

# Cardiomyocytes Multi Electrode Array (MEA) for Comprehensive *In Vitro* Proarrythmic Evaluation

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## Introduction

The human heart is an electrically driven pump with high fidelity auto-rhythmic, which beats about 3 billion times in a normal life span. If normal pumping rhythm and function is severely interrupted for more than a few minutes (arrhythmia) irreversible multi-organ damage and death occurs. Consequently, cardiotoxicity is one of the major causes of failed drug development and withdrawals (Stevens and Baker, 2009). Hence, consistent preclinical in vitro cardiotoxicity tests are required to improve drug safety and reduce the attrition and/or the cost of drug development.

Human stem cell-derived or human induced pluripotent stem cell derived ventricular cardiomyocytes offer an integrated system to study drug impact on cardiomyocyte function. Stem cell myocytes present an opportunity to move beyond single-component analysis into a rich, dynamic system and thus provide a more appropriate preclinical model for cardiac liability assessment.

We measured field potential duration (FPD) using Axion BioSystems MEA with eleven (11) drugs, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nifedipine, nitrendipine, quinidine, sotalol and verapamil which are well characterized for their cardiac safety liability.

## Methods

16 and/or 48-well multielectrode array (MEA) plates (Axion BioSystems) were prepared by pipetting 4µl droplets of fetal bovine serum (FBS) onto each electrode and incubating for 1.5h at room temperature. Following adsorption of FBS to the surface the droplet was replaced with 4µl fibronectin solution (12.5µg/ml, BD Biosciences) and plates incubated at 37°C for 2h with 90% humidity to prevent the drying of fibronectin droplet. Cytiva Plus CMs or CDI ICell<sup>2</sup> cardiomyocytes were seeded direct from thawed cryovials onto prepared MEA plates using 4µl of cell suspension at 1.5 x 10<sup>7</sup> viable cells/ml (60,000 cells per well) in RPMI 1640/B27 (Life Technologies) medium for Cytiva Plus cells and plating medium for ICell<sup>2</sup> (CDI) with 90% humidity to prevent the drying of cardiomyocyte droplet. Once cell attachment was complete (2–3h) wells with Cytiva Plus cells were filled with RPMI 1640/B27 medium and wells with ICell<sup>2</sup> were filled with CDI maintenance medium taking care not to disturb the plated cells. The plates were then incubated at 37°C/5% CO<sub>2</sub>. FP measurements were recorded from spontaneously beating hESC/hIPSC-CM monolayers using a Maestro MEA system (Axion BioSystems) at 37°C. Data was sampled at 12.5 kHz and filtered with a Butterworth 1 Hz–2 kHz band-pass filter.

