dHealth 2023
B. Pfeifer et al. (Eds.)

doi:10.3233/SHTI230010

A Novel Algorithmic Approach to the Analysis of Multi-Electrode Array Signals of hiPSC-Derived Cardiomyocytes

Christoph VOUTSINAS[a,1], Laura Sophie FROMMELT[b], Chiara VOLANI[b], Alessandra ROSSINI[b] and Johannes OBERZAUCHER[a]
[a] *Fachhochschule Kärnten, Klagenfurt, Austria*
[b] *Eurac Research, Bolzano, Italy*

Abstract. In order to perform in vitro cardiotoxicity screening of pharmacological substances, multi-electrode array systems are increasingly used to measure the extracellular field potentials of cell layers of human induced pluripotent stem cell cardiomyocytes. The analysis of the field potentials is usually performed using complex analysis software provided by the hardware manufacturers. In the case of the Cardiac Analysis Tool software from Axion Biosystems, inconsistencies were found in the results, which can significantly influence the cardiotoxicity screening results. In order to obtain more reliable results, a new algorithm was developed and implemented in an easy-to-use software tool, the INCardio Data Analysis Tool, which, due to its high degree of automation, can also be used by inexperienced users. The validation reveals differences in the results of the two tools both in depolarization spike amplitudes and in the time course of the field potential durations. The manual analysis of all signals affected by deviations shows that the results of the newly developed Data Analysis Tool are correct in all cases and can therefore be classified as more accurate and reliable than the reference analysis software.

Keywords. multi-electrode array, cardiomyocytes, cardiotoxicity, electrophysiology, field potential

1. Introduction

Newly developed pharmacological drugs not only offer opportunities for more effective disease treatment, but also potential risks in the form of undesirable side effects, such as the development of drug-induced arrhythmias of the heart. For this reason, drug developers are obliged under the rules of the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to test new active substances for their cardiotoxicity according to the guidelines of the Comprehensive In Vitro Proarrhythymia Assay (CiPA) collaboration which, among other things, requires the in vitro investigation of the influence of new drug-candidates on the electrophysiological properties of stem cell derived cardiomyocytes (CM) [1].

Within the framework of the INCardio project funded by the European Union through the Interreg Italia-Austria funding scheme and the partnering institutions

[1] Corresponding Author: Christoph Voutsinas, Fachhochschule Kärnten, Klagenfurt, Austria, E-Mail: c.voutsinas@fh-kaernten.at

International Centre for Genetic Engineering and Biotechnology, Trieste, Eurac Research, Bolzano, Fachhochschule Kärnten, Klagenfurt, and Medizinische Universität Innsbruck, Eurac Research is investigating the cardiotoxicity of the 320 active substances in the Prestwick phytochemical drug library [2] using a Maestro Edge multi-electrode array device and the associated signal analysis software Cardiac Analysis Tool (CAT) provided by the manufacturer, Axion Biosystems Inc.

1.1. Multi-Electrode Arrays

Since in many cases entire libraries of active substances have to be investigated, Multi-Electrode Arrays (MEAs) have been increasingly used in recent years. Although they are not as precise as the patch clamping technique, they are much more efficient. [3].

MEAs consist of microelectrodes arranged in a two-dimensional grid that non-invasively record changes in the extracellular electric field potential (FP) of CM cell layers [3][4]. Unlike the action potentials resulting from patch clamping, FPs resemble classic electrocardiogram (ECG) curves, with a pronounced depolarization spike and a T-wave, which are used to calculate the field potential duration (FPD) corresponding to the ECG QT-interval [4].

1.2. MEA-Data Analysis

Following the analysis of the initial data by the CAT, a review of the results showed that in some cases there are discrepancies between calculated and actual depolarization spike amplitudes and FPDs as determined by graphical signal analysis.

These discrepancies provided the impetus for a cooperation between Eurac Research and CUAS to develop an analysis tool that uses a new algorithmic approach to improve the results.

2. Methods

The CAT was created using the MATLAB scripting language, which, in addition to appropriate libraries for signal analysis, also offers an efficient application for creating standalone applications.

2.1. Data Pre-Processing

Due to the sampling rate of f_s =12.5 kHz at a typical measurement duration of t =120 s for the 24 wells per measurement plate and 16 electrodes per well, the Maestro Edge MEA system produces around half a billion data points, which are stored in a proprietary binary file format. In order to make this data readable and processable, it must be exported to a CSV-file with a conversion tool from the manufacturer, which increases the file size to several gigabytes. Since the ad-hoc conversion of such large CSV-files is not possible with MATLAB, a file splitting concept was implemented to read in and convert the data block by block into the binary .MAT file format.

