



Simultaneous electro-dynamic stimulation accelerates maturation of engineered cardiac tissues generated by human iPSC cells

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ABSTRACT

Electrical and dynamic stimulation are commonly employed to enhance the maturation of engineered cardiac tissue (ECT) derived from human induced pluripotent stem cells (iPSCs), reflecting the physiological environment of the heart. While electrical stimulation mimics natural bioelectrical signals and dynamic stimulation replicates mechanical forces, the combined effects of these stimuli on ECT maturation have not been thoroughly explored. We hypothesized that simultaneous electro-dynamic stimulation would enhance ECT maturation and function more effectively than either stimulus alone. Human iPSC-derived cardiovascular cells were co-cultured with Collagen I and Matrigel for 2 weeks, followed by a comparative analysis of four groups: no stimulation, dynamic stimulation, electrical stimulation, and simultaneous electro-dynamic stimulation. The functionality of ECTs was assessed by measuring contractile capacity and calcium indicators, and histological assessments examined structural maturation. Our results demonstrated that simultaneous electro-dynamic stimulation significantly increased the CM component, elevated *TNNT2* mRNA expression levels, and enhanced calcium transient capacity. Additionally, ECTs subjected to simultaneous stimulation exhibited a positive force-frequency relationship in contractility and an elevation in peak calcium flux, indicative of advanced tissue maturation. Moreover, simultaneous stimulation promoted vascular network formation within the ECTs, suggesting improved structural organization. These findings underscore the importance of simultaneous stimulation for developing effective cardiac tissue engineering strategies.

1. Introduction

Cardiac tissues generated from pluripotent stem cells (PSCs) hold significant promise as platforms for patient-specific studies of physiology and disease. However, the predictive power of these models remains constrained due to the immature state of the derived cardiac tissues. Efforts to enhance the maturation process are essential to improve the fidelity and reliability of these models for more accurate physiological and pathological studies [1–3].

Biophysical stimuli, such as electrical stimulation, have recently been recognized as critical factors in promoting cardiac tissue maturation. The native environment of the heart is inherently dynamic, characterized by continuous electrical impulses, suggesting that replicating these conditions in vitro could enhance the maturation process [4–6]. Dynamic rocking culture with cyclic see-sawing stimuli using digital rockers has been reported to promote the tissue maturation of human

PSC-engineered cardiac tissues [7,8]. Although both electrical and dynamic stimulation are standard methods of physical stimulation in cardiac construct development, to date, there have been no direct simultaneous combinations of electrical and dynamic stimulation to cardiac tissue derived from human PSCs. In the present study, we hypothesized that simultaneous electro-dynamic stimulation would accelerate human induced pluripotent stem cell (iPSC)-derived cardiac tissue maturation to resemble in vivo conditions.

2. Results

2.1. Optimizing rocking conditions to enhance maturation of hiPSC-derived engineered cardiac tissues (ECTs)

We generated human iPSC-derived ECTs using a co-culture approach involving cardiovascular cells derived from human iPSCs (including

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vascular endothelial cells and vascular mural cells) and extracellular matrix proteins as previously described (Fig. 1A) [9,10]. The composition of cells at the time of ECT formation is shown in Extended Fig. 1A and B. To determine the optimal rocking frequency for cardiomyocyte maturation, we tested control (no rocking), 30 rpm, and 60 rpm. The 30-rpm condition significantly increased cardiac isoform of troponin-T (cTnT) staining intensity, indicating better cell alignment and tissue organization. Apoptosis was minimal at 30 rpm, whereas control and 60-rpm showed higher prevalence of TdT-mediated dUTP Nick End Labeling (TUNEL) staining-positive cells, particularly in the tissue center (Extended Fig. 1C and D). The 30-rpm rocking condition showed an elevation in the expression levels of *TNNT2* compared to control and 60-rpm (Extended Fig. 1E). Based on these results, the 30-rpm rocking condition was determined to be more effective for promoting cardiac tissue maturation.

