Phenotypic screening of iPSC-derived cardiomyocyte toxicity in a novel 3D cell culture system

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Introduction
There is growing evidence to suggest that cells grown in 3-dimensional (3D) cultures offer a more physiologically relevant model and demonstrate different pharmacological responses to those grown in 2-dimensional (2D). Refinement of human cardiotoxicity modelling has significant implications for the improvement of compound screens, mechanistic studies and drug development.

In this study we have evaluated three different sources of human iPSC-derived cardiomyocytes; two commercially available (Cellular Dynamics International iCell3 and AxioGenesis Cor4U) & one non-commercial source. Cells were grown in either a classic 2D culture or on 3D aligned nanofibre plates and treated with a range of pharmacological agents representing different targets or modes of action.

The FLIPR Tetra system was used to measure the potential of a number of pharmacological agents to modulate calcium mobilisation in both environments. Specifically, we analysed beat count & frequency, amplitude of contractions and peak rise and decay to quantify changes in the phenotypic profile.

Comparing baseline parameters of 2D & 3D cardiomyocyte models
Cardiomyocytes from different manufacturers show significant differences in baseline BPM and amplitude in 2D and 3D culture. Human cardiomyocytes were sourced from Cellular Dynamics International (CDI), AxioGenesis and a non-commercial partner. All cells were grown in classic 2D tissue culture or on electrop spun nano-fibre plates (3D) prior to measurements on the FLIPR Tetra. We observed that for all cells, growth in 3D resulted in increased baseline BPM but a reduced beat wave amplitude. Analysis of FLIPR wave profiles (right) indicates that the beat pattern of 2D and 3D are very different and 3D is more cyclical in nature.

Materials and Methods
Cardiomyocytes were selected from some current manufacturers of iPSC-derived cardiomyocytes. All cells were prepared and plated as per the original manufacturer’s recommended protocol on either standard tissue culture or 3D aligned nanofibre plates (Nanofibre Solutions). All cells were monitored for synchronous beating prior to treatment.

To measure changes in the phenotypic profile of the cardiomyocytes we utilized the FLIPR™ Tetra with the EarlyTox™ cardiotoxicity kit (Molecular Devices). This assay is based on a calcium sensitive dye that monitors Ca2+ fluxes during cardiomyocyte beating.

After an initial baseline reading, all cardiomyocytes were treated in a dose-dependent manner with a range of pharmacological compounds known to affect the cellular phenotype. Compounds were prepared and added simultaneously to all wells in the FLIPR Tetra instrument.

Plates were then measured (for 60 seconds) every 15 minutes for up to 2 hours post-compound addition. All data was acquired and analysed using FLIPR ScreenWorks Peak Pro software.

Increased sensitivity of phenotypic assays using 3D culture of human iPSC derived cardiomyocytes
CDI iCell3 cardiomyocytes show increased sensitivity and observable phenotypic difference in response to compounds on 3D nanofibre plates.

Cardiomyocytes were treated with sotalol or propranolol and measured every 15 minutes for 45 minutes. Sotalol treated cells in 3D show a decrease in BPM in a concentration dependent manner, compared to 2D. The effect of sotalol was transient and the cells recovered to baseline levels after 45 minutes. In contrast, propranolol treated cells did not recover after treatment. However, we did observe a significant increase in assay sensitivity and range in 3D nanofibre grown cells.

Dose response to pharmacological agents
Cardiomyocyte response to astemizole varies depending on source of cells and growth conditions. Human iPSC-derived cardiomyocytes were either grown in a standard tissue culture plate (2D; top panel) or electrop spun nanofibre plate (3D; bottom panel) and subsequently treated with various concentrations of astemizole. Data expressed as beats per minute (BPM +/- 5D).

Overall, cardiomyocytes responded in a dose dependent manner with increasing concentrations of astemizole having a negative effect on the BPM of all cardiomyocytes tested, compared to untreated controls. The non-commercial cells proved to be more sensitive to astemizole treatment and toxic at the higher concentrations. In both CDI and AxioGenesis derived cells, growth in 3D improved the sensitivity and dynamic range of the assay compared to cardiomyocytes in 2D conditions.

Summary and Conclusion
• There is significant variation between the resting BPM and beat amplitude in 2D cardiomyocytes compared to 3D.
• 3D cardiomyocytes were more effective for observing the potentially subtle changes in beat peak profile caused by pharmacological compounds.
• The source and model used in cardiomyocyte testing protocols should be carefully considered when screening compounds or carrying or mechanistic studies with varying or unknown modes of action.

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