Biomedical Signal Processing and Control 45 (2018) 202–212

Contents lists available at ScienceDirect

Biomedical Signal Processing and Control

journal homepage: www.elsevier.com/locate/bspc

A novel statistical signal processing method to estimate effects of compounds on contractility of cardiomyocytes using impedance assays

L. Batista[a,b,d], T. Bastogne[a,b,c,d,*], A. Delaunois[e], J.-P. Valentin[e], F. Atienzar[e]

[a] Université de Lorraine, CRAN, UMR 7039, Vandœuvre-lès-Nancy, France
[b] CNRS, CRAN, UMR 7039, Vandœuvre-lès-Nancy, France
[c] INRIA, BIGS, France
[d] CYBERnano, 193 Avenue Paul Muller, 54602 Villers-lès-Nancy, France
[e] UCB Biopharma SPRL, Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium

ARTICLE INFO

Article history:
Received 8 October 2017
Received in revised form 16 February 2018
Accepted 28 May 2018

Keywords:
Signal processing
Biostatistics
High-content screening
Impedance
Label-free technology
Cardiomyocyte
Cardiotoxicity

ABSTRACT

Label free methods such as cell impedance assays are *in vitro* tests increasingly used in drug development and producing large and high-content data files. Since the current commercial software is not suited for fully automated analysis, there is a need to develop validated and rapid solutions to extract relevant information for biologists. This need is particularly obvious in the case of impedance signals analysis from cardiomyocytes. The proposed solution is based on three main steps. The first one consists in calculating five indices informing about the time variations of frequency (F), amplitude (A), shape (S) of beatings, trends (T) of the cardiomyocyte dependent on spreading, viability and attachment as well as irregularity (I) of the contractility. In a second phase, two summary statistics are proposed to test the concentration effect of drugs on the five FASTI indices. Results of the statistical tests are finally aggregated in a cardio-effect grade to compare the tested molecules in a cardio-impact scale graduated from 0 (no influence) to 10 (highly disturbed effects in cardiomyocytes). This innovative approach was tested using *in vitro* data obtained from cell impedance analysis of three known molecules (2 cardiotoxic and 1 non-cardiotoxic compounds). Results have clearly shown the ability of the proposed approach to identify significant effects on the contractility of cardiomyocytes. This solution speeds up the analysis of cardiomyocyte impedance data, takes into account all the kinetic data generated and is now available for biologists on a web-platform: i-Cardio™ developed by CYBERnano™.

1. Introduction

The CiPA[1] initiative [1,2] aims at developing a new approach to assess the proarrhythmic potential of new chemical entities. So far, cardiovascular safety assessment was mainly focused on QT interval prolongation, which is used as a marker for predicting the risk of a compound to cause potentially fatal ventricular cardiac arrhythmia called Torsade de Pointes (TdP). The current preclinical safety guideline (ICH[2] S7B) relies on preclinical electrophysiology tests against hERG (*human-ether-à-go-go Related Gene*) and an *in vivo* QT measurement. Nevertheless, this approach may increase the number of false positive drugs. In addition, to increase the likelihood of success, an effective derisking strategy should evaluate proarrhythmia liability, hemodynamic cardiac contractility assessment, taking into account both functional and structural aspects of cardiotoxicity. All this situation has motivated ICH to reconsider approaches to the evaluation of drug-induced cardiac injuries. To that aim, *in silico* action potential simulations based on the O'Hara-Rudy model [3] have been integrated in the comprehensive in vitro proarrhythmia assay (CiPA) effort to better assess human cardiotoxicity risks. A second main component of the CiPA project relies on stem cell-derived human myocyte studies. Two technologies were suggested for evaluation, namely the multielectrode array (MEA) and the voltage-sensing optical (VSO) platforms. However, other emerging techniques exist, such as the cardiomyocyte impedance analysis, which proved to be useful to identify cardiotoxic compounds [4].

* Corresponding author at: Université de Lorraine, CRAN, UMR 7039, Vandœuvre-lès-Nancy, France.
E-mail address: thierry.bastogne@univ-lorraine.fr (T. Bastogne).

[1] Comprehensive In Vitro Proarrhythmia Assay.
[2] International Conference on Harmonisation.