2.2. Algorithm Development

In order to detect peaks in signals algorithmically, peak finding functions such as the internal MATLAB function *findpeaks()* are usually applied. However, the diverse forms of MEA signals make it difficult for algorithms to correctly identify the peaks that occur and assign them to the corresponding features, such as depolarization spike peaks (DSPs) or T-wave peaks (TWPs).

To circumvent this, a bandpass filter with lower and upper passband of f_L = 350 Hz and f_U = 550 Hz, respectively, is used to localize DSPs. The Fourier transformed box-car spectrum of this filter results in a sinc function in the time domain whose maximum is located on the time axis in the immediate vicinity of the respective peak, which is then precisely identified with a peak finder function.

The time duration between DSP and TWP is used to determine the FPD. However, the special significance of the FPD lies not in the exact determination of this distance, but in the difference of the FPDs over time, as this provides information about a possible QT prolongation. However, CM T-waves in MEA signals often have two peaks with opposite orientations, which makes it difficult to clearly identify the correct peak and leads to inconsistencies when determining the temporal development of the FPD. For this reason, the T-Wave Point of Interest (TPOI) was introduced, which is determined with the help of the maximum of the first derivative of the signal in the area of the occurring T-wave and which can be reliably determined independently of the number of peaks occurring.

2.3. INCardio Data Analysis Tool

The INCardio Data Analysis Tool (DAT) combines the functionalities of data conversion to convert CSV to MAT files, data loading to load the data from up to three MEA plates, data analysis with analysis and evaluation of the data by the algorithm and data export to export the results, in one software with an easy-to-use interface. The tool is designed in such a way that it requires neither special training nor the manual input of parameters such as signal thresholds.

2.4. Validation

Data from five MEA plates were used for validation, each containing one well of DMSO-treated and one well of E4031-treated cells. These acted as negative and positive controls, respectively, with respect to QT prolongation, and their FPs were recorded at five consecutive time points (baseline/QC, 30 min, 2h, 24h, 48h).

Bland-Altman plots (BA) and linear regression models were used to validate the analysis results of the DAT against those of the CAT, with the restriction that only electrodes that gave results with both tools were considered. For the validation of the Depolarization Spike Amplitude Mean Values (DSAM), a Mean of Differences (MoD) of ±0.1 mV, the exceedance of the Limits of Agreement (LoA) as well as an R^2-score of 0.98 and for the validation of the FPD Differences, a MoD of ±5 ms, the exceedance of the LoAs and an R^2-score of 0.97 were set.

3. Results

3.1. Depolarization Spike Amplitude Mean Values

The validation of the DSAM values of the electrodes of all wells shows that the results of 11 of the 36 measurements performed with CAT and DAT show significant disagreement or differences (Table 1). These were subsequently investigated in detail for their causes.

Table 1. Evaluation of the results of the Bland-Altman plots applied to the DSAM values of Axion CAT and INCardio DAT as well as the coefficient of determination (CoD) of those 11 measurements that show a too weak agreement or a too low R^2-score [5].

	Bland-Altman Results [mV]			CoD	
Well_Subst_Time	**MoD**	**LoA_{Lower}**	**LoA_{Upper}**	**R^2-Score**	**Significant**
Plate 8					
D6_DMSO_2h	-0.0749	-0.6003	0.4506	0.0498	Y
D6_DMSO_24h	0.0187	-0.0520	0.0894	0.9771	Y
Plate 9					
A3_DMSO_30min	-1.4990	-5.6640	2.6650	0.0670	Y
Plate 10					
D2_E4031_30min	-0.0743	-0.2086	0.0601	0.9598	Y
D2_E4031_2h	-0.1029	-0.3193	0.1135	0.9757	Y
Plate 12					
B4_DMSO_baseline	-0.0019	-0.0660	0.0622	0.9981	Y
C1_E4031_baseline	-0.5961	-1.8800	0.6873	0.0002	Y
C1_E4031_30mın	-0.4978	-1.6700	0.6745	0.9934	Y
C1_E4031_2h	-1.1340	-2.0880	-0.1790	0.9860	Y
C1_E4031_48h	-0.7059	-2.1990	0.7870	0.7683	Y
Plate 14					
C5_E4031_30min	-2.0770	-3.6260	-0.5276	1.0000	Y

Table 2. Results of the examination of all wells whose SAMs show significant differences in the results of CAT and DAT. The values of the column True are the mean values of all manually measured spikes of an electrode signal against which the electrode mean values determined by CAT and DAT are compared [5].

