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Cardiac microtissues from human pluripotent stem cells recapitulate the phenotype of long-QT syndrome

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ABSTRACT

Background: Human induced pluripotent stem cells (hiPSCs) and their derivative cardiomyocytes (hiPSC-CMs) have been successfully used to study the electrical phenotype of cardiac ion channel diseases. However, strategies to mature CMs and more comprehensive systems recapitulating the heart complexity are required to advance our ability to capture adult phenotypes.
Methods: We differentiated wild-type (WT) and long QT syndrome type 1 (LQT1) hiPSCs into CMs, endothelial cells and cardiac fibroblasts. The three cell types were combined to form three-dimensional (3D) spheroids, termed "cardiac microtissues" (cMTs) and the electrophysiological properties were measured using 96-well multi-electrode arrays.
Results: LQT1 cMTs displayed prolonged field potential duration compared to WT controls, thus recapitulating the typical feature of LQTS. Isoprenaline caused a positive chronotropic effect on both LQT1 and WT cMTs. The 96-well multi-electrode array format proved suitable to detect electrical changes directly in the 3D tissues.
Conclusions: 3D hiPSC cMTs are a scalable tool that can be used to identify LQT electrical hallmarks and drug responses. We anticipate this tool can be adopted by pharmaceutical companies to screen cardioactive compounds.

1. Introduction

Upon their discovery, human induced pluripotent stem cells (hiPSCs) have become crucial human *in vitro* tools providing significant advancement in the study of human diseases. Differentiation of hiPSCs into cardiomyocytes (hiPSC-CMs) has allowed the investigation of disease mechanisms and the quantification of the functional consequences of inherited cardiac disorders in patient-specific cells. One of the first disorders modelled *in vitro* with hiPSC-CMs is the long QT syndrome (LQTS) [1], one of the most common and best described cardiac ion channel diseases (or channelopathies) [2]. LQTS is characterized by a delay in the repolarization phase of the heart, visible as a pathological prolongation of the QT interval at the electrocardiogram (ECG); this is primarily the consequence of variants in genes encoding for ion channels or proteins involved in the cardiac excitation-contraction coupling, and it may lead to life-threatening arrhythmias called "Torsade de Pointes" (TdP) [3]. LQTS can also be "acquired", when triggered by exogenous factors such as drugs which inadvertently block cardiac ion channels involved in the repolarization phase of the action potential (AP) as side-effect. This has ultimately caused multiple compounds to be withdrawn from the market after commercialization [4].

LQTS is also characterized by an incomplete penetrance, with carriers of identical pathogenic variants often exhibiting a broad spectrum in the severity of the disease phenotype. This significantly complicates the identification of subjects who may be potentially at risk, and the choice of treatments that may be more appropriate for these patients. In this context, hiPSC-CMs have allowed the identification and functional validation of protective [5] or detrimental [6] genetic modifiers capable to shape the

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functional role of disease-causing variants, thus modifying the penetrance of LQTS in individuals carrying the same variant [7]. As such, it is imperative to identify which patients are at risk of incurring in QT prolongation and TdP and significant benefits might come from hiPSC-based platforms for disease modelling and drug testing over classical experimental models based on small animals or heterologous systems.

Importantly, two-dimensional (2D) monolayer of CMs from hiPSCs still carry inherent limitations due to their incomplete maturation status [8]. Furthermore, there is a strong need for more advanced *in vitro* models that are capable to recapitulate the most complex features of human adult cardiac electrophysiology. Three-dimensional (3D) cardiac models from hiPSCs, like engineered cardiac tissues and cardiac microtissues (cMTs), offer unique opportunities to study complex, multilineage cardiovascular disorders, and to model adult-onset disease phenotypes that were previously not feasible in 2D monolayer settings. hiPSC-CMs in both engineered cardiac tissues and cMTs showed postnatal electrophysiological properties [9,10]. We have recently developed a scaffold-free cMT model that incorporates small cell numbers (5000 cells per tissue), which makes it ideal for medium- and high-throughput drug screening pipelines and electrophysiology readouts [10,11]. We have shown that hiPSC-CMs in our 3D cMT model are more structurally and functionally mature than 2D monolayers of hiPSC-CMs, including their electrophysiological profile, which was assessed by dissociating cMTs to single-cell CMs and measured at low-throughput by patch-clamp electrophysiology [10]. However, it would be ideal to 1) assess the electrophysiological profile of hiPSC-CMs within the 3D environment, without the need of tissue dissociation, and 2) take advantage of the high-throughput potential of the cMT format.

