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Interpretation of field potentials measured on a multi electrode array in pharmacological toxicity screening on primary and human pluripotent stem cell-derived cardiomyocytes

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ABSTRACT

Multi electrode arrays (MEAs) are increasingly used to detect external field potentials in electrically active cells. Recently, in combination with cardiomyocytes derived from human (induced) pluripotent stem cells they have started to become a preferred tool to examine newly developed drugs for potential cardiac toxicity in pre-clinical safety pharmacology. The most important risk parameter is proarrhythmic activity in cardiomyocytes which can cause sudden cardiac death. Whilst MEAs can provide medium- to high- throughput noninvasive assay platform, the translation of a field potential to cardiac action potential (normally measured by low-throughput patch clamp) is complex so that accurate assessment of drug risk to the heart is in practice still challenging. To address this, we used computational simulation to study the theoretical relationship between aspects of the field potential and the underlying cardiac action potential. We then validated the model in both primary mouse- and human pluripotent (embryonic) stem cell-derived cardiomyocytes showing that field potentials measured in MEAs could be converted to action potentials that were essentially identical to those determined directly by electrophysiological patch clamp. The method significantly increased the amount of information that could be extracted from MEA measurements and thus combined the advantages of medium/high throughput with more informative readouts. We believe that this will benefit the analysis of drug toxicity screening of cardiomyocytes using in time and accuracy.

1. Introduction

Multi electrode arrays (MEAs) have been developed to measure electrical activity in neural and cardiac cells. They are now being increasingly used specifically to analyze pharmacological toxicity of newly developed or combinations of compounds on cardiomyocytes of the heart. Most recently, in combination with cardiomyocytes derived from human (induced) pluripotent stem cells (hiPSC-CMs) they have started to emerge as powerful tools to examine the ability of certain compounds to induce arrhythmias in the heart, which can lead to “Sudden Cardiac Death”. This represents a major toxic hazard for new drugs in pre-clinical evaluation [1]. In this context, the Food and Drug Administration recently initiated the “Comprehensive in Vitro Proarrhythmia Assay” which aims to establish robust methods to assess cardiac drug safety using hiPSC-CMs. Crucial will be the ability to measure electrical responses of the cardiomyocytes in an accurate and predictive yet medium- to high- throughput platform.

Classically, the cardiac action potential (AP) is measured using patch clamp electrophysiology in current clamp mode. All ionic currents of which the AP is composed can be measured individually in whole cell voltage clamp and have been studied in detail over several decades [2–4]. Alterations of one or more of these currents can lead to serious dysfunction of the heart, such as Torsade de Pointes (TdP) or prolongation of the “QT interval” on the standard electrocardiogram. Drug induced changes in the AP can be caused by modulations in Na^+ and Ca^{2+} inward currents or several of the

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outward K^+ currents, such as the rapidly activated (I_{Kr}) and slow activated (I_{Ks}) currents in human cardiomyocytes (Fig. 1A). Prolongation or shortening of repolarization can lead to concomitant modulation of the QT interval [5–7]. Parameters involved in determining the magnitude of these AP effects are the AP amplitude (APA; mV), the resting membrane potential (RMP; mV), the maximal rate of depolarization (V_{max}; Vs^{-1}) and AP duration at 50% and 90% of repolarization (APD_{50}, APD_{90} respectively; ms). Although these parameters can be accurately measured using patch clamp electrophysiology, this is labour intensive and requires highly skilled experienced operators. By contrast, MEAs are much more user-friendly, are medium- to high- throughput in use but record the cardiac field potential (FP) instead of the AP. Prolongation or shortening of FP duration (FPD) are routinely measured on MEAs and are considered a measure of the APD_{90} [1], but other parameters are difficult to extract from FPs although they may actually contain a high level of information. In practice extraction of this information is hampered by poor knowledge of the underlying relationship (transfer function) between the AP and the FP. Here we present a robust basis for more informative MEA analysis by comparing simulated APs with their resulting FPs using an electrical circuit model. We validated our findings by analysis of FPs recorded on MEAs in hPSC-CMs exposed to drugs with known effects.

2. Material and methods

2.1. Patch clamp electrophysiology

Patch clamp electrophysiology was essentially done as described previously [8]. Microelectrodes with a resistance between 2 and 4 MOhm were made from Borosilicate glass (Warner Instruments, GC-150 T) with Flaming/Brown Micropipette Puller Model 97 (Sutter Instruments, CA). The sampling frequency was 5 kHz. During electrophysiological measurements, cells were kept in a buffer containing 145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 10 mM D-glucose and 10 mM HEPES, adjusted to pH 7.30 with NaOH. The pipette contained buffer consisting of 145 mM KCl, 5 mM NaCl, 2 mM $CaCl_2$, 4 mM EGTA, 2 mM $MgCl_2$, and 10 mM HEPES, adjusted to pH 7.30 with KOH.

