

Impedance and EFP recordings of Pluricyte® Cardiomyocytes on the CardioExcyte 96

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Pluricyte® Cardiomyocytes kindly provided by Ncardia.



Summary

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) are gaining interest in cardiac safety screening. Given their recapitulation of native behavior, availability, ease of use and standardized production, they are likely to provide a viable alternative to acutely isolated cardiomyocytes to assess the pro-arrhythmic potentials of drug candidates. Although automated patch clamp can provide excellent information about the effects of compounds on cardiac ion channels and possible effects on the cardiac action potential¹, other outputs such as extracellular field potential (EFP) and impedance, also provide crucial and complementary information about complex physiological parameters such as beat rate, amplitude and duration. The CardioExcyte 96 is a hybrid screening tool combining impedance (cell contractility) and EFP recordings^{2,3}. These measurements are non-invasive, label-free and have a temporal resolution of 1 ms. The recordings are made from cells within a network thus providing a physiologically relevant environment for measuring drug-induced changes in contractile parameters. This hybrid technology is an easy-to-use screening tool which permits the reliable investigation of short- and long-term pharmacological effects.

Here we present data recorded on the CardioExcyte 96 using Pluricyte® Cardiomyocytes from Ncardia. The effects of the Ca_v blocker, nifedipine, hERG blockers, dofetilide and E4031, and Na_v blocker, mexiletine, on EFP and impedance parameters are shown.

Results

Pluricyte® Cardiomyocytes were grown on the NSP-96 plates of the CardioExcyte 96. The cardiomyocytes formed a monolayer and started to beat synchronously, at which point they were used for pharmacological experiments, typically 7 - 12 days after plating. Figure 1 shows the effect of nifedipine, a Ca_v channel blocker, and dofetilide, a hERG channel blocker, on the impedance and EFP signals of Pluricyte® Cardiomyocytes.

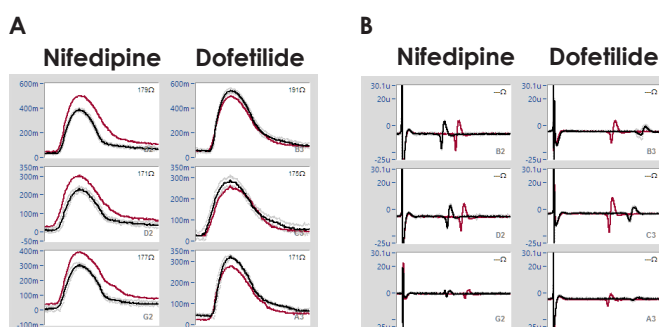


Figure 1: Effect of nifedipine and dofetilide on the impedance and EFP signals of Pluricyte® Cardiomyocytes. **A** Impedance traces of six example wells in control solution (red trace) and in the presence of 10 nM nifedipine (left, black trace) or 10 nM dofetilide (right, black trace). **B** EFP traces of six example wells in control solution (red trace) and in the presence of 10 nM nifedipine (left, black trace) or 10 nM dofetilide (right, black trace).

Application Note

Nifedipine is an L-type calcium channel blocker which causes QT shortening in isolated heart⁴. In impedance mode nifedipine caused a concentration-dependent decrease in amplitude and pulsewidth 20 and an increase in beat rate as previously reported^{3,5,6} and shown in Figure 2A & C. Figure 2C shows the impedance parameters: amplitude, pulsewidth 20, beat rate and beat rate regularity displayed as a spider chart for better visualization of the effect of the compound. Nifedipine had no effect on beat regularity. In EFP mode, nifedipine caused a decrease in field potential duration (FPD) and an increase in beat rate in agreement with the literature^{2,3,5,6} and shown in Figure 2B & D. Figure 2D shows the EFP parameters: amplitude, FPD, beat rate and beat rate regularity displayed as a spider chart for better visualization of the effect of the compound.

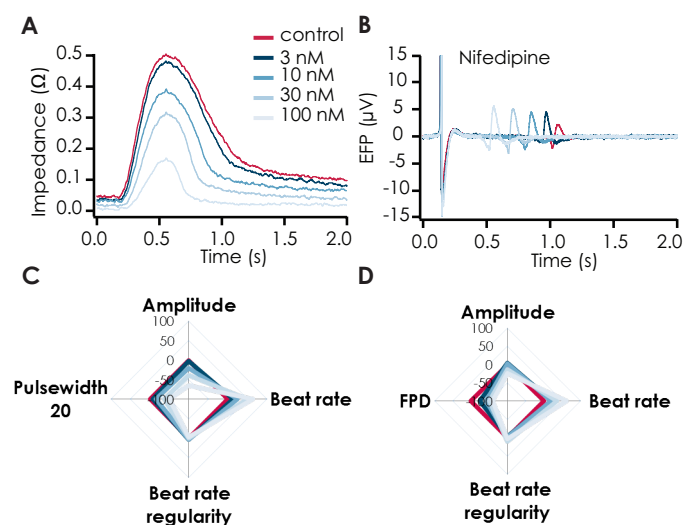


Figure 2: Effect of nifedipine on the impedance and EFP signals of Pluricyte® Cardiomyocytes. **A** Impedance traces of an example well in increasing concentrations of nifedipine. **B** EFP traces of an example well in increasing concentrations of nifedipine. **C** Effect of nifedipine on impedance parameters: amplitude, Pulsewidth 20, beat rate and beat rate regularity shown as a spider chart. **D** Effect of nifedipine on EFP parameters: amplitude, FPD, beat rate and beat rate regularity shown as a spider chart.