Here, we present an alternative to the gold standard method to model LQTS *in vitro* based on 2D monolayers by using a 3D multicellular cMT system generated from wild-type (WT) and long-QT syndrome type 1 (LQT1) hiPSC derivatives. Utilizing the Multi-Electrode Array (MEA), which enables 2D and 3D electrophysiological analysis in a medium throughput range, we show that this platform is capable to capture the electrophysiological profile of LQT1 cMTs and detect the positive chronotropic effect of isoprenaline in this 3D culture format and disease setting.

2. Material and methods

2.1. hiPSC generation and culture

Wild-type (WT) hiPSC line LUMC0020iCTRL-06 (female [12], LUMCi028-A), and long-QT syndrome type 1 (LQT1) hiPSC line LUMC0021iKCNQ-30 (female [12], LUMCi039-A) carrying the KCNQ1-R594Q variant in heterozygous were previously generated from primary skin fibroblasts using Sendai virus and characterized [12]. Both lines were routinely tested for mycoplasma and genomic integrity by karyotyping. hiPSCs were cultured on vitronectin recombinant human protein-coated plates in E8 medium as described previously [13]. Cells were passaged twice a week using PBS containing EDTA (0.5 mM). RevitaCell Supplement (1:200) was added during passaging.

2.2. hiPSC differentiation into cardiomyocytes, cardiac endothelial cells and cardiac fibroblasts

Cardiomyocyte (CM) and cardiac fibroblast (cF) differentiations were induced in monolayer as described previously [10,11]. Briefly, 25×10^3 cells per cm^2 (WT) and 30×10^3 cells per cm^2 (LQT1) were seeded on plates coated with 83.3 g/ml growth factor reduced Matrigel (Corning) the day before differentiation (day −1). On day 0, cardiac mesoderm was induced by changing E8 to BPEL medium (Bovine Serum Albumin [BSA] Polyvinyl alcohol Essential Lipids [14] supplemented with a mixture of cytokines (20 ng/ml BMP4, R&D Systems; 20 ng/ml ACTIVIN A, Miltenyi Biotec; 1.5 mM GSK3 inhibitor CHIR99021, Axon Medchem).

For CM differentiation: on day 3 (cardiac mesoderm), cytokines were removed and the Wnt inhibitor XAV939 (5 mM, Tocris) was added. BPEL medium was refreshed every 3 days. On day 14–21, CMs (WT, LQT1) that showed > 80 % purity, measured as the percentage of cardiac troponin T-positive cells by FACS, were dissociated using the Multi Tissue Dissociation Kit 3 (Miltenyi Biotec) following the manufacturer's instructions and used for generation of cardiac microtissues (cMTs).

For cF differentiation: on day 3 (cardiac mesoderm), cytokines were removed and the WNT inhibitor XAV939 (5 mM) was added for 3 days with BMP4 (30 ng/ml) and Retinoic Acid (RA; 1 mM; Sigma Aldrich). On day 6, BPEL medium supplemented with BMP4 (30 ng/ml) and RA (1 mM) was refreshed. On day 9, 15×10^3 per cm^2 (WT) and 20×10^3 per cm^2 (LQT1) were seeded on plates coated with 2–5 mg/ml of fibronectin from bovine plasma (fibronectin; Sigma Aldrich) in BPEL medium supplemented with the TGFb inhibitor SB431542 (10 mM; Tocris). When confluent (~day 12), 25×10^3 cells per cm^2 (WT, LQT1) were seeded on plates coated with vitronectin in BPEL medium supplemented with FGF2 (10 ng/ml; R&D Systems). On day 13 and every 2 days thereafter, medium was refreshed with BPEL supplemented with FGF2 (10 ng/ml). After 6 days (day 19), cFs were matured by changing BPEL to Fibroblast Growth Medium 3 (FGM3; PromoCell). FGM3 was refreshed every 2 days for approximately 10 days in total. After 10 days (day 29), cFs were confluent and ready to be passaged at 1:2 ratio. FGM3 was refreshed the day after passaging and every 2 days thereafter. When confluent, cFs (WT, LQT1) were cryopreserved (10 cm^2 per vial) in CryoStor CS10 medium (0.5 ml per vial; Stem Cell Technologies), or used for generation of cMTs.

Cardiac endothelial cell (cECs) differentiation was induced in monolayer as described previously [10,11]. Briefly, 15×10^3 cells per cm^2 (WT) and 20×10^3 cells per cm^2 (LQT1) were seeded on Matrigel at day −1. On day 0, cardiac mesoderm was induced as described above. On day 3, cytokines were removed and VEGF (50 ng/ml, R&D Systems) and XAV939 (5 mM) were added. cECs were isolated as described previously [13,15] using a Human cord blood CD34 Positive selection kit II (StemCell Technologies) following the manufacturer's instructions. Following isolation, 15×10^3 cells per cm^2 (WT) and 20×10^3 cells per cm^2 (LQT1) were seeded on fibronectin-coated plates and cultured in BPEL medium supplemented with VEGF (50 ng/ml). After ~4 days, cECs were confluent and cryopreserved (30 cm^2 per vial) in CryoStor CS10 medium (0.5 ml per vial; StemCell Technologies), or used for generation of cMTs.

