Highly efficient immunomagnetic purification of cardiomyocytes derived from human pluripotent stem cells

Kristin Noack, Andreas Bosio, and Dominik Eckardt
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Pure cardiomyocytes (CMs) derived from human pluripotent stem cells (hPSCs) are of high interest for heart disease modeling, drug safety studies, and development of cellular therapies. Although several protocols for cardiac differentiation of hPSCs have been developed, major limitations are, e.g., the high variability in differentiation efficacy due to clone-to-clone variations and the heterogeneity of generated CM populations. For that reason we have developed several tools to improve the workflow starting from CM differentiation and monolayer dissociation via MACS® Technology–based CM purification to downstream analysis using flow cytometry, immunofluorescence stainings, and calcium imaging. Additionally, a novel cryopreservation method for enriched CMs was established.

Results

1 hPSC cultivation and cardiac differentiation

hPSCs were maintained under xeno-free conditions in StemMACS™ iPS-Brew XF medium (left) and differentiated to CMs with efficiencies of up to 80% using a monolayer protocol[1] with consecutive activation (CHIR99021) and inhibition (IWR-1) of Wnt signaling. First contractions were observed at day 9 of differentiation and CMs showed a typical morphology and sarcomeric structure (right).

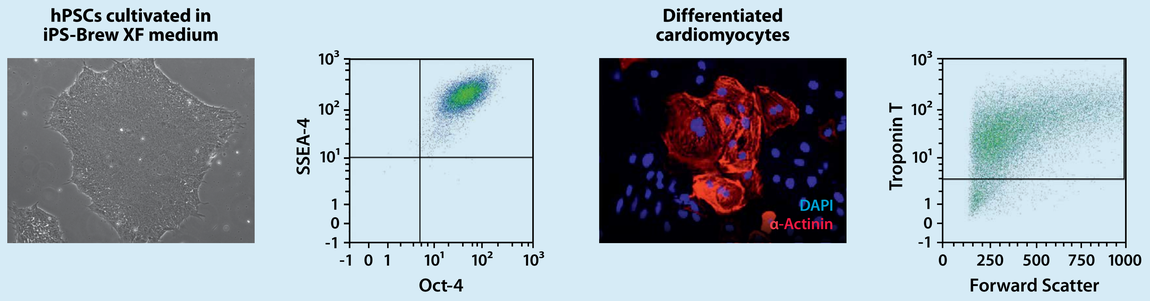

Figure 1

2 Purification of hPSC-derived CMs

In order to identify antibodies suitable for enrichment of CMs or depletion of non-myocytes, we performed a surface marker screening with more than 400 antibodies between days 10 and 20 of differentiation. Although no exclusive CM markers were identified, we found several markers that either labeled CMs and non-myocytes or subpopulations of CMs. Based on these data we developed a novel magnetic cell separation procedure that consistently delivered CM purities of >90%, regardless of the differentiation protocol, hPSC line used, time point and efficiency of differentiation. Depending on the hPSC differentiation efficiency different magnetic cell sorting strategies for the enrichment of hPSC-derived CMs were applicable. For cell populations with low differentiation efficiencies, e.g., at CM ratios of <50%, strategy A (depletion of non-myocytes) could be combined with strategy B (CM enrichment). For samples showing a higher differentiation efficiency strategy A alone was sufficient.

