A simple and efficient culture system for the large-scale expansion of human pluripotent stem cell-derived cardiomyocytes

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INTRODUCTION

The in vitro expansion of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) is limited, and the ability to generate large numbers of cardiomyocytes would benefit drug discovery, toxicology, and cell therapy research. Here, we present the STEMdiff™ Cardiomyocyte Expansion Kit, the first commercially available and optimized culture system amenable to large-scale expansion of hPSC-CMs. The STEMdiff™ Cardiomyocyte Expansion Kit provides a standardized and scalable expansion workflow to generate large numbers of functional hPSC-derived cardiomyocytes that are ready for use in downstream applications.

METHODS

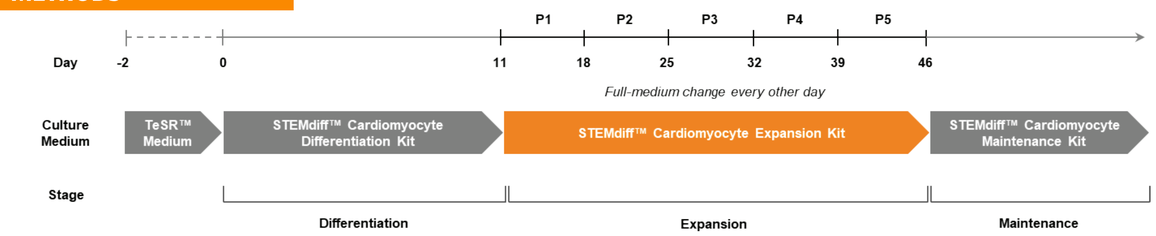

FIGURE 1. STEMdiff™ Cardiomyocyte Expansion Kit Workflow

Cardiomyocyte differentiation: 2 embryonic stem (ES) cell lines (H1 and H9) and 2 induced pluripotent stem (iPS) cell lines (WLS-1C and STiPS-M001) cultured in mTeSR™1 on Corning® Matrigel® were seeded as single cells (3.5 - 8 x 10^5 cells/well) onto Matrigel®-coated 12-well plates in mTeSR™1 medium containing Y-27632 and maintained for two days. On day 0, cardiomyocyte differentiation was initiated using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit and the standard differentiation protocol was performed, up to day 11.

Expansion: Day 11 early-stage hPSC-CMs were harvested using STEMdiff™ Cardiomyocyte Dissociation Kit and expanded with STEMdiff™ Cardiomyocyte Expansion Kit, which comprises STEMdiff™ Cardiomyocyte Passaging Medium and STEMdiff™ Cardiomyocyte Expansion Medium. Day 11 hPSC-CMs were replated onto Matrigel®-coated cultureware at a low density (5.2 x 10^5 hPSC-CMs/cm^2) in STEMdiff™ Cardiomyocyte Passaging Medium. After 24 hours, a full-medium change was performed with STEMdiff™ Cardiomyocyte Expansion Medium, and every 2 days onwards. On day 18, after 7 days of expansion (end of passage 1 [P1]), a confluent beating monolayer of expanding hPSC-CMs was observed. The expanding hPSC-CMs were then dissociated and replated as described above for subsequent passages. After 5 passages, the hPSC-CMs were cultured for 7 days using STEMdiff™ Cardiomyocyte Maintenance Kit.

Characterization: Fold expansion data was collected at the end of every passage for the expanding hPSC-CMs. Cumulative fold expansion was calculated by multiplying the fold expansion from P1 to P5. Flow cytometry was performed on day 11 and from P1 to P5 for expression of definitive cardiomyocyte marker cardiac troponin T (cTnT) and cell cycle marker Ki67. After 5 passages, electrophysiology of the expanded hPSC-CMs was recorded using a Maestro microelectrode array (MEA) plate.

RESULTS

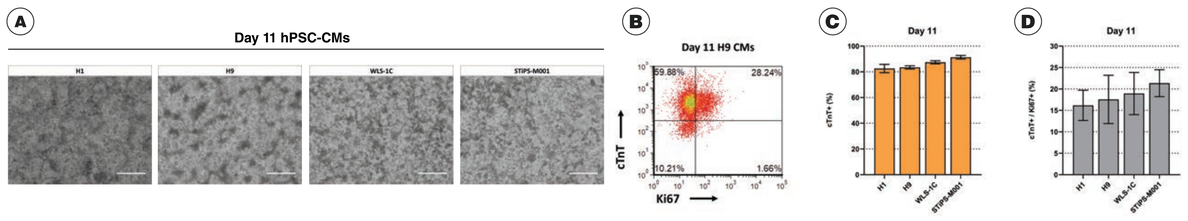

FIGURE 2. Early-stage hPSC-CMs exhibited confluent beating monolayers and expressed cTnT and Ki67.

