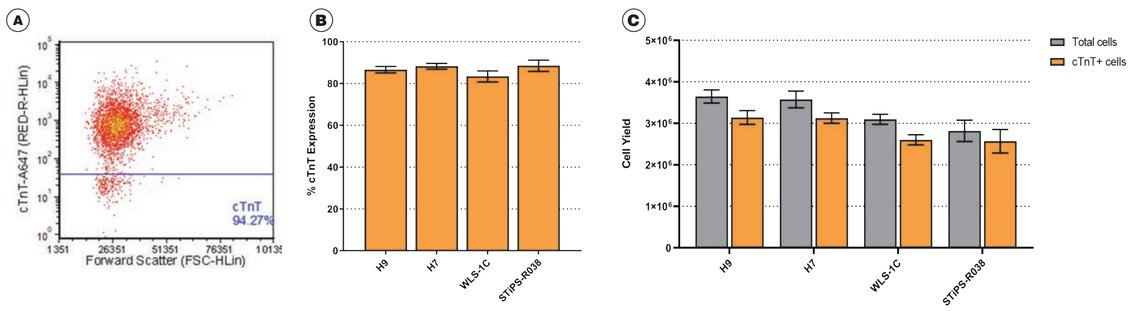


FIGURE 3. STEMdiff™ Atrial Cardiomyocyte Differentiation Kit Generated a High Percentage and Yield of cTnT-Positive Cells Across hPSC Lines

To demonstrate the efficiency of the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit, four hPSC lines were differentiated to atrial cardiomyocytes and were harvested for flow cytometry. Differentiation was performed in a 12-well plate format. **(A)** Flow cytometry histogram of cTnT expression for cardiomyocytes generated with the STiPS-R038 hiPSC line. Bar graphs showing **(B)** > 80% cTnT expression and **(C)** > 2 million cardiomyocytes were produced from four hPSC lines. Data are shown as mean ± SEM, n = 18.

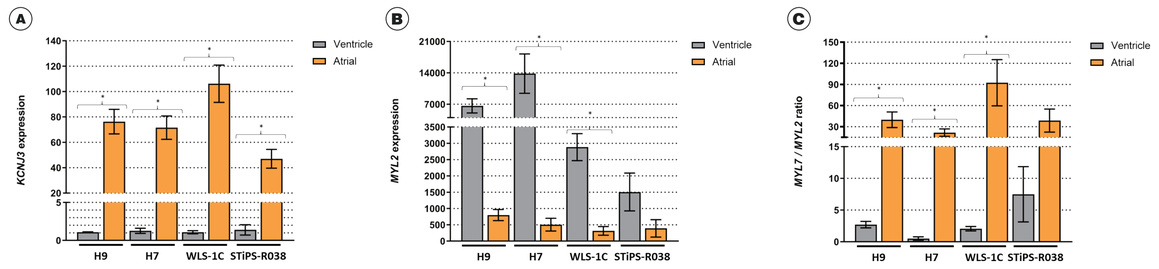


FIGURE 4. Quantitative PCR Revealed Upregulation of *KCNJ3* and Downregulation of *MYL2* in hPSC-derived Atrial Cardiomyocytes

Four hPSC lines were differentiated to either atrial cardiomyocytes using the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit or ventricular cardiomyocytes using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. Cardiomyocyte subtypes were assessed by qPCR for expression of **(A)** *KCNJ3*, an atrial-specific potassium ion channel gene, and **(B)** *MYL2*, a ventricle-specific regulatory light chain of myosin gene. **(C)** The ratio of *MYL7/MYL2* expression can be used as an indicator for an atrial-like gene expression. Across four hPSC lines, atrial cardiomyocytes showed significantly increased *KCNJ3* expression (> 30-fold, n = 40), significantly decreased *MYL2* expression (< 0.3-fold, n = 63), and significantly increased *MYL7/MYL2* ratio (> 2-fold, n = 63) compared to ventricular cardiomyocytes. Data are shown as mean ± SEM. Asterisk indicates significance of $p < 0.05$.

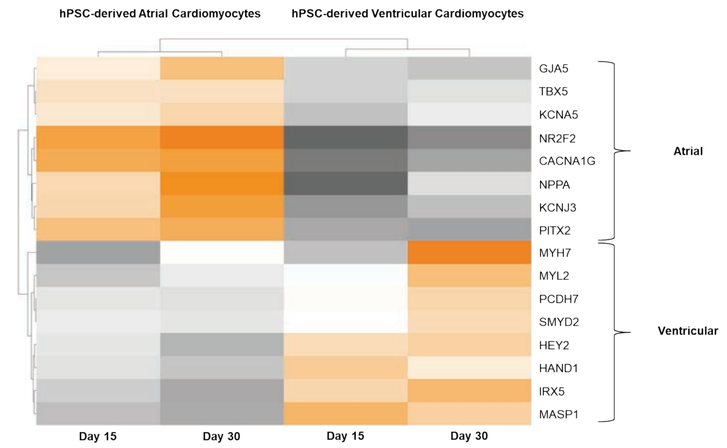


FIGURE 5. RNAseq Timepoint Analysis Revealed Upregulation of Atrial-Specific Genes and Downregulation of Ventricle-Specific Genes in hPSC-Derived Atrial Cardiomyocytes

Four hPSC lines were differentiated to either atrial cardiomyocytes using the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit or ventricular cardiomyocytes using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. Cardiomyocyte subtypes were harvested on day 15 and day 30 for RNAseq analyses. hPSC-derived atrial cardiomyocytes showed upregulation of atrial-specific genes (*GJA5, TBX5, KCNA5, NR2F2, CACNA1G, NPPA, KCNJ3, PITX2*) and downregulation of ventricle-specific genes (*MYH7, MYL2, PCDH7, SMYD2, HEY2, HAND1, IRX5, MASP1*) on day 15 and day 30 compared to hPSC-derived ventricular cardiomyocytes. RNAseq data was generated from 3 hPSC lines (H9, WLS-1C, and STiPS-M001).

