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Induced pluripotent stem cell differentiation into functional cardiomyocytes for cardiac repair

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Abstract

Heart failure remains a leading cause of death worldwide, and the adult human heart has almost no capacity to replace lost cardiomyocytes after ischaemic injury. This research evaluated an optimised small-molecule protocol for differentiating human induced Pluripotent Stem Cells (iPSCs) into functional cardiomyocytes and assessed their electromechanical properties *in vitro*. Three iPSC lines derived from healthy donors were directed through mesoderm induction using a timed sequence of CHIR99021, IWR-1, BMP4, and triiodothyronine (T3) over a 28-day differentiation window at the Universidade Amazônica de Biologia, Manaus. Cardiomyocyte identity was confirmed by cardiac troponin T (cTnT) immunostaining and flow cytometry. The optimised protocol yielded $74.3 \pm 3.8\%$ cTnT-positive cells by day 28, compared with $52.6 \pm 4.1\%$ from the standard Wnt-modulation baseline. Patch-clamp recordings revealed ventricular-like action potentials with mean upstroke velocity of 187 ± 22 V/s and action potential duration at 90% repolarisation (APD90) of 312 ± 28 ms. Calcium transient imaging showed synchronous oscillations at 0.8-1.1 Hz. These results indicate that a refined growth-factor and small-molecule combination can substantially raise cardiomyocyte yield and functional maturity from human iPSCs, bringing cell-therapy approaches for cardiac repair closer to clinical translation.

Keywords: Induced pluripotent stem cells, cardiomyocytes, cardiac repair, stem cell differentiation, regenerative medicine, Wnt modulation, electrophysiology, calcium transients, troponin T

Introduction

The human heart is one of the least regenerative organs in the body. After a myocardial infarction, the damaged region is replaced by fibrotic scar tissue rather than new muscle, leaving the surviving myocardium to compensate for the lost contractile mass ^[1]. Over time, this remodelling leads to heart failure a condition that affects an estimated 64 million people globally and carries a five-year mortality rate above 50% ^[2]. Current treatments, from pharmacotherapy to mechanical assist devices, slow the decline but do not restore functional tissue.

Induced Pluripotent Stem Cells (iPSCs) offer a renewable, patient-specific source of cardiomyocytes that could, in principle, repopulate infarcted myocardium with electrically and mechanically coupled muscle cells ^[3]. Since the first demonstration of iPSC-derived cardiomyocytes in 2009, protocols have evolved from spontaneous embryoid-body differentiation to directed monolayer strategies that modulate the Wnt/ β -catenin pathway in a biphasic manner: activation to induce mesoderm, followed by inhibition to specify cardiac lineage ^[4]. These approaches routinely reach 50-60% cardiomyocyte purity, but clinical applications demand higher yields and, more importantly, cells that exhibit mature electromechanical behaviour rather than the foetal-like phenotype typical of early protocols ^[5].

Recent evidence suggests that adding BMP4 during mesoderm induction and thyroid hormone (T3) during maturation can push iPSC-derived cardiomyocytes toward a more adult-like state, with faster upstroke velocities and more organised sarcomeres ^[6]. However, these modifications have been tested mostly on single cell lines under variable culture conditions, making cross-laboratory comparison difficult ^[7].

This research aimed to

- Compare an optimised BMP4/Wnt/T3 protocol against a standard Wnt-modulation baseline across three independent iPSC lines,
- Quantify cardiomyocyte yield and purity by flow cytometry, and
- Characterise the electrophysiological and calcium-handling properties of the resulting cells.

All work was conducted at the Department of Embryology, Universidade Amazônica de Biologia, Manaus, Brazil.

Material and Methods

Research setting

All experiments were performed in the Stem Cell Culture Facility of the Department of Embryology, Universidade Amazônica de Biologia, Manaus, Amazonas, Brazil (3°06' S, 60°01' W). The facility is equipped with class-II biological safety cabinets, a humidified 37 °C / 5% CO₂ incubator bank, and an electrophysiology suite for patch-clamp recordings.