2.2. Primary cardiomyocytes

Hearts of mouse embryos at embryonic day (E)17.5 were isolated by micro scalpel and washed in low calcium (Ca^{2+}) medium for 30 min at room temperature. Tissue fragments were then incubated in enzyme-containing medium for 25–35 min at 37 °C. Dissociation of the tissue was completed in King's B medium by gentle shaking at room temperature for 1 h. The isolated cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% fetal calf serum (FCS) and incubated at 37 °C. MEA chips were plasma cleaned and coated with fibronectin (40 μg/mL) for 1 h at 37 °C. The isolated cardiomyocytes were allowed to attach to the surface of the MEA for 24 h in DMEM containing 5% FCS. Mouse experiments were performed conform EU Directive 2010/63/EU for animal experiments (Leiden University Medical Center).

2.3. hPSC-CM and differentiation

The human embryonic stem cell (hESC) line HES3 [9] was routinely cultured on 129SV mouse embryonic fibroblasts (MEFs) and induced to differentiate to cardiomyocytes as described previously [8]. Large numbers of contracting areas were obtained

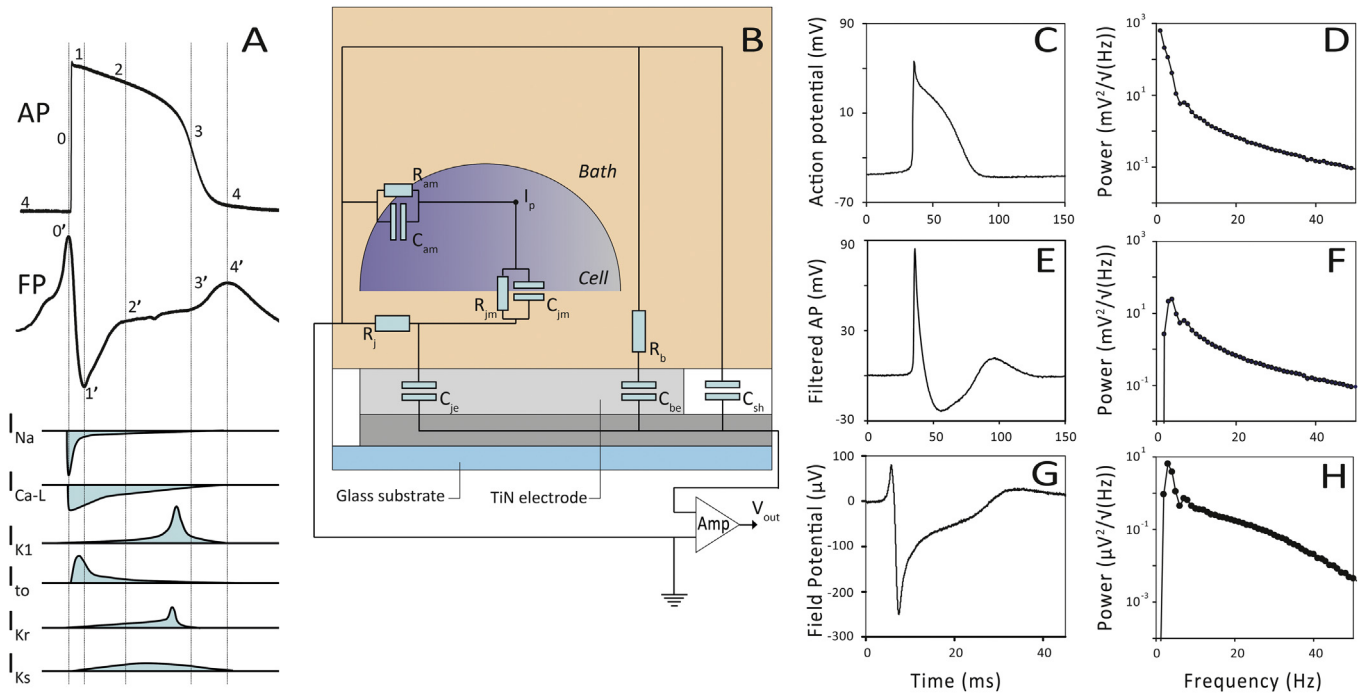

Fig. 1. (A) A ventricular hPSC-CM AP and FP. Composing active currents are schematically depicted during time. (B) Equivalent circuit of a cell on a MEA. R_{am}) apical cell membrane resistance to the bath; C_{am}) apical membrane capacitance to the bath; C_{jm}) cell membrane capacitance to junction; R_{jm}) membrane resistance to the junction; R_b) bath resistance; C_{be}) electrode capacitance via the bath resistor; C_{sh}) shunt capacitance of the electrode; R_j) junction resistance; C_{je}) junction capacitance of the electrode; I_p) injection point of the AP (V_m) in the simulation circuit. (C) Measured AP of a spontaneous beating mouse E17.5 cardiomyocyte. (D) Power spectrum of panel C, peak frequency at 1 Hz. (E) Result after Chybechev IIR filtering ($F_c = 30$ Hz) of the AP in panel C. (F) Power spectrum of panel E, peak frequency at ~3.5 Hz. (G) Measured FP of a mouse E17.5 cardiomyocyte on a MEA. (H) Power spectrum of panel G, peak frequency at ~3 Hz.

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