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Differentiation and functional characterization of human iPSC-derived cardiomyocytes

StemMACS™ CardioDiff Kit XF, human

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Background

Directed differentiation of human-induced pluripotent stem cell (human iPSC) lineages is a major tool for the development of cell-based disease models, drug screening platforms, and cell therapies. Each of these applications requires a consistent, efficient, and reliable differentiation protocol that generates cells that are functionally and phenotypically consistent.

The generated human iPSC-derived model must show characteristics that resemble those of the primary cells, such as morphology, gene, and protein expression, as well as functional characteristics of the primary cells.

Moreover, having a standardized and reliable method for differentiation ensures comparable results with varying cell lines between experiments.

In this application note, we show a detailed phenotypical and functional characterization of differentiated human iPSCs into cardiomyocytes using StemMACS CardioDiff Kit XF, human.

Materials and methods

- StemMACS CardioDiff Kit XF, human (130-125-289)
- Multi Tissue Dissociation Kit 3 (130-110-204)
- StemMACS Cardiac Cultivation Medium XF, human (130-125-287)
- Inside Stain Kit (130-090-477)
- Matrigel® coated plates
- Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity™ (130-119-674)
- α-Actinin (Sarcomeric) Antibody, anti-human/mouse/rat, PE, REAfinity (130-123-996)
- Myosin Heavy Chain Antibody, anti-human/mouse/rat, APC, REAfinity (130-122-968)
- MLC2a Antibody, anti-human/mouse/rat, APC, REAfinity (130-118-674)
- MLC2v Antibody, anti-human/mouse/rat, PE, REAfinity (130-119-680)
- REA Control Antibody (I), human IgG1, APC, REAfinity (130-120-709)
- REA Control Antibody (I), human IgG1, FITC, REAfinity (130-118-354)
- REA Control Antibody (I), human IgG1, PE, REAfinity (130-118-347)

Human iPSC differentiation into cardiomyocytes

Using StemMACS CardioDiff Kit XF, human, human iPSCs were differentiated into cardiomyocytes following the protocol. To begin, the human iPSCs were seeded as single cells on Matrigel® coated plates in Mesoderm Induction Medium from the kit. Four different cell densities were used: 125,000 cells/cm^2; 250,000 cells/cm^2; 300,000 cells/cm^2; and 400,000 cells/cm^2. On day 1, cells were maintained in Cardiac Cultivation Medium, on day 2 media was switched to Cardiac Induction Medium, and from day 3 to 8 changed back to Cardiac Cultivation Medium with daily media changes.

Cell harvest

The content of 3 wells in a 12-well plate were harvested using the Multi Tissue Dissociation Kit 3. The enzyme mix was prepared by mixing Enzyme T and Buffer X (Multi Tissue Dissociation Kit 3) in a 1:10 ratio, with 400 µL of the mix then added to each well. After 10 minutes of incubation at 37 °C, the reaction was stopped by adding 600 µL of StemMACS Cardiac Cultivation Medium XF, human containing 20% fetal calf serum (FCS) to each well.

Cells were dislodged by gently pipetting up and down using a 1 mL pipette and applied to a 100 µm cell strainer. To increase cell yield, both the wells and strainer were further washed with 1 mL StemMACS Cardiac Cultivation Medium XF, human containing 20% FCS.

Flow cytometry analysis

A total of 1.54×10^7 cells with a viability of 89% were harvested. Cells were resuspended in Inside Fix Solution (Inside Stain Kit) and incubated in the dark for 20 minutes at room temperature. Cells were then collected by centrifugation (at 300×g for 5 min), resuspended in 1 mL PEB (DPBS [-/-], 0.5% BSA, and 2mM EDTA), and collected again after a second centrifugation (at 300×g for 5 min).

The samples for the isotype controls were obtained by resuspending the cells in 98 µL Inside Perm solution (Inside Stain Kit) plus 2 µL of the appropriate REA Control Antibody. Additionally, the samples for the double stain were obtained by resuspending the cells in 96 µL Inside Perm solution plus 2 µL of each antibody.

After 10 minutes of incubation at room temperature, 1 mL of Inside Perm solution was added and samples were centrifuged (at 300×g for 5 min). The pellet was resuspended in 500 µL of PEB buffer and analyzed using BD FACSCalibur™.

Exposure to cardiotropic compounds

Human iPSC-derived cardiomyocytes were treated with 30 mM isoproterenol or 30 mM propranolol in the StemMACS Cardiac Cultivation Medium XF, human. Cells were visually monitored and beatings per minute were manually scored.

Results

Human iPSCs were successfully differentiated into cardiomyocytes with the first visible beating patches at day 7. After 8 days of differentiation, human iPSC-derived cells showed changes in morphology, protein expression, and beating properties comparable to cardiomyocyte fate-specification. The extent of such change was, as expected, dependent on the initial seeding density.

