Measuring iPSC-CM Ca^{++} & Contractility

Effect of maturation medium on iPSC-CM calcium and contractility with CytoCypher CytoMotion measurements

Patient-derived human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) hold great promise as a model system for various applications [1,2]. Firstly, when obtained from patients suffering from (genetic) cardiac disease, hiPSC-CMs can be used to evaluate whether the genetic abnormality is causal to the disease phenotype [3,4]. Secondly, these cells can be used for screening drugs that would be effective for treatment while having limited side-effects [5]. Finally, hiPSC-CMs are an promising system for disease modeling and exploring the molecular mechanisms underlying diseases that affect the heart [4,6]. Current methods available to measure contractility in hiPSC-CMs are limited to video based analysis which results in high data storage and slow computation [7-9]. Our CytoMotion Pixel Correlation real-time measurements of hiPSC-CMs is fast and enables higher throughput to assess contractility. Here we demonstrate how to measure calcium and contractility in hiPSC-CMs to characterize the effect of a maturation medium.

Methods

hiPSC-CM differentiation

hiPSCs derived from fibroblast of a healthy donor were differentiated to cardiomyocytes as described previously [10]. In short, first iPSCs were subjected to GSK3 inhibitors, followed by Wnt inhibitors. Once spontaneously beating cardiomyocytes could be observed, the cells were metabolically selected based on lactate metabolism (day 11). Next, half of the hiPSC-CMs were subjected to maturation medium[10] while the other half remained in RPMI medium for 3 weeks. hiPSC-CMs were measured on day 40 after start of differentiation.

System setup

Cardiac contractility was assessed on spontaneously beating hiPSC-CMs in a 24 well culture plate using the CytoCypher MultiCell High Throughput System (CytoCypher BV). The CytoCypher MultiCell system is a motorized stage microscope combined with a high-speed, high-resolution camera and an objective that moves in x-y-z position. hiPSC-CM contraction kinetics were measured at 37°C and were based on CytoMotion Pixel Correlation changes relative to the reference frame taken at diastole at 250Hz sampling frequency (IonOptix LLC). Simultaneously with contractility measurements, calcium transients were measured by loading the cells with Fura-2, AM. Each area (~100x100um) was measured for 10s, in which 4-10 contraction traces could be recorded. For an example of a full-length trace of an area, see Figs. 2B and 3B. We measured n=20 different areas per well and N=3 well per medium type. We measured spontaneous contractions and the same locations in response to isoprenaline (500nM).

Loading protocol

To load hiPSC-CM with the calcium indicator Fura-2, Fura-2, AM was dissolved in DMSO to a final concentration of 1mM and kept as stock solution at -20°C. Fura-2, AM stock solution was further diluted in 1ml Tyrode solution to a final concentration of 0.5uM. Culture medium was aspirated from the dish and replaced with the Tyrode-dye mixture. After 15 min incubation in the dark at 37°C, the dye containing mix was removed and cells were washed 2x with warm Tyrode.

Analysis

The CytoSolver Transient Analysis Tools package from CytoCypher BV was used to yield averaged contractile and calcium kinetic parameters from each area. For the purposes of this study, we have analyzed four key parameters of calcium and contractility: beat frequency (Hz), contraction time or time to peak (s), relaxation time or time to baseline 50% (s) and 50% peak width (s).

Results

Data collection in each well took ~20min from start to finish (excluding incubation time with Fura-2, AM) and allowed pairwise statistical comparisons. First, we compared the parameters at baseline. In general, it can be observed that between the wells there is significant variation in beat frequency, 50% peak width, and Time to baseline 50, but less variation within wells (Figs. 1, 2 and 3). The maturation medium had no effect on beat frequency, but relaxation kinetics (Time to baseline 50) was significantly lower in maturation medium compared to RPMI medium for contractility and almost significantly lower in calcium kinetics (Table 1).

Table 1.