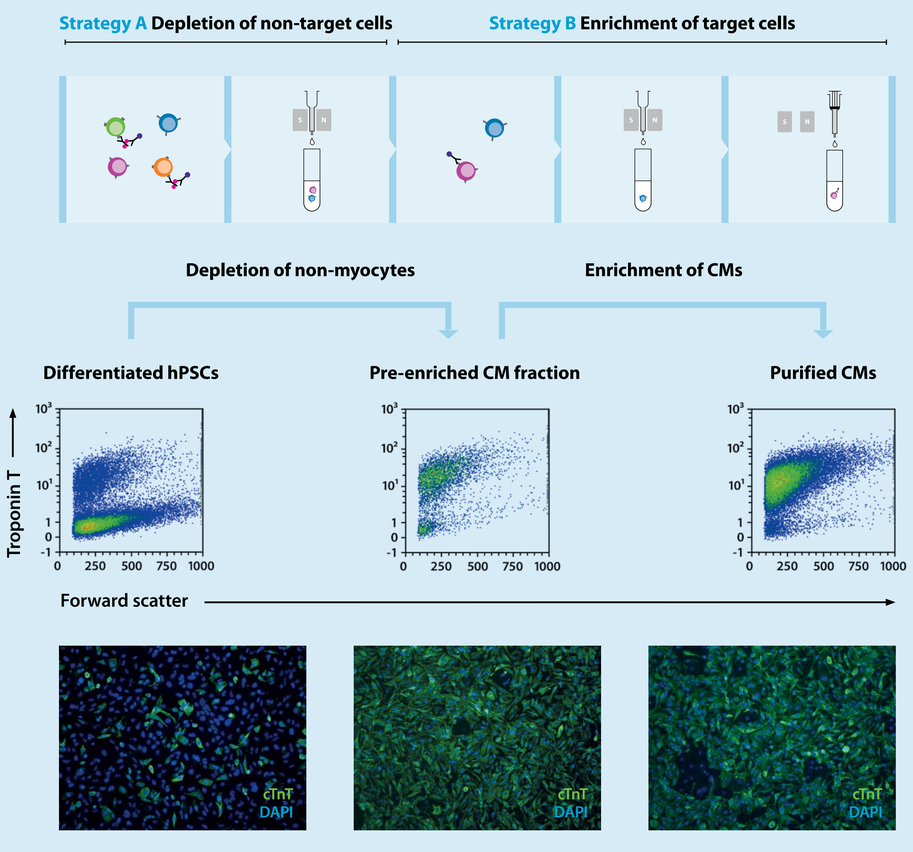

Figure 2

3 Purified CMs are functional and express CM-specific markers

Regardless of the strategy chosen, magnetically enriched CMs attached well, initiated contractions, and could be stably maintained in culture. They showed a typical morphology and sarcomeric structure. Moreover, flow cytometry analysis demonstrated the expression of CM-specific markers, such as α-Actinin, Myosin Heavy Chain (MHC), MLC2v, MLC2a, and Troponin T, indicating that the newly developed cell separation strategy is suitable for enrichment of CMs and different CM subtypes. Labeling with the Ca^{2+}-sensitive dye FLUO-8® indicated functionality of purified CMs, as typical Ca^{2+} fluxes were observed.