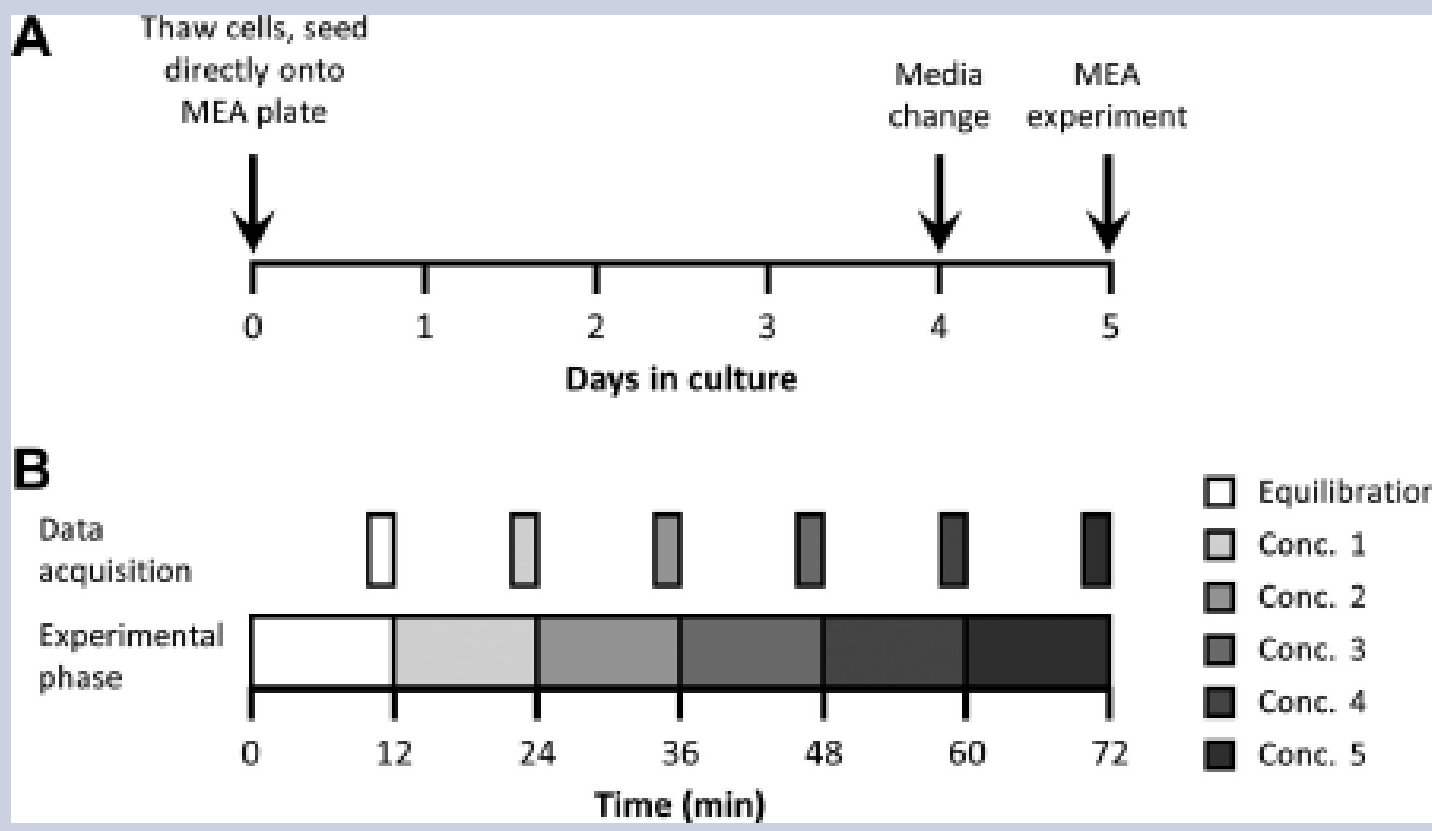


FIG. 1. MEA experimental protocol. (A) Time-line depicting cell seeding and maintenance for MEA experiments. (B) Schematic depicting the time line for hESC-CM or hIPSC-CM MEA assays. MEA recordings were made on day 5 post-thaw at 37°C following the addition of increasing drug concentrations as shown in the key.

The MEA data were analyzed off-line using the cardiac beat detector and statistics compiler of Axion BioSystems and the data analysis exported in CSV format. A mean-based beat filter with  $\pm$  2SD was used to isolate the beats that showed beat consistency and well consistency. Cardiac beats from multiple electrodes and multiple wells were averaged for each experimental condition and Inter-spike interval (beat period), Na spike amplitude and field potential duration. Cardiac field potential duration is dependent on the beat rate therefore the field potential durations were corrected for the beat rate using Fridericia Correction:  $cFPD = FPD \sqrt[3]{RR}$

Where cFPD is Fridericia correction, FPD is filed potential duration and RR is R-R interval (beat interval). Pharmacological effects of test compounds were compared to baseline vehicle control using a paired t-test. Percent change in beat period, sodium spike amplitude and the corrected field potential duration for the compound concentrations over vehicle control values are listed in the summary table.

## hESC-CM Cardiac Field Potential Waveform Data

Figure 2: Graphical representation of the effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nifedipine, nitrendipine, quinidine and sotalol additions on interspike interval, spike amplitude and cFPD.

