Deriving parameters from calcium transient in human-induced pluripotent stem cell-derived cardiomyocytes and its applications in cardiotoxicity prediction

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Introduction

Cardiovascular toxicity is one of the most common causes for both preclinical and clinical safety closures in the pipeline of drug discovery and development. Despite the usefulness of ion channel blockade assays, such as the inhibition assay of the voltage-gated potassium channel Kv11.1 (also known as human Ether-a-go-go-Related Gene or hERG), such molecular assays do not provide coverage of all cardiac liability and may fail in identification of abnormalities caused by other mechanisms such as changes in cardiomyocyte contractility.

Human induced pluripotent stem cell derived cardiomyocytes (hiPS-CMs) have become promising and have been widely used in recent years. Detection of changes in cardiomyocyte contraction and other assays such as patch-clamp recording from isolated cardiomyocytes are time consuming and technically challenging to increase throughput. Calcium transient detected by fast kinetic fluorescence imaging in spontaneously beating hiPS-CMs provides a higher throughput, which is very promising for cardiotoxicity screening.

Conventional calcium transient analysis mostly focused on the peak frequency/beat rate, peak amplitude and peak width of the waveforms[1,2]. Though these commonly used parameters are sensitive enough to detect compounds with the risk of cardiotoxicity, new parameters derived from the waveforms could be more relevant and potentially reveal different mechanisms of toxicity of different compounds.

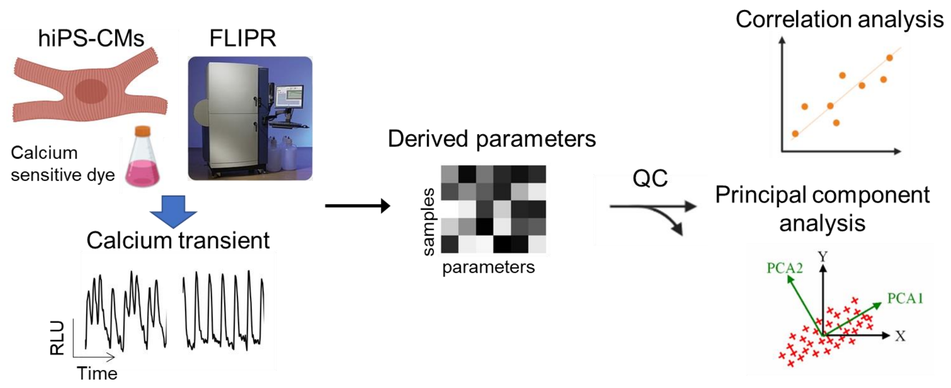


Figure 1: Workflow for the analysis of calcium transient of hiPS-CMs
Calcium transients in hiPS-CMs were assessed using FLIPR 5 Assay Kit, a calcium sensitive fluorescent dye. Then parameters were derived from the waveforms, followed by a quality control process removing samples with low quality. Correlation analysis and principal component analysis were then applied to understand their relationship to cardiac activity.

Objectives

The objectives of this project are to:

- Develop a toolkit to derive parameters from the waveforms of calcium transient
- Understand the importance of the parameters in terms of their correlation with cardiac activity

Methods

Peak detection is implemented attempting to get the cycle number of each signal and to derive parameters from each period. Peaks of which the prominence is lower than 20 or 10% of maximum amplitude of the waveform will be regarded as a false peak. For the boundary of the waveform, the prominences lie on the inner side of the peaks. The leftmost peak bases only on the right side of the peak to measure the prominence.

For each cycle of a waveform, we can derive parameters according to the key points, including the rising point, peak point, tail starting point and valley point. As shown in Figure 2a, by calculating the distance between the key points, we can get durations of “Rise”, “Decay”, “Peak to Tail”, “Tail”. We also derived peak widths at different prominences, including 10%, 25%, 50%, 80%, and 90%, which depict the shape of the peaks. In addition to frequency related parameters, amplitude related ones were also derived from each cycle, including “Amplitude”, “Intensity”, “Valley” as indicated in Figure 2.