2.2. Structural maturation of engineered cardiac tissues under simultaneous electro-dynamic stimulation

Next, we conducted a comparative analysis of four groups: no stimulation (E-R-), dynamic stimulation (E-R+), electrical stimulation (E + R-), and simultaneous electro-dynamic stimulation (E + R+) (Fig. 1B). To reproduce the synchronization of the timing between the electrical stimulation from the sinoatrial node and the volume change pattern associated with blood ejection from the ventricles of the native heart,

this study introduced a specially designed sensor to provide simultaneous electrical and dynamic stimulation. This sensor generates electrical pulses timed with the end of the see-sawing dynamic motion for mechanical stimulation (Fig. 1C) (Video 1). Both E-R+ and E + R-stimuli increased cTnT staining levels, with the most pronounced effects seen in E + R+. TUNEL staining results indicated that E-R- showed the highest apoptosis, while both E-R+ and E + R-significantly reduced apoptosis individually. The E+R+ condition increased apoptosis slightly compared to single stimuli but less than the control, likely due to higher cell density in the center compared to E-R+ and E + R- (Fig. 1D and E). The immunofluorescence staining results for cTnT indicated that E+R+ stimulation enhanced the alignment, elongation, and organization of cardiomyocytes and sarcomere structures in cardiac tissues (Fig. 1F).

2.3. Enhanced positive force-frequency relationship and Gene Expression in ECTs under simultaneous electro-dynamic stimulation

Next, we evaluated contractile force using MUSCLEMOTION, a versatile video-based system that quantifies the motion amplitude of the ECTs [11]. In the E-R- group, we observed a reduction in motion amplitudes across the entire beating cycle as the frequency of external electrical stimulation increased from 1000 ms (1 Hz) to 500 ms (2 Hz), indicating a negative force-frequency relationship (FFR) (Video 2). Similarly, the E-R+ group showed a negative FFR under the same conditions. In the E + R-group, we initially observed an increase in motion