	RPMI medium	Maturation medium	P
Contractility			
Beat frequency	0.66±0.10	0.7±0.23	0.66
Time to Peak	0.239±0.117	0.202±0.058	0.42
Time to Baseline 50	0.262±0.114	0.147±0.056	0.043
PeakWidth50	0.456±0.039	0.295±0.085	0.094
Calcium			
Time to Peak	0.207±0.074	0.177±0.068	0.62
Time to Baseline 50	0.305±0.037	0.233±0.040	0.063
PeakWidth50	0.410±0.034	0.331±0.058	0.11

The average contractility and calcium traces (Figures 2B and 3B) show the different transient shapes in RPMI versus maturation medium and their response to isoprenaline. The response to isoprenaline was different in maturation medium compared to RPMI medium as shown by a significant increase in beat frequency in maturation medium (Fig. 1).

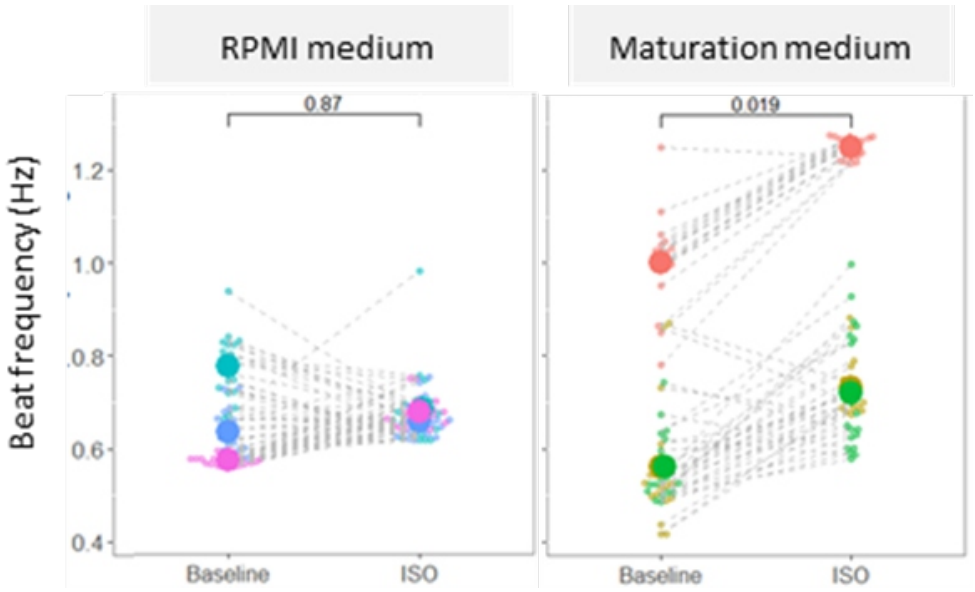

Figure 1: Maturation medium results in an increase in beat frequency upon isoprenaline (ISO). No change in beat frequency was observed in RPMI medium, while beat frequency increased significantly in maturation medium after 500nM of ISO. Each color represents a well (N=3), small dots indicate individual areas per well (n=20), big dot indicates average of each well. Lines connect measurements of the same areas before and after ISO.

Contraction kinetics were faster in response to ISO in RPMI medium (Fig. 2C), while relaxation kinetics were unaffected by ISO in both RPMI and maturation medium (Fig. 2D). Peak width was smaller in RPMI medium in response to ISO (Fig. 2E). Calcium kinetics were faster in response to ISO in both RPMI and maturation medium (Fig. 3C and 3D), which resulted in a substantial reduction in peak width in both media in response to ISO (Fig. 3E).