2.3. In vitro generation and culture of cardiac microtissues from hiPSCs

Three-cell-type cMTs were generated as described previously [10]. Prior to cMT formation, cECs and cFs derived from WT and LQT1 hiPSCs were prepared as follows: 2–3 days before cMT formation, a vial of cryopreserved cECs and a vial of cryopreserved cFs were thawed and cultured either in BPEL medium supplemented with VEGF on plates coated with fibronectin (cECs), or in medium supplemented with FGM3 on un-coated plates (cFs). Alternatively, fresh, confluent cECs and cFs were used.

On the day of cMT formation (day 0), fresh or previously thawed cECs and cFs (WT, LQT1) were detached using TrypLE 1X for 5 min at 37 °C, 5 % CO_2, centrifuged for 3 min at 1100 rpm, resuspended in BPEL medium and counted. On day 14–21, CMs derived from WT

and LQT1 hiPSCs that showed >80 % purity, measured as the percentage of troponin positive cells by FACS, were dissociated using the Multi Tissue Dissociation Kit 3 (Miltenyi Biotec) following the manufacturer's instructions, resuspended in BPEL medium and counted. Cell suspensions were combined to a total of 5000 cells (70 % CMs, 15 % cECs, 15 % cFs) per 50 ml BPEL medium supplemented with VEGF (50 ng/ml) and FGF2 (5 ng/ml). Cell suspensions were seeded on v-shaped bottom 96-well plates (Greiner bio-one) and centrifuged for 10 min at 1100 rpm. cMTs were incubated at 37 °C, 5 % CO2 for three weeks with media refreshed every 3–4 days. Analysis of cMTs was performed after three weeks from tri-culture initiation.

2.4. Whole mount immunofluorescence of cardiac microtissues

Whole mount immunofluorescence staining of cMTs was performed as described previously [10,13,15]. After three weeks from tri-culture initiation, cMTs were transferred manually (using a P-1000) from v-shaped bottom 96-well plates to 1.5 ml eppendorf tubes and staining was performed in suspension. cMTs were washed in PBS (Calcium, Magnesium) and fixed for 1 h at 4 °C with 4 % paraformaldehyde, washed 3 times in PBS (Calcium, Magnesium) and stored at 4 °C until processing (no later than 2 weeks). cMTs were permeabilized for 30 min with PBS (Calcium, Magnesium) containing 0.2 % Triton X-100 and blocked for 2 h (minimum) in PBS (Calcium, Magnesium) containing 10 % FCS. All incubations were done at room temperature. Primary antibodies were added overnight at 4 °C. The following day, cMTs were washed 3 times with PBS (Calcium, Magnesium) at room temperature, each time incubated for 10 min. Secondary antibodies were added overnight at 4 °C. The following day, cMTs were washed 3 times with PBS (Calcium, Magnesium) at room temperature, each time incubated for 20–30 min and then stained with DAPI for 1 h at room temperature. cMTs were mounted with ProLong Gold antifade Mountant (Thermo Fisher Scientific) onto microscope slides on top of 12 mm round, glass coverslips. Images were captured with a Leica SP8 WLL confocal laser scanning microscope, using a 100x objective, 0.75 and 1.5 zoom.

2.5. Bright field images

Bright field images were acquired with a Nikon DS- 2 MBW camera connected to a Nikon Eclipse Ti–S microscope, controlled by the Nikon NIS-Element BR software. Lens magnification was 4x with a PhL contrast filter. Images of cMTs seeded on MEA plates were acquired with a smartphone camera microscope adapter attached to a stereo microscope.

2.6. Multi-Electrode Arrays measurements and analyses

Single-well Multi-Electrode Arrays (MEAs; Multichannel Systems) and 96-well multiwell MEAs (Multichannel Systems) were coated with 166-333 μg/ml growth factor reduced Matrigel (Corning) for more than 30 min. After removing the coating, cMTs were individually transferred from v-shaped bottom 96-well plates on MEA wells dropwise; each MEA plate was positioned in a custom-made humidified chamber inside the incubator at 37 °C and 5 % CO_2 for ~1 h to prevent a rapid evaporation and to promote cMTs attachment. Subsequently, 200 μL (1 ml) of BPEL medium were added to each multiwell (single-well) MEA to allow long-term culture on MEA chips. The measurements were performed after at least three days from the seeding. FPs were acquired from multiwell MEAs with Multiwell-Screen (Multichannel Systems) at 20 kHz and filtered with a Butterworth 1 Hz high-pass and a Butterworth 3.5 kHz low-pass filters. A 15-min adaptation phase was done before each recording. The analyses of FP parameters were performed offline with Multiwell-Analyzer (Multichannel Systems), with Field Potential Duration (FPD) and beating period (RR interval) being quantified as previously described [16]. Exported raw MEA data were further analyzed using custom-made R scripts (R 4.0; R Foundation for Statistical Computing). Isoproterenol hydrochloride (I6504 Sigma-Aldrich) powder was dissolved in water to create a 1 mM stock solution.