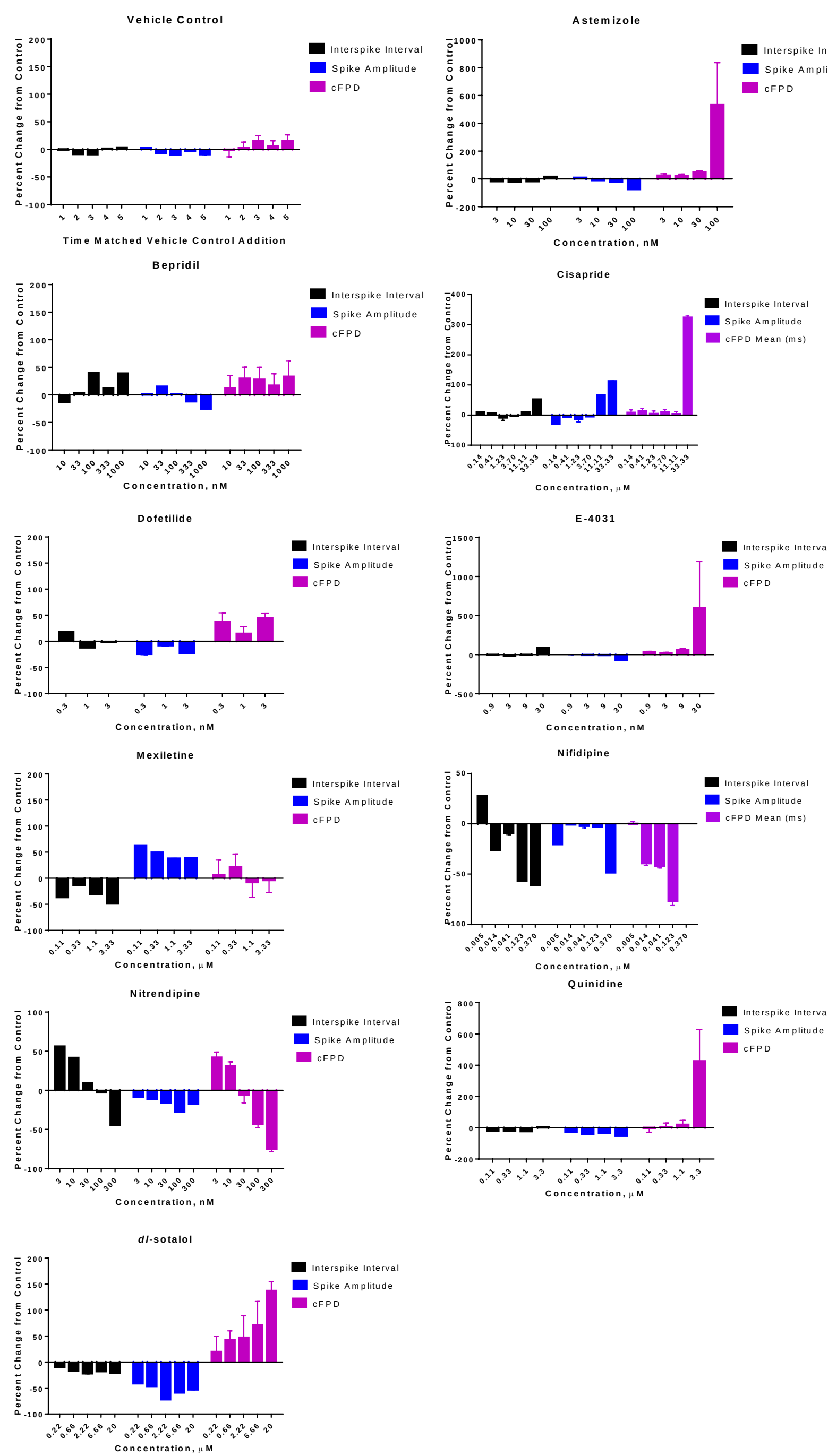


Table 1: Effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nifedipine, nitrendipine, quinidine and sotalol additions on interspike interval, spike amplitude and cFPD.