Well_Subst_Time	Electrode	CAT [mV]	DAT [mV]	True [mV]	Correct
Plate 8					
D6_DMSO_2h	23	0.306	1.312	1.312	DAT
D6_DMSO_24h	31	0.618	0.501	0.501	DAT
Plate 9					
A3_DMSO_30min	22	1.050	5.877	5.877	DAT
Plate 10					
D2_E4031_30min	31	1.583	1.682	1.682	DAT
D2_E4031_2h	33	3.378	3.531	3.531	DAT
Plate 12					
B4_DMSO_baseline	34	4.182	4.275	4.275	DAT
C1_E4031_baseline	21	2.865	4.166	4.166	DAT
C1_E4031_30min	14	9.908	11.292	11.292	DAT
C1_E4031_2h	13	9.439	11.044	11.044	DAT
C1_E4031_48h	14	5.152	6.898	6.898	DAT
Plate 14					
C5_E4031_30min	32	0.799	3.415	3.415	DAT

Since the DSAM values of almost all active electrodes in the affected wells showed differences, only the electrode with the largest deviation per well is listed in Table 2. The

exact graphical measurement of the affected FP signals leads to the result that in all cases the values of the INCardio DAT are correct.

3.2. Field Potential Duration Differences

The results for the FPD differences in Table 3 show that out of five wells investigated, there is only one case, Plate 8/D6, where there is strong agreement between the two tools.

In two cases, Plate 10/D1 and Plate 11/B4, the agreement is weaker because the FPD values of one and two is weaker, as the FPD values of one and two electrodes respectively had a high standard deviation. However, replacing the FPD mean values with the median values of these electrodes leads to strong agreement between the tools, which is why the agreement can be considered acceptable.

Weak or no agreement is found for Plate 9/A3 and Plate 14/B6. The cause for this was found in the results of the Axion CAT and christened "peak switching". This effect (Figure 1) occurs when a T-wave shows two distinct peaks with opposite orientation and the CAT algorithm does not follow the same peak at the different measurement times. This leads to a false shortening or lengthening of the FPD and thus to an incorrect representation of the development of the FPD over time.

Table 3. Results of the FPD Differences validation with the strength of agreement between the results of the two tools, CAT and DAT, and the main causes of deviations [5].

Plate	Well	Agreement	Cause	By Tool
8	D6	Strong	-	-
9	A3	Poor	Peak switch	CAT
10	D1	Acceptable	High standard deviation	CAT
12	B4	Acceptable	High standard deviation	CAT
14	B6	Poor	Peak switch	CAT

4. Discussion

The mandatory in vitro cardiotoxicity screening of already established and new pharmacological substances [1] leads to an increased use of MEA systems due to the higher throughput and higher efficiency [3].

Since the results of the CAT from Axion Biosystems used by Eurac Research show deviations from the true values in some cases with regard to both DSAM and FPD, a new MEA analysis tool was developed by CUAS as part of the Interreg INCardio project. In addition to a novel algorithm to increase the accuracy and reliability of the results, which does not require any manual input of parameters like DSAM thresholds, the tool also features a user interface that is easy to use for inexperienced users.

The validation data of the INCardio DAT show that the algorithm developed for the detection and calculation of DSAMs works more reliably and accurately than the reference system CAT, which is important when Sodium blockers are involved. Also, in cardiotoxicity screening, special attention must be paid to the correct identification of the respective T-wave features, such as TWPs or TPOIs, to determine the temporal development of the FPD. If an algorithm is subject to inconsistent behavior such as peak switching (Figure 1), this can lead to false conclusions about the mode of action of a drug. The validation has shown that the DAT has no peak-switching effect and thus provides more accurate and robust results than the reference analysis software CAT.