Dofetilide is a hERG inhibitor which has been shown to prolong cardiac action potential duration and induce Torsade de Pointes (TdP)^{7,8,9}. High concentrations (1 μ M) induce early afterdepolarizations (EADs) in isolated rabbit cardiomyocytes⁹. In impedance mode, dofetilide caused an increase in pulsewidth 20 which resulted in a decrease in beat rate (Figure 3A, C). In EFP mode, dofetilide caused a dramatic and concentration-dependent increase in FPD which also resulted in a decrease in beat rate (Figure 3B, D).

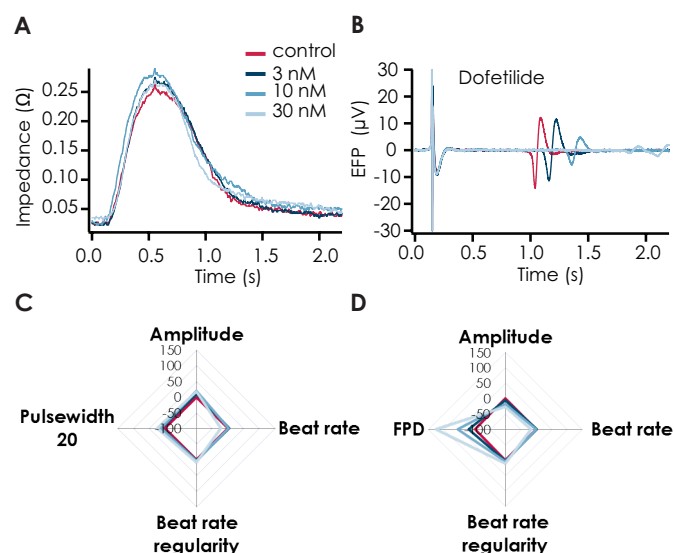


Figure 3: Effect of dofetilide on the impedance and EFP signals of Pluricyte® Cardiomyocytes. **A** Impedance traces of an example well in increasing concentrations of dofetilide. **B** EFP traces of an example well in increasing concentrations of dofetilide. **C** Effect of dofetilide on impedance parameters: amplitude, Pulsewidth 20, beat rate and beat rate regularity shown as a spider chart. **D** Effect of dofetilide on EFP parameters: amplitude, FPD, beat rate and beat rate regularity shown as a spider chart.

Application Note

Similarly, E4031 is a specific hERG inhibitor shown to block hERG in patch clamp experiments at low concentrations¹⁰. Furthermore, E4031 causes EADs in stem cell-derived cardiomyocytes¹¹. The effects of E4031 in impedance and EFP modes are complex. In EFP mode, there is an increase in FPD and a moderate increase in beat rate, whereas little effect is seen on beat parameters in impedance mode (Figure 4). This may be due to the irregular beating patterns induced by E4031. We have implemented new CiPA-approved protocols where we chose endpoints for analysis taken 30 min after compound addition. The effects of individual compounds develop over time and the discrepancies between the specific hERG blockers, dofetilide and E4031, as depicted in the spider charts may be thus explained. To further investigate this, analysis at multiple timepoints may be implemented and coupled with additional patch clamp experiments where hERG inhibition could be analyzed directly in the voltage-clamp mode. This highlights the importance of implementing complementary assays for cardiac safety screens.

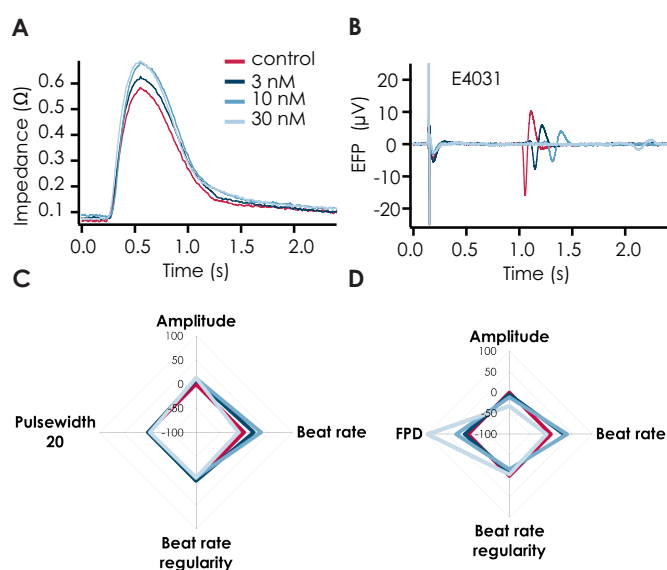


Figure 4: Effect of E4031 on the impedance and EFP signals of Pluricyte® Cardiomyocytes. **A** Impedance traces of an example well in increasing concentrations of E4031. **B** EFP traces of an example well in increasing concentrations of E4031. **C** Effect of E4031 on impedance parameters: amplitude, Pulsewidth 20, beat rate and beat rate regularity shown as a spider chart. **D** Effect of E4031 on EFP parameters: amplitude, FPD, beat rate and beat rate regularity shown as a spider chart.