| Test Article | Concentration | Percent Change in Interspike Interval |      | Percent Change in Spike Amplitude |      | Percent Change in cFPD |        |
|--------------|---------------|---------------------------------------|------|-----------------------------------|------|------------------------|--------|
|              |               | Mean                                  | sem  | Mean                              | sem  | Mean                   | sem    |
| Vehicle      | Addition 1    | -6.14                                 | 0.03 | 2.22                              | 0.02 | -0.66                  | 12.78  |
|              | Addition 2    | -8.22                                 | 0.04 | -6.10                             | 0.06 | 2.96                   | 10.35  |
|              | Addition 3    | -8.58                                 | 0.03 | -9.46                             | 0.08 | 14.94                  | 10.05  |
|              | Addition 4    | 1.28                                  | 0.02 | -2.79                             | 0.88 | 5.73                   | 9.88   |
|              | Addition 5    | 3.33                                  | 0.02 | -8.51                             | 0.94 | 45.99                  | 10.74  |
| Astemizole   | 3nM           | -15.64                                | 0.01 | 8.23                              | 1.19 | 22.67                  | 14.26  |
|              | 10nM          | -19.61                                | 0.01 | -8.06                             | 1.09 | 20.64                  | 14.64  |
|              | 20nM          | -15.15                                | 0.01 | -17.29                            | 1.09 | 47.17                  | 13.04  |
|              | 100nM         | 14.85                                 | 0.78 | -72.37                            | 0.86 | 534.15                 | 302.17 |
|              | 300nM         | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
| Bepridil     | 12.5nM        | -12.68                                | 0.36 | 0.88                              | 0.35 | 12.11                  | 23.22  |
|              | 30nM          | 3.49                                  | 0.18 | 14.69                             | 0.39 | 29.25                  | 21.09  |
|              | 100nM         | 39.21                                 | 0.20 | 1.39                              | 0.35 | 27.36                  | 22.66  |
|              | 330nM         | 11.63                                 | 0.19 | -11.52                            | 0.34 | 16.69                  | 21.64  |
|              | 1000nM        | 38.64                                 | 0.19 | -24.73                            | 0.31 | 32.84                  | 28.40  |
| Cisapride    | 0.41          | 6.21                                  | 0.11 | -5.93                             | 0.11 | 12.87                  | 9.66   |
|              | 1.23          | -7.93                                 | 0.65 | -13.17                            | 0.66 | 3.91                   | 9.67   |
|              | 3.7           | -2.32                                 | 0.03 | -4.35                             | 0.11 | 9.15                   | 9.64   |
|              | 11.11         | 9.74                                  | 0.03 | 65.48                             | 0.07 | 2.41                   | 9.65   |
|              | 33.33         | 51.94                                 | 0.02 | 112.24                            | 0.95 | 323.35                 | 5.71   |
| Dofetilide   | 1nM           | 17.52                                 | 0.00 | -24.24                            | 1.33 | 36.70                  | 17.98  |
|              | 3nM           | -11.68                                | 0.00 | -7.62                             | 1.45 | 14.44                  | 13.66  |
|              | 10nM          | -1.38                                 | 0.00 | -22.05                            | 1.42 | 44.54                  | 9.42   |
|              | 30nM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
|              | 100nM         | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
| E-4031       | 0.9nM         | -2.04                                 | 0.01 | 0.25                              | 0.95 | 31.09                  | 11.79  |
|              | 3nM           | -15.44                                | 0.01 | -3.75                             | 0.90 | 20.19                  | 11.09  |