3. Results

3.1. Generation of cardiac microtissues form wild-type and long-QT syndrome type 1 hiPSCs

To investigate the disease phenotype of long-QT syndrome type 1 (LQT1) in a humanized system that uniquely incorporates the heart complexity, we generated *in vitro*, 3D cMTs combining hiPSC-CMs, hiPSC-cECs and hiPSC-cFs derived as previously from common cardiac mesoderm precursors [10,11,13,15]. Two types of cMTs were produced (Fig. 1A). These were WT cMTs, combining hiPSC-CMs, hiPSC-cECs and hiPSC-cFs differentiated from WT hiPSCs (WT CMs, WT cECs, WT cFs, respectively), and LQT1 cMTs, combining hiPSC-CMs, hiPSC-cECs and hiPSC-cFs differentiated from LQT1 hiPSCs (LQT1 CMs, LQT1 cECs and LQT1 cFs, respectively) (Fig. 1A). Each cMT was generated by aggregation of 70 % CMs, 15 % cECs and 15 % cFs, for a total of 5000 cells per cMT (Fig. 1A) [10,11]. 3D spheroid cMTs were cultured for three weeks from tri-culture initiation, with media refreshment every 3–4 days (Fig. 1B, top panel). Following three weeks in culture, WT and LQT1 cMTs were used for electrophysiological evaluation (Fig. 1B, bottom panel). For assessment of cardiac sarcomeric proteins TNNI and ACTN2, three-weeks-old cMTs were transferred manually to 1.5 ml eppendorf tubes and used for immunofluorescence (IF) staining as shown in Fig. 1C.

3.2. LQT1 cMTs recapitulate in vitro field potential prolongation

cMTs from WT and LQT1 hiPSC lines were manually seeded on different MEA plate configurations to identify the most suitable system for assessing i) long-QT phenotype and ii) response to pharmacological challenges (Fig. 2A). We successfully measured electrical signal from cMTs plated in all the tested formats (single well MEA with 64 electrodes and multiwell MEAs up to 96-wells with 3 electrodes/well, Fig. 2A). A 96-well MEA configuration was eventually chosen since it successfully allowed medium-throughput accurate electrophysiological evaluations despite the reduced number of electrodes per well (Fig. 2A, right panel). cMTs from both hiPSC lines exhibited field potentials (FPs) characterized by clear depolarization and repolarization peaks (Fig. 2B), with a good signal-to-noise ratio (especially considering the reduced number of cells composing each cMTs and the small surface contact area between 3D spheroids and the electrodes). LQT1 cMTs exhibited a significantly prolonged field potential duration (FPD) (Fig. 2C and D) compared to WT cMTs, demonstrating the suitability of this experimental model to capture *in vitro* a clinically relevant disease phenotype characterized by FPD prolongation (**WT:** 0.48 ± 0.02 s; **LQT1:** 0.61 ± 0.02 s $p = 3.0 \times 10^{-7}$). No differences were found in the spontaneous beating frequency of the two groups (RR, **WT:** 3.12 ± 0.48 s, **LQT1**: 3.02 ± 0.19 s, $p = 0.8452$).

3.3. Isoprenaline induces a positive chronotropic effect in WT and LQT1 cMTs

cMTs were subsequently stimulated with the β-adrenergic agonist isoprenaline (ISO, 1 μM) to assess their response to a