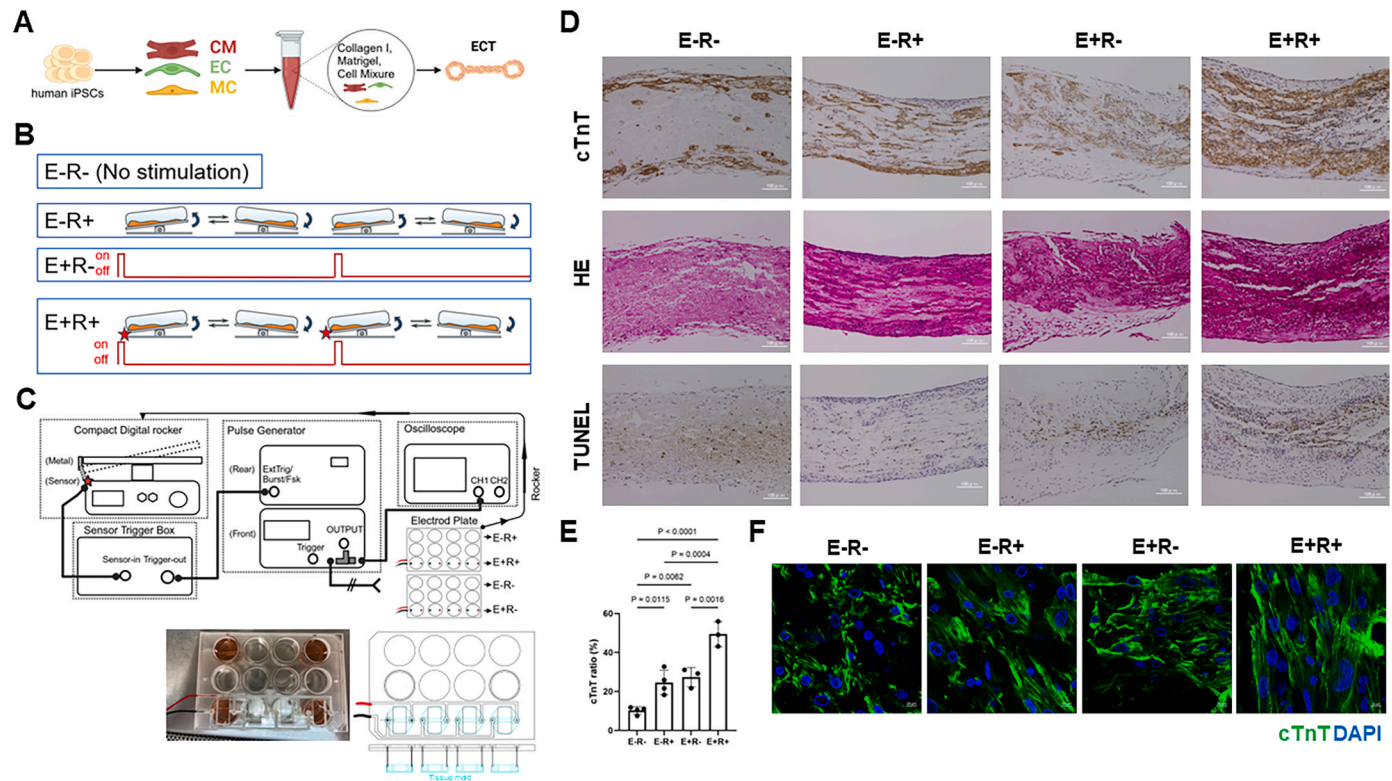


Fig. 1. Overview of Differentiation, Stimulation Protocols, and Histological Analysis of Engineered Cardiac Tissues (ECTs).

(A) Schematic representation of the differentiation and generation process for engineered cardiac tissues (ECTs). Human induced pluripotent stem cells (iPSCs) are differentiated into cardiomyocytes (CM), vascular endothelial cells (EC), and vascular mural cells (MC). ECT, Engineered cardiac tissue. (B) Schematic representation of experimental conditions for ECT stimulation. E-R-: No stimulation condition. E-R+: Rocking stimulation without electrical stimulation. E + R-: Electrical stimulation without rocking. The red lines indicate the on-off pattern of electrical stimulation. E + R+: Combined electrical and rocking stimulation. The red lines represent the on-off pattern of electrical stimulation, synchronized with the rocking motion applied to the ECT. The red stars indicate specially designed sensors that generate electrical pulses timed with dynamic stimulation. (C) Schematic of the experimental setup for simultaneous combined electrical and mechanical stimulation of ECTs. (D) Histology of the cardiac isoform of troponin-T (cTnT) immunostaining, Hematoxylin and Eosin (HE), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) under conditions E-R-, E-R+, E + R-, and E + R+. Scale bars = 100 μ m. (E) Quantitative analysis of the cTnT positive ratio among the whole tissue area evaluated by cTnT immunostaining. (F) Representative immunofluorescent staining of cTnT for ECTs by confocal microscopy. DAPI: 4',6-diamidino-2-phenylindole. Scale bars = 10 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

amplitudes, indicating a positive FFR from 1000 ms (1 Hz) to 800 ms (1.25 Hz) (or 700 ms = 1.43 Hz), but it became negative as the frequency increased further to 500 ms (2 Hz). In contrast, the E+R+ group consistently demonstrated an increase in motion amplitudes across the entire beating cycle from 1000 ms (1 Hz) to 500 ms (2 Hz), indicating a clearly positive FFR (Fig. 2A and B) (Video 3). The peak FFR observed was approximately 2.0 Hz, which is similar to the previously reported peak frequency of 2.0–2.5 Hz in human myocardial tissue [12]. Additionally, in the E+R+ group, we observed significantly increased expression levels of *TNNT2*, *TNNI1*, and *TNNI3* compared to the E-R group and even higher than in the single stimulation groups. However, the *TNNI3/TNNI1* ratio did not reach its maximum in the E+R+ condition and was not significantly different from other groups. These results indicate that we have not necessarily achieved full myocardial cell maturation, but achieved significant maturation of the myocardial tissue (Fig. 2C). This suggests that the combination of electrical and dynamic stimulation works better in promoting cardiac tissue maturation and function.

