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Generation of purified human iPSC derived cardiomyocytes using clinically relevant workflows

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Dr. Herron's research focuses on the complex interplay between cardiac electrical excitation and contractile force generation – a process known classically as excitation-contraction coupling. His lab was among the first to utilize human induced pluripotent stem cells cardiomyocyte (hiPSCs-CM) monolayers to study human cardiac arrhythmia mechanisms *in vitro*. His current research is concentrated on the development of patient specific cardiomyopathy disease models *in vitro* using hiPSC-CM technology as well as the development of high throughput screening platforms to test for compound effects on human cardiac function.

Dr. Herron was featured in our LabRoots webinar in May 2017. To see the webinar, please go to: **www.miltenyibiotec.com/hecm**

"In the UM CVC Cardiovascular Regeneration Core Laboratory, we rely on products from Miltenyi Biotec for generation of clinically relevant cells. Currently we utilize xeno-free stem cell maintenance media from Miltenyi for iPSC derivation and maintenance. The use of the new PSC-Derived Cardiomyocyte Isolation Kit, human has enabled us to create very large numbers of purified human cardiomyocytes for high throughput pro-arrhythmia and cardiotoxicity screening of pre-clinical compounds in development. The new isolation kit has also enabled us to generate 3D cardiac micro-tissues with defined CM and non-CM populations."

Cell culture

StemMACS™ iPS-Brew XF: xeno-free cell culture medium for optimal maintenance of clinically relevant cells in feeder-free conditions.

Sample preparation

Multi Tissue Dissociation Kit 3: pure isolation of high numbers of human cardiomyocytes for high throughput screenings.

Cell separation

autoMACS® Pro and PSC-Derived Cardiomyocyte Isolation Kit: true walk-away cell isolation with automated labeling and separation allows enrichment of vital cardiomyocytes in less than an hour.

Figure 1: Workflow for the generation of purified human iPSC-derived cardiomyocytes.

Experimental setup

Differentiated cardiomyocytes (CMs) from human iPSCs were dissociated into single-cell suspensions using the Multi Tissue Dissociation Kit 3. Using the PSC-Derived Cardiomyocyte Isolation Kit, non-CMs were then magnetically labeled with the Non-Cardiomyocyte Depletion Cocktail and Anti-Biotin MicroBeads. The labeled cells were subsequently depleted by separation using a MACS® Column, placed in the magnetic field of a MACS Separator (Strategy A). Depending on the initial differentiation efficiency, further purification could be obtained by magnetically labeling the pre-enriched CMs with Cardiomyocyte Enrichment Cocktail and positively selecting the pre-enriched PSC-derived CM fraction (Strategy B).

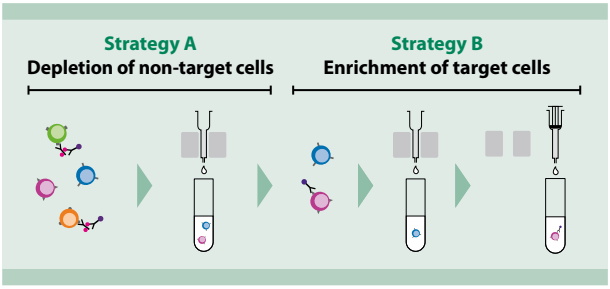

Figure 2: Innovative purification approach: the non-CM population is targeted for depletion (Strategy A) and consequently the CM population can be enriched for further purification (Strategy B).

Results

This single depletion step alone (Strategy A) was sufficient to obtain a highly efficient purification of CMs with purities of up to 90% (Strategy A, fig. 3A). Further enrichment of the PSC-derived CM population was achieved by an optional second separation step (Strategy B, fig. 3B). Almost no residual CMs were detected in the non-CM fraction, demonstrating the high efficacy of this separation method. CMs isolated with the kit displayed high expression levels of the CM-specific markers, N-Cadherin and cardiac troponin T (cTnT). Confocal imaging showed that CMs are intact, healthy and have good circular structure (fig. 4). These data suggest that CMs isolated by depletion of non-CMs are functional and possess the appropriate phenotype.

Curious to learn more? Download the application note at:
www.miltenyibiotec.com/cardioappnote

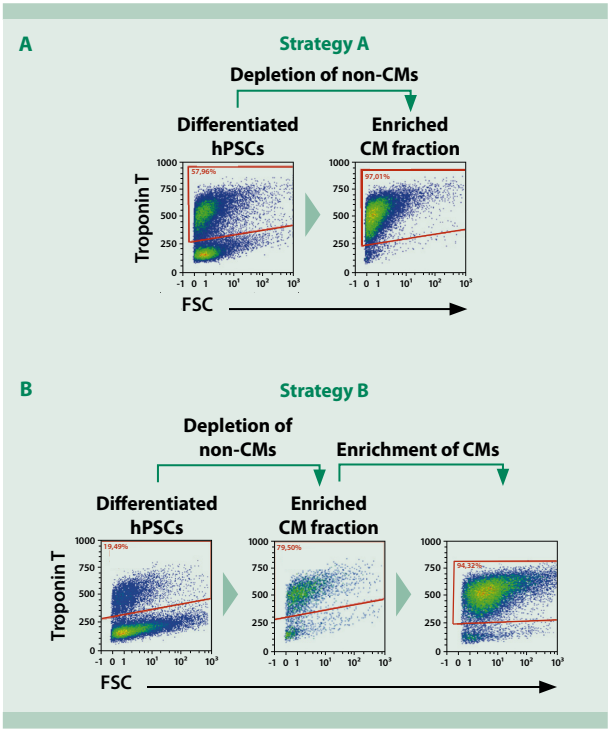

Figure 3: Starting with CM ratios of 20–80%, magnetic enrichment of PSC-CMs using the PSC-CM isolation kit, human, gives purities of up to 97%. Troponin T is a specific marker for cardiomyocytes.

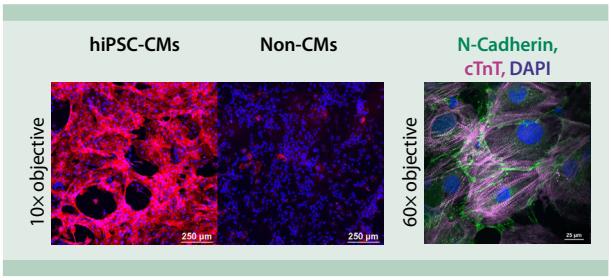

Figure 4: Confocal image of the positive and negative fraction after cell separation, stained for cardiac TnT (red) and nucleus stained with DAPI (blue). A) In the hiPSC-CM fraction CMs can be seen by the expression of cTnT while B) in the non-CM fraction no significant loss of CMs is detected. C) Using N-cadherin for junctional protein staining (green), tight junctions in between the cTnT positive cells have formed.

Summary

The development of highly efficient cardiac-directed differentiation methods makes it possible to generate large numbers of hiPSC-CMs. The PSC-Derived Cardiomyocyte Isolation Kit, human by Miltenyi Biotec, is a fast and cost-effective approach to purify CMs derived from human PSCs. The PSC-derived CMs are highly functional and display the appropriate structural characteristics. Furthermore, the process is gentle on the cells, resulting in high viability. This magnetic separation method for enriching CMs from PSCs is a highly efficient alternative to currently used methods.

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