|              | 9nM           | -2.89                                 | 0.01 | -5.00                             | 0.88 | 42.32                  | 14.81  |
|              | 30nM          | 88.47                                 | 1.53 | -67.40                            | 0.72 | 594.85                 | 596.31 |
|              | 90nM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
| Mexiletine   | 0.11µM        | -36.13                                | 0.44 | 62.88                             | 0.55 | 6.08                   | 28.73  |
|              | 0.33µM        | -12.45                                | 0.48 | 49.10                             | 0.50 | 11.52                  | 24.98  |
|              | 1.1µM         | -29.92                                | 0.46 | 37.75                             | 0.48 | -7.48                  | 29.21  |
|              | 3.3µM         | -48.49                                | 0.41 | 39.07                             | 0.44 | -3.56                  | 23.64  |
|              | 10µM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
| Nifedipine   | 0.005µM       | 27.70                                 | 0.04 | -20.29                            | 0.11 | 0.16                   | 2.16   |
|              | 0.01µM        | -25.96                                | 0.11 | -0.58                             | 0.12 | -19.17                 | 2.03   |
|              | 0.04µM        | -9.38                                 | 0.16 | -1.89                             | 2.05 | -41.36                 | 2.02   |
|              | 0.132µM       | -56.40                                | 0.01 | -2.98                             | 0.12 | -76.93                 | 4.52   |
|              | 0.37µM        | -60.96                                | 0.08 | -48.39                            | 0.05 | ND                     | ND     |
| Nitrendipine | 3nM           | 55.90                                 | 0.00 | -7.91                             | 1.09 | 41.83                  | 7.15   |
|              | 10nM          | 41.37                                 | 0.00 | -11.10                            | 0.80 | 30.85                  | 5.62   |
|              | 30nM          | 9.22                                  | 0.00 | -15.98                            | 0.77 | -5.89                  | 10.42  |
|              | 100nM         | -2.63                                 | 0.00 | -27.41                            | 0.81 | -43.03                 | 4.62   |
|              | 300nM         | -44.09                                | 0.01 | -17.20                            | 0.80 | -74.20                 | 3.67   |
| Quinidine    | 0.11µM        | -20.52                                | 0.30 | -25.05                            | 0.64 | 0.65                   | 27.71  |
|              | 0.33µM        | -19.80                                | 0.36 | -37.82                            | 0.61 | 3.47                   | 26.53  |
|              | 1.1µM         | -21.73                                | 0.41 | -32.86                            | 0.60 | 19.11                  | 28.37  |
|              | 3.3µM         | 2.31                                  | 0.71 | -51.50                            | 0.56 | 434.87                 | 203.09 |
|              | 10µM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
| Sotalol      | 30µM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |
|              | 0.02µM        | -9.49                                 | 0.39 | -40.96                            | 0.43 | 19.41                  | 30.53  |
|              | 0.66µM        | -16.75                                | 0.44 | -46.17                            | 0.42 | 42.04                  | 17.93  |
|              | 2.22µM        | -21.67                                | 0.10 | -71.78                            | 0.40 | 46.97                  | 42.06  |
|              | 6.66µM        | -17.49                                | 0.61 | -58.60                            | 0.60 | 19.38                  | 46.27  |
| Verapamil    | 20µM          | -21.32                                | 0.36 | -52.79                            | 0.40 | 136.95                 | 18.19  |
|              | 60µM          | ND                                    | ND   | ND                                | ND   | ND                     | ND     |

