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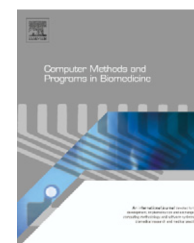

Averaging *in vitro* cardiac field potential recordings obtained with microelectrode arrays

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ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form
13 February 2011
Accepted 9 April 2011

Keywords:
Cardiomyocyte
Field potential
Averaging
Microelectrode array

ABSTRACT

Extracellular field potential (FP) recordings with microelectrode arrays (MEAs) from cardiomyocyte cultures offer a non-invasive way of studying the electrophysiological properties of these cells at the population level. Several studies have examined the FP properties of cardiomyocytes of various origins, including stem cell-derived cardiomyocytes. This focus reflects growing importance and interest in the field of MEA. High-quality cardiac FP signals are often difficult to obtain, especially from stem cell-derived cardiomyocyte cultures, which represent an important new field in cardiac electrophysiology. One way to improve the quality of these recordings is to average the cardiac FP signals. To date, however, no studies have examined the effect of averaging on cardiac FP signals. We report here that cardiac FP averaging can yield higher-quality signals than original individual FPs, and therefore promise more accurate detection of different phases and analysis of the cardiac FP signal. Averaged signals improved the signal-to-noise ratio (SNR), and obtaining reliable averages required approximately 50 cardiac cycles. We therefore propose that routine cardiac FP averaging can serve as a tool to compare the effects of different experimental conditions or stimuli on the properties of cardiac FPs.

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1. Introduction

Extracellular field potential (FP) recordings of cardiomyocytes with microelectrode arrays (MEA) enable the study of cardiac electrophysiological properties at the population level [1]. Previous studies have shown cardiac FPD on MEA to correspond with QT interval properties in the electrocardiogram [2]. Thus, FP recordings of beating cardiomyocyte aggregates offer insight into the electrical function of myocardial tissue *in vitro* [3]. The MEA platform has served extensively in the study of cardiomyocytes of various origins, such as the chick heart [4], mouse embryonic stem cell (ESC)-derived cardiomyocytes [5], mouse induced pluripotent stem (iPS) cell-derived cardiomyocytes [6], human ESC-derived cardiomyocytes [7–9] and human induced pluripotent stem (iPS) cell-derived cardiomyocytes [10].

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doi:10.1016/j.cmpb.2011.04.001

Signals of good quality, especially from pluripotent stem cell-derived cardiomyocytes, are sometimes difficult to obtain. In the case of human embryonic stem cell-derived cardiomyocytes (hESC-CMs), the quality of the signal is often limited by low numbers of cardiomyocytes in the clusters. Irregular beating rhythms can also present a challenge in analysing the signals [11]. Obtaining a clear signal is crucial for determining different parameters, especially the end-point of the field potential duration (FPD), which indicates the end of repolarisation and thus the end of electrical activation in one cardiac cycle. Low signal-to-noise ratio (SNR) recordings can yield higher quality via averaging. To date, however, no studies have investigated the direct effects of averaging on cardiac FP signals.

We hypothesised that averaging several FP cycles from recordings with a stable beating rhythm would yield more accurate and reliable results than would measuring the parameter values from one or a few representative individual FP cycles. This would be especially important when poor quality due to a low signal-to-noise ration obscures identification of the end of the cardiac replolarisation phase in the FP signals. Indeed, our data suggest that averaging can serve to produce reliable results from the cardiac field potential recordings of neonatal rat cardiomyocytes (NRC) and hESC-CMs.

2. Materials and methods

2.1. *Human embryonic stem cell culture*

H7 hESCs (WiCell) were cultured on mitomycin C inactivated mouse embryonic fibroblasts (MEF) in hES medium, which consisted of DMEM/F-12 (Invitrogen) supplemented with 20% KnockOut serum replacement (Invitrogen), 1% non-essential amino acids (Lonza), 2 mM Glutamax (Invitrogen), 50 U/ml penicillin/streptomycin (Lonza), 0.1 mM beta mercaptoethanol (Invitrogen), and 7.8 ng/ml basic fibroblast growth factor (R&D Systems). The medium was refreshed daily, and the hESC colonies were passaged onto a new MEF layer once a week using 1 mg/ml collagenase IV (Invitrogen).

