

Assessment of Drug Effects on Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CiPA) Results

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Abstract

The on-going comprehensive in vitro pro-arrhythmia assay (CiPA) initiative aims to improve drug safety testing and help more beneficial chemical entities reach the market. The project consists of three main parts, which include 1) electrophysiological investigation of drug effects on human ventricular ion channels, 2) characterization of the in-silico cardiosafety model predictions, 3) assessment of discrepancies and gaps in fully integrated biological systems (human induced pluripotent stem-cell derived cardiac myocytes (iPSC-CMs) and the human ECG). To assess and validate drug effects on hiPSC-CMs in a combined measurement of cardiac excitation and contraction, a subset of compounds which are part of the Phase II CiPA study were tested. A prospective comparison study using combined extracellular field potential (EFP) and impedance technology was conducted across 4 independent laboratories testing 12 reference compounds in 4 independent commercially available hiPSC-CMs. Our data demonstrates that hERG channel blockers, such as Sotalol and Dofetilide prolonged field potential duration (FPD) at low concentration and induced arrhythmia as measured by field potential (FP) recording and impedance (IMP) recordings at higher concentrations. The cross-cell comparison displayed different minimal effective concentrations regarding FPD prolongation and cessation of beating. On the contrary, Diltiazem, a calcium channel inhibitor, weakened cell contractile activity and shortened FPD. Multichannel inhibitors, such Quinidine increased FPD, weakened the contraction force and induced arrhythmia. Furthermore, validation on low risk proarrhythmia compounds such as Tamoxifen and intermediate risk compounds such as Droperidol will be presented. Comparison of the compound effects across the four different sites showed the consistent trend of the effect. Taken together, commercially available hiPSC-CMs in conjunction with combined measurements of ion channel activity and contractility is a reliable approach for risk assessment of proarrhythmic compounds.

Label-free toxicity and cardiac safety screening

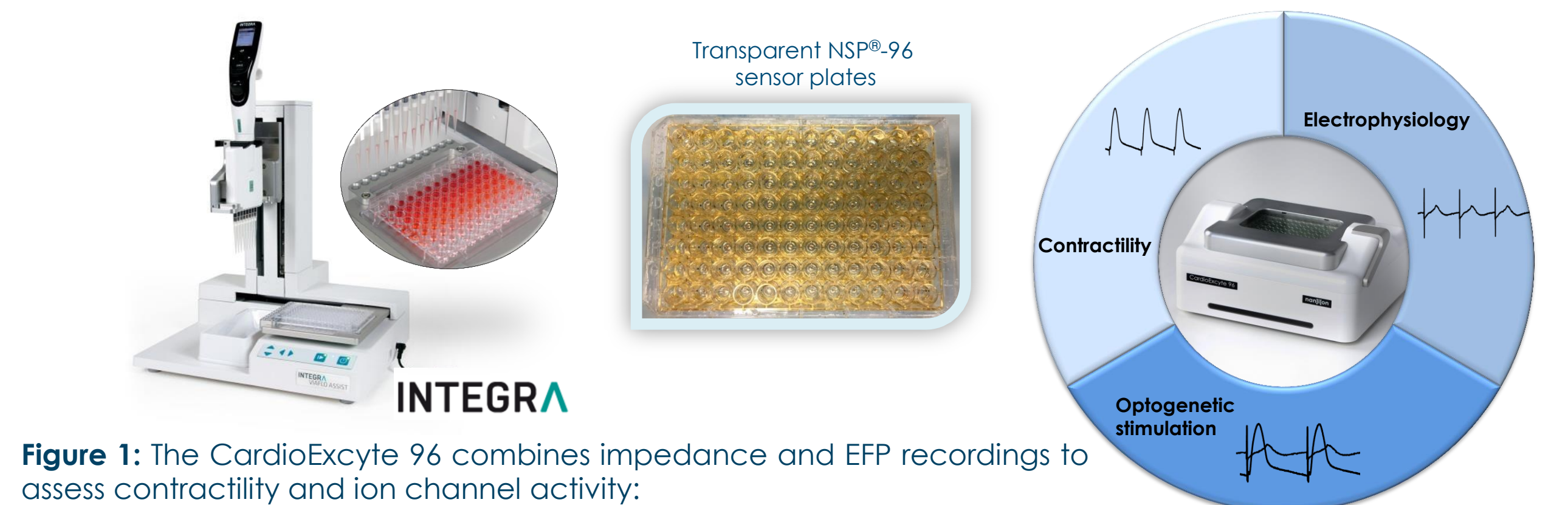


Figure 1: The CardioExcyte 96 combines impedance and EFP recordings to assess contractility and ion channel activity:

- **Electrical and Optical Stimulation**
- Independent system – no incubator required
- Automated liquid handling robot for cell seeding, medium exchange and compound addition (Integra Viaflo Assist)
- Long-term recording capabilities