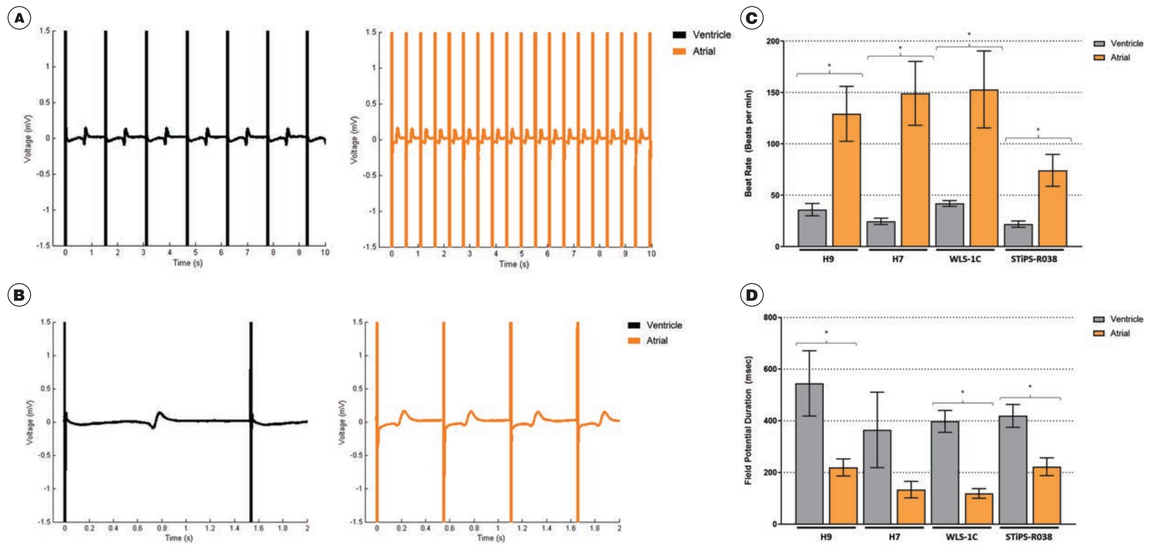
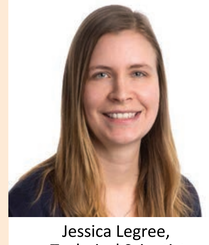


FIGURE 6. Microelectrode Array of hPSC-Derived Atrial Cardiomyocytes Showed Increased Beat Rate and Shorter Field Potential Duration Compared to hPSC-Derived Ventricular Cardiomyocytes

Microelectrode Array (Maestro MEA, Axion BioSystems) recordings of hPSC-derived atrial cardiomyocytes (orange) and hPSC-derived ventricular cardiomyocytes (black). hPSC-derived atrial cardiomyocytes showed **(A)** increased beat rate and **(B)** decreased field potential duration (FPD) compared to hPSC-derived ventricular cardiomyocytes. **(C)** Beat rate recordings from 4 hPSC lines demonstrated that hPSC-derived atrial cardiomyocytes consistently beat faster compared to hPSC-derived ventricular cardiomyocytes. Across 4 cell lines, the average atrial beat rate was 123 ± 14 beats per minute (mean ± SEM; n = 21), and the average ventricular beat rate was 32 ± 3 beats per minute (mean ± SEM; n = 16). **(D)** Across 4 cell lines, FPD values were consistently shorter in hPSC-derived atrial cardiomyocytes compared to ventricular cardiomyocytes. Average atrial FPD was 181 ± 18 msec (mean ± SEM; n = 21), and average ventricular FPD was 443 ± 49 msec (mean ± SEM; n = 16). Asterisk indicates a significance of $p < 0.05$.

Summary

- STEMdiff™ Atrial Cardiomyocyte Differentiation Kit is a standardized, defined, and serum-free differentiation workflow for simple and efficient generation of functional hPSC-derived atrial cardiomyocytes.
- hPSC-derived atrial cardiomyocytes generated using the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit produced uniform beating monolayers and generated a high percentage and yield of cTnT-positive cells across hPSC lines.
- hPSC-derived atrial cardiomyocytes demonstrated upregulation of atrial-specific genes (*KCNJ3, GJA5, NPPA, PITX2*) and downregulation of ventricle-specific genes (*MYL2, MYH7, HEY2, HAND1*).
- Electrophysiology profile of hPSC-derived atrial cardiomyocytes showed increased beat rate and shorter field potential duration (FPD) compared to hPSC-derived ventricular cardiomyocytes.



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