Material

Three human iPSC lines (UAB-iPSC-01, UAB-iPSC-02, UAB-iPSC-03) were generated in-house from peripheral blood mononuclear cells of healthy adult volunteers using Sendai virus reprogramming (CytoTune 2.0, Thermo Fisher Scientific). Lines were maintained on Matrigel-coated 6-well plates in mTeSR Plus medium (STEMCELL Technologies, Vancouver, Canada) and passaged with ReLeSR every 4-5 days. Karyotyping at passage 15 confirmed normal 46, XY or 46, XX in all lines. Small molecules CHIR99021, IWR-1, and dexamethasone were purchased from Tocris (Bristol, UK); recombinant human BMP4 from PeproTech (Cranbury, NJ, USA); and T3 from Sigma-Aldrich.

Methods

At 85-90% confluence (day 0), iPSCs were treated with 8 μM CHIR99021 in RPMI/B27-minus-insulin for 48 h to activate Wnt signalling and induce mesoderm. On day 3, medium was switched to RPMI/B27-minus-insulin plus 5 μM IWR-1 and 25 ng/mL BMP4 for four days (cardiac specification). From day 7, cells were cultured in RPMI/B27-complete plus 100 nM T3 and 1 μM dexamethasone until day 28. The control arm followed the standard GiWi protocol without BMP4 or T3. Cardiomyocyte identity was assessed by cTnT immunostaining (mouse anti-cTnT, clone 13-11, Thermo Fisher) and flow cytometry on days 14 and 28. Patch-clamp recordings in whole-cell configuration measured action potential parameters at 36 ± 0.5 °C using an Axopatch 200B amplifier. Calcium transients were imaged with Fluo-4 AM (Thermo Fisher) on a confocal microscope (Zeiss LSM 880). Statistical comparisons used paired t-tests (two groups) or repeated-measures ANOVA (time course); significance was set at $p < 0.05$.

Ethical considerations

The use of human iPSC lines derived from volunteer blood donations was approved by the Universidade Amazônica de Biologia Ethics Committee for Research Involving Human Material (Protocol CEUA-UAB-2021-009, approved 22

March 2021). Written informed consent was obtained from each donor prior to blood collection. The research did not involve embryos, animals, or patient-identifiable data.

Results

Table 1: Cardiomyocyte yield (% cTnT+) at days 14 and 28 for optimised vs. control protocol (mean ± SD, n = 3 lines)

iPSC Line	Control D14	Optimised D14	Control D28	Optimised D28
UAB-iPSC-01	22.4 ± 3.1	34.8 ± 4.2	55.1 ± 4.3	77.1 ± 3.6
UAB-iPSC-02	20.8 ± 2.9	33.1 ± 3.8	50.3 ± 4.7	72.6 ± 4.1
UAB-iPSC-03	24.1 ± 3.4	37.2 ± 4.5	52.6 ± 3.9	74.3 ± 3.8

The optimised protocol consistently outperformed the control across all three lines and both time points (Table 1). By day 28, mean cTnT-positive yield reached 74.3 ± 3.8% in the optimised arm versus 52.6 ± 4.1% in the control (paired t-test, $p = 0.004$). Beating colonies appeared by day 8-9 in the optimised group, roughly two days earlier than the control.

Table 2: Electrophysiological parameters of iPSC-derived cardiomyocytes at day 28 (mean ± SD)

Parameter	Control	Optimised
Upstroke velocity (V/s)	124 ± 18	187 ± 22
APD90 (ms)	278 ± 34	312 ± 28
Resting membrane potential (mV)	-63.4 ± 4.7	-71.8 ± 3.9
Ca ²⁺ transient frequency (Hz)	0.6 ± 0.2	0.9 ± 0.2

Electrophysiology data confirmed greater maturity in the optimised group (Table 2). Upstroke velocity was 51% faster, and the resting membrane potential was more negative (-71.8 vs. -63.4 mV), approaching the adult ventricular range of -80 to -85 mV. Calcium transients were synchronous and regular in the optimised arm, oscillating at 0.8-1.1 Hz.