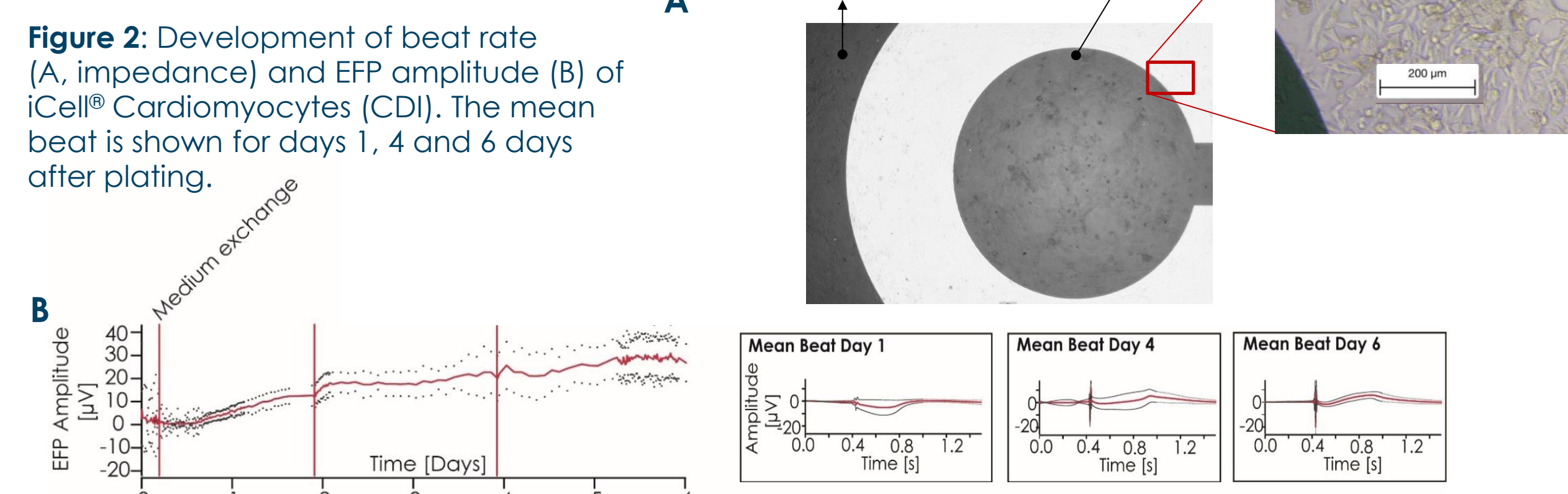


Figure 2: Development of beat rate (A, impedance) and EFP amplitude (B) of iCell® Cardiomyocytes (CDI). The mean beat is shown for days 1, 4 and 6 days after plating.

Automated Field Potential Duration and Arrhythmia Detection

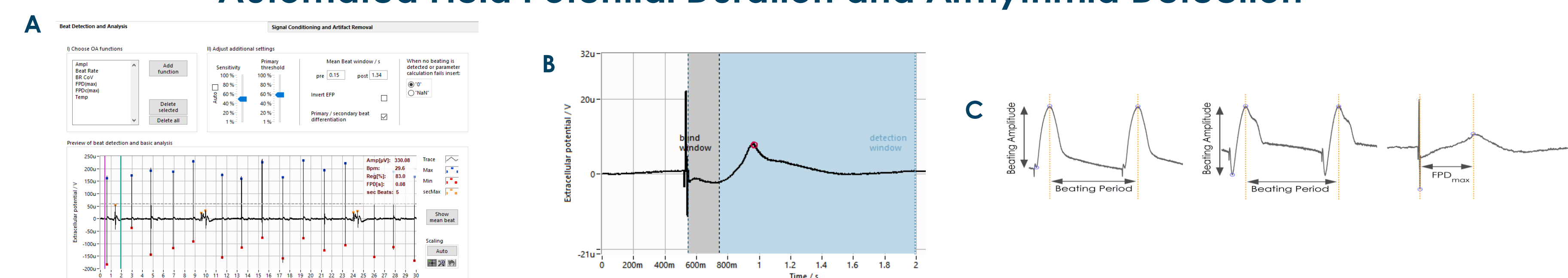


Figure 3: A. Secondary beat detection allows quantification of number of arrhythmic events for each recorded sweep. B. Automated detection of T-wave in an interval specified by user. C. Field potential duration maximum (FPD_{max}) is measured from initial spike depolarization to the maximum value of the repolarization (left). Automated detection of beat period, impedance amplitude in case of monophasic (left figure) and biphasic (middle) impedance traces.