Establishing the most successful seeding density for differentiation is vital when differentiating a human iPSC line for the first time into cardiomyocytes. This is then used to set starting cell numbers for future differentiation rounds. For the selected Human iPSC line, a high seeding density of 300,000 cells/cm² resulted in the best functional performance. Human iPSC-derived cardiomyocytes from high cell density wells showed wave-like beating patterns through the whole well. Furthermore, the cells showed a high expression of the cardiomyocyte marker, cardiac muscle troponin T (cTnT) (fig.1).

Furthermore, human iPSC-derived cardiomyocytes showed expression of additional markers typical of cardiac fate, such as α-actinin and myosin heavy chain (MHC). Moreover, the higher cell expression of the atrial isoform of myosin regulatory light chain 2 (MLC2a) (27.3%) versus the lower expression of the ventricular isoform (MLC2v) (0.48%), indicated that cells started to express atrial-like characteristics just after 8 days of differentiation. (fig. 2).

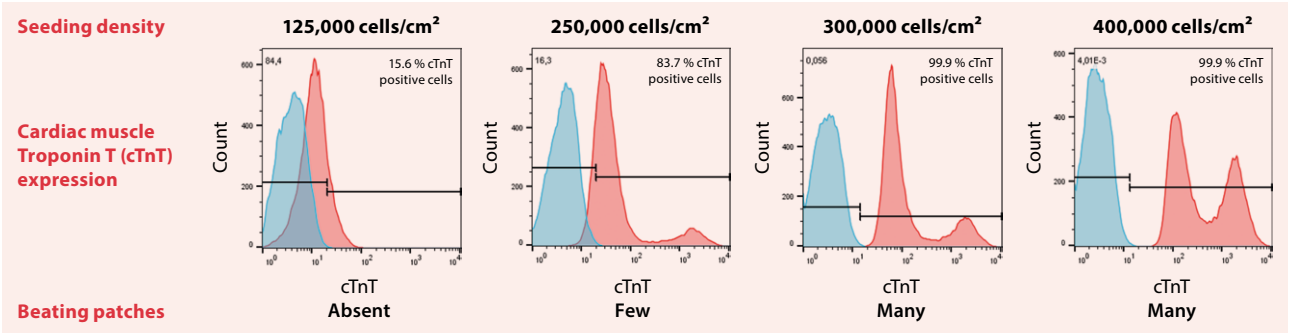

Figure 1: Impact evaluation of different seeding densities on cardiomyocyte functional performance by analyzing cardiomyocyte marker expression and the number of beating patches in culture after 8 days of differentiation. For the selected human iPSC line, the seeding density of 300,000 cells/cm² was found to be the optimal seeding density, as it resulted in high expression of cTnT and numerous beating patches in culture.

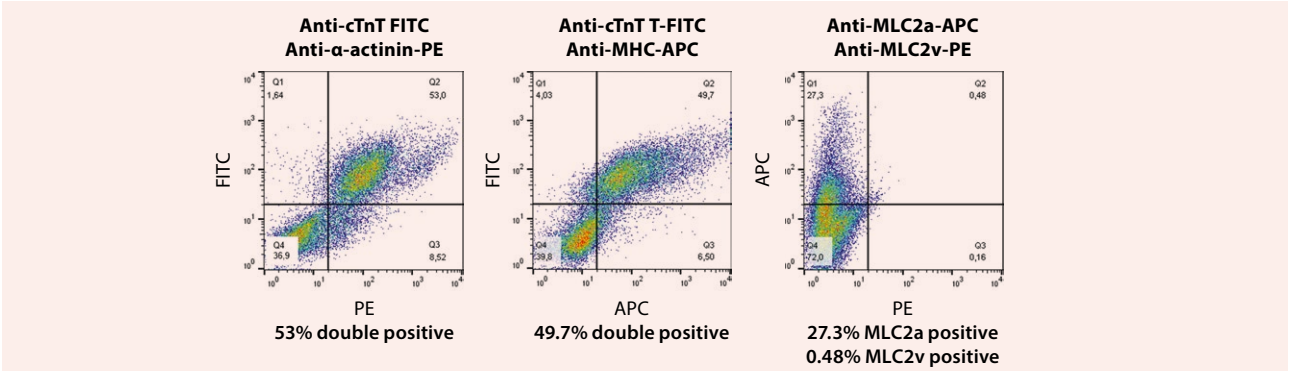

Figure 2: Flow cytometry analysis showed a high expression of cardiac markers in human iPSC-derived cardiomyocytes after only 8 days of differentation using the StemMACS CardioDiff Kit XF, human. 53% of cells expressed both cTnT and α-actinin while 49.7% were positive for both cTnT and MHC. Furthermore, 27.3% of the cells expressed the atrial isoform (MLC2a), whereas only 0.48% of the cells expressed the ventricular isoform (MLC2v).