(A) Robust, uniform beating monolayers of early-stage hPSC-CMs were observed in all four hPSC lines on day 11 of ventricular cardiomyocyte differentiation. Scale bar = 500 µm. **(B)** Flow cytometry analysis of day 11 H9-derived hPSC-CMs showed a cTnT+ population (top left + top right quadrant), as well as a subpopulation of expanding cTnT+/Ki67+ CMs (top right quadrant). **(C)** 86 ± 1% of day 11 early-stage hPSC-CMs expressed cTnT (n = 14, 4 hPSC lines). Early-stage hPSC-CMs should express > 80% cTnT on day 11 before proceeding with expansion. **(D)** A double-positive population of cTnT+/Ki67+ expanding CMs was also observed on day 11, which constituted an average of 18 ± 2% of the total sample (n = 14, 4 hPSC lines). Data are shown as mean ± SEM.

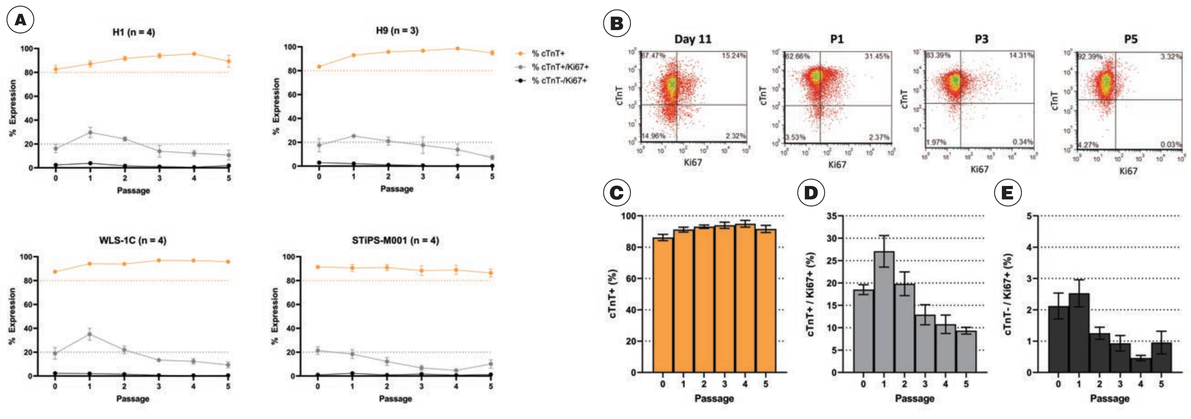

FIGURE 3. Cardiomyocyte purity increased during expansion in STEMdiff™ Cardiomyocyte Expansion Medium.

(A) Expression of cTnT and Ki67 was tracked across 4 hPSC lines during expansion. Cardiomyocyte purity (cTnT+; orange line) remains above 80% throughout expansion. The population of expanding CMs (cTnT+/Ki67+; grey line) peaked at P1 (> 20%) and subsequently decreased with each passage. A background population of cTnT-/Ki67+ cells was observed (black line), and remained < 5% throughout expansion (n = 4, 4 hPSC lines). **(B)** Representative flow cytometry plots of H9-derived hPSC-CMs before expansion on day 11, and during expansion at P1, P3, and P5. **(C)** On day 11 (P0), 86 ± 2% of hPSC-CMs expressed cTnT (n = 14) and peaked at 95 ± 1% (n = 15) by P4. After 5 passages, expression remained high at 92 ± 2% (n = 14). **(D)** On day 11, 18 ± 2% of expanding CMs expressed cTnT+/Ki67+ (n = 13), and peaked at P1 with 27 ± 2% of cTnT+/Ki67+ cells (n = 15). **(E)** The expanding background population peaked at P1 with 2.6 ± 0.5% of cTnT-/Ki67+ cells (n = 15). Data are shown as the mean of 4 cell lines ± SEM.

