Application Note 002

Benchtop Automated Differentiation of hiPSCs into Cardiomyocytes Using Our Scientific Co-Pilot, *Emmet*.

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Abstract

Induced pluripotent stem cells (iPSCs) offer a versatile foundation for regenerative and personalised medicine and cardiac research due to their ability to differentiate into cardiomyocytes. However, there are significant challenges in the complexity of both maintaining and differentiating iPSCs into various cell types, including cardiomyocytes. These challenges reduce consistency, scalability, and cost-efficiency, ultimat4ely limiting translation of iPSC technology into clinical applications. We applied a published cardiomyocyte differentiation protocol using *Emmet*, our closed-loop automated cell culture system, to address these limitations. The system was user-programmed to perform media exchanges and reagent delivery according to the published workflow, eliminating the need for manual handling. Assessments using flow cytometry, gene expression analysis, and immunostaining confirmed the generation of high-purity cardiomyocytes. *Emmet* enables efficient, reproducible, and scalable cardiomyocyte production for cardiac research and therapeutic development.

Introduction

Human induced pluripotent stem cells (hiPSCs) are central to advancements in drug development, disease modeling, and cell-based therapies. Their ability to self-renew and differentiate into specialised cells, such as cardiomyocytes, makes them a valuable resource. However, conventional manual culture methods are often labour-intensive and susceptible to variability, posing challenges to reproducibility and scale. To overcome these obstacles, *Emmet* was developed as a cell culture system that streamlines cell culture tasks including media changes, temperature and CO_2 regulation, and reagent addition. This automation reduces human error, improves reproducibility, and increases throughput.

In this application note, we demonstrate the use of *Emmet* to execute a cardiomyocyte differentiation protocol with a hiPSC line. The quality and identity of the resulting cardiomyocytes were verified through immunostaining, flow cytometry, and gene expression analysis.

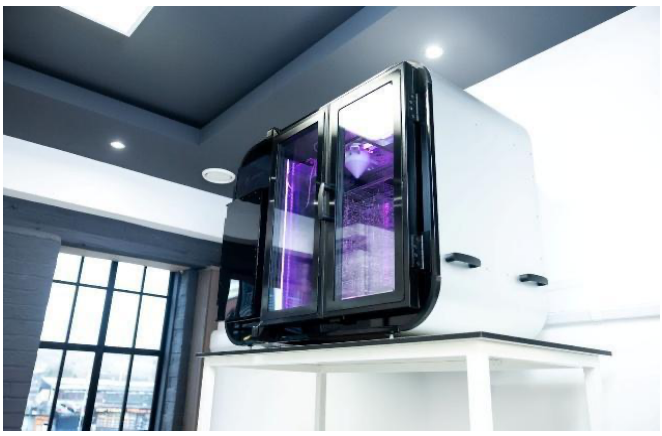

Image 1: *Emmet* in a Unicorn Bio tissue culture (TC) Lab.

Methods

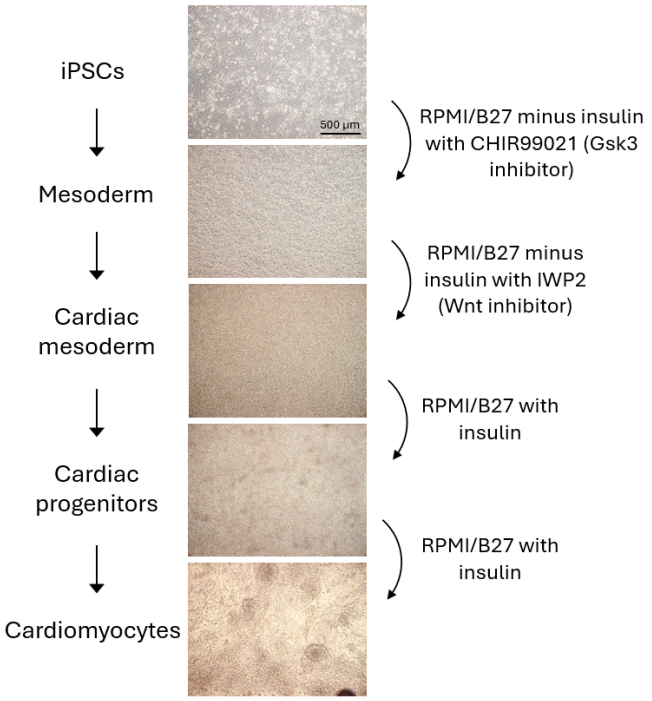

Figure 1: Schematic of cardiomyocyte differentiation from hiPSCs. Overview of the process of hiPSC differentiation into cardiomyocytes using inhibitors for Gsk3 and Wnt. The scale bar represents 500 µm.