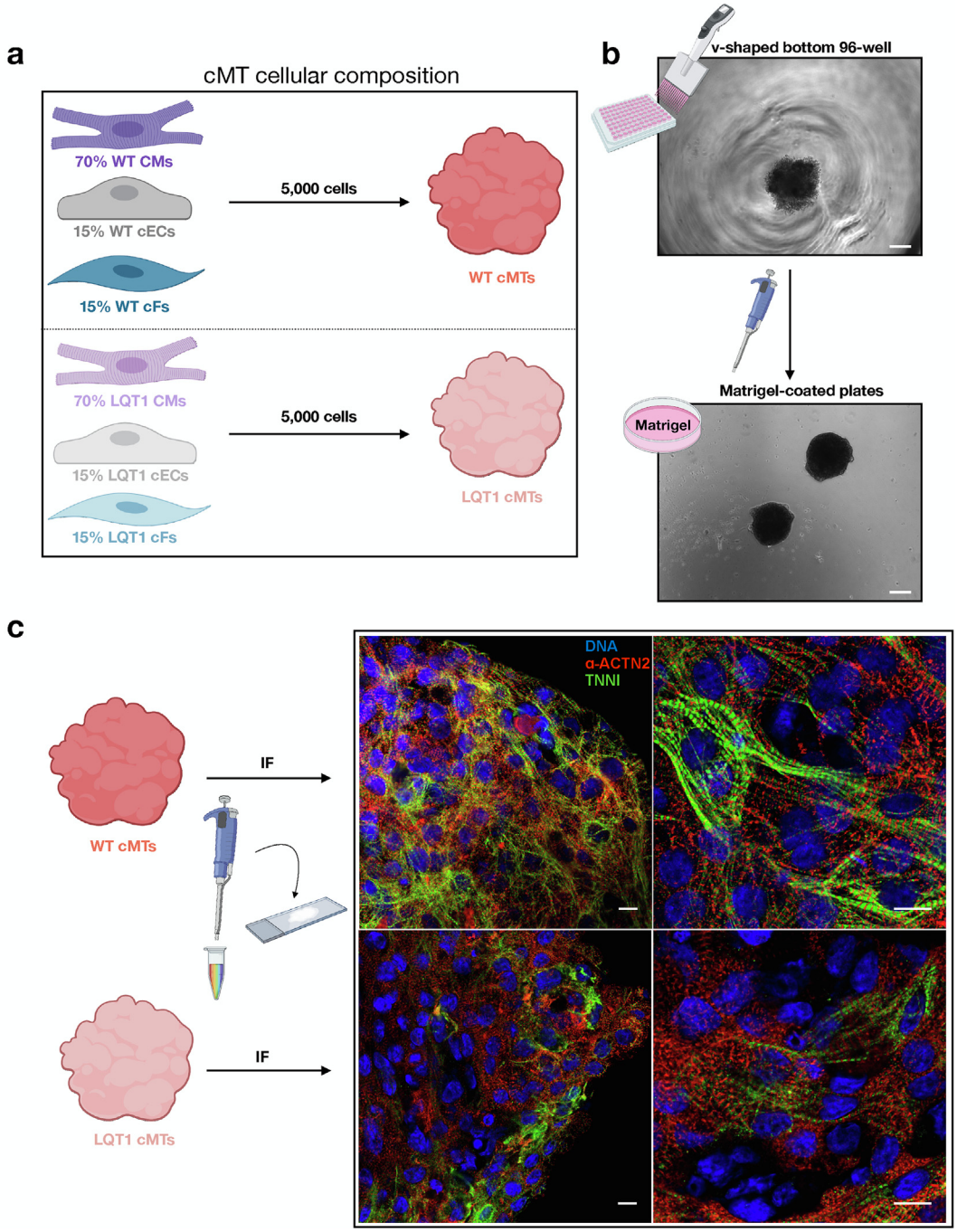

Fig. 1. Generation of cardiac microtissues from wild-type control- and LQT1-hiPSCs:
A) Schematic showing cellular composition of three-dimensional (3D), *in vitro*
cardiac microtissues (cMTs) generated by aggregation of 70 % cardiomyocytes (CMs), 15 % cardiac endothelial cells (cECs) and 15 % cardiac fibroblasts (cFs), differentiated from wild-type (WT, upper panel) and Long QT Syndrome 1 (LQT1, bottom panel) human induced pluripotent stem cells (hiPSCs). Each cMT is made of 5000 cells.
B) cMTs are cultured in v-shaped bottom 96-well plates for three weeks from cMT formation (upper panel). Following three weeks in culture, cMTs are transferred to Matrigel-coated MultiElectrode Array (MEAs) plates for field potential (FP) measurements. Scale bars: 200 μm
C) Representative immunofluorescence (IF) images for cardiac sarcomeric proteins TNNI (green) and ACTN2 (red) in WT cMTs (upper panel) and LQT1 cMTs (bottom panel). Nuclei are stained with DAPI (blue). Scale bar: 10 μm. Zoom: 0.75 (left); 1.5 (right). For IF, cMTs are transferred from v-shaped bottom 96-well plate to 1.5 ml Eppendorf tubes, stained in suspension and then mounted on top of microscope slides for imaging.

positive inotropic and chronotropic compound (Fig. 3). As expected, β-adrenergic stimulation shortened the RR interval (**WT**: from 2.27 ± 0.8 s to 1.21 ± 0.54 s, $p = 1.1284 \times 10^{-3}$; **LQT1**: from 1.79 ± 0.44 s to 1.09 ± 0.54 s, $p = 1.088 \times 10^{-5}$) and also decreased the FPD (**WT**: from 0.52 ± 0.18 s to 0.29 ± 0.13 s, $p = 2.118 \times 10^{-3}$; **LQT1**: from 0.74 ± 0.18 s to 0.47 ± 0.24 s, $p = 1.094 \times 10^{-7}$). This demonstrated that cMTs can be used to measure FPD changes in response to active compounds and are therefore suitable to assess pharmacological responses at a medium-throughput scale.