2.4. Enhanced calcium handling and sarcoplasmic reticulum function in ECTs through simultaneous electro-dynamic stimulation

We posited that one of the mechanisms behind tissue maturation could involve the enhancement of calcium handling capacity, which is a known indicator for cardiomyocyte maturation. To assess this, we visualized intracellular calcium transients using Calbryte 520, a calcium indicator. Our findings revealed a marked improvement in calcium turnover capacity in the E+R+ group, evidenced by measurements of peak calcium flux and the rates of increase and decrease in intracellular calcium concentration of spontaneously beating ECTs (Fig. 2D and E) (Video 4). Furthermore, we observed a significant elevation in the

expression levels of key components of the sarcoplasmic reticulum (SR), namely *SERCA2* and *PLN*, following simultaneous electro-dynamic stimulation (Fig. 2F).

2.5. Enhanced vascular network formation in ECT through simultaneous dynamic and electro-dynamic stimulation

Vascular network formation is reported to be one of the fundamental factors for the establishment of mature heart organoids derived from human pluripotent stem cells [13]. Dynamic culture has been reported to enhance vascular network formation in hiPSC-derived cardiac tissue containing vascular cells [14–16]. Building on this finding, we used confocal imaging in the static stimulation group (E-R-, E+R-) and could not observe vascular network formation. However, in the dynamic stimulation group (E-R+, E+R+), better vascular network formation was observed, especially in the E+R+ group (Fig. 2G).

3. Discussion

The physical stimulation of bioengineered cardiac tissue has gained significant interest as a method to develop constructs that closely mimic healthy myocardium. Mechanical and electrical stimulations have been standard methods for tissue maturation, and there are several studies of the combination of electrical stimulation and mechanical stretch stimulation for bioengineered cardiac tissues utilizing mesenchymal stem cells or primary cardiac cells [17,18]. However, no study has previously investigated combined electrical and dynamic stimulation of cardiac tissue from human PSCs to mimic the in vivo environment. We hypothesized that simultaneous electro-dynamic stimulation would closely mimic the mechanical and electrical environment of the native heart. To study this, we developed a novel sensor system to precisely control the

