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Integrating Heart-on-Chip Technology and 3D Porcine Living Myocardial Slices for Cardiotoxicity Screening

Dual pace for MPSlms

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Abstract: Cardiotoxic events are a major challenge in drug development and a leading cause for market withdrawal. Cardiotoxicity frequently originates from electrophysiological alterations within cardiomyocytes, therefore increasing risk of life-threatening arrhythmias. Current preclinical 2D cell culture models lack the structural and functional complexity of mature myocardium. To address this limitation, we validated a microphysiological system (MPS) with porcine *ex vivo* living myocardial slices (LMS) incorporating a dual-pacing strategy (S1S2) that was developed for direct assessment of effective refractory period (ERP) duration of action potential. *Ex vivo* cultivated porcine LMS responded to dual pacing and displayed action potential durations comparable to healthy human hearts. Specific human ether-a-go-go-related gene (hERG) potassium channel blockade with Dofetilide prolonged ERP in a dose-dependent manner. Of great interest, our model was validated by mimicking the clinically cardiotoxic side effect of Cisapride. Titration of Cisapride triggered an elongated ERP, thus confirming cardiotoxicity screening power. Our newly developed MPSlms Tox platform enhances translatability by incorporating mature cardiac tissue and potentially allows multi-dose drug testing from a single donor heart thereby elegantly reducing animal studies.

Keywords: cardiotoxicity, heart-on-chip, living myocardial slices, hERG channel

1 Introduction

Cardiotoxicity remains a major concern in drug development and is one of the leading causes for drug withdrawal from the market [1]. A key factor of drug-induced cardiotoxicity is the interaction between compounds and ion channels (e.g. hERG K⁺ channel, L-type Ca²⁺ channel), which contributes to the action potential duration in cardiomyocytes. Unintended adverse effects on cardiac function can lead to severe clinical outcomes, including QT interval alterations and increased risk of life-threatening arrhythmias.

Current preclinical 2D testing models are predominantly cell lines (e.g. transfected HEK293 or CHO cells) that express the hERG potassium channel or induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM). The activity of the hERG channel in these models is determined by patch clamp measurements [2]. However, these techniques lack the structural and functional complexity of the mature 3D myocardium with native action potential of cardiomyocytes therefore hampering clear prediction to the *in vivo* situation.

To overcome these limitations and enhance clinical translations, we extended a modular microphysiological system for 3D cultivation of *ex vivo* living myocardial slices (MPSlms) [3] by integrating a dual-pacing strategy enabling evaluation of cardiac electrophysiology.

LMS are ultrathin (300µm) cardiac tissue sections which retain three-dimensional multicellular composition and extracellular matrix of the heart. Electrical pacing of LMS allows the evaluation of contractile parameter of cardiomyocytes. Recently, LMS have emerged as a promising *ex vivo* platform for cardiotoxicity testing to study drug-induced effects on electrophysiological properties [4].

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Applying a dual-pacing strategy to LMS enables the assessment of drug-induced cardiotoxicity by monitoring the action potential duration. For instance, hERG channel blockage prolongs the action potential, while non-responding compounds trigger no change of action potential duration. 3D LMS can thus bridge initial hERG assay data from patch clamp technique to a more relevant *in vivo* situation reflecting drug binding towards any cardiac ion channel. In line, 3D LMS offer the potential to reduce animal testing strategies by allowing multiple screening conditions from a single porcine donor heart.

2 Methods

2.1 Processing of porcine LMS

Anaesthesia of pigs was induced with Tiletamin/Zolazepam (10 mg/kg) and maintained with i.v. application of Propofol (10 mg/kg/h). Fentanyl (10 µg/kg bolus and adjusted dosing every 20 min) was used for analgesia and heparin (400 IE/kg) for anticoagulation treatment. After accessing the thorax organ preparation and retrieval was conducted according to standard procedure for human organ donations. A perfusion canula was inserted into the ascending aorta and used to flush the heart with cold cardioplegic (Custodiol) solution while blood was drained by opening the vena cava. After cold storage of the heart in Tyrode's solution, LMS were prepared from pigs following previously published protocols [5]. Briefly, left ventricular tissue was sectioned using a high precision vibratome (Model 7000SMZ2, Campden Instruments LTD) to obtain 300 µm myocardial slices. For modified cultivation in the MPS, slices were trimmed to 4 × 4 mm format and secured within isometric tissue holder [3].

2.2 Heart-on-chip cultivation

LMS were cultured in MPS as described in previous publication [3]. The pump speed was adjusted to 80 bpm on MPScontrol unit and LMS were paced for cultivation with electrical parameters set to a pulse duration of 10 ms, amplitude of 6 V, and frequency of 0.2 Hz. LMS were cultured in M199 medium (Pan, P04-07050) supplemented with 1:100 insulin-transferrin-selenium (Pan, P07-03210) and 3% penicillin/streptomycin (Gibco, 15140-122) at 37 °C and 5% CO₂.