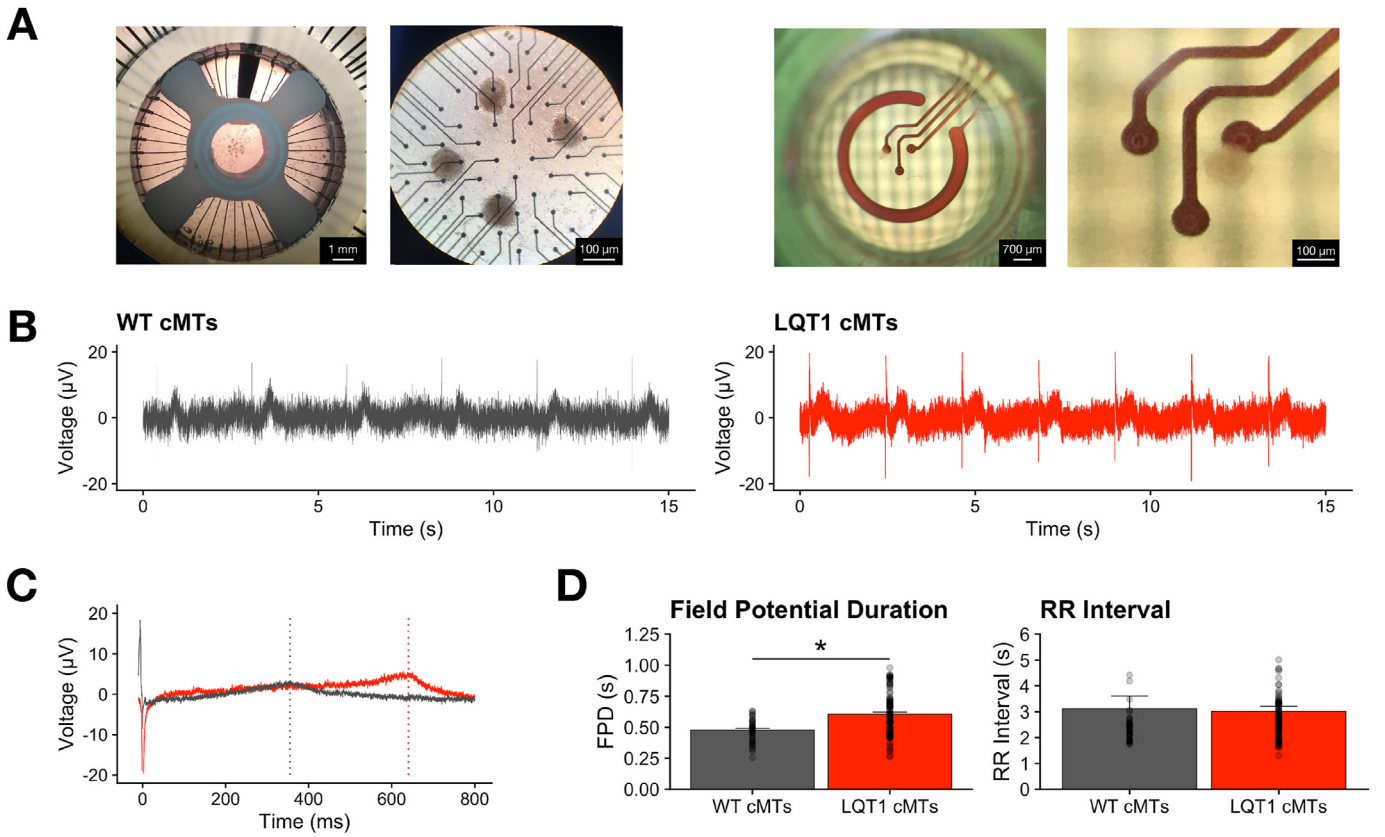

Fig. 2. CMTs recapitulate LQT1 disease phenotype on multiwell MEAs:
A) cMT plated on single-well (left) and multiwell MEAs, respectively, for low and medium throughput electrophysiological analyses.
B) Time-course of field potentials measured in WT and LQT1 cMTs.
C) Representative overlaid field potential traces show that LQT1 cMTs display prolonged FPD compared with WT cMTs, thus recapitulating the LQT1 diseased phenotype. Dotted bars indicate the repolarization peaks used to calculate FPD.
D) Average FPD and RR quantification from WT and LQT1 cMTs. N = 52, 131; p = 3.0×10^{-7} for FPD; p = 0.8452 for RR.