Automated Cardiomyocyte Differentiation

hiPSCs were expanded to 80–90% confluence in mTeSR™ Plus (StemCell Technologies) in *Emmet*. For differentiation, cells were dissociated using Accutase (Gibco) and seeded onto Cultrex-coated T25 flasks (ThermoFisher) in mTeSR™ Plus (Stem Cell Technologies) with 5 µM Y-27632 (Tocris). Daily medium exchanges with mTeSR™ Plus were performed until day 5.

On day 5, the medium was replaced with RPMI (Gibco) supplemented with B27 minus insulin (Gibco) and 12µM CHIR99021 (Tocris). After 24 hours (day 6), the media was replaced with fresh RPMI/B27 without insulin (Gibco). On day 8, cells were fed with RPMI/B27 without insulin (Gibco) containing 5µM IWP2 (Tocris), prepared using a 50:50 mixture of conditioned media and fresh RPMI/B27 without insulin (Gibco). From day 10 onward, cultures were maintained in RPMI with full B27 supplement (Gibco), and media was exchanged every other day.

Flow Cytometry

On day 18, cells were dissociated using Accutase (Gibco). Then manually fixed with 4% paraformaldehyde and permeabilised with 0.2% Triton X-100. Cells were stained with antibodies against cardiac troponin T (cTnT) (ThermoFisher, 701620, 1:1000) and myosin heavy chain (MHC) (R&D Systems, MAB4470, 1:100). Samples were analysed using the Aurora flow cytometer (Cytek®).

Gene Expression Analysis

RNA extraction was performed manually using the NEB Monarch Total RNA miniprep kit. 2µg RNA was reverse transcribed into 2µg cDNA using the Applied Biosystems high-capacity cDNA reverse transcription kit. 25ng cDNA was used per well of a 96-well PCR plate. qPCR was performed with primers targeting OCT4, TNNT2, and ISL1. Reactions were run on the QuantStudio 1 real-time PCR system (ThermoFisher) and analyzed using the ΔΔCt method.

Immunostaining

Cells were fixed in 4% paraformaldehyde, stained with DAPI (Invitrogen, D1306, 1:10,000) and phalloidin (Invitrogen, A12380, 1:400), then immunofluorescently imaged using the EVOS FLoid (ThermoFisher).

Results

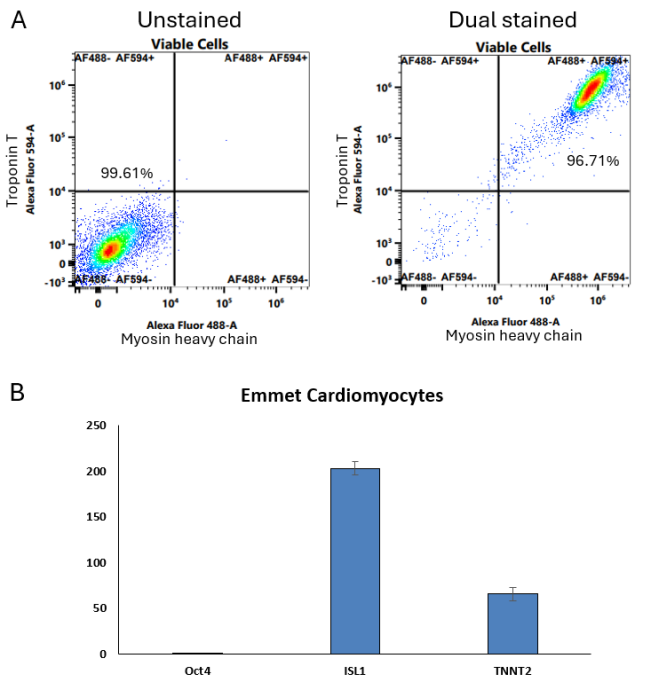

Figure 2. Molecular and phenotypic characterisation of cardiomyocytes generated with *Emmet*. A) Flow cytometry plots showing 99.61% of unstained cells do not express cardiac troponin T (cTnT) and myosin heavy chain (MHC) and 96.71% of dual stained cells co-express cardiac troponin T (cTnT) and myosin heavy chain (MHC). (B) qPCR analysis showing upregulation of TNNT2 and ISL1, and downregulation of OCT4 in cardiomyocytes cultured in *Emmet*.