\* indicate P<0.05

## hIPSC-CM Cardiac Field Potential Waveform Data

Figure 3: Graphical representation of the effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, sotalol and verapamil additions on interspike interval, spike amplitude and cFPD.

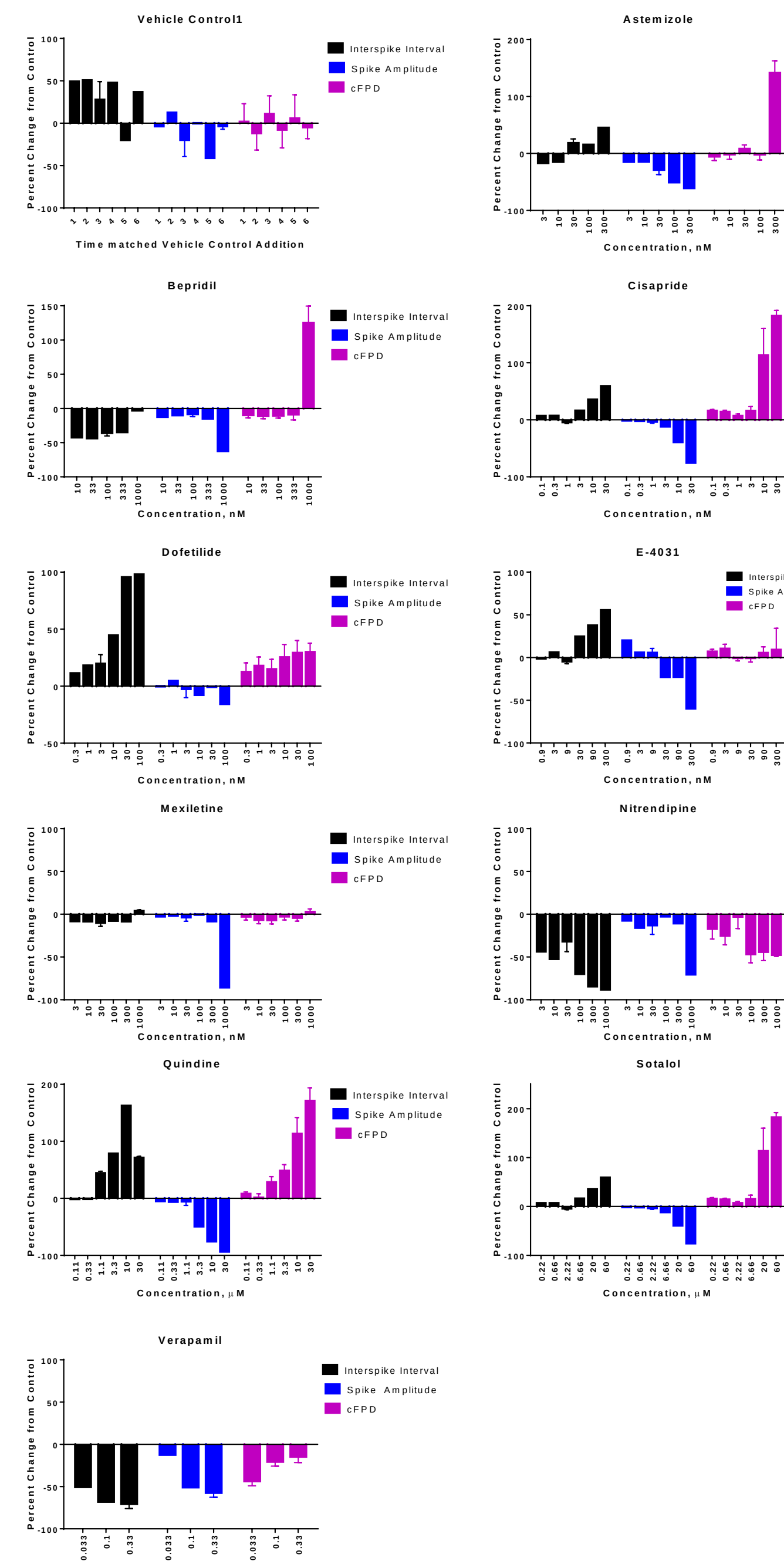


Table 2: Effect of time-matched vehicle control, astemizole, bepridil, cisapride, dofetilide, E-4031, mexiletine, nitrendipine, quinidine, sotalol and verapamil additions on interspike interval, spike amplitude and cFPD.