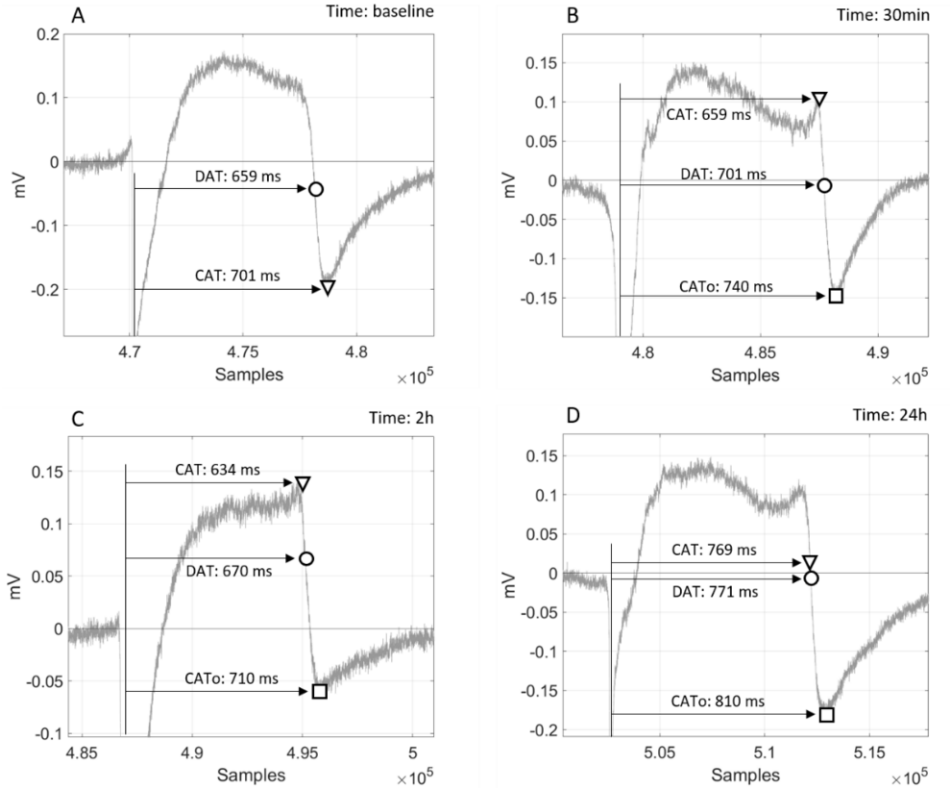

Figure 1. Explanation of the "peak switching" effect that leads to the incorrect representation of the evolution of the FPD over time by the Axion CAT. (A) TWP identified by CAT (triangle) and TPOI (circle) determined by DAT as well as their respective FPDs at the initial measurement. (B) Occurrence of peak switching by selection of the positive (triangle) instead of the originally negative (square) peak by the CAT with the consequence of a shorter instead of longer FPD. (C) Retention of the positive peak and the false FPD by CAT. (D) Identification of a point on the slope between the two peaks as a TWP by CAT instead of the originally selected peak in negative direction.

References

[1] Food and Drug Administration, HHS, "International Conference on Harmonisation; guidance on S7A safety pharmacology studies for human pharmaceuticals; availability. Notice," eng, Federal Register, vol. 66, no. 135, pp. 36 791–36 792, Jul. 2001, https://pubmed.ncbi.nlm.nih.gov/12356097, last access: 11/06/2022.

[2] Prestwick Phytochemical Library, https://www.prestwickchemical.com/screening-libraries/prestwick-phytochemical-library, last access: 16/06/2022.

[3] S. Kussauer, R. David, and H. Lemcke, "hiPSCs Derived Cardiac Cells for Drug and Toxicity Screening and Disease Modeling: What Micro- Electrode-Array Analyses Can Tell Us," en, Cells, vol. 8, no. 11, p. 1331, Oct. 2019, https://www.mdpi.com/2073-4409/8/11/1331, last access: 12/06/2022.

[4] P. Pradhapan, J. Kuusela, J. Viik, K. Aalto-Setälä, and J. Hyttinen, "Cardiomyocyte MEA Data Analysis (CardioMDA) – A Novel Field Potential Data Analysis Software for Pluripotent Stem Cell Derived Cardiomyocytes," en, PLoSONE, vol. 8, no. 9, A. B. Pant, Ed., e73637, Sep. 2013, https://dx.plos.org/10.1371/journal.pone.0073637, last access: 12/06/2022.

[5] C. Voutsinas, "Automated Analysis of Multi-Electrode Array Signals of hiPSC-Derived Cardiomyocytes", Master Thesis, Carinthia University of Applied Sciences, Master Program Healthcare IT, Oct. 2022.