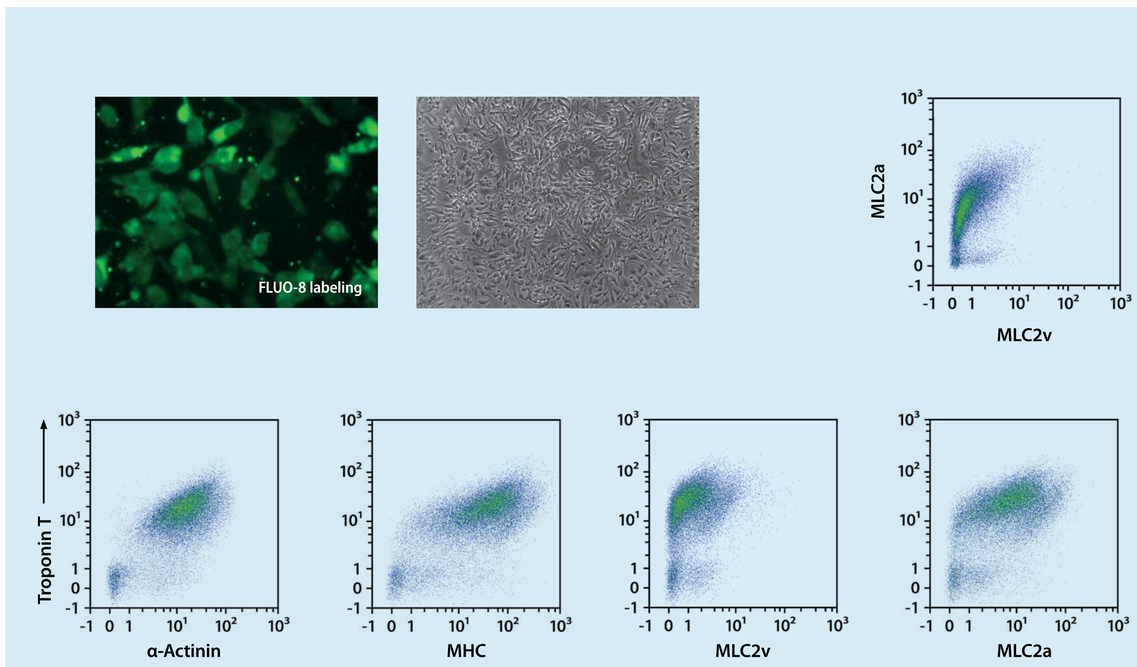

Figure 3

4 Cryopreserved CMs maintain high viability

Enriched CMs could be cryopreserved in StemMACS Cryo-Brew medium. The optimal freezing density was 5×10^6 cells/250 µL. Thawed CMs showed high viabilities and were cultured most efficiently in the presence of StemMACS Thiazovivin and at a cell density of 3×10^5 cells/cm². Cells initiated contractions 24–48 h after thawing.

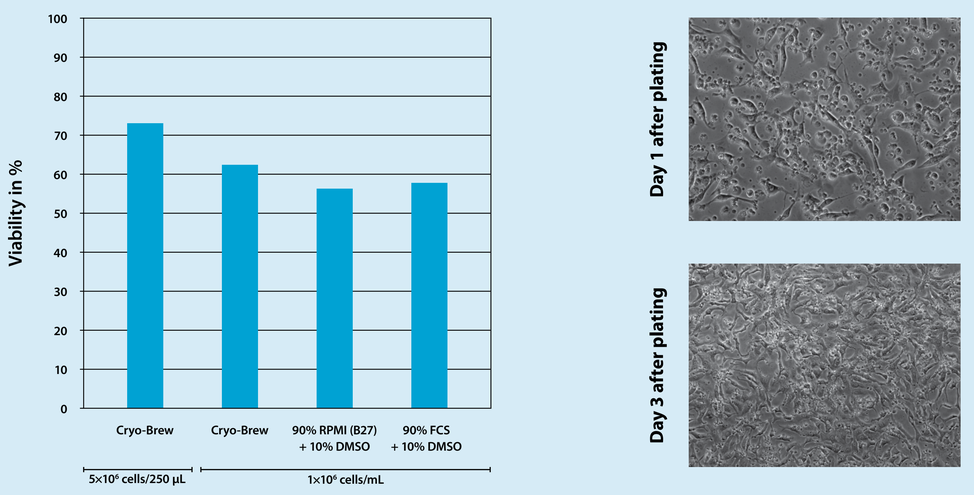

Figure 4

Conclusion

We developed novel tools supporting the workflow for efficient generation, magnetic purification, flow cytometry or immunofluorescence-based characterization, and cryopreservation of hPSC-derived CMs.

- The PSC-derived Cardiomyocyte Isolation Kit, human enables the enrichment of hPSC-derived CMs to high purities regardless of the differentiation efficiency, stem cell clone, differentiation time point, or differentiation protocol used.
- Purified CMs were analyzed using our newly developed recombinant antibody conjugates labeling intracellular cardiac muscle proteins, such as α-Actinin, Troponin T, MHC, MLC2a, and MLC2v for precise detection of CMs and distinction between CM subtypes.
- Magnetically purified CMs start rhythmic contractions 24 h after plating, can be stably maintained in culture, show regular Ca^{2+} fluxes and can be cryopreserved with good viabilities.

Reference
1. Lian, X. *et al.* (2013) Nat. Protoc. 8: 162–175.