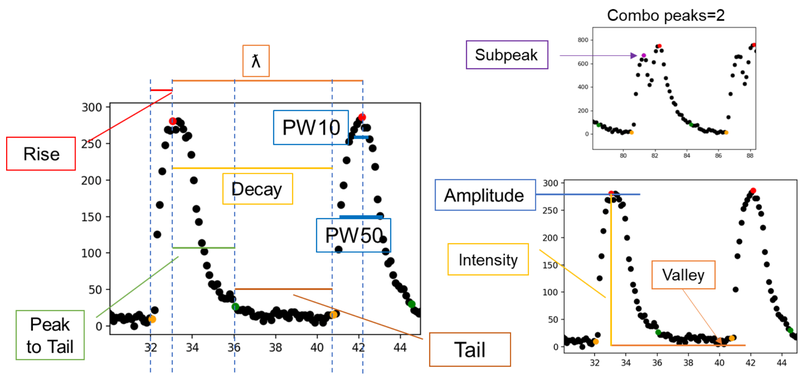


Figure 2: Frequency and amplitude related parameters derived from each cycle in a waveform

We combined the data from two sources. One contains 39 compounds, tested with measurements taken 30 minutes after compound addition with three parallel biological replication and two technical replication. For the other source, there are two plates, tested 30 minutes and 3 days after adding compound respectively without replication. Hence, there are 64 compounds (with 11 overlaps) in total and we labelled a ground truth of cardiac hazard for each compound according to the public and internal information we can access. For each compound there are at least 8 concentrations ranging from 0.01 μM to 300 μM. The raw data went through the quality control and normalisation procedures (Figure 3) before further analysis.

Point-biserial correlation coefficient was used to evaluate the correlation between the parameters under different concentrations and the hazard label. M_+ and M_- are mean values of the parameters of cardiac active/inactive samples, n_+ and n_- are the number of active/inactive samples. S_n is the standard deviation of the parameters of different samples.

$$r_{pb} = \frac{M_+ - M_-}{s_n}\sqrt{\frac{n_- \cdot n_+}{n_{total}}}$$

To understand the correlation within the parameters, Pearson correlation was used to calculate the distance between two parameters. The distance is a complement of the absolute value of Pearson’s correlation coefficient. Then hierarchical clustering was applied to visualise the relationship between the parameters.

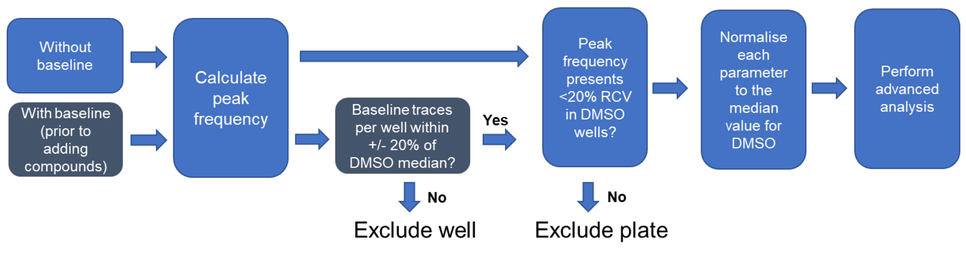


Figure 3: Workflow of quality control and normalisation

Results

The absolute correlations between the parameters and the ground truths are shown in Figure 4A. In general, the correlation becomes higher when the concentration increases but the correlation at 50 μM is not as high as 30 μM and is similar to 10 μM. The top parameters include RMS, Amplitude, Shoulder/Tail, and PW10, of which the correlation values ($|r_{pb}|$) are around 0.5. The distance heatmap (Figure 4B) shows that the amplitude relevant parameters such as RMS and Amplitude are highly inter-correlated (d<0.1), which is not unexpected due to the similar definition. But the other parameters are generally independent.