4. Discussion

In this work, we have demonstrated that cMTs incorporating hiPSC-CMs, hiPSC-cECs, and hiPSC-cFs are capable to reproduce the clinically-relevant hallmark feature of LQT1 *in vitro*. Although this might not represent an advantage *per se* over 2D experimental models based on confluent monocultures of hiPSC-CMs when the cardiac ion channel is expressed early in the differentiation and does not undergo fetal-to-adult regulation (reviewed in Ref. [17]), the cMTs model carries additional advances.

The role of the maturation status of hiPSC-CMs is of fundamental importance in modelling LQTS. Albeit the electrophysiological immaturity can be partially circumvented in isolated hiPSC-CMs by the injection of an electronic I_{K1} via dynamic clamp [5], this issue still cannot be addressed on 3D and multicellular constructs and thus the need for more intrinsically mature experimental models is compelling. As we showed recently [10], compared to 2D hiPSC-CMs, our cMT model provides for the expression of ancillary proteins and ion channel accessory subunits, more organized networks of t-tubules, more hyperpolarized resting membrane potential, and the presence of crosstalk between CMs and other relevant cell types of the heart. Here, we have generated cMTs in which the three cell types combined were all derived from WT or LQTS hiPSC sources. However, a major advantage of this paradigm is the ease with which it can be manipulated. Cell types derived from WT or diseased cell lines can be mixed to interrogate which cell drivers a particular phenotype, which is crucial to appropriately model complex multilineage cardiovascular diseases and arrhythmogenic conditions. This process could be easily adapted to high throughput pipelines for drug screenings (also due to the modest price per datapoint [11]). In this view, the development of more predictive and advanced platforms for drug screening based on human cells is also relevant in the context of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA [18]). The focus of CiPA is to identify a combination of technologies which can be used to predict and classify the arrhythmogenic potential of compounds and, among these, platforms based on human stem-cell derived ventricular myocytes can offer a superior or complementary translational relevance in terms of drug screening when compared to individual heterologous assays or animal models, respectively.

The FPDs recorded from cMTs were more prolonged than the FPDs measured in 2D monolayer settings [19]. Beside the impact of the 3D conformation of the cMT, this is likely to be attributed to the coculture of CMs with cECs and cFs, which might passively contribute to buffer conduction velocity and repolarization speeds. The presence of cECs and cFs seem to influence the baseline beating frequencies (RR intervals) of cMTs, which are significantly longer than those from the same cell lines cultured in 2D monolayers. It is still not clear whether this has to be attributed to a more mature, and thus silent-population of ventricular-like CMs, or to the direct influence of cECs and cFs on the firing of cMTs. To answer this question, a series of LQTS hiPSC lines with different mutations and corresponding controls should be analyzed in parallel by comparing 2D versus cMTs configurations.