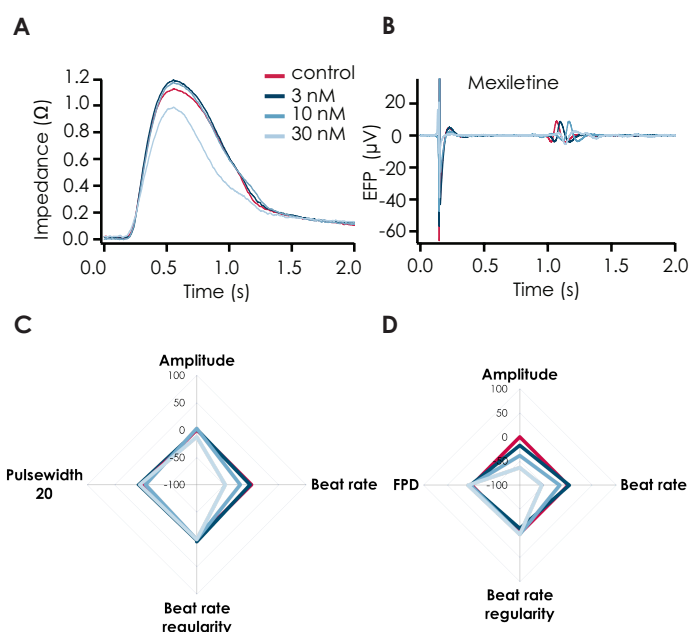


Figure 5: Effect of mexiletine on the impedance and EFP signals of Pluricyte® Cardiomyocytes. **A** Impedance traces of an example well in increasing concentrations of mexiletine. **B** EFP traces of an example well in increasing concentrations of mexiletine. **C** Effect of mexiletine on impedance parameters: amplitude, Pulsewidth 20, beat rate and beat rate regularity shown as a spider chart. **D** Effect of mexiletine on EFP parameters: amplitude, FPD, beat rate and beat rate regularity shown as a spider chart.

Mexiletine is a non-selective Na_v channel blocker, similar to lidocaine, used as an anti-arrhythmic compound¹². It is used to treat arrhythmias within the heart and may also be useful as an analgesic. Mexiletine caused a moderate decrease in amplitude in impedance mode at higher concentrations (30 nM) and concentration-dependent decrease in beat rate (Figure 5A, C). In EFP mode, mexiletine caused a dramatic, concentration-dependent decrease in spike amplitude, presumably due to block of the Na_v channels, and a decrease in beat rate (Figure 5B, D) as previously reported⁶.

Application Note

In conclusion, the CardioExcyte 96 can be used to reliably perform EFP and impedance measurements on hiPSCs from Ncardia (Pluricyte® Cardiomyocytes). A variety of compounds were used, for example the Ca_v blocker, nifedipine, decreased amplitude in impedance mode and increased beat rate in both impedance and EFP modes. The hERG blocker, dofetilide, dramatically increased FPD in EFP mode and decreased beat rate in both impedance and EFP modes. The effects of different compounds on the impedance and EFP signals can be complex, even though they may exert their actions on the same ion channels, eg. dofetilide and E4031 inhibiting hERG. This highlights the importance of combining experiments on the CardioExcyte 96 with

complementary techniques such as automated patch clamp to obtain a more complete profile for cardiac safety and cardiotoxicity studies.

We are confident that the CardioExcyte 96 in combination with hiPSCs will have a great impact on the field of pharmacology, toxicology, and cardiac safety screening in the light of the CiPA (Comprehensive In Vitro Pro-Arrhythmia Assay) guidelines. This initiative aims to define a new, integrated pre-clinical in vitro/in silico paradigm in which the potential pro-arrhythmic risk of a new drug will be assessed using hiPSCs.



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Methods

Cardiomyocytes

Pluricyte® Cardiomyocytes were kindly provided by Ncardia.

Impedance and EFP measurements

Impedance and EFP measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. Cardiomyocytes were seeded at 30,000 viable cells per well on fibronectin-coated Sensor Plates. Pluricyte Cardiomyocyte Medium was exchanged every other day until the conclusion of the assay. The cells were typically used 7 - 12 days after plating. This enabled the formation of a dense monolayer and a suitable network of cell-cell interconnections to ensure a steady propagation of the excitation and synchronized beating. At least 4 hours before drug application the medium was completely removed from the wells and 100 µl fresh medium was added. For the experiments, a 2x concentration of compound was prepared, 50 µl of solution was removed from the well and 50 µl of the 2x compound was added to the wells. Measurements were taken after 30 minutes incubation. All signals were normalized to a group of control measurements (n=5-11) on the same plate using DMSO (0.01%) as vehicle.