Scalable process for standardized generation of human PSC-derived cardiomyocytes

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

Introduction

Fast and highly efficient generation of pure pluripotent stem cell–derived cardiomyocytes (CMs) is a prerequisite for therapeutic applications, drug development, and heart disease modeling. All these applications require controlled and standardized processes to allow for cost-effective, large-scale production of CMs. Nevertheless, cardiovascular differentiation of human pluripotent stem cell (PSC) cultures does not generate homogeneous cell populations, but a rather heterogeneous mixture of CM subpopulations and non-cardiomyocytes. The final cell composition and differentiation efficiencies currently depend, e.g., on the stem cell clone, its passage number, and the differentiation protocol used. To circumvent these experimental variations and prepare for standardized processes suitable for automation and clinical scale-up, we now established a complete workflow from controlled cardiac differentiation to CM harvesting, purification, storage, and analysis (fig. 1A).

Results

1 Cardiovascular differentiation of PSCs

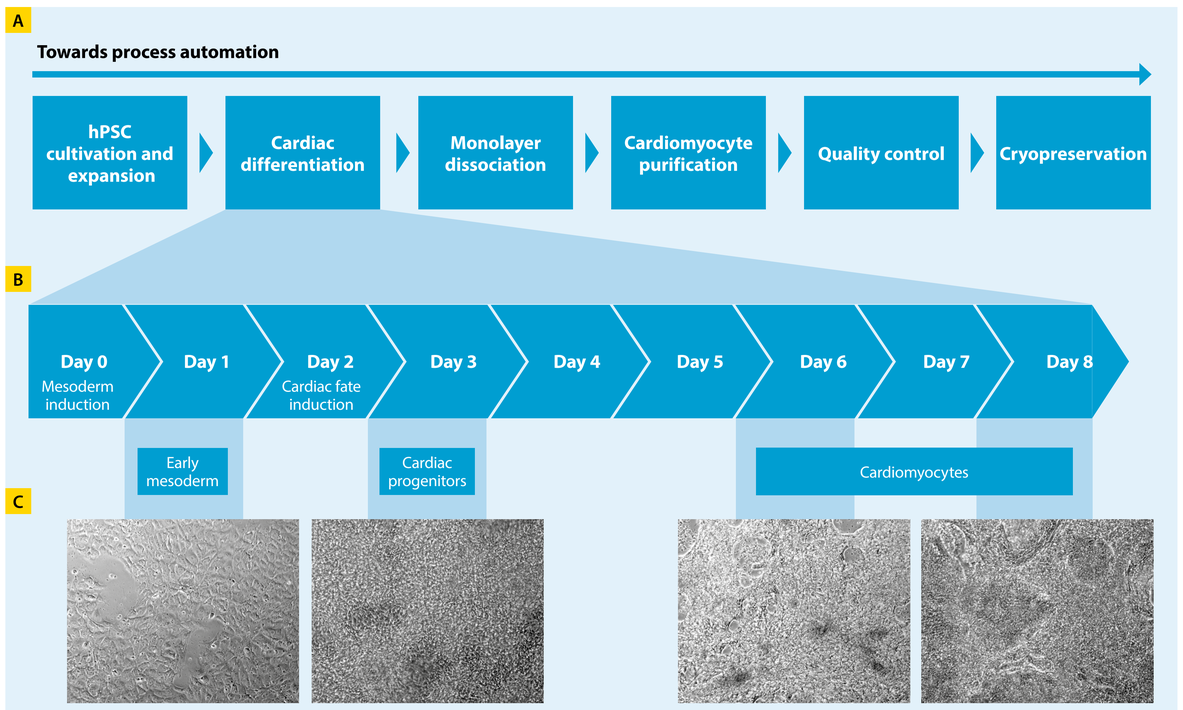

Figure 1

Cardiac differentiation of PSCs was induced stepwise by using three different media (fig. 1B). First, PSCs were seeded as single cells in mesoderm induction medium on plates coated with Matrigel®. Cardiac fate was induced on day 2 of differentiation. Depending on the time point of differentiation, morphological changes could be observed. On day 1, cells started to form clusters, which disappeared on day 3 of differentiation. From days 4 to 6, cardiac clusters started to form (fig. 1C). First contracting cells were observed on days 6–8 of differentiation. This new workflow protocol allows for robust, highly efficient, and scalable generation of CMs within less than 10 days of differentiation, thereby solving several technical issues related to the generation of PSC-derived CMs.