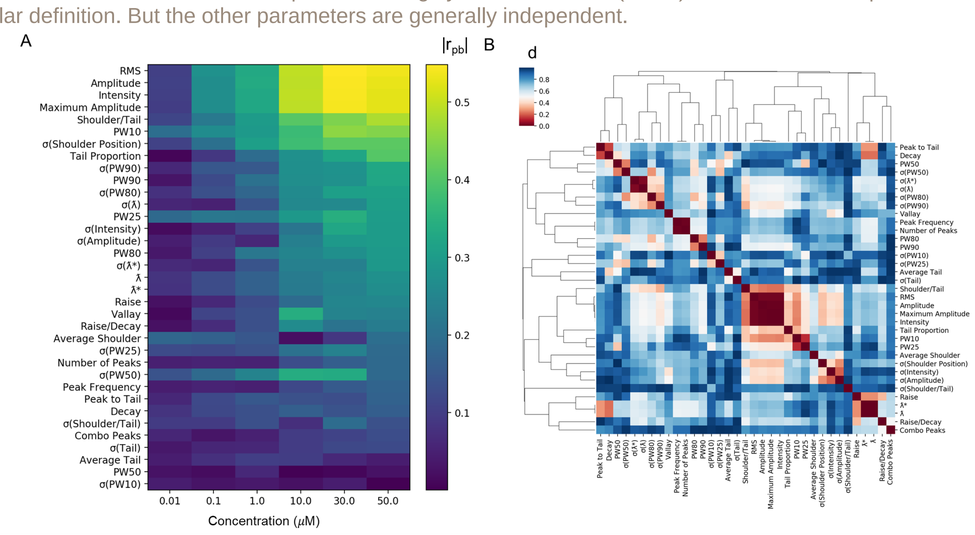
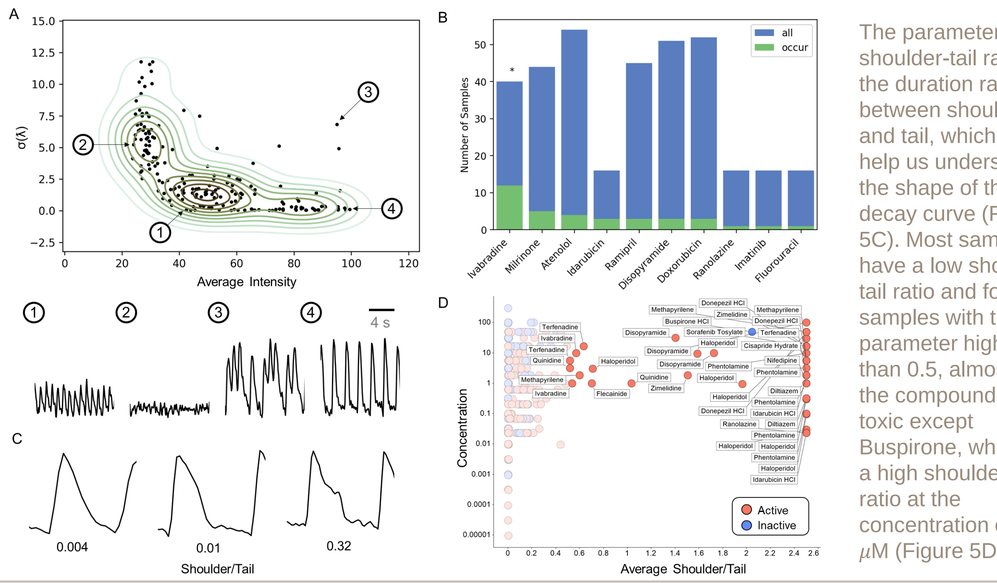


Figure 4: Correlation between parameters and cardiac activity

Standard deviation of wavelength ($\sigma(\lambda)$) and average intensity can be used to recognise beat stop or other irregular calcium transient in hiPS-CMs. Example 2 in Figure 5A is a typical beat stop signal where the amplitude is very low and $\sigma(\lambda)$ is high.

