Abstract

A recent examination of drug failures in clinical trials reveals that the number of post-market withdrawals and black box labelling of drugs has concomitantly increased. This resulting increase of unforeseen toxicity in late-stage clinical development and post-market release highlights the need for highly predictive models of adverse clinical outcomes attributable to targeted and off-target drug toxicity. To address these concerns and enable a better clinical development path for drug safety as it relates to toxicity prediction and mechanistic investigations, the use of the induced pluripotent stem cell (iPSC) technology has been an intense area of research interest. This technology allows access to human cell types not otherwise available to the research community, however the traditional 2-dimensional (2D) cell culture still presents challenges in representing the in vivo tissue conditions.

Here we report recent advancements towards exploring 3-dimensional (3D) culture systems for human iPSC-derived cell types (cardiomyocytes and hepatocytes) and the potential impact of these systems on functionality and toxicity studies. 3D spheroid formation was accomplished by seeding the cells into ultra-low attachment (ULA) culture plates. Functional assessment of the 3D spheroids was accomplished with various endpoint assays and performed using commercially available reagents.

iCell Cardiomyocytes

Human iPSC-derived cardiomyocytes have been used previously in numerous applications to measure compound toxicity in 2D format (a complete list of iCell Cardiomyocytes and iCell Cardiomyocytes publications can be found at www.cellulardynamics.com). Here, we illustrate that iCell Cardiomyocytes spontaneously formed 3D spheroids when plated in ULA plates and baseline beat rate parameters, as well as responsiveness to known cardio-active and cardio-toxic compounds could be detected and measured.

iCell Hepatocytes 2.0

The potential for iPSC-derived cells to engender reproducible donor diversity in unlimited long-term supply along with stability and consistency is already being realized. However, there is still room for improvement in these model systems as it is applied to predictive toxicity beyond compound assessment in monocultured cells in a 2D format. The combination of iPSC and bioengineering technologies can synergize to advance predictive and HTS-compatible in vitro models for toxicity screening and ADME-T studies.

Compound Responses 2D vs 3D

<table>
<thead>
<tr>
<th>Compound</th>
<th>2D</th>
<th>3D</th>
</tr>
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<tbody>
<tr>
<td>Lidocaine</td>
<td>3.07 µM</td>
<td>9.00 µM</td>
</tr>
<tr>
<td>Grapxide</td>
<td>2.4 µM</td>
<td>0.07 µM</td>
</tr>
<tr>
<td>Valsecare</td>
<td>6.0 µM</td>
<td>6.0 µM</td>
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Figure 2: iCell Cardiomyocytes in 3D Exhibit Reproducible Responses to Compounds Across Different Endpoint Assays. Analysis of compound effects in 3D cultures of Cell Cardiomyocytes using the ImageXpress Micro-Confocal or FLIPR Tetra System from Molecular Devices demonstrated reproducible responses in 3D spheroids compared to 2D format. We show below dose-response curves and example traces recorded on both platforms.

Summary

Discussion

We have shown here the ability to form robust iPSC-derived spheroids in multiple tissue types. These spheroids can be maintained in culture over time enabling high throughput screening and testing compounds in vitro. The microtissue models presented here provide 3-dimensional biological complexity with high reproducibility.

Conclusion

Figure 1: Phase Images of 2D and 3D Cultures of iCell Cardiomyocytes 2.0
(A) Cells were thawed and plated in the collagen-coated cell culture plate to form a 2D confluent monolayer. (B) Cells were then detached and seeded in the ULA plate to form 3D spheroids within 2 days.

Figure 3: iCell Hepatocytes 2.0 Spheroids Exhibit Highly Reproducible CYP3A4 and CYP1A2 Induction
CYP3A4 activity can be induced in response to a rifampicin in 3D spheroids. The activity was measured using a luminescent assay (P450 Glo, Promega).

Figure 4: iCell Hepatocytes 2.0 Spheroids Enable Assay Miniaturization for High-Throughput
The hepatotoxic response to FCP was detected with consistent EC50 for liver spheroid sizes. Even the smallest spheroid (50 cells/well) showed a good assay window. The viability was measured using a luminescent assay (Cell Titer Glo, Promega).

Figure 5: iCell Hepatocytes 2.0 in 3D Exhibit Expected Response to Compounds
Analysis of compounds effects in 3D cultures of Cell Hepatocytes 2.0 showed expected responses to hepatotoxic compounds. The differences in sensitivity depending on incubation time and 2D vs 3D is currently under investigation.

Figure 6: iCell Hepatocytes 2.0 Spheroids Enable Multiplexed High Content Analyses
Expected responses to hepatotoxic compounds were detected and quantified in iCell Hepatocytes 2.0 spheroids using the ImageXpress Micro-Confocal from Molecular Devices. The analysis software of this system allows segmentation of nuclei and cell/vial scoring. Multiparametric analysis provides the ability to characterize cells marked with different dyes. Staining for viability markers was performed using a simple no-wash 1-step protocol.

Figure 7: 3D Spheroid Formation

Figure 8: Control of iCell Hepatocytes 2.0 Spheroid Size
(A) The spheroid size was tuned by the number of cells seeded into low-attachment wells. (B) Viability correlated well and numbers from per aggregate, suggesting that the microtissues maintain cell health throughout.

3D Culture of Human iPSC-derived Cell Types for Toxicity Testing

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