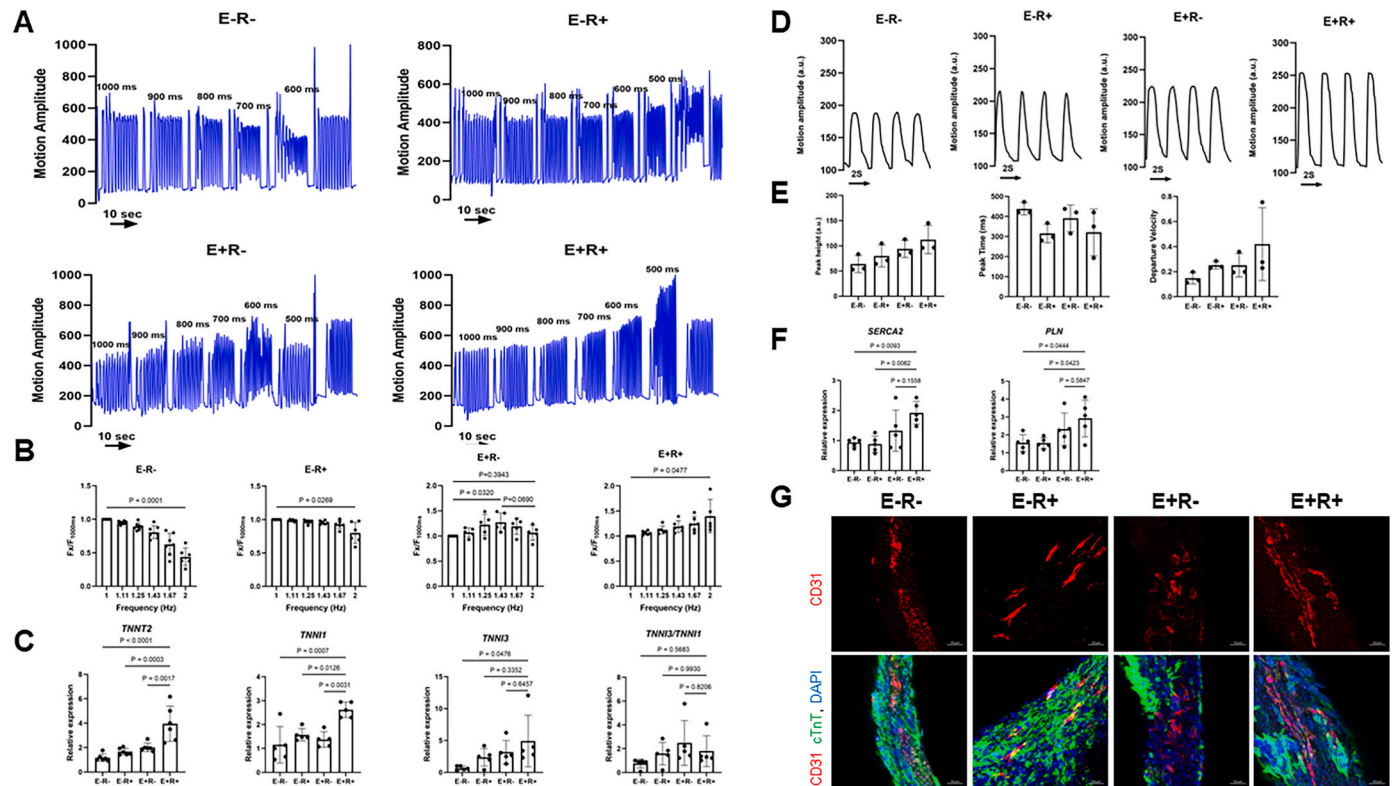


Fig. 2. Assessment of Motion Amplitude, Gene Expression, Calcium Oscillation, and Vascular Network Formation in ECTs. (A) Representative motion amplitude and (B) Quantification in the motion amplitude in accordance with the interval and frequency of external electrical stimulation. (C) RT-qPCR. *TNNT2*: cardiac troponin T2; *TNNI1*: troponin I1, *TNNI3*: troponin I3. (D) Representative calcium oscillation in spontaneous beating, and (E) Peak height, peak time and departure velocity. (F) RT-qPCR. *CACNA1C*: calcium voltage-gated channel subunit alpha1 C, *SERCA2*: sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase, *PLN*: phospholamban. (G) Representative CD31 and cTnT confocal immunofluorescent imaging of the ECTs. Scale bars = 50 μm .

timing of electrical stimulation at the end of the see-sawing motion which highly recapitulates normal human heart physiology.

Our results indicated that the E+R+ condition showed higher cTnT-positive area, higher cell density, and better tissue organization, indicating that simultaneous electro-dynamic stimulation enhanced tissue structural maturation. On the other hand, in the E+R+ group, we observed increased apoptosis in the center of the tissue. This suggests that under these conditions, as in vitro tissue formation progressed, cell density increased, and the supply of oxygen and nutrients, which relies solely on direct diffusion from the tissue surface in an in vitro culture environment, was insufficient to keep all the cells healthy. However, considering that only under the E+R+ condition was a rich vascular network formed within the ECT, it is expected that when this ECT under the E+R+ condition is used for transplantation therapy into the heart, it can quickly achieve blood supply from the host after transplantation. As a result, central apoptosis observed in the in vitro study may not occur, leading to more efficient cardiac regenerative therapy.

A key observation was that our engineered heart tissue demonstrated a positive FFR and maximum capture rate at 2 Hz under simultaneous electro-dynamic stimulation. This characteristic, fundamental to native heart muscle, indicates progress in developing realistic human myocardium models. A positive FFR in the native heart can be induced by several factors: proper cardiomyocyte alignment, organized sarcomeres enhancing contractions, efficient SR function ensuring rapid calcium handling, high mitochondrial density providing necessary ATP, and a well-structured extracellular matrix (ECM) supporting structural integrity and force transmission [12,19]. In line with these reports, our data showed increased *TNNT2* expression, greater cardiomyocyte alignment, better sarcomere organization, and enhanced SR function, contributing to a positive FFR.

