Real-time calcium flux measurements in iPSC derived 3D heart tissues

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- **Calcium flux was measured every 0.01 seconds using Fluo-4 and the CLARIOstar®**
- **As a model system for analyzing compound-induced cardiotoxicity, the effects of Cisapride, Thapsigargin, E4031, Nifedipine, are shown**

Introduction

Achieving an approval for a new drug involves extensive testing. While the developers of new drugs not only need to prove that their candidate exhibits a positive effect to treat disease, they also need to confirm that the drug is not dangerous to the overall health of people. Before testing potential drug candidates on humans, a lot of preclinical testing is required to predict potential unwanted side effects that may be caused by taking the drug candidate. Although test results using laboratory animals can predict those side effects, animal models may respond differently to drugs. Researchers are seeking suitable *in vitro* test systems that mimic the physiological state of human tissues and/or organs as close as possible to the *in vivo* response(s).
Traditional test methods involve two-dimensional (2D) cell cultures that can be useful in a first screen. However, a 3D cell culture approach can provide data closer to those obtained in an *in vivo* tissue and minimizes misleading results (false positives and negatives) obtained in 2D cultures.

Engineered heart tissues (EHTs) were fabricated using cardiac myocytes derived from human induced pluripotent stem cells (iPSCs). We show that both human cardiac muscles as well as the EHTs beat at similar rates spontaneously. In this application note we will present how this test model can be used to analyze compound induced changes in calcium handling of cardiac myocytes in 3D tissue, which can indicate potential side-effects of drug candidates.
When an action potential arrives at the cell membrane, the membrane potential (-80mV) gets depolarized. As a result voltage gated L-type calcium (Ca^{2+}) channels open and Ca^{2+} influxes into the cell, which triggers a large release of Ca^{2+} from its internal stores, the Sarcoplasmic Reticulum (SR). This increase in Ca^{2+} initiates a contraction by activating Ca^{2+}-dependent contractile proteins.

The CLARIOstar multi-mode microplate reader from BMG LABTECH can measure rapid changes (100 data points per second or 1 data point every 0.01 seconds) in fluorescent intensity of the cytoplasmic Ca^{2+} indicator, Fluo-4 (Life Technologies). This was used to quantitatively measure Ca^{2+}-transients.

Materials & Methods

- CLARIOstar, BMG LABTECH
- Fluo-4 (Life Technologies)
- 3D tissue culture micro chamber or plates, MC-8™ or MC-96™ respectively (InvivoSciences)

Hydrogel solution preparation and tissue formation
A general tissue fabrication protocol was published previously. For specific protocols for various tissue types please contact InvivoSciences.

Calcium-indicator measurements with the CLARIOstar
Calcium Transient analysis is performed using commercially available dyes (Fluo-4-AM, Life Technologies). Tissues are bathed in Tyrodes solution containing Fluo-4-AM (15 µM) and Pluronic F127 at 37 °C for 45-60 minutes. Once dye loading is observed by fluorescent microscopy, the loading solution is replaced with just Tyrodes solution and incubated for an additional 20 minutes at 37 °C after which time the samples are ready for study using the CLARIOstar (see instrument settings below). The same approach can be used for tracking 2D Ca-transients optically using our protocol.

CLARIOstar instrument settings

Measurement Method:	Fluorescence Intensity, Well Mode
Monochromator :	Ex: 483-14 and Em: 530-30
Reading Mode:	Bottom Reading
Gain:	1750
Focal height:	2.0 mm
Target temperature:	37°C

Kinetic settings

No. of intervals:	1000
No. of flashes per interval:	1
Interval time:	0.01 sec
Total measurement time per well:	10 sec

Results & Discussion

Periodic changes in fluorescent intensity from the calcium indicator were apparent (Fig. 1A). After smoothing data by filtering using adjacent averaging or Fast Fourier Transfer (FFT) analysis, profiles of Ca-transients from ten (or more) cardiac contractions overlap (Fig. 1B) and their average (Fig. 1C) shows Ca-transients similar to those obtained using trabeculae of human nonfailing myocardium.

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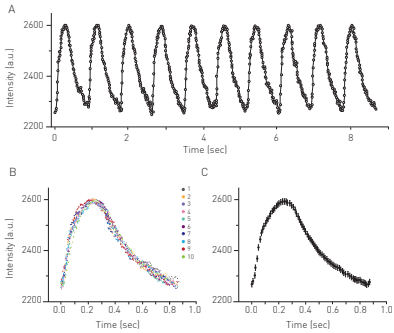

Fig. 1: Calcium (Ca^{2+}) transient measurements using iPSC derived 3D heart tissue and Fluo-4. (A) CLARIOstar microplate reader captures periodic Ca^{2+}-transients that induce cardiac contractions. (B) Overlapping 10 Ca^{2+}-transients of each single cardiac twitch. (C) An average profile of Ca^{2+}-transient (bar=standard error).

The changes in profiles of calcium transients were also apparent when EHTs were treated with various compounds that are known to influence the excitation-contraction coupling (ECC) (Fig. 2) that regulates cardiac contraction through calcium as a second messenger.

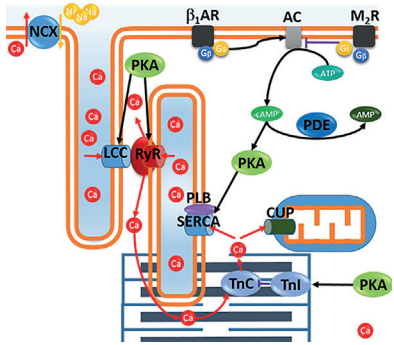

Fig. 2: EC-coupling & regulation. LCC: L-type Ca channel, RyR: Ryanodine receptor NCX: Na-Ca exchanger, PDE: Phosphodiesterase III, PKA: Protein kinase A, TnC/I: Troponin C/I, SERCA: SR Ca pump, PLP: Phospholamban, β1AR: beta adrenergic receptor, AC: Adenylyl cyclase, M2R: M2 Receptor, CUP Ca uniporter.

The results in Figure 3 show similar calcium transient profiles for different concentrations of Cisapride and Thapsigargin, two components that are thought not to affect calcium transients. In contrary, two known effectors (E4031 and Nifedipine) show a clear concentration dependent effect on the shape of the calcium transients.

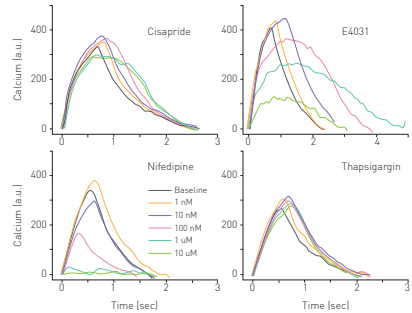

Fig. 3: Calcium Transient Profiles. Human EHTs were treated with increasing concentrations of Cispride, E4031, Nifedipine, and Thapsigargin.

These data suggest that drugs withdrawn from the market changed calcium transients in human EHTs, which may lead to arrhythmia in long-term treatments or in myocardium with significant fibrosis. The analysis of ECC using human EHTs is shown to predict potential safety concerns in drug candidates at the early stage of drug discovery.

Conclusion

The ability to generate EHTs using cardiac myocytes derived from various individuals with different susceptibility to toxicants and drugs could become an important tool to develop safer and more effective personalized treatments.

With the CLARIOstar microplate reader it is possible to measure 100 data points per second. This is very important in these fast kinetic reactions, allowing calcium transient measurements in real time to profile drug-induced changes in excitation-contraction coupling.