2.3 Dual-pacing strategy

MPS-related dual pacing (S1S2) protocol provides the determination of action potential duration of stimulated cardiomyocytes. In general, the dual-pacing strategy involves the stimulation with two pulses in quick succession, with the interval between the pulses continuously shortening (see Figure 1). The following parameters can be specified:

- Amplitude (M_S1) and length (t_S1) of the first pulse
- Amplitude (M_S2) and length (t_S2) of the second pulse
- The pulse interval is shortened from the initial pulse interval (t_Pmax) to the final pulse interval (t_Pmin) in defined steps (dt_P).
- The double pulse stimulation can be repeated automatically with period duration (T).

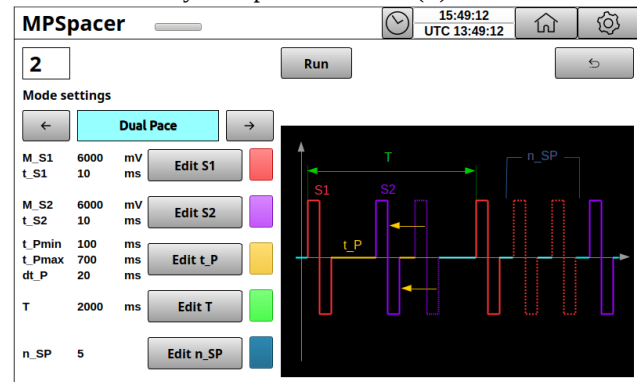


Figure 1: Graphical user interface of the MPSpacer stimulation system for duals pace mode.

S1 stimulation parameters were set at 6 V amplitude and 10 ms pulse duration every 2 s, after 5 S1 stimuli one additional S2 stimulation occurred. S2 stimulus was set at 6 V amplitude and pulse duration of 10 ms. Initial distance between S1 and S2 stimulus was 700 ms and interval was continuously decreased by 20 ms until interval reached 200 ms. The effective refractory period (ERP) of action potential was defined as the longest interval at which two individual contractions from S1S2 pacings failed to be present (see Figure 2) LMS contractions were documented by recording 4 min 30 s videos (30 fps) with an Axiovert 25 (Zeiss) and MikrOkular Full HD (Bresser GmbH) using the Open Broadcaster Software (OBS). The extraction of between-frame displacement fields from the video data was conducted using a matrix-free image registration technique, based on the methodology outlined by König et al. [6], and averaged into motion vectors.

Contractions were afterwards determined as the maximum differences of two subsequent motion vectors.

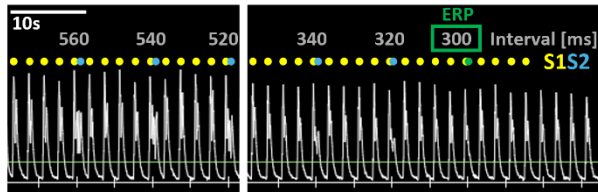


Figure 2: S1S2 dual pacing strategy for determination of effective refractory period (ERP) of action potential of ex vivo LMS.

2.4 Drug testing

The specific hERG channel blocker Dofetilide (Selleckchem, S1658) and unspecific hERG channel blocker Cisapride hydrate (Selleckchem, S4751) were dissolved in DMSO with a stock concentration of 100 mM. Before drug treatment, baseline of contractile behavior of LMS was recorded by performing the dual-pacing strategy. LMS were treated with compounds and after 15 min incubation at 37 °C and 5% CO₂ dual-pacing strategy was performed (see Figure 3)

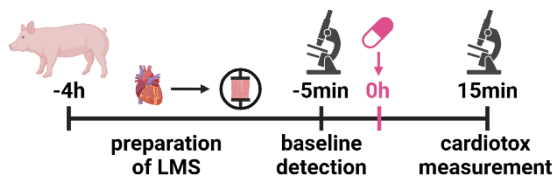


Figure 3: Timeline of drug testing on 3D porcine LMS ex vivo.

3 Results

3.1 Dual-pacing strategy enables determination of action potential duration

Functional analysis of LMS mainly focusses on contractile performance and often neglects the organomimetic electrophysiology. Clinically, drug-induced alterations of electrophysiological capacity by ion channel binding enhances the risk for life-threatening arrhythmias. Myocardial action potential is a highly regulated process involving numerous voltage-gated ion channels in cardiomyocytes, including hERG K⁺ and L-type Ca²⁺ channels. For our approach, we integrated a dual-pacing strategy with the heart-on-chip model enabling detection of effective refractory period in 3D LMS. Experiments were performed by utilizing porcine LMS underlining that the action potential and its ion channel

expression are highly translatable to the human myocardium. Initial testing with the on-chip dual pacing strategy validated the ventricular effective refractory period of action potential in a physiological range of 240-340 ms (see Figure 4) comparable to human myocardium.