2 Analysis of cardiac differentiation

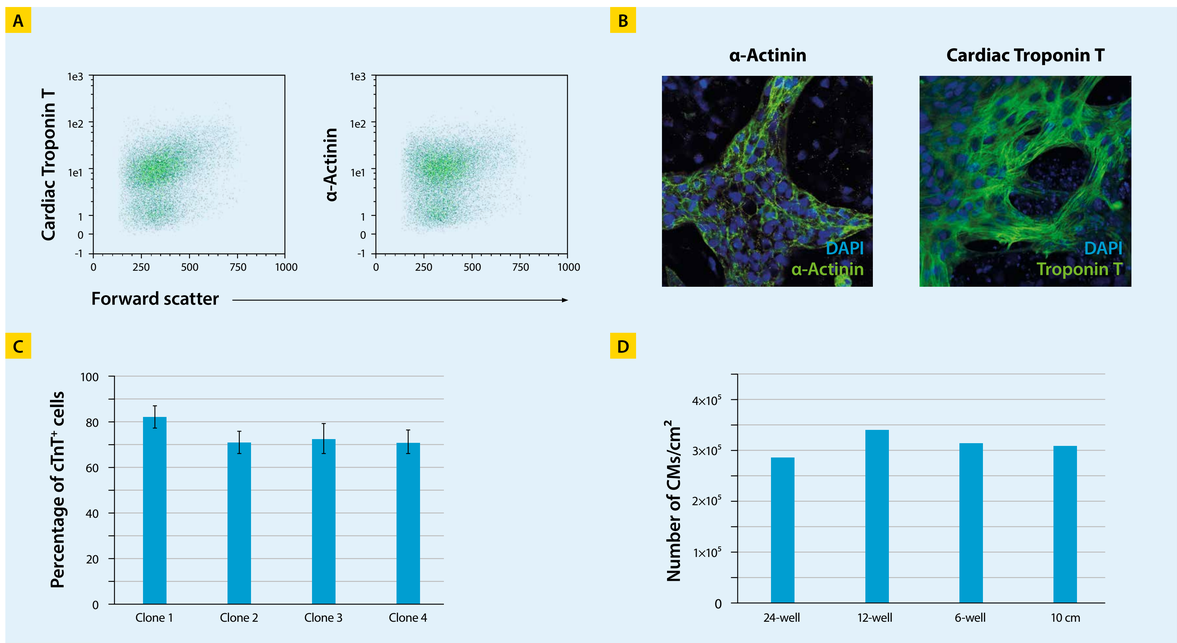

Figure 2

CM cultures were analyzed by flow cytometry and immunofluorescence (fig. 2A, B). Cells expressed specific cardiac markers such as cardiac Troponin T (cTnT) and α-actinin. Transferring the protocol to different stem cell clones yielded differentiation efficiencies of up to 85% with at least 2.5×10^5 CMs/cm^2 (fig. 2C). Further experiments have shown that the protocol can be scaled up to different multi-well plate formats or 10-cm dishes (fig. 2D), thus making it suitable for automation experiments.

3 Purification of PSC-derived cardiomyocytes

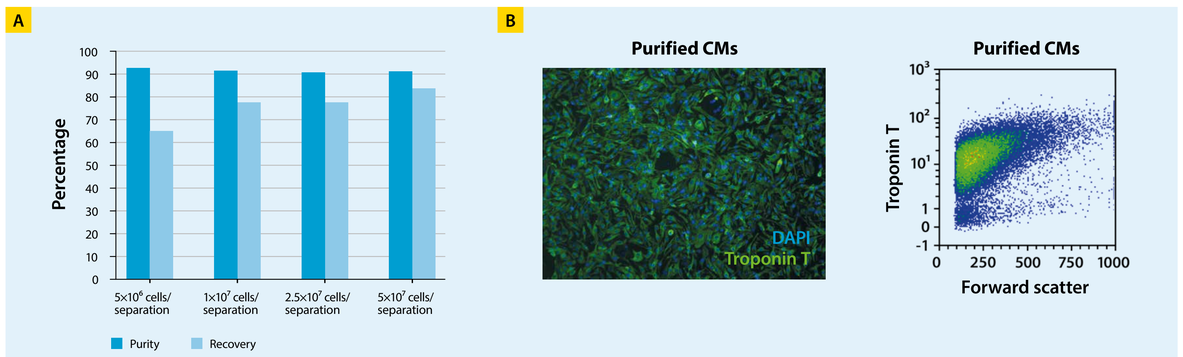

Figure 3

PSC-derived CMs were magnetically purified to >90% using the PSC-Derived Cardiomyocyte Isolation Kit, human (fig. 3). This cell isolation strategy enables the standardized generation of CM populations with reproducibly high purity. The cell separation process could be scaled up for the use of 5×10^7 cells per separation (fig. 3A), thus making it suitable for large cell numbers and therefore applicable for automated processes. Enriched CMs attached well, initiated contractions, and could be stably maintained in culture. They showed the typical morphology and sarcomeric structure. Moreover, flow cytometry analysis demonstrated the expression of CM-specific markers, such as α-actinin, Myosin Heavy Chain (MHC), Myosin Light Chain (MLC) 2a and 2v, and cardiac Troponin T (fig. 3B), indicating that the 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 (not shown).