Vascularized organoids are particularly valuable in regenerative medicine because the development of vasculature within organoids enhance nutrient and oxygen delivery, facilitate waste removal, and integrate more effectively with host tissues. These improvements promote more effective and sustained tissue repair and regeneration, making vascularized organoids a critical advancement in the field. In our study, the dynamic stimulation groups (E-R+, E + R+) exhibited better vascular network formation, with the most significant improvements observed in the E+R+ group. The ability of our system to create vascularized organoids offers great benefits to regenerative medicine by improving tissue function and repair outcomes. These findings highlight the critical role of simultaneous electro-dynamic stimulation protocols in enhancing the effectiveness of cardiac tissue engineering strategies.

4. Methods

4.1. Human iPSC culture and differentiation

In this study, we used the human iPSC line 201B6 [20]. The expansion, maintenance, and differentiation of the iPSCs into cardiovascular cells were carried out following our previously established protocols with certain modifications [15]. The iPSCs were cultured and maintained in StemFit AK02 N medium (AJINOMOTO, Tokyo, Japan). When the cells reached confluence, they were dissociated using TrypLE Select (Thermo Fisher Scientific, Waltham, MA, USA), resuspended in 0.5 mM ethylenediaminetetraacetic acid in PBS (1:1), and passaged as single cells at a density of 5000–8000 cells/cm² every 7 days in AK02 N medium. This medium included iMatrix-511 silk (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) at 0.125 µg/cm², an uncoated laminin fragment, along with the ROCK inhibitor Y-27632 (10 µM, FUJIFILM Wako) [21]. For differentiation into cardiovascular cells, single iPSCs were seeded onto dishes coated with Matrigel (diluted 1:60) at a density of 360,000–400,000 cells/cm² in AK02 N medium containing Y-27632 (10 µM). After the cells reached confluence, a Matrigel overlay (1:60 dilution in AK02 N) was applied one day before initiating differentiation. The culture medium was then switched to RPMI + B27 (RPMI

1640, Thermo Fisher; 2 mM L-glutamine, Thermo Fisher; 1 × B27 supplement without insulin, Thermo Fisher) with the addition of 100 ng/mL Activin A (R&D, Minneapolis, MN, USA) and 5 µM CHIR99021 (Tocris Bioscience, Bristol, UK) for a period of 24 h, designated as day 0 (d0). On day 1 (d1), the medium was supplemented with 10 ng/mL bone morphogenetic protein 4 (BMP4; R&D) and 10 ng/mL basic fibroblast growth factor (bFGF), and this condition was maintained for 4 days without medium change. To promote differentiation into cardiomyocytes (CM) and endothelial cells (EC) (CM + EC protocol), on day 5 (d5), the medium was replaced with RPMI1640 supplemented with 50 ng/mL vascular endothelial growth factor (VEGF) (FUJIFILM Wako), 2.5 µM IWP4 (Stemgent, Cambridge, MA, USA), and 5 µM XAV939 (Merck, Kenilworth, NJ, USA). The medium was subsequently refreshed every other day with RPMI1640 insulin-minus medium supplemented with 50 ng/mL VEGF. Beating cells were observed between days 11 and 13. For mural cell (MC) differentiation (MC protocol), the process on day 0 followed the same steps as in the CM + EC protocol, except CHIR99021 was not included. Starting from day 3 (d3), the medium was switched to RPMI + FBS (RPMI 1640 with 2 mM L-glutamine and 10 % fetal bovine serum), with the medium being refreshed every other day.