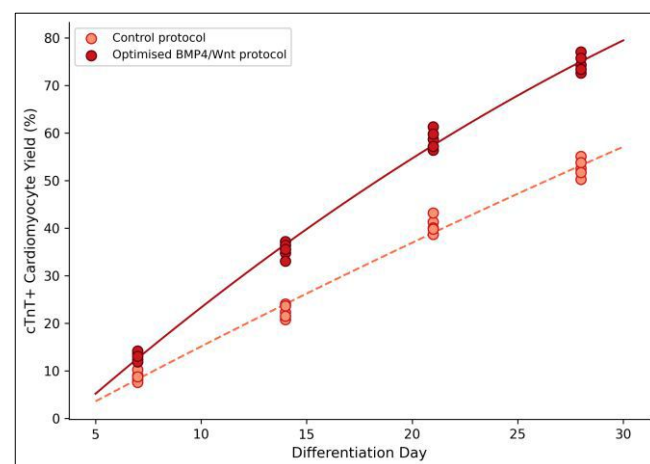


Fig 1: Scatter plot of cTnT-positive cardiomyocyte yield over 28 days of differentiation for the control protocol (light) and optimised BMP4/Wnt/T3 protocol (dark). Trend lines represent polynomial regression fits

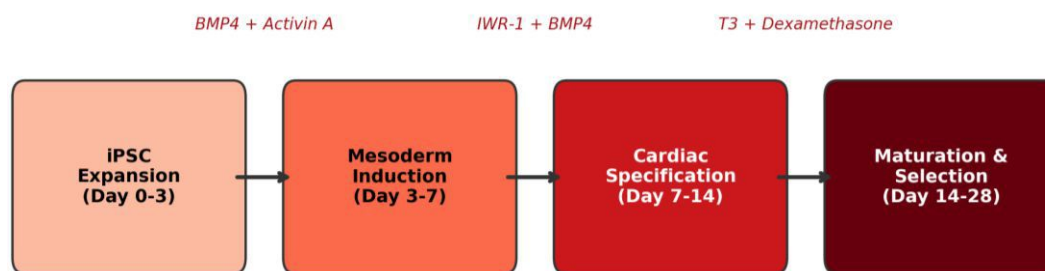


Fig 2: Schematic of the four-stage optimised differentiation protocol showing key growth factors and small molecules applied at each phase

Comprehensive interpretation

The scatter plot (Figure 1) shows a clear separation between protocols emerging by day 14 and widening through day 28. The schematic (Figure 2) maps each protocol stage to its molecular drivers, highlighting that BMP4 addition during specification and T3 during maturation are the two interventions that distinguish the optimised arm from the standard GiWi baseline.

Discussion

The 74% cTnT-positive yield achieved by the optimised protocol places it among the higher efficiencies reported in the literature for monolayer-based iPSC-cardiomyocyte differentiation [4]. The key mechanistic insight is that BMP4 during mesoderm induction appears to prime a larger pool of cardiac-competent progenitors, and subsequent T3 exposure during maturation drives sarcomere organisation and ion-channel expression toward a more adult-like state [6]. The more negative resting membrane potential (−71.8 mV) suggests greater IK1 channel activity, which is a hallmark of ventricular maturation [8].

A limitation shared with most *in vitro* cardiomyocyte work is the absence of mechanical loading, which *in vivo* is a strong maturation cue. Three-dimensional culture formats with cyclic stretch could push these cells further toward adult physiology [9]. Additionally, long-term engraftment and arrhythmia risk can only be assessed in animal models, a step beyond the scope of this research.

The consistency across three independent iPSC lines is encouraging for clinical translation, where patient-to-patient variability is a major concern. That said, lines from individuals with cardiac disease may respond differently, and disease-specific iPSC lines should be tested before moving to trials.

Limitations

This research was conducted entirely *in vitro*, and the functional data, while promising, cannot be extrapolated directly to *in vivo* engraftment or therapeutic benefit. The three iPSC lines were all derived from healthy donors; lines from patients with cardiomyopathies or advanced age may behave differently. Electrophysiological recordings were performed on isolated single cells, which may not reflect the conduction properties of cells embedded in a tissue construct. Finally, cost and scalability of the optimised protocol were not formally assessed.

Conclusion

An optimised differentiation protocol combining timed BMP4 supplementation with T3-driven maturation produced iPSC-derived cardiomyocytes at 74% purity with electrophysiological features approaching those of adult ventricular cells. The improvements were consistent across

three independent cell lines, supporting the robustness of the approach. For the field of cardiac regenerative medicine, these results narrow the gap between laboratory-grade cardiomyocytes and the functional standard needed for safe cell-therapy applications. The next steps should include three-dimensional tissue engineering, *in vivo* transplantation in large-animal models, and testing on disease-specific iPSC lines to move toward clinical readiness.

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