Human iPSC-derived cardiomyocytes expressed cardiac associated marker while losing pluripotency marker expression

Gene expression analysis showed a high expression of cardiac muscle troponin T *(TNNT2)* after 8 days of differentiation which was accompanied by a complete loss of expression of the pluripotency-associated marker *POU5F1 (POU Class 5 Homeobox 1, OCT4)*. These results demonstrated the absence of undifferentiated human iPSCs in the wells that underwent differentiation with StemMACS CardioDiff Kit XF, human (fig. 3).

Human iPSC-derived cardiomyocytes respond to treatment with cardiotropic compounds by altering their beating frequency

Functional human iPSC-derived cardiomyocytes must show contractile properties and be able to react to external stimuli in a similar fashion to the primary cells. To evaluate their functionality, the iPSC-derived cardiomyocytes were exposed to two well-known cardiotropic compounds: isoproterenol and propranolol.

Isoproterenol, also known as isoprenaline, is a medication used for the treatment of bradycardia (slow heart rate) and induces an increase in beating frequency. Conversely, propranolol opposes this effect, slowing down beat frequency.

Human iPSC-derived cardiomyocytes exposed to 30 mM isoproterenol or 30 mM propranolol responded by modifying their beating patterns accordingly compared to the control, in line with the expected effects of the compounds (fig. 4).

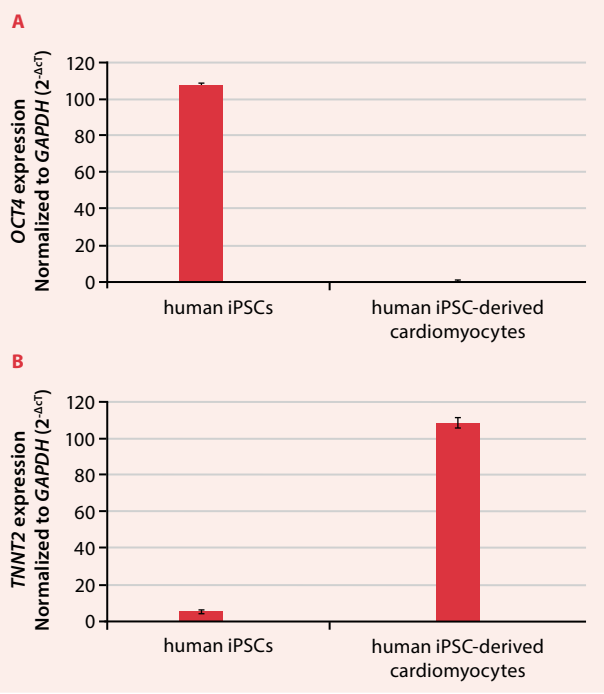

Figure 3: Gene expression analysis showed a high expression of the stem cell-specific marker *OCT4* in undifferentiated human iPSCs which, as expected, was lost after differentiation. Moreover, expression of the cardiac marker *TNNT2* increased after just 8 days of differentiation using the StemMACS CardioDiff Kit XF, human within human iPSC-derived cardiomyocytes.

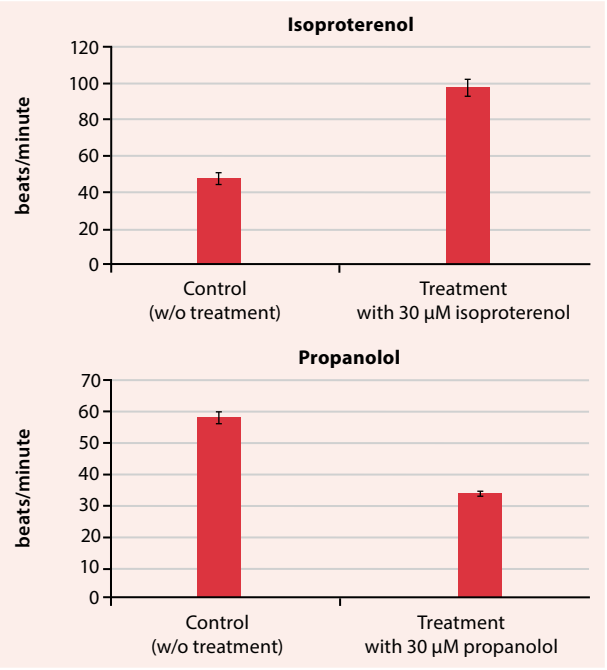

Figure 4: Human iPSC-derived cardiomyocytes responded to cardiotropic compounds by modifying their beating patterns.

Conclusions

Human iPSC lines differentiated with StemMACS CardioDiff Kit XF, human show:

- gene and protein expression typical of cardiomyocytes;
- absence of remnant undifferentiated human iPSCs; and
- appropriate beating responses to treatment with cardiotropic compounds.

Combined, these results suggest the good quality of the human iPSC-derived cellular model generated.

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