| Test Article | Concentration | Percent Change in Interspike Interval |       | Percent Change in Spike Amplitude |       | Percent change in cFPD |       |
|--------------|---------------|---------------------------------------|-------|-----------------------------------|-------|------------------------|-------|
|              |               | Mean                                  | sem   | Mean                              | sem   | Mean                   | sem   |
| Vehicle      | Addition 1    | -49.83                                | 0.01  | -3.39                             | 0.18  | 1.35                   | 21.48 |
|              | Addition 2    | 50.50                                 | 0.18  | 12.38                             | 0.19  | -11.62                 | 19.97 |
|              | Addition 3    | 27.58                                 | 21.48 | -19.45                            | 19.97 | 10.76                  | 21.55 |
|              | Addition 4    | 47.58                                 | 0.00  | -10.10                            | 0.17  | -7.45                  | 21.72 |
|              | Addition 5    | -19.64                                | 0.01  | -40.75                            | 0.07  | 5.37                   | 27.07 |
| Astemizole   | 3nM           | -16.68                                | 0.00  | -14.44                            | 0.20  | -5.02                  | 7.49  |
|              | 10nM          | -14.37                                | 0.20  | -14.11                            | 0.20  | -1.43                  | 8.80  |
|              | 30nM          | 17.98                                 | 7.49  | -28.30                            | 8.80  | 7.84                   | 7.17  |
|              | 100nM         | 15.07                                 | 0.00  | -50.29                            | 0.18  | -1.59                  | 9.72  |
|              | 300nM         | 44.81                                 | 0.00  | -60.41                            | 0.01  | 140.91                 | 21.60 |
| Bepridil     | 30nM          | ND                                    | ND    | ND                                | ND    | ND                     | ND    |
|              | 10nM          | -42.11                                | 0.00  | -11.97                            | 0.29  | -9.41                  | 4.52  |
|              | 30nM          | -43.35                                | 0.29  | -9.65                             | 0.29  | -11.01                 | 4.03  |
|              | 100nM         | -35.70                                | 4.52  | -7.96                             | 4.03  | -10.40                 | 3.66  |
|              | 330nM         | -34.41                                | 0.00  | -14.80                            | 0.29  | -8.61                  | 8.14  |
| Cisapride    | 1000nM        | -2.84                                 | 0.04  | -62.02                            | 0.03  | 124.59                 | 25.01 |
|              | 0.3nM         | 6.76                                  | 0.00  | -1.01                             | 0.14  | 15.44                  | 2.65  |
|              | 1nM           | 6.79                                  | 0.14  | -1.51                             | 0.14  | 13.90                  | 2.84  |
|              | 3nM           | -3.87                                 | 2.65  | -3.04                             | 2.84  | 6.73                   | 3.43  |
|              | 10nM          | 15.90                                 | 0.00  | -11.36                            | 0.13  | 15.05                  | 8.20  |
| Dofetilide   | 30nM          | 35.34                                 | 0.00  | -38.81                            | 0.02  | 113.09                 | 47.02 |
|              | 100nM         | 58.78                                 | 0.00  | -75.04                            | 0.01  | 181.83                 | 10.19 |
|              | 0.3nM         | 13.19                                 | 0.00  | -0.01                             | 0.24  | 12.32                  | 8.16  |
|              | 1nM           | 17.96                                 | 0.24  | 4.44                              | 0.25  | 17.75                  | 7.84  |
|              | 3nM           | 19.56                                 | 8.16  | -2.56                             | 7.84  | 14.80                  | 8.71  |
| E-4031       | 10nM          | 44.54                                 | 0.01  | -7.47                             | 0.23  | 25.22                  | 11.37 |
|              | 30nM          | 65.44                                 | 0.00  | -0.54                             | 0.01  | 29.12                  | 10.97 |
|              | 100nM         | 97.90                                 | 0.00  | -15.47                            | 0.01  | 29.84                  | 7.93  |
|              | 0.9nM         | -0.98                                 | 0.00  | 19.80                             | 0.13  | 6.87                   | 2.86  |
|              | 3nM           | 6.01                                  | 0.13  | 5.68                              | 0.11  | 10.37                  | 5.28  |