Molecular Characterization

Emmet generated cardiomyocytes with high purity. Flow cytometry revealed that 96.71% of differentiated cells co-expressed cardiac troponin T (cTnT) and myosin heavy chain (MHC), confirming successful cardiomyocyte differentiation. Gene expression analysis showed marked upregulation of TNNT2 and ISL1 and downregulation of OCT4, indicating a transition from a pluripotent to a cardiomyocyte-specific profile.

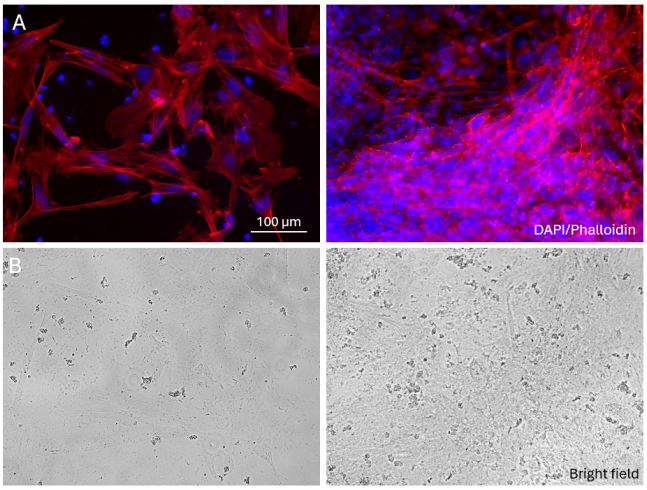

Figure 3. Fluorescence and brightfield imaging of cardiomyocytes cultured in *Emmet*. A) Cardiomyocytes cultured using *Emmet* were stained with DAPI (blue) to visualise nuclei and Phalloidin (red) to visualise filamentous actin. B) Brightfield images provide structural context and overall cell morphology. Scale bars represent 100 µm.

Structural and Functional Maturity

Immunostaining with phalloidin showed well-organised actin filaments. Spontaneous beating was observed consistently by day 18, confirming the functional maturation of the cardiomyocytes. Together, these structural and functional indicators support the successful implementation of cardiomyocyte differentiation in *Emmet*.

Discussion

The automated *Emmet* workflow generated high-purity cardiomyocytes from hiPSCs using a small-molecule Wnt-modulation protocol executed entirely without manual handling. Quantitative analyses confirmed robust lineage specification and maturation: more than 96 percent $cTnT^{+}/MHC^{+}$ cells by flow cytometry, upregulation of *TNNT2* and *ISL1*, and spontaneous contractility with organized sarcomeric structure by day 18. These results confirm that *Emmet* can reproduce multistage differentiation workflows with consistency comparable to conventional manual systems.

Automation of this process offers more than efficiency. Standardized media exchanges, temperature control, and reagent timing minimize user-dependent variability, ensuring consistent exposure to differentiation cues across experiments. This precision enhances reproducibility and improves data comparability between replicates, cell lines, and laboratories. Once a protocol is validated, it can be digitally transferred to other *Emmet* systems, allowing identical differentiation profiles to be achieved across sites and teams.

These findings demonstrate that *Emmet* supports reliable and scalable production of functional cardiomyocytes, bridging laboratory research and preclinical manufacturing. As workflows expand to other lineages, *Emmet* provides a foundation for reproducible, high-quality stem-cell differentiation in regenerative medicine, disease modeling, and high-throughput screening.

References

1. Lian, X., Hsiao, C., Wilson, G., Zhu, K., Hazeltine, L. B., Azarin, S. M., Raval, K. K., Zhang, J., Kamp, T. J., & Palecek, S. P. (2012). Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 109(27), E1848–E