Application Note

Cells:
iCell® Hepatocytes 2.0
Tools:
CardioExcyte 96
AtlaZ

Comprehensive impedance-based hepatotoxicity assay for metabolically active iPSC-derived hepatocytes

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Summary

Hepatotoxicity and drug-induced liver injury (DILI) are leading reasons for drug failure to market, leading to approximately 18% of market withdrawals of drugs in the last decade[1]. Additionally, only half of drugs with a potential to induce hepatotoxicity are actually identified during preclinical animal studies[2]. This highlights the importance of generating more advanced cell-based models and experimental strategies to enhance the predictivity of these assays. Current existing *in vitro* models employed to predict DILI mostly focus on hepatocytes, though primary hepatocytes do not maintain their phenotype. iCell® Hepatocytes 2.0 (FUJIFILM Cellular Dynamics, FCDI) are human iPSC-derived cells with a wide variety of basic and functional characteristics which make them amenable to applications such as compound-mediated ADME-T and DILI toxicity. In addition to displaying characteristic hepatocyte morphology, (i.e., polygonal shape, polynucleation and formation of bile canalicular channels), these cells also express liver cell markers, including albumin, A1AT, and HNF4a, and exhibit basic and induced P450 functions, as observed in primary human hepatocytes. iCell® Hepatocytes 2.0 maintain morphology, marker expression, and metabolic function in culture over a longer time frame compared to primary human hepatocytes, rendering these cells useful for investigations of acute and chronic DILI responses in a 2D culture system using impedance. Combining these cells with the planar gold-film electrodes on the impedance systems reveal alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells, thereby providing a measure of toxicity. Dose-dependent harmful effects of drugs could be evaluated over time in a functional 2D cell-based model without the need for 3D spheroid formation.

Results

iCell® Hepatocytes 2.0 were recorded on the Nanion´s impedance systems and changes in base impedance, showing confluency and toxicity, were used to measure DILI. The impedance-based continuous and multi-parametric cell monitoring, an advantage over individual endpoint assays, was used to investigate the kinetics of cell behavior in real-time. Hepatocytes were cultured for 7 days at confluence, before the compound addition series started. Cells were treated according to the manufacturer's protocol. Acute and chronic liver toxic effects (dose-dependent) were tested for 5 relevant compounds: acetaminophen, diclofenac, troglitazone, chlorpromazine, and aflatoxin B1. Compound-containing medium was exchanged every 2