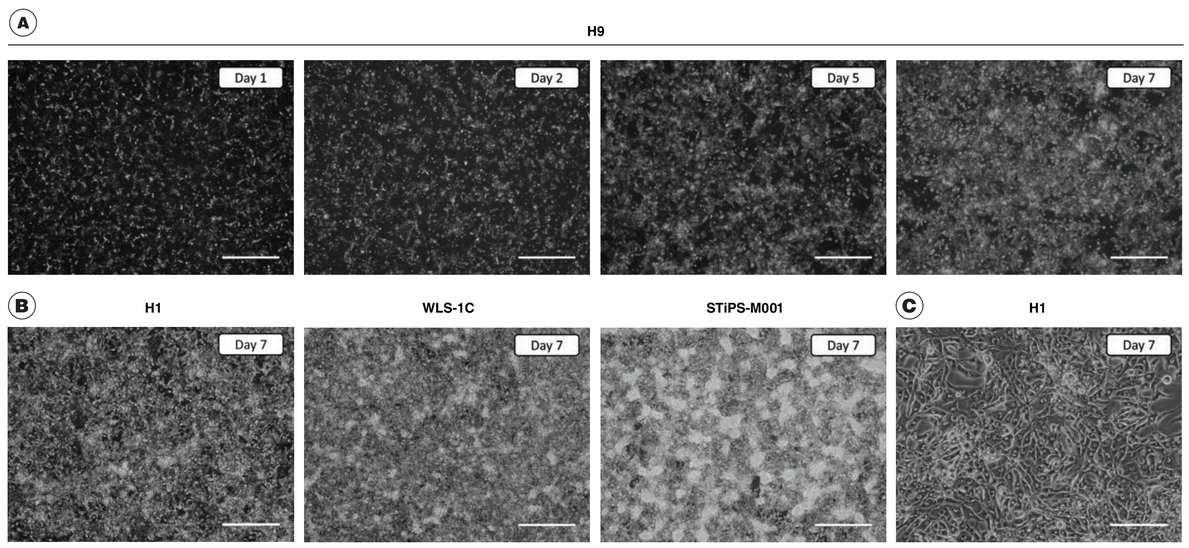

FIGURE 4. Early-stage hPSC-derived CMs reached confluency 1 week after low-density replating using STEMdiff™ Cardiomyocyte Expansion Kit.

(A) Representative images (2X magnification) of an expanding P1 H9-derived hPSC-CM culture. On day 1, hPSC-CMs are at a low confluency and ready for expansion. From day 2 onwards, hPSC-CMs increase in confluency. On day 7, a confluent, beating monolayer of expanding hPSC-CMs was observed. Scale bar = 1 mm. **(B)** Representative images (2X magnification) of confluent H1, WLS-1C, and STiPS-M001-derived hPSC-CM monolayers on day 7. Scale bar = 1 mm. **(C)** 10X magnification image of day 7 expanded H1-derived hPSC-CM culture. Scale bar = 200 µm.