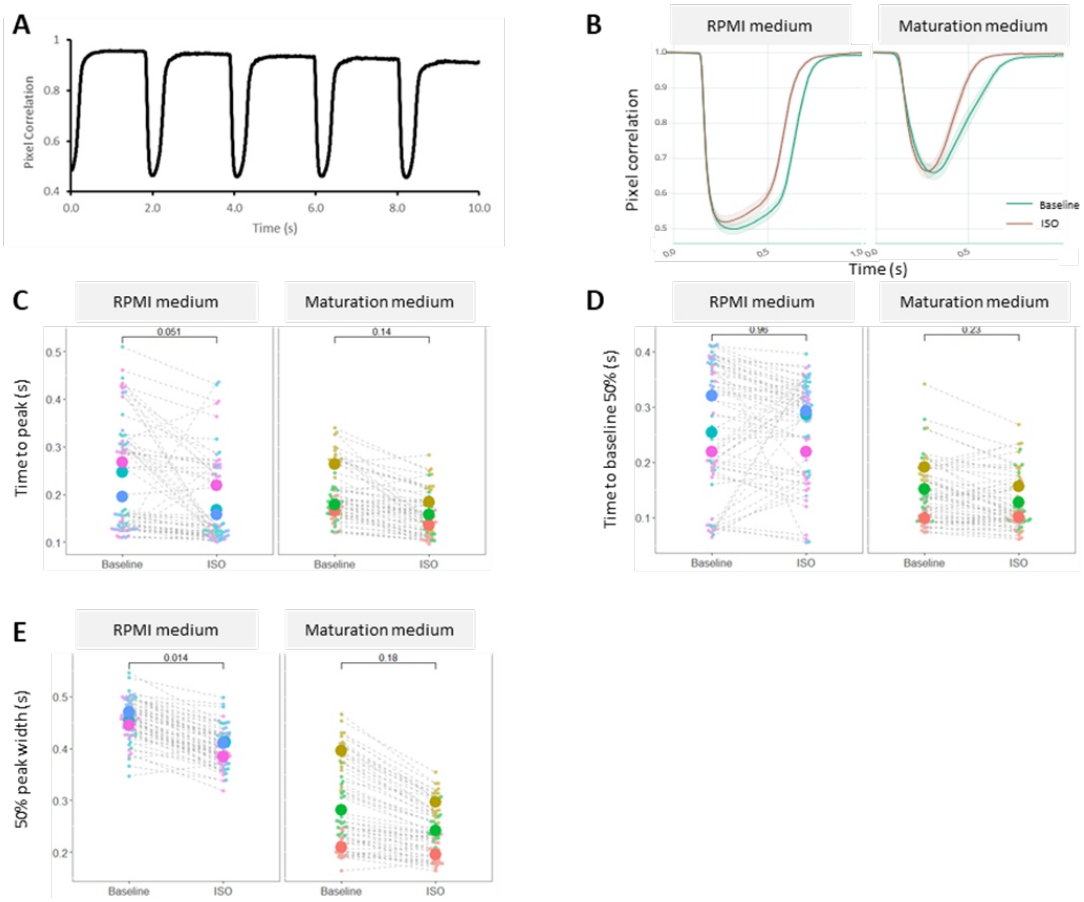

Figure 2: Contraction kinetics increase after isoprenaline (ISO) in RPMI-medium. (A-B) Example of a contractility trace of one area (maturation medium) and average contractility traces in RPMI and maturation medium before and after ISO. (C) Contraction kinetics (time to peak) was slightly reduced in response to ISO in RPMI medium. (D) Relaxation kinetics (time to baseline 50%) were unaffected by ISO in both RPMI and maturation medium. (E) 50% peak width was significantly reduced in RPMI medium in response to ISO. Each color represents a well (N=3), small dots indicate individual areas per well (n=20), big dot indicates average of each well. Lines connect measurements of the same areas before and after ISO.