4 Automation of cardiomyocyte generation

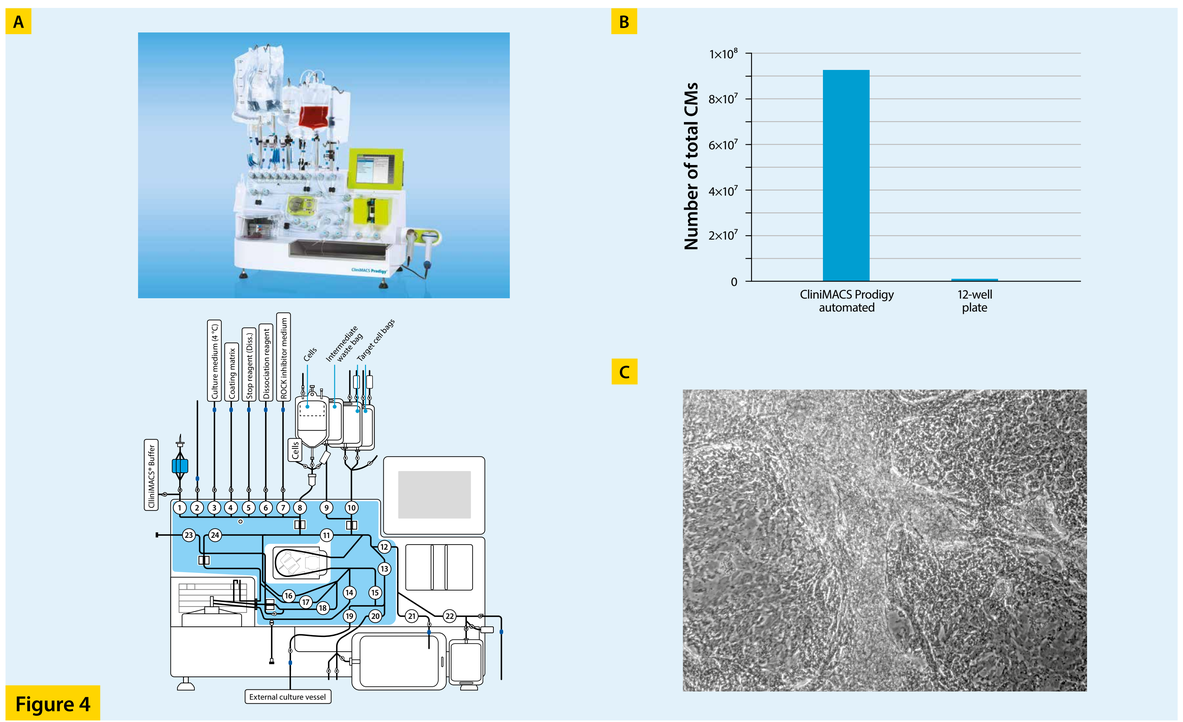

Figure 4

Previously, we developed a PSC cultivation and expansion workflow using the CliniMACS Prodigy® (fig. 4A). In a next step, our workflow protocol for CM generation will be transferred to this functionally closed system enabling the scale-up of CM manufacturing. First data indicated that 9×10^7 CMs could be generated in a single automated production run of the CliniMACS Prodigy connected to a one-layer cell stack system (fig. 4B). Cells formed contracting cardiac clusters after 8 days (fig. 4C), comparably to the small-scale culture. CMs generated with this system showed an expression pattern similar to small-scale differentiations (not shown). Adaptation of the complete workflow and reagents to the integrated cell processing platform of the CliniMACS Prodigy will pave the way for standardized, large-scale manufacturing of PSC-derived CMs.

Conclusion

We developed a novel protocol for standardized CM differentiation, which can be conveniently embedded into a workflow encompassing the standardized differentiation, magnetic and microchip-based purification, flow cytometry– or immunofluorescence-based quality control, and cryopreservation of hPSC-derived CMs. This workflow is a prerequisite for future clinical scale-up and process automation.

- The differentiation protocol enables fast and efficient generation of PSC-derived CMs within less than 10 days.
- The protocol is applicable for different stem cell clones, consistently yielding high differentiation efficiencies.
- The protocol is scalable and can therefore be applied to different plate formats.
- Initial tests showed that the protocol can be transferred to a functionally closed system for large-scale CM manufacturing. First data indicate the possibility of generating 9×10^7 CMs in a one-layer cell stack system.