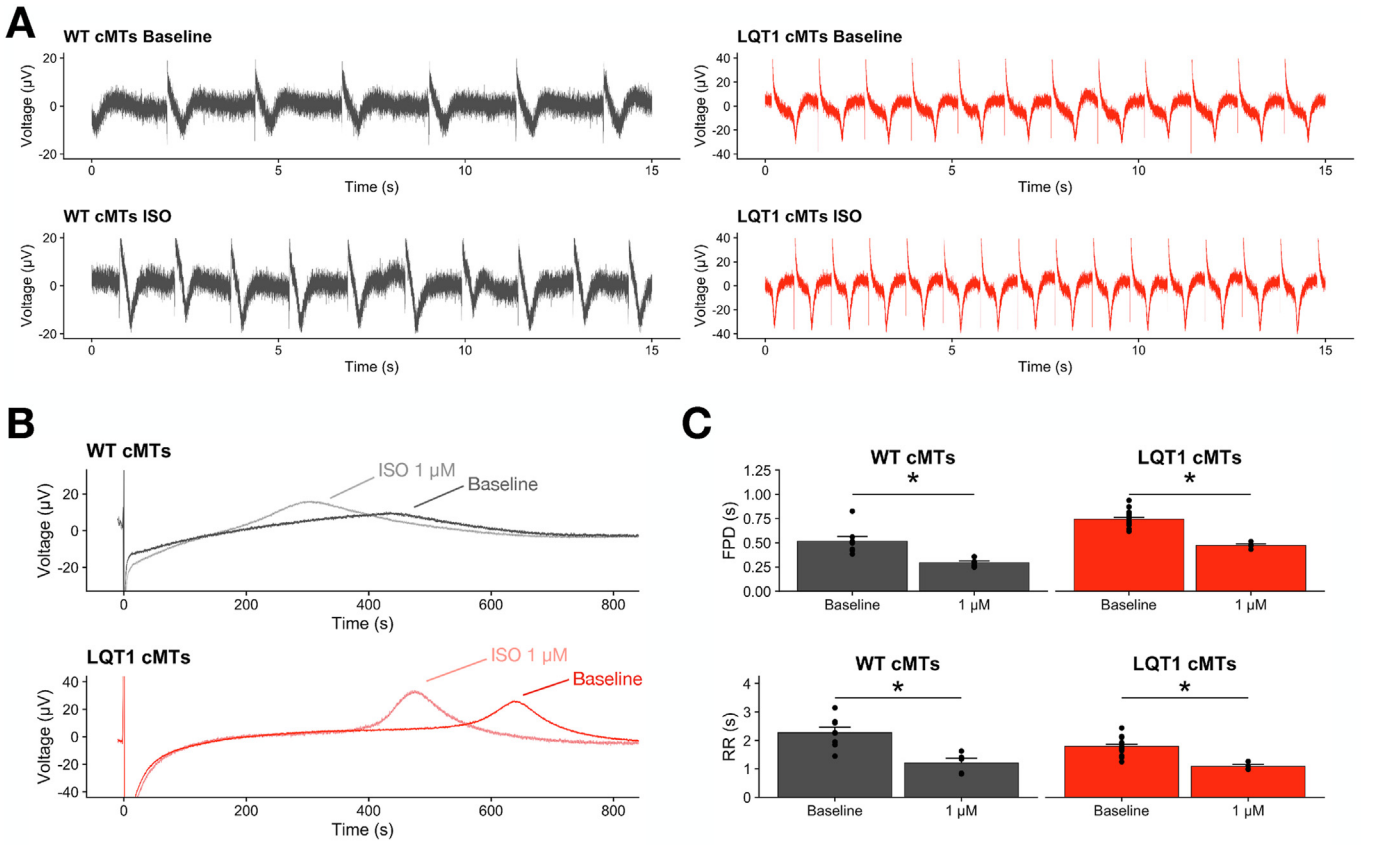

Fig. 3. CMTs recapitulate physiological response to isoprenaline on multiwell MEAs:
A) Time-course of field potentials measured in WT (gray) and LQT1 (red) cMTs under baseline conditions and in the presence of 1 μM isoprenaline (ISO)·
B) Representative overlaid field potential traces show that both WT and LQT1 cMTs recapitulate FPD shortening upon ISO treatment.
C) Average FPD quantification from WT (gray) and LQT1 (red) cMTs. N WT: 5–8; FPD: $p = 2.118 \times 10^{-3}$; RR: $p = 1.284 \times 10^{-3}$; N LQT1: 4–17. FPD: $p = 1.094 \times 10^{-7}$; RR: $p = 1.088 \times 10^{-5}$.

Of note, MEA signals from cMTs have a smaller amplitude compared to those from 2D monolayers in terms of Na-peak amplitude and repolarization peak(s), which is caused by the lower number of cells and smaller footprint of cMTs compared to 2D monolayers. Although this might complicate the quantification of FP parameters in very specific scenarios (e.g. in the presence of negative inotropes), the smaller 3D form factor compared to a 2D monolayer includes an additional dimension that can be further captured through other technologies (e.g. epifluorescence) to improve signal recording and achieve a higher throughput in multiwell plates (>384 wells).

Compared to other 3D cardiac tissue systems used to model LQTS, our cMT model presents both advantages and disadvantages. For example, compared to the engineered heart tissue (EHT) model from Goldfracht and colleagues [20] and Lemoine and colleagues [21], our cMT incorporates a significant smaller number of cells (5000 vs 2 million and 1 million, respectively), which makes it more amenable to high-throughput. On the other hand, Goldfracht and colleagues [20] could measure the absolute force of contraction and conduction analysis in their EHTs, while contraction force measurements and optical mapping cannot be performed in our cMT system. That said, these types of analysis are not critical to study LQTS phenotypes, whilst FPD and arrhythmia are critical parameters which are clinically relevant to the disease. Therefore, our cMT represents a platform suitable to study and identify novel therapeutically relevant targets for LQTS.

5. Conclusion

The tri-cellular 3D cMT, entirely derived from a single hiPSC source, is a model that can be used to capture disease-related and drug-induced field potential changes in a multiwell MEA plate and it is therefore a useful tool not only for modelling disease displaying a prolongation of the repolarization but also to assess the pro- or anti-arrhythmic effect of molecules. These are important conclusions to help moving forward both fields of drug discovery and precision medicine.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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