2.2. *Cardiomyocyte sources and field potential recordings*

hESCs were differentiated into cardiomyocytes by co-culturing them with mouse visceral endoderm-like (END-2) cells as described elsewhere [12]. The spontaneously beating cardiomyocyte aggregates were mechanically excised from the cell cultures and plated onto fetal bovine serum (30 min, Invitrogen)-coated and 0.1% gelatine (1 h, Sigma–Aldrich)-coated MEAs (Multi Channel Systems MCS GmbH). The culture medium was refreshed three times a week.

Neonatal rat cardiomyocytes (NRCs) were extracted from the hearts of newborn Sprague-Dawley rats as described elsewhere [13]. The neonatal rats were quickly decapitated, and their hearts were harvested. Cardiomyocytes were extracted with multiple rounds of collagenase treatment, preplated for 1 h at +37 °C, 5% CO_2 and plated on coated MEAs; 800,000 NRCs were plated per MEA well. The culture medium was refreshed daily.

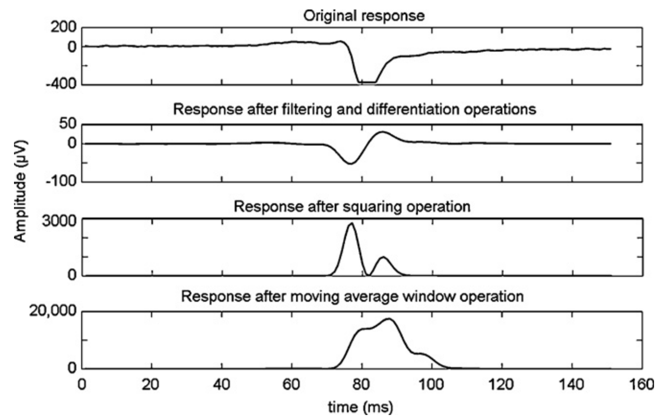

Fig. 1 – The stepwise process of the Pan–Tompkins QRS peak detection algorithm.

The FP recordings took place in room air with an MEA1060-Inv-BC amplifier using a 20-kHz sampling rate and MC_rack software (both from Multi Channel Systems MCS GmbH). Standard 200/30iR-Ti-gr MEAs were covered with a gas-permeable membrane (ALA Scientific) to keep the cultures sterile. During recordings, the temperature was kept at +37 °C using the heating element of the MEA amplifier. In drug test E-4031 (Alomone labs) was diluted in the cell culture medium for a final concentration of 600 nM. The FP signals were recorded from MEAs with NRCs ($n = 9$) and from MEAs with hESC-CMs ($n = 4$).

2.3. *Field potential averaging*

The Matlab-based analysis program was designed to obtain an average FP complex in order to calculate specific parameters from this average. The recorded files were imported into the Matlab (The Mathworks, Inc.)-based in-house programmed analysis program.

The program contains two different peak detection algorithms from which users can select the appropriate one to align the individual FPs correctly for calculation of the average field potential. Because of the variation in the shape of the cardiac FP signals, providing two different peak detection algorithms that correctly detect different forms yielded more accurate results. Both algorithms have a tenth-order FIR low-pass filter with a cut-off frequency of 1 kHz. The files were analysed using the algorithm that detected the peaks correctly.

The first peak detection algorithm is based on the QRS peak detection originally presented by Pan and Tompkins [14]. Fig. 1 illustrates process of the Pan–Tompkins algorithm. Briefly, the algorithm squares the signal after which the edges of the squared signal are detected. The peak is identified as the maximum of the original signal between the samples under these squares.

The second peak detection algorithm applies first- and second-order derivatives of the signal to obtain the signal's local maxima. The signals are divided into sections of 0.05 s, and the local maxima within these sections are identified with the Matlab implementation provided by Vargas Aguilera (http://www.mathworks.com/matlabcentral/fileexchange/

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