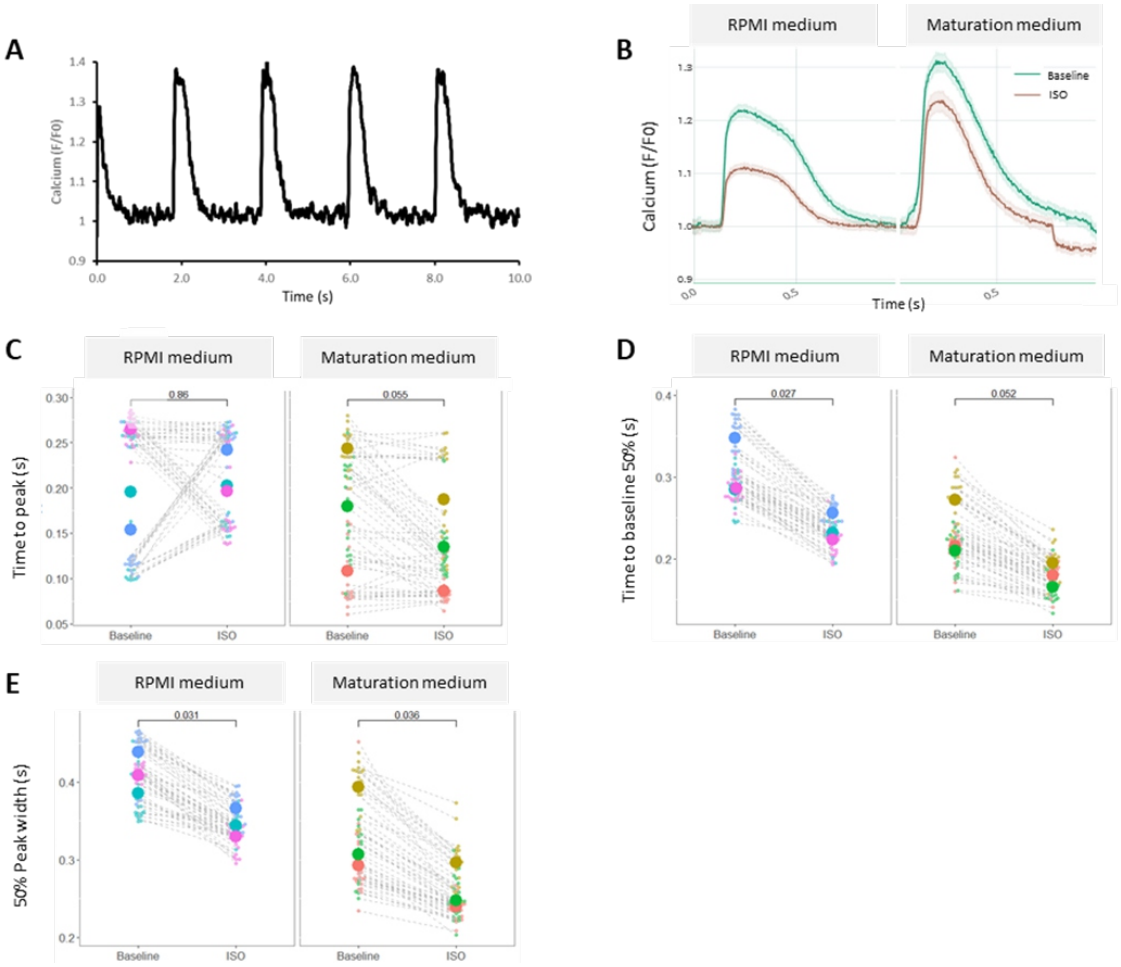

Figure 3: Calcium kinetics increases after isoprenaline (ISO). (A-B) Example of a calcium trace of one area (maturation medium) and average calcium traces in RPMI and maturation medium before and after ISO. **(C)** Time to peak was slightly reduced in response to ISO in maturation medium. **(D)** Relaxation kinetics (Time to baseline 50%) was reduced in both RPMI and maturation medium in response to ISO. **(E)** 50% peak width was significantly reduced in RPMI and maturation medium in response to ISO. Each color represents a well (N=3), small dots indicate individual areas per well (n=20), big dots indicates average of each well. Lines connect measurements of the same areas before and after ISO.

Discussion

We have shown that simultaneous measurements of calcium and contractility is feasible in hiPSC-CMs in a relatively high throughput matter. In total, it took ~2hours to perform 240 measurements in these preparations. We used the time to measure 1 well to load another well with Fura-2, thereby minimizing waiting time. The ability to perform repeated measurements at the same locations greatly improved our statistical power. There was variation between wells, but the response to ISO was comparable between wells (Fig. 2 and Fig. 3).

Maturation mostly improved calcium re-uptake and relaxation kinetics, previously described by Feyen et al [10]. Beat frequency did not change in RPMI medium in response to ISO, however changes in calcium and contractility kinetics were larger in RPMI medium compared to maturation medium.

In conclusion, CytoMotion Pixel Correlation in combination with Fura-2 calcium measurements revealed significant differences between RPMI and maturation medium and their response to isoprenaline.

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