Figure 5B shows the number of samples with combo peaks (such as a double peak), in green, and the number of all samples, in blue, for the compounds of high occurrence. Compared to the background of DMSO samples (about 10% occurrence rate), only Ivabradine is significantly high in occurrence (p=0.013), which indicates that there might be an intrinsic mechanism of Ivabradine leading to the combo peaks in the waveforms.



The parameter shoulder-tail ratio is the duration ratio between shoulder and tail, which can help us understand the shape of the decay curve (Figure 5C). Most samples have a low shoulder-tail ratio and for the samples with this parameter higher than 0.5, almost all the compounds are toxic except Buspirone, which has a high shoulder-tail ratio at the concentration of 50 μM (Figure 5D).

Figure 5: Examples of the usage of parameters

PCA shows that cardiac inactive compounds tend to be clustered together and overlap with active compounds under lower concentrations (Figure 6A). We can also discover some false negatives which are blue but located in red areas in Figure 6B. The distance distribution plots (Figure 6C-F) show that the Euclidean distances of the parameters between inactive compounds are ranging from 0 to 50, similar to the distances between two (biological or technical) replicates. Distances between cardiac active compounds are larger even for replicates.

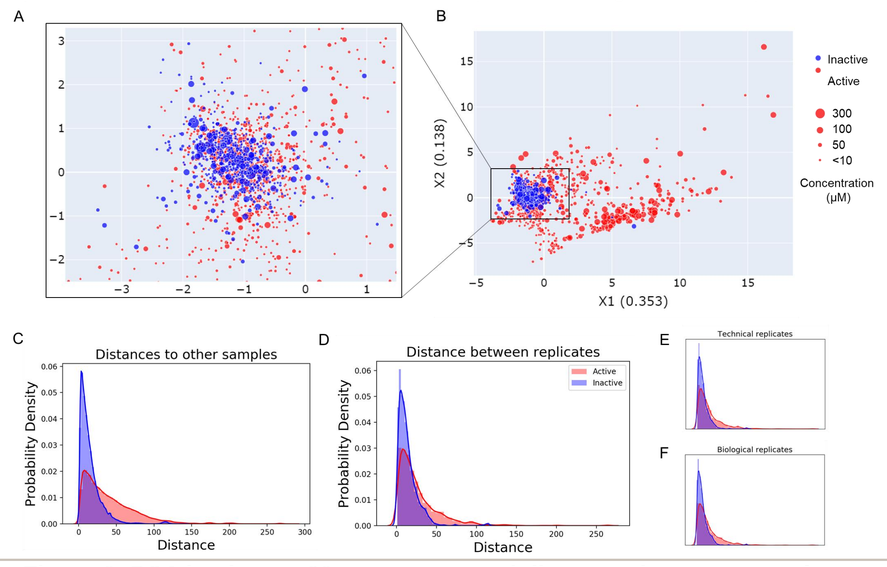


Figure 6: PCA by the top 20 parameters and distances between samples

Conclusions

Results to date indicate that the new parameters calculated by our toolkit can help us understand the shape of the waveforms of calcium transient. Parameters about amplitude and peak width play key roles in illustrating the potential cardiovascular toxicity of a compound. Other parameters such as combo peaks and shoulder-tail ratio are also informative indicators for cardiac active compounds, though it is still difficult to assess the cardiac risk under a low concentration. This toolkit offers the potential to gain more from hiPS-CM calcium transient data than traditional parameters offer.

References

1. Kopljar, I et al. (2018). Stem Cell Reports, 11(6), 1365–1377.
2. Pointon, A. et al. (2015). Toxicol. Sci., 144, 227–237