https://doi.org/10.1016/j.bspc.2018.05.038
1746-8094/

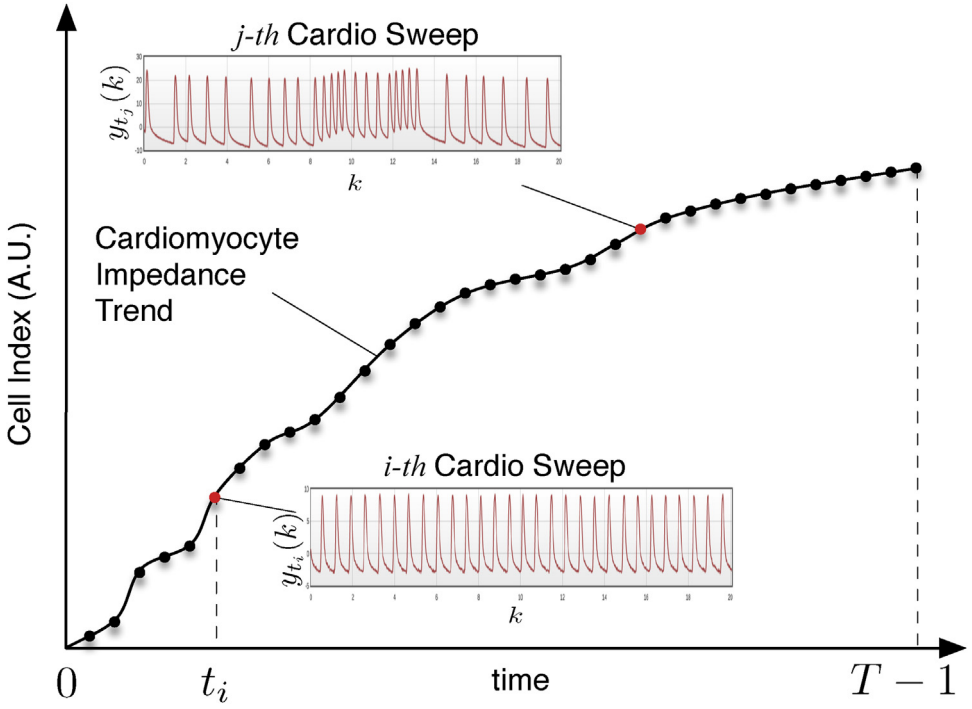

Fig. 1. Real-time measurement of cardiomyocyte impedance responses. The trend response is measured the cell index (arbitrary unit) with a sampling period of about 5 mn. This increasing trend signal provides information about the cardiomyocyte attachment and spreading during the experimentation period. Zooming into the cell index response allows to monitor the contraction of the cardiomyocytes. At each time point t_i, a cardio-sweep is measured during 20 s with a sampling rate of 77.35 Hz to observe the cardiomyocyte beating pattern. k is the time index used in each cardio-sweep.

In this context, in the present paper, we evaluated the real-time cell analysis (RTCA) platform, measuring cell impedance, to assess effects on contractility of cardiomyocytes exposed to a small panel of drugs. By analogy, this approach may be compared with the detection and characterization of abnormalities in ECG signals. Mahmoud et al. [5] reported that the time-frequency distribution is employed to reveal the exact multicomponent structure of surface electromyogram with the presence of ECG noise and abnormal cardiac signals. Martis et al. (2014) compared four methods to diagnose ECG abnormalities [6]. They associate two different transforms (wavelet *vs* cosinus) with statistical techniques of reduction such as the Principal Component Analysis followed by clustering analysis involving K-nearest neighbor, decision tree and artificial neural networks. Since that time, a large spectrum of methods have been tested such as sequential Bayesian methods [7], multiscale energy and eigenspace approach [8], multiresolution time-dependent entropy method [9], signal decomposition model-based Bayesian approach [10] and continuous wavelet transform [11]. However, several main differences may be emphasized between the ECG and cardiomyocyte impedance analysis. Firstly, PQRST waves do not exist in cell index (CI) signals and their beating shape may change completely depending on the drugs used [12,13]. Secondly, conversely to usual ECG that are recorded during about 10 s only, CI responses can be measured during one week of experimentation and generate larger data files to be processed.

MEA, VSO and RTCA platforms are increasingly used for drug discovery applications. In such a context, large number of molecules can be tested on cardiomyocytes and several parameters can be analyzed to assess cardiotoxicity risks. This computational task is time-consuming and user dependent. Our objective was to develop an automatic, reliable and fast method to compare the effects of tested drugs on cell impedance (CI) responses in cardiomyocytes. To address this issue, we propose a triple-stage method. The first step is based on the estimation of five time-variant parameters of CI responses. In a second phase, a concentration-effect modeling of the five indices is performed and statistical tests are used to assess the relevance of all variations in comparison with the control group. Finally, a score of critical effects is proposed to compare the alteration effects on the CI responses of tested molecules in a new cardio-impact scale. The second main intended purpose of this article is to assess the practical relevance of this new approach applied to real cardiomyocyte impedance data.

This paper is organized as follows. In Section 2, the cardiomyocyte impedance analysis is introduced. Section 3 describes the methodology and the impedance data corresponding to three molecules are presented in Section 4. Finally, as a proof of concept, results are discussed with a set of two cardiotoxic and one non-cardiotoxic compounds to demonstrate the potential of the

Table 1
Description of the parameters and variables used in the proposed statistical signal processing method.

Symbols	Legend
$t \in [0, \ldots, T-1]$	Moment of measurement of the $(t+1)$th sweep
$k \in [0, \ldots, K-1]$	Moment of CI measurement within each sweep
$y_t(k)$	$(k+1)$th CI value in the tth sweep
T	Number of recorded sweeps
K	Number of time points in a sweep signal
P	Number of time points in a beating period
t_m	Time of drug administration
$d \in [1, \ldots, D]$	Index of the drug concentration
D	Number of concentration (dose) levels
$r \in [1, \ldots, R]$	Index of the replicated well
R	Number of assay replications
Δt	Time range of analysis after compound addition
$i \in [F, A, S, T, I]$	FASTI index
$F(t)$	Beating mean frequency characteristic of the tth sweep
$A(t)$	Beating mean amplitude characteristic of the tth sweep
$S(t)$	Beating pattern characteristic of the tth sweep
$T(t)$	tth value of the CI trend, mean value of the tth sweep
$I(t)$	Beating irregularity feature of the tth sweep