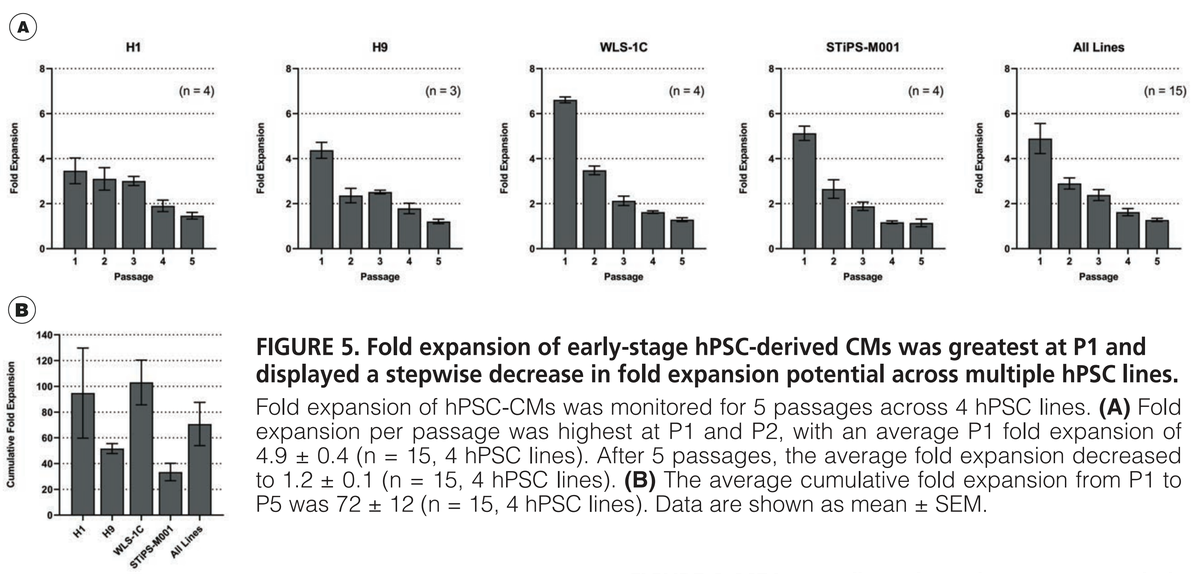

FIGURE 5. Fold expansion of early-stage hPSC-derived CMs was greatest at P1 and displayed a stepwise decrease in fold expansion potential across multiple hPSC lines.

Fold expansion of hPSC-CMs was monitored for 5 passages across 4 hPSC lines. **(A)** Fold expansion per passage was highest at P1 and P2, with an average P1 fold expansion of 4.9 ± 0.4 (n = 15, 4 hPSC lines). After 5 passages, the average fold expansion decreased to 1.2 ± 0.1 (n = 15, 4 hPSC lines). **(B)** The average cumulative fold expansion from P1 to P5 was 72 ± 12 (n = 15, 4 hPSC lines). Data are shown as mean ± SEM.

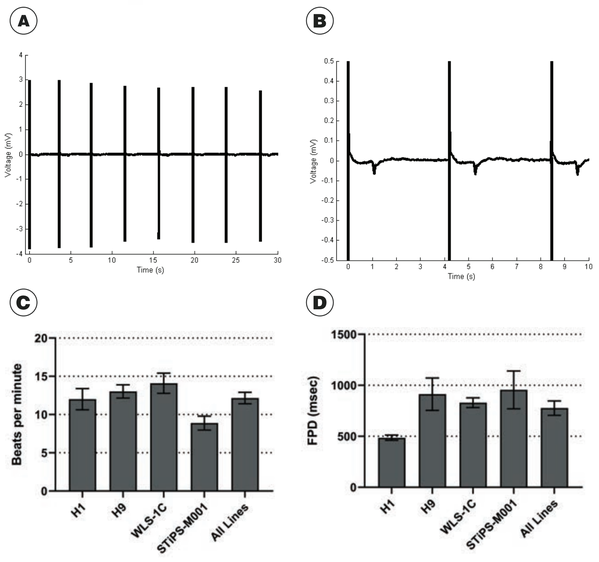

FIGURE 6. MEA recordings show that post-expanded hPSC-derived CMs exhibited a stable electrical profile.

After 5 passages with STEMdiff™ Cardiomyocyte Expansion Kit, hPSC-CMs were seeded onto MEA plates at high density and cultured for 7 days using STEMdiff™ Cardiomyocyte Maintenance Kit. **(A)** The post-expanded hPSC-CMs were beating spontaneously and retained a stable beat rate. **(B)** The post-expanded hPSC-CMs exhibited a characteristic field potential duration (FPD), with a large depolarization spike, followed by a small repolarization wave. **(C)** Across 4 hPSC lines, post-expanded hPSC-CMs had an average beat rate of 12 ± 1 bpm (n = 14). **(D)** Across 4 hPSC lines, post-expanded hPSC-CMs had an average FPD of 776 ± 70 msec (n = 14). Data are shown as mean ± SEM.

Summary

- STEMdiff™ Cardiomyocyte Expansion Kit provides a standardized and scalable workflow to expand early-stage hPSC-derived cardiomyocytes.
- Cardiomyocyte purity (cTnT+) increased during expansion from 86% on day 11 to 92% after 5 passages.
- An expanding subpopulation of cardiomyocytes (cTnT+/Ki67+) was observed on day 11 (18%) that peaked after 1 passage (27%).
- STEMdiff™ Cardiomyocyte Expansion Kit successfully expanded multiple human ES and iPS lines, with an average cumulative fold expansion of 72-fold over 5 passages. A total yield of 3.7 x 10^6 hPSC-CMs/cm^2 can be obtained when starting from 5.2 x 10^5 hPSC-CMs/cm^2.
- Post-expanded hPSC-CMs retain a stable electrical profile and beat rate, with an average of 12 beats per minute.

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