| Mexiletine   | 9nM           | -4.35                                 | 2.86  | 5.49                              | 5.28  | -0.26                  | 3.54  |
|              | 30nM          | 24.55                                 | 0.00  | -22.47                            | 0.10  | -0.28                  | 4.96  |
|              | 100nM         | 27.53                                 | 0.01  | -22.31                            | 0.04  | 5.29                   | 7.33  |
|              | 300nM         | 55.39                                 | 0.00  | -59.40                            | 0.01  | 9.01                   | 25.28 |
|              | 3nM           | -7.92                                 | 0.03  | -2.48                             | 0.27  | -2.53                  | 4.33  |
| Nitrendipine | 10nM          | -8.19                                 | 0.27  | -1.53                             | 0.27  | -6.35                  | 4.77  |
|              | 30nM          | -9.84                                 | 4.33  | -3.47                             | 4.77  | -6.72                  | 4.67  |
|              | 100nM         | -7.43                                 | 0.03  | -0.47                             | 0.27  | -2.49                  | 4.23  |
|              | 300nM         | -8.22                                 | 0.03  | -8.14                             | 0.24  | -3.94                  | 4.13  |
|              | 1000nM        | 3.55                                  | 1.51  | -85.46                            | 0.15  | 2.52                   | 3.66  |
| Quinidine    | 3nM           | -43.41                                | 0.00  | -7.23                             | 0.23  | -16.91                 | 12.16 |
|              | 10nM          | -52.01                                | 0.23  | -15.57                            | 0.24  | -24.97                 | 10.81 |
|              | 30nM          | -31.63                                | 12.16 | -12.66                            | 10.81 | -2.74                  | 13.93 |
|              | 100nM         | -69.63                                | 0.07  | -2.42                             | 0.26  | -46.50                 | 10.33 |
|              | 300nM         | -84.13                                | 0.00  | -10.51                            | 0.01  | -43.86                 | 10.37 |
| Sotalol      | 1000nM        | 88.00                                 | 0.02  | -70.19                            | 0.02  | -47.28                 | 1.97  |
|              | 0.11µM        | -1.00                                 | 0.02  | -4.56                             | 0.24  | 7.59                   | 3.51  |
|              | 0.33µM        | -0.89                                 | 0.24  | -5.66                             | 0.23  | 0.96                   | 7.10  |
|              | 1.1µM         | 43.98                                 | 3.51  | -5.18                             | 7.10  | 28.07                  | 10.02 |
|              | 3.3µM         | 78.21                                 | 0.02  | -49.01                            | 0.19  | 48.14                  | 11.15 |
| Verapamil    | 10µM          | 162.09                                | 0.02  | -75.23                            | 0.20  | 113.05                 | 28.85 |
|              | 30µM          | 70.89                                 | 2.64  | -93.22                            | 0.12  | 170.72                 | 23.22 |
|              | 0.1µM         | 6.76                                  | 0.00  | -1.01                             | 0.14  | 15.44                  | 2.65  |
|              | 0.66µM        | 6.79                                  | 0.14  | -1.51                             | 0.14  | 13.90                  | 2.84  |
|              | 2.22µM        | -3.87                                 | 2.65  | -3.04                             | 2.84  | 6.73                   | 3.43  |
| Verapamil    | 10nM          | 15.90                                 | 0.00  | -11.36                            | 0.13  | 15.05                  | 8.20  |
|              | 30nM          | 35.34                                 | 0.00  | -38.81                            | 0.02  | 113.09                 | 47.02 |
|              | 100nM         | 58.78                                 | 0.00  | -75.04                            | 0.01  | 181.83                 | 10.19 |
|              | 0.1µM         | -67.78                                | 0.11  | -50.64                            | 0.09  | -20.41                 | 5.34  |
|              | 0.33µM        | -70.32                                | 5.66  | -57.25                            | 5.34  | -14.29                 | 7.26  |
| 1.1µM        | ND            | ND                                    | ND    | ND                                | ND    | ND                     |       |
| 3.3µM        | ND            | ND                                    | ND    | ND                                | ND    | ND                     |       |
| 10µM         | ND            | ND                                    | ND    | ND                                | ND    | ND                     |       |