Transmembrane Potential Measurements in Cardiac 3D Microtissues Derived from Human Stem Cells

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Introduction

The electrical characteristics of mammalian adult myocardium depend critically on the geometry of the tissue. Production of large quantities of human cardiac myocytes differentiated from induced pluripotent stem cells (hiPSC-CMs) is now available commercially. These cells, normally cultured on 2D surfaces, are increasingly used to assess the electrophysiological effects of drugs. This study examined the electrical activity of a commercially available hiPSC-CM cell line in both 2D culture and in 3D culture of microtissues.

Methods

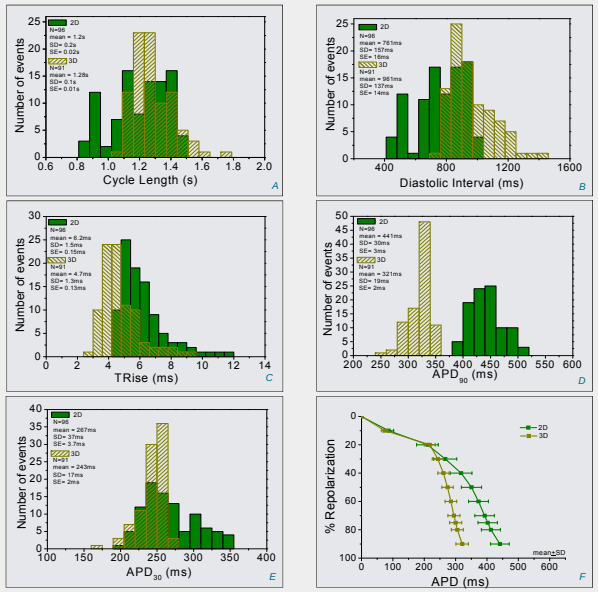
iCell Cardiomyocytes were purchased from Cellular Dynamics Inc (USA) and either (i) seeded onto wells of a 96 well plate (25,000 cells/well) and cultured for 10 days or (ii) formed into micro tissues by creating hanging drops of approximately 0.3mm diameter in a 96 well format using the a patented automated hanging drop platform (InSphero AG).

In both cases the cells were transferred to serum free media and transiently exposed to the voltage sensitive dye (Di-4-ANEPPS 6μM). The Di-4-ANEPPS fluorescence was recorded at 10KHz from regions 2D and 3D cultures for periods up to 15s in the 96 well plates on the CellOPTIQ electrophysiology platform (Clyde Biosciences Ltd). The records were subsequently analysed off-line using proprietary software (Clyde Biosciences).

Two drugs, Nifedipine (L-Type Ca^{2+}-Channel -LTCC- blocker) and Dofetilide (IK_R Blocker), were tested. The electrical activity was recorded before (Baseline) and after drug/vehicle treatment (30min). The data were plotted as % change of baseline.

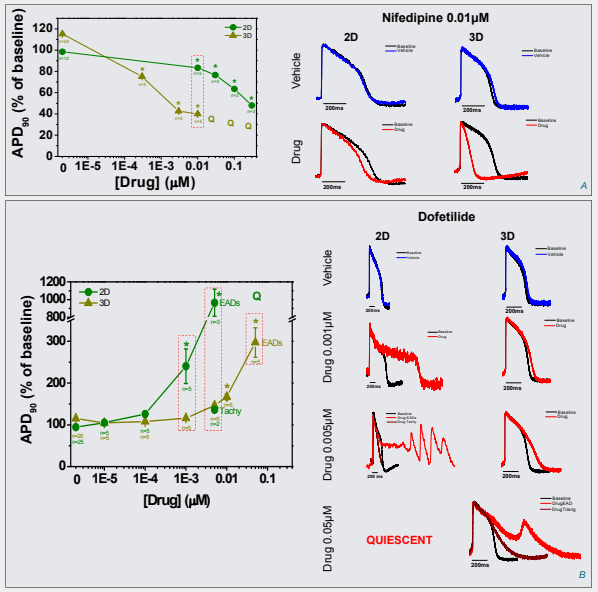
Results

Electrophysiological Characteristic of 2D and 3D hiPSC-CMs



The average time between spontaneous action potential (AP) firing in the two culture formats was similar (1.2±0.2s vs. 1.28±0.1s; 2D vs. 3D, *Panel A*), as was the rate of depolarisation of the AP (6.2±1.5ms vs. 4.7±1.3ms; 2D vs. 3D, *Panel C*). But the action potential duration (APD) in 3D microtissues was significantly shorter than 2D format at the late repolarization phase (*Panel F*). At 90% repolarisation APD was 441±30ms vs. 321±19ms (2D vs. 3D P<0.01, *Panel D*), whereas APD at 30% repolarisation was close (267±37ms vs. 243±17ms 2D vs. 3D, *Panel E*). N=96 and 91 for 2D and 3D, respectively.

LTCC and HERG Blockade on 2D and 3D hiPSC-CMs



Different sensitivities to drug were shown in the two culture format when blocking L-type Ca^{2+} channel (LTCC) –Panel A– or IK_R –Panel B– with the well-known drugs Nifedipine and Dofetilide respectively. In both cases the % change of baseline for APD at 90% repolarization is plotted as well as the representative traces for some concentrations (Black Line = Baseline; Red Line = Drug; Blue Line = Vehicle Control). The data are given as the mean ± SE. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's test. Significant differences (*) from vehicle control ([Drug]=0) were considered when p<0.05.

Conclusions

- *3D Microtissues of human iPSC-CMs exhibit a significantly shorter APD than comparable 2D cultures, the difference may arise from increased electronic interactions within microtissues.*
- *Different sensitivity to LTCC and HERG blockade is shown between 2D and 3D cultures.*

References

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