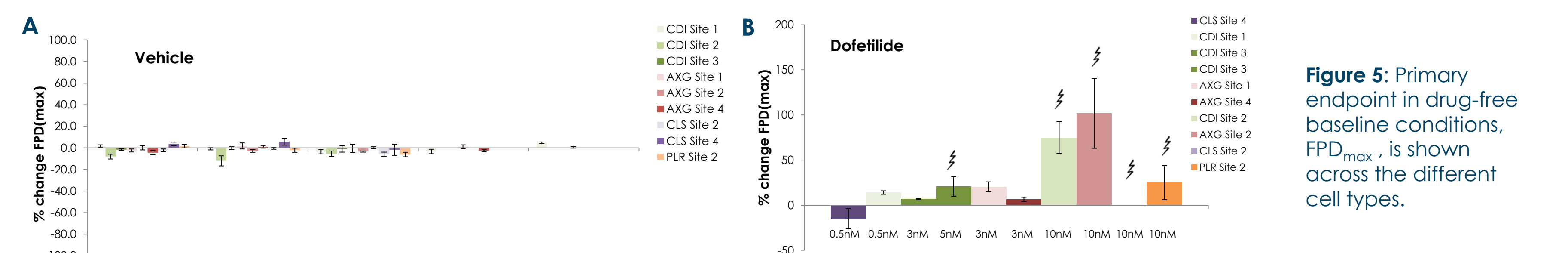


Figure 4: A. No significant changes are present in % change of FPD_{max} when comparing vehicle control across sites. B. An increase in repolarization prolongation is observed post application of 3, 5 or 10nM Dofetilide, when compared to vehicle control.

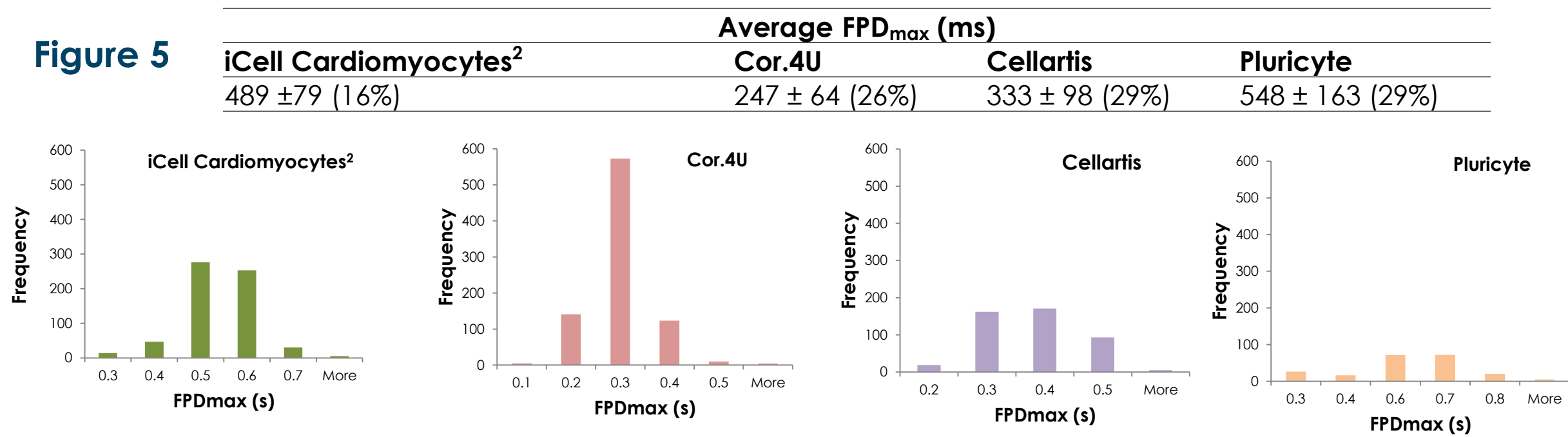


Figure 5: Primary endpoint in drug-free baseline conditions, FPD_{max}, is shown across the different cell types.