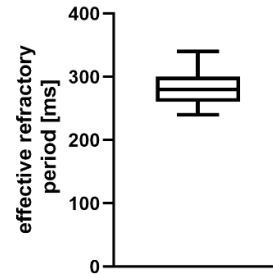


Figure 4: Physiological ERP duration of healthy porcine LMS is between 240 and 340 ms. *n* = 12

3.2 Specific hERG-blocker Dofetilide prolongs action potential duration in dose-dependent manner

The voltage-gated hERG K⁺ channel is a pivotal factor in action potential repolarization. In pathological conditions, loss of function or drug-induced blockage of the hERG channel can cause an elongated action potential of cardiomyocytes, resulting in a long QT syndrome in patients [2]. In order to translate these clinical observations into the heart-on-chip model, we conducted a proof-of-concept experiment by applying the specific hERG blocker Dofetilide to the LMS and performed dual-pacing strategy ex vivo in minutes after drug treatment. Dofetilide treatment immediately increased the effective refractory period in a dose-dependent manner of 1 nM, 10 nM and 100 nM (see Figure 5). These findings highlight rapid detection of the 3D electrophysiological capacity and consequently validation of potential cardiotoxicity by drug-induced action potential alterations.

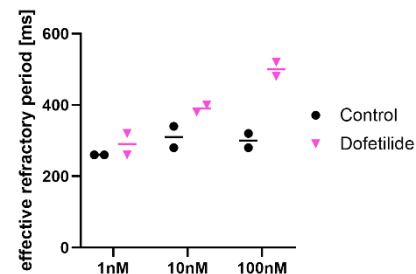


Figure 5: Specific potassium channel blocker Dofetilide treatment prolongs ERP of action potential in porcine LMS. *n* = 2

3.3 Dual pacing strategy detects cardiotoxicity of Cisapride in nM range

In line with detectable shifts of LMS electrophysiology, we investigated if the on-chip dual pacing strategy is able to unravel unspecific voltage-gated channel interference. For this approach, LMS were treated with Cisapride, which was originally developed for the treatment of heartburn and gastrointestinal diseases. However, due to severe cardiotoxic side effects resulting from off-target high-affinity binding to hERG and resulting arrhythmias [7], Cisapride was withdrawn from the market. Application of Cisapride on LMS at 10 and 100 nM concentrations resulted in an increased effective refractory period (see Figure 6). These findings clearly underline the opportunity for screening off-target cardiotoxicity to possibly prevent life-threatening cardiac side effect in clinical settings.

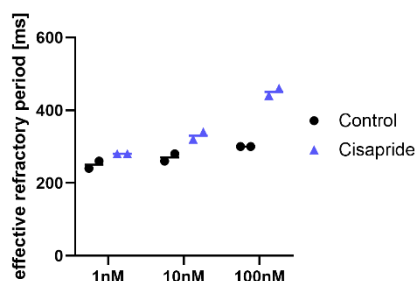


Figure 6: Cardiotoxic effect by ERP elongation after Cisapride treatment in porcine *ex vivo* LMS. *n* = 2

4 Discussion

We herein present the integration of a dual-pacing strategy within the MPSlms system. This study setup enables cardiotoxicity screening at the electrophysiological level of *ex vivo* 3D LMS within minutes following treatment. Direct translatability between porcine LMS and clinical conditions was highlighted by the specific hERG blockage with Dofetilide and the unspecific off-target effect by Cisapride as a valuable proof-of-concept. In line, the use of adult porcine LMS provides an elegant opportunity for comprehensive drug testing monitoring cardiomyocyte voltage-gated ion channels involved in the action potential regulation. Future testing of L-type Ca^{2+} channel blocker Nifedipine and Na^{+} channel blocker Propafenone should underline the benefit of the LMS setup in comparison to the standard hERG channel analyses from 2D cell culture systems. This platform could also provide

functional insights for the testing of psychopharmaceutical drugs targeting ion channels, which intrinsically have a high potential of cardiac ion channel affinity. Age-related, a great option would be to extend the adult cardiotoxicity screening to neonatal porcine tissue, thereby ensuring the safety of drugs administered to newborns. Multi-dimensional combination of functional evaluation and molecular analyses upon drug testing, such as transcriptomics of cardiac tissue, will facilitate a deeper understanding for the mode of action.

Author Statement

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