4.2. Flow cytometry

Flow cytometry was conducted following our previous study with certain modifications. On day 13, HiPSC-derived cardiovascular (CV) cells were dissociated using Accutase, and 500,000 cells were collected for flow cytometry analysis. Dead cells were excluded by staining with the LIVE/DEAD fixable Aqua dead cell staining kit (Thermo Fisher). The cells were then labeled with specific surface markers to distinguish different cell types. For intracellular proteins, staining was carried out in cells fixed with 4 % paraformaldehyde (PFA) in PBS. Mural cells were identified using anti-PDGFRβ conjugated with phycoerythrin (PE) (clone 28d4, 1:100, BD, Franklin Lakes, NJ, USA), while endothelial cells were marked with anti-VE-cadherin conjugated with allophycocyanin (APC) (clone 55-7h1, 1:100, BD). Cardiomyocytes were detected by staining with anti-cardiac troponin T (cTnT) (clone 13-11, Thermo Fisher), which was labeled with APC using Zenon technology (Thermo Fisher) at a dilution of 1:50. The stained cells were analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA, USA), and the data were processed with CytExpert software (Beckman Coulter).

4.3. Tissue mold fabrication

Tissue molds were fabricated using polydimethylsiloxane (PDMS) (Strex, Osaka, Japan). The PDMS molds were first autoclaved to ensure sterility. Following sterilization, the molds were coated with 1 % Pluronic F127 for 1 h to prevent cell adhesion to the mold surface. After the coating period, the Pluronic F127 solution was removed, and the molds were thoroughly rinsed with PBS to eliminate any residual Pluronic F127. The cleaned molds were then ready for tissue molding.

4.4. ECT generation

To generate engineered cardiac tissues (ECTs), we modified methods from our previous studies [9]. Following the flow cytometry results, differentiated CM, EC, and MC were combined to reach a final MC concentration of 10 %, as shown in Extended Fig. 1B. A total of six million cells were suspended in a culture medium composed of high glucose Dulbecco's modified essential medium (Life Technologies) with 20 % fetal bovine serum (Life Technologies). The matrix solution consisted of acid-soluble collagen type I (2 mg/mL, pH 3), an alkali buffer (0.2 M NaHCO₃, 0.2 M HEPES, 0.1 M NaOH), and Matrigel (15 % of the total volume). The cell suspension and matrix solution were combined to reach a final collagen type I concentration of 0.67 mg/mL in a total volume of 200 µL. This mixture was then poured onto Pluronic F127-coated PDMS molds, which were placed in a twelve-well culture

plate and allowed to polymerize in a CO₂ incubator (37 °C, 5 % CO₂) for 60 min. Afterward, the molds were soaked with alpha minimum essential medium (αMEM; Life Technologies) supplemented with 10 % fetal bovine serum (FBS), 0.05 mM 2-mercaptoethanol (Sigma), and 100 U/mL Penicillin-Streptomycin (Life Technologies). The ECTs were subsequently cultured for 14 days, with the medium being refreshed every other day before they were subjected to physical stimulation.

4.5. Histological analysis and fluorescence assay

Three-dimensional ECTs were fixed in 4 % paraformaldehyde (PFA) and embedded in paraffin, followed by staining with hematoxylin-eosin, cTnT, and TUNEL. For fluorescence microscopy, the 3D engineered cardiac tissues were stained for cTnT (Abcam, ab45932) and CD31 (monoclonal mouse IgG1, clone 9G11) (R&D) at a dilution of 1:500, along with 4',6-diamidino-2-phenylindole (DAPI) (Thermo Fisher). Secondary antibodies used included anti-mouse Alexa 546 (Thermo Fisher) and anti-rabbit Alexa 488 (Thermo Fisher). Images of the tissues were captured using a Zeiss LSM880 confocal microscopy system (Carl Zeiss, Jena, Germany). Quantitative analysis of the cTnT-positive area was performed using ImageJ software.

4.6. Electrical stimulation test

After two weeks of stimulation, we carried out electrical stimulation of the 3D ECTs using a custom-made electrical stimulation system connected to an electrical pulse generator (Strex, Osaka, Japan). The tissues were subjected to electrical pulses at 30 V (peak-to-peak) with a pulse width of 4 ms. The stimulation frequency began at a 1000 ms interval (1 Hz) and gradually increased to 400 ms (2.5 Hz). The response of the 3D ECTs to the electrical stimulation was verified through simultaneous microscopic observations.