Extracellular Field Potential and Arrhythmia

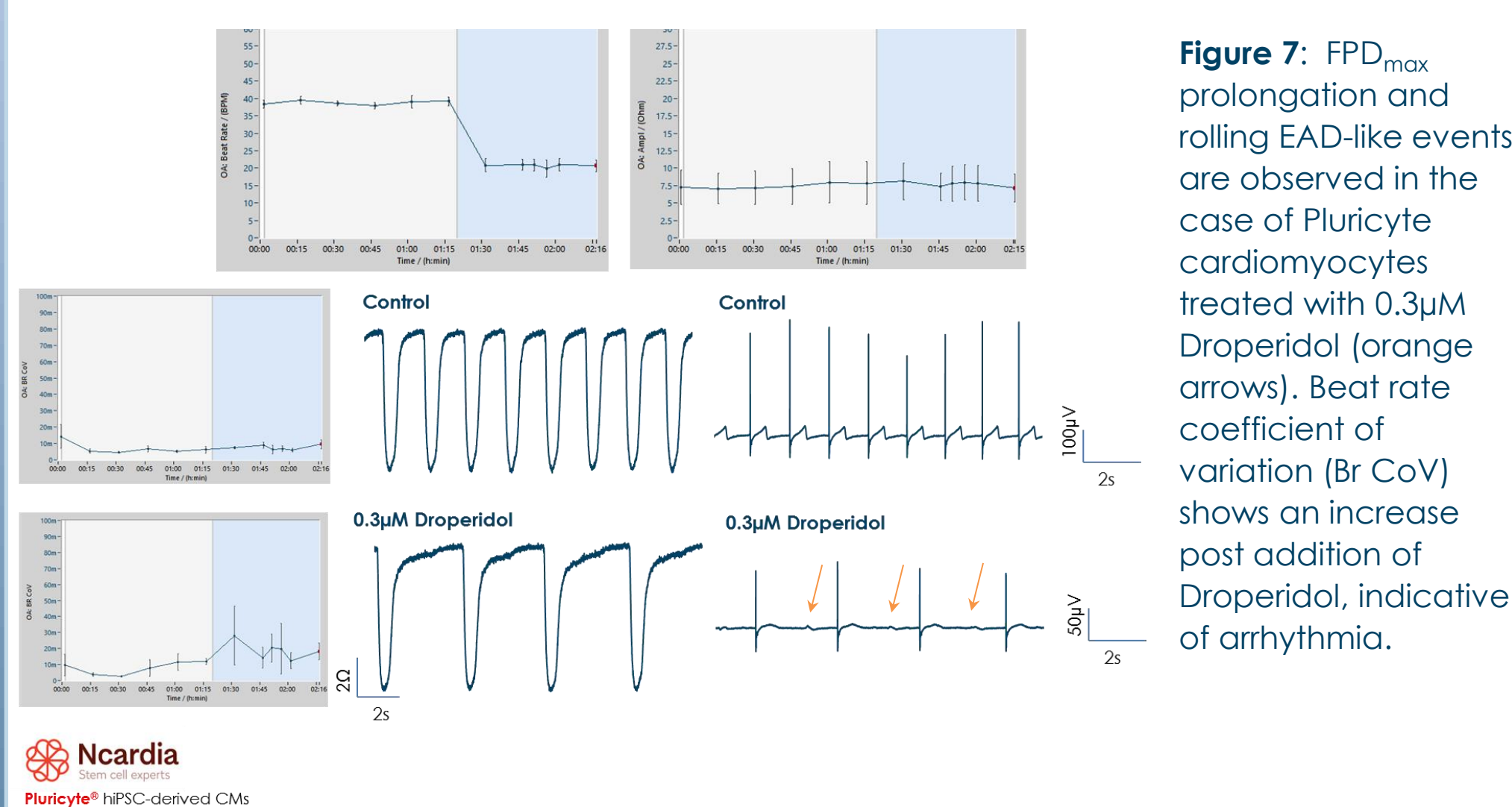


Figure 7: FPD_{max} prolongation and rolling EAD-like events are observed in the case of Pluricyte cardiomyocytes treated with 0.3µM Droperidol (orange arrows). Beat rate coefficient of variation (Br CoV) shows an increase post addition of Droperidol, indicative of arrhythmia.