4.7. MUSCLEMOTION analysis

MUSCLEMOTION is a flexible open-source software that utilizes a video-based system to assess contractile functions [11]. We adhered to the provider's guidelines for using the software. Briefly, MUSCLEMOTION was installed as a plug-in within ImageJ software, and the motion amplitude was used as the key parameter for analysis.

4.8. Calcium transient assay

Calbryte™ 520 AM (AAT Bioquest, Pleasanton, CA, USA; final concentration of 5 μM) loading solution, containing 0.04 % PF-127, was added to the tissue molds. The 3D ECTs were incubated with the dye-loading buffer for 60 min in a 5 % CO₂ incubator at 37 °C. After incubation, the dye-loading solution was replaced with FluoroBrite™ DMEM for analysis. Videos of the signal intensity were captured using fluorescence microscopy (CKX53, Evident, Tokyo, Japan) equipped with a FITC filter, a camera system (DP27), and software (cellSens, Evident). Data were analyzed using ImageJ software, with manually selected regions of interest (ROIs) and the "Plot Z-axis Profile" function.

4.9. RNA extraction, cDNA synthesis, quantitative RT-PCR

Total RNA was extracted using the Qiashredder (Qiagen, Hilden, Germany) and subsequently purified with the RNeasy Mini Kit (Qiagen), following the manufacturer's guidelines for both procedures. The purification process included a DNase treatment using the RNase-Free DNase Set (Qiagen). RNA yield and purity were assessed with a NanoDrop One spectrophotometer (Thermo Fisher). First-strand cDNA was synthesized from 200 ng of total RNA for each sample using the ReverTra Ace® qPCR RT Master Mix (FSQ-201, TOYOBO, Osaka, Japan) as per the manufacturer's instructions. RNA was then analyzed by quantitative real-time polymerase chain reaction (RT-PCR) using the Applied Biosystems

StepOne Plus system (Thermo Fisher) with the PowerUp™ SYBR Green Master Mix (Thermo Fisher), following the manufacturer's protocol. Data analysis was performed using the fold change method, normalized to the expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The primer sequences are as follows:

human-GAPDH-Forward: AGAAGGCTGGGGCTCATTTG
 human-GAPDH-Reverse: AGGGGCCATCCACAGTCTTC
 human-TNNT2- Forward: ACAGAGCGGAAAAGTGGGAAG
 human-TNNT2- Reverse: TCGTTGATCCTGTTTCGGAGA
 human-TNNI1- Forward: CCGGAAGTCGAGAGAAAACCC
 human-TNNI1- Reverse: TCAATGTCGTATCGCTCCTCA
 human-TNNI3- Forward: TTTGACCTTCGAGGCAAGTTT
 human-TNNI3- Reverse: CCCGGTTTTCTTCTCGGTG
 human-SERCA2- Forward: CTGACGGTGGTCCAAGAGTG
 human-SERCA2- Reverse: CATGGACAGGCAGATGGAC
 human-PLN- Forward: AAATCCCGAGCTAAACACC
 human-PLN- Reverse: GAACTTCAGAGAAGCATCACGATGATA

4.10. Statistical analysis

All results are presented as mean – standard deviation. Differences between conditions were compared using a one-way ANOVA with Tukey's post hoc test for all measurements. A repeated measures ANOVA was performed to account for the within-sample variability and assess the differences in contractile force across the different stimulation frequencies. All statistical analyses were conducted using GraphPad Prism version 10.2.3 (Boston, MA, USA). Statistical significance was determined as $P < 0.05$.

CRediT authorship contribution statement

Wusiman Maihemuti: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kozue Murata:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Mosha Abulaiti:** Validation, Investigation, Data curation. **Kenji Minatoya:** Supervision, Investigation, Conceptualization. **Hidetoshi Masumoto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized ChatGPT to enhance the language and readability of this work, with careful consideration. Following the use of this tool/service, the authors thoroughly reviewed and revised the content as necessary, taking full responsibility for the final version of the publication.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2024.150605>.

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