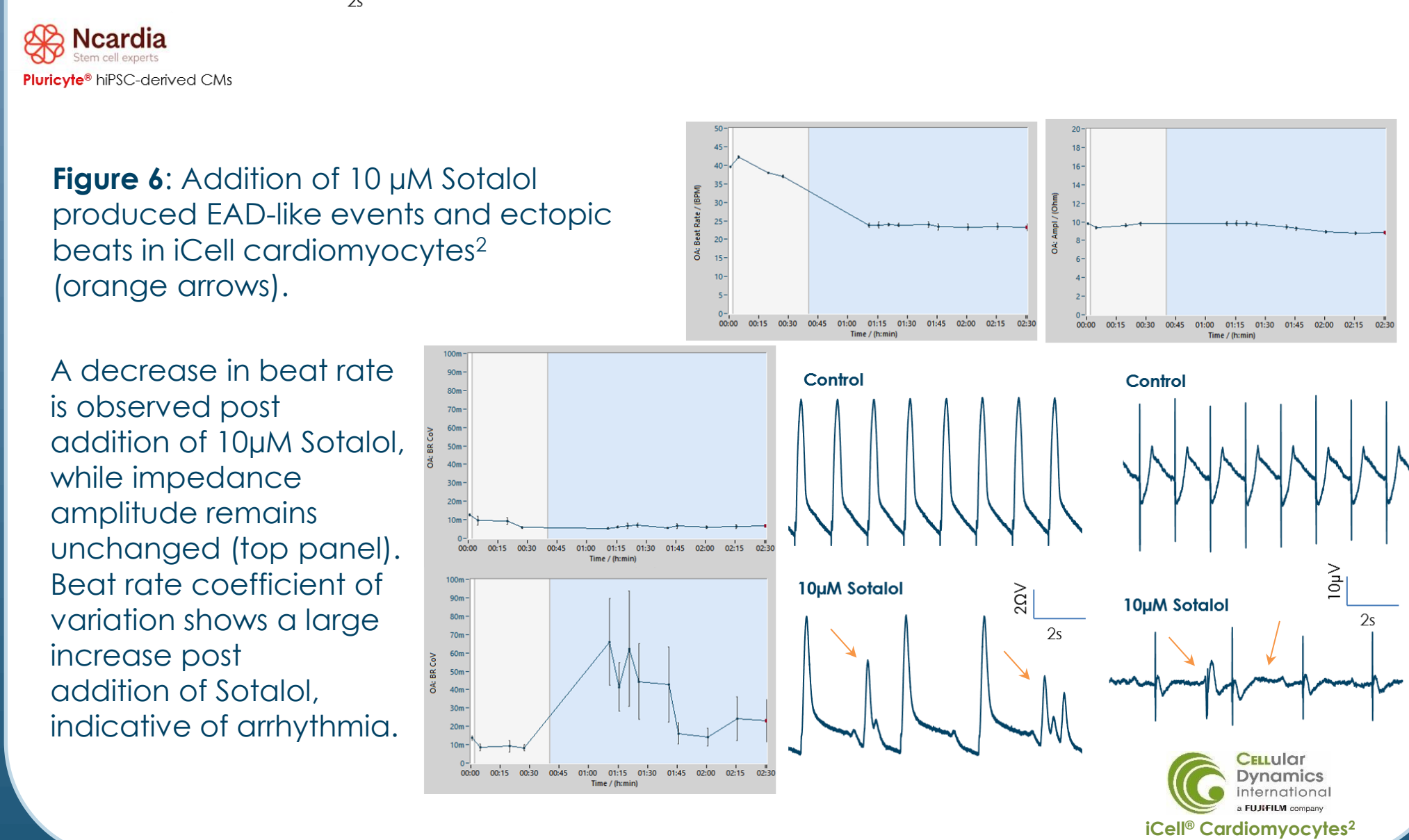


Figure 6: Addition of 10µM Sotalol produced EAD-like events and ectopic beats in iCell cardiomyocytes² (orange arrows).

A decrease in beat rate is observed post addition of 10µM Sotalol, while impedance amplitude remains unchanged (top panel). Beat rate coefficient of variation shows a large increase post addition of Sotalol, indicative of arrhythmia.

Disopyramide M.I.C.

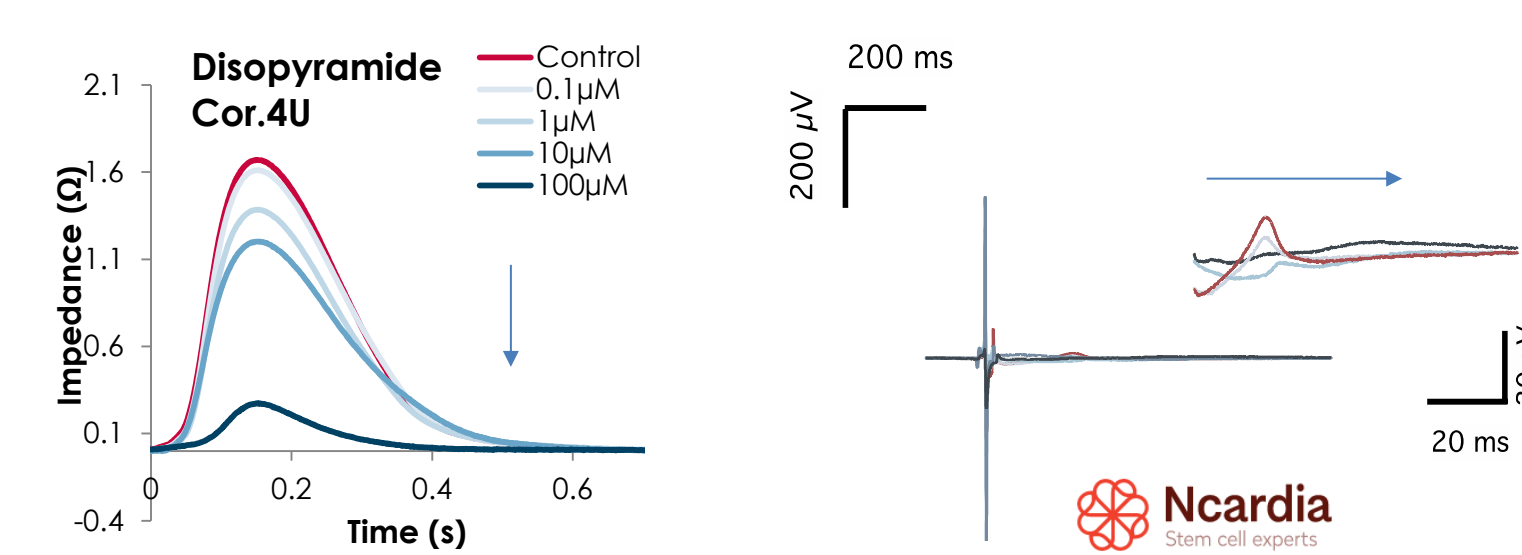


Figure 8: Disopyramide affects contractility and has multiple ion channel effects (MIC). Example raw impedance and EFP waveforms (Cor.4U®, AXG cells) (left) are presented before (red) and after (shades of blue) dosing with increasing concentrations of Disopyramide. Most prominent effects of Disopyramide were decreased contractility together with a reduction of EFP spike amplitude across all cell types.

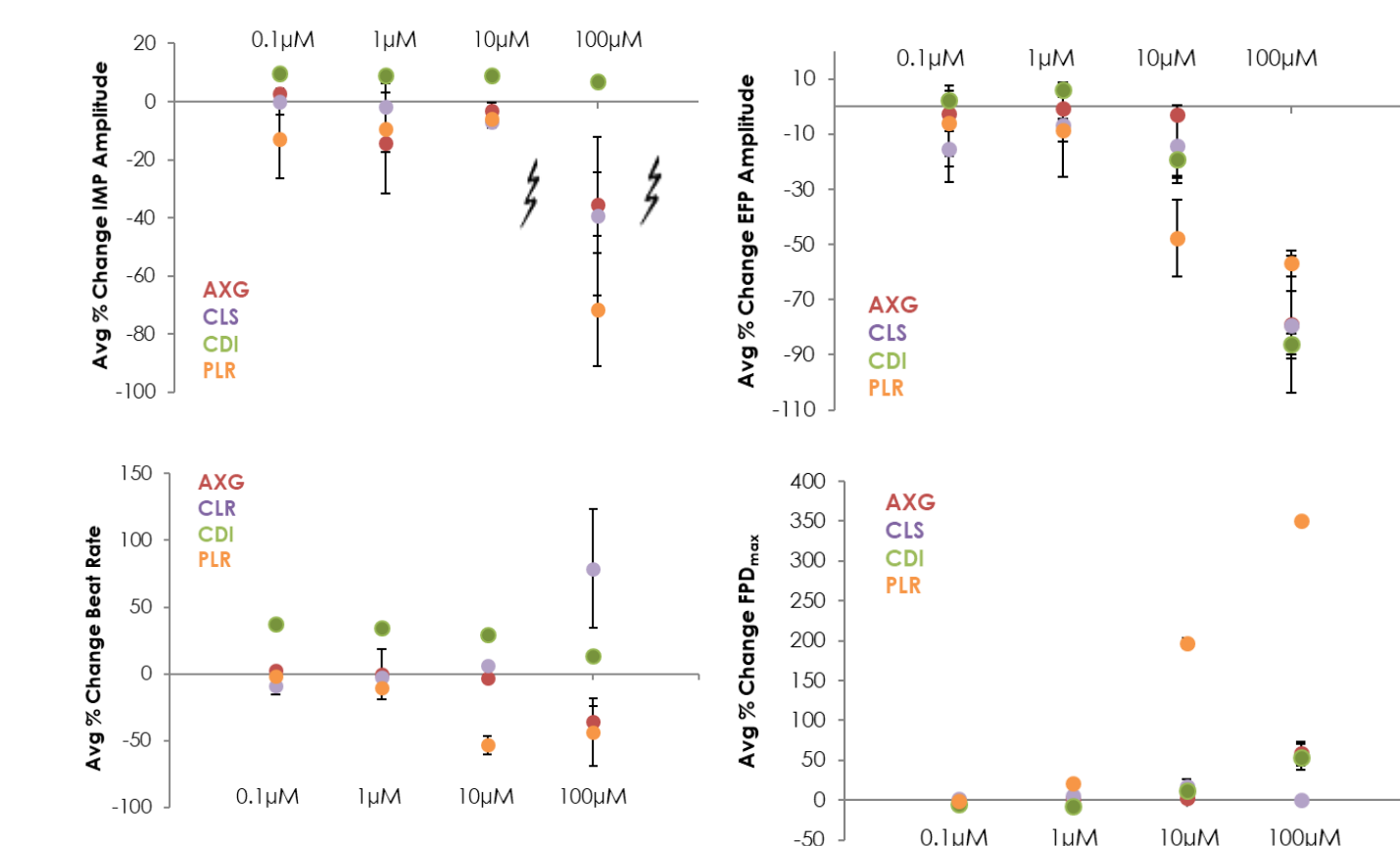


Figure 9: Disopyramide demonstrates expected electrophysiological responses at 0.1, 1, 10, 100µM. The primary endpoints (right) are quantified across replicates at x sites (n=4 or 5 replicates). Error bars indicate +/- standard error across the replicates.

Extracellular Field Potential (Voltage)

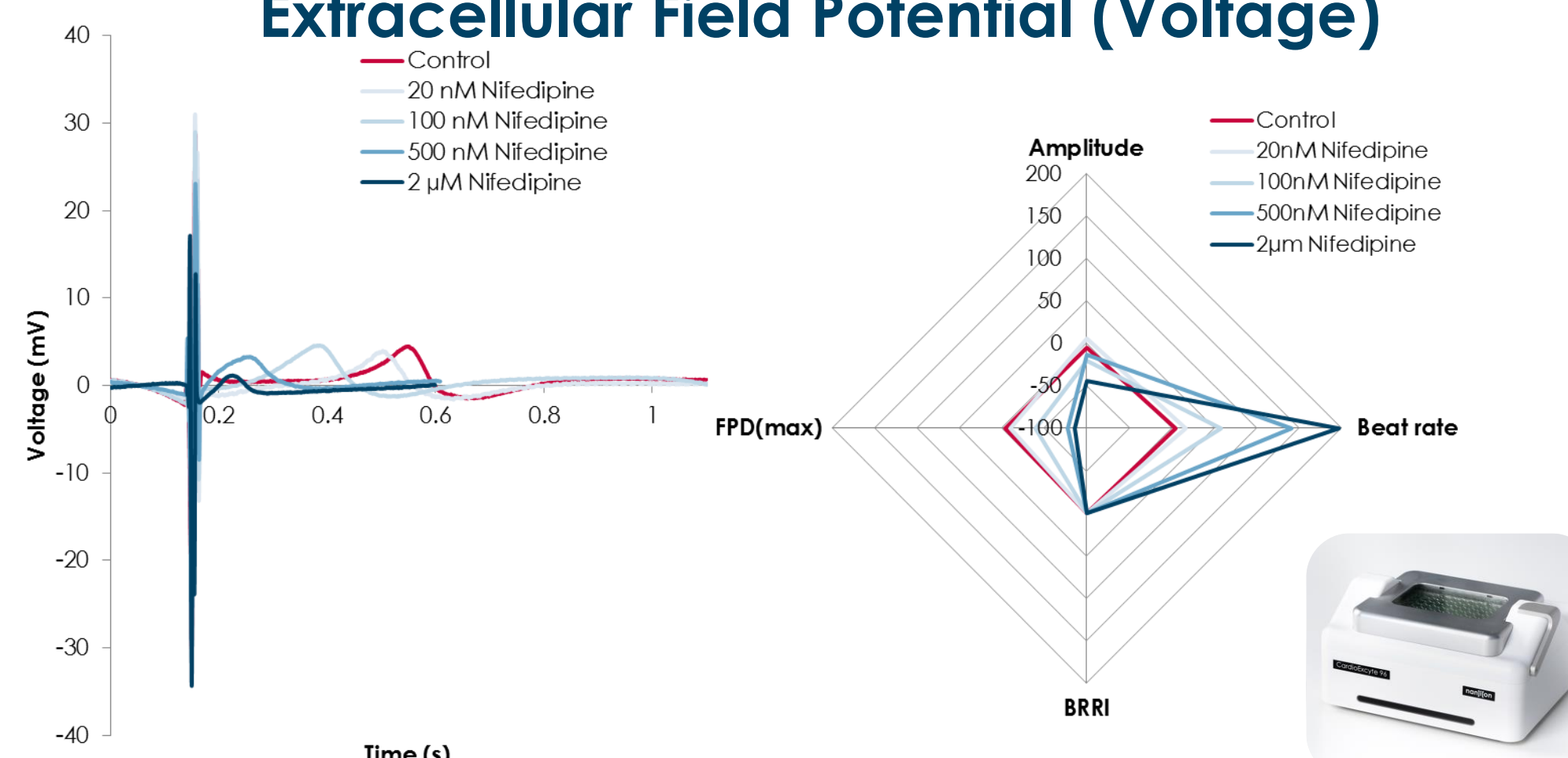


Figure 10: Effect of Nifedipine on the EFP signals of iCell cardiomyocytes: mean beat view of overlaid voltage traces (left graph). Effect of Nifedipine on EFP parameters: amplitude, field potential duration (FPD_{max}), beat rate and beat rate regularity shown as a spider chart (right graph).

Impedance

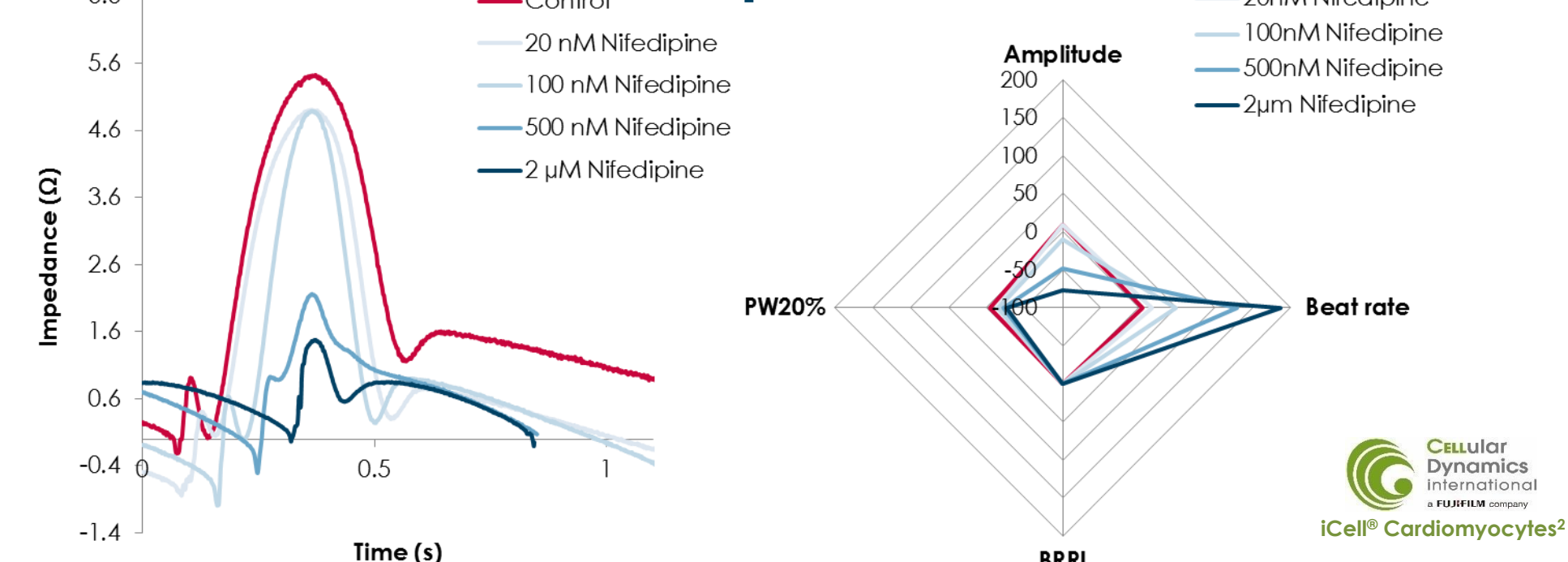


Figure 11: Effect of increasing concentrations of Nifedipine on the impedance signals of iCell cardiomyocytes: mean beat view of overlaid impedance traces (left graph). Effect of Nifedipine on impedance parameters: amplitude, Pulse width 20, beat rate and beat rate regularity shown as a spider chart (right graph).

Test article concentrations (µM)									
Compound	Site / Cell type	Trend	ETPC-Free (µM)	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6
HIGH RISK									
Disopyramide	Site 1, CDI	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 2, CDI	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 3, CDI	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 1, AXG	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 2, AXG	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 3, CLS	0.7	0.1	1	-	10	†	100	††
Disopyramide	Site 2, PLR	0.7	0.1	1	-	10	†	100	††
INTERMEDIATE RISK									
Droperidone	Site 2, CDI	0.02	0.003	0.03	-	0.3	††	3	††
Droperidone	Site 2, AXG	0.02	0.003	0.03	-	0.3	††	3	††
Droperidone	Site 3, CLS	0.02	0.003	0.03	-	0.3	††	3	††
Droperidone	Site 2, PLR	0.02	0.003	0.03	-	0.3	††	3	††
LOW RISK									
Tamoxifen	Site 1, CDI	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 2, CDI	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 3, CDI	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 1, AXG	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 2, AXG	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 2, CLS	0.02	0.1	0.3	-	0.95	-	3	-
Tamoxifen	Site 2, PLR	0.02	0.1	0.3	-	0.95	-	3	-

Figure 12: A summary of % FPD_{max} changes at 30min post compound addition compared to baseline is summarized for one compound from each risk category, all cell types and across all participating sites. "Trend" column displays actual average data per compound, n= 4 or 5 replicates each, with min (blue dot) and max (red dot) values pointed out. Arrhythmia classification was simplified by marking the occurrence of EAD-like events with † (type A arrhythmia listed under arrhythmia category specified in the HESI Myocyte Phase II Validation Study Protocol), next to each test article concentration. All other types of / combinations of arrhythmia observed are marked with †† (type B, C, D arrhythmias listed in Myocyte Phase II Validation Study Protocol)

Summary

Impedance data confirms arrhythmia detected in EFP modes. Arrhythmia occurrence was consistent with previous literature findings, and hiPSC-CMs tested exhibited prolongation of FPD_{max} in agreement with mechanism of action reported for compounds under testing. Altogether, these results demonstrate the potential of predicting torsadogenic risk with hiPSC-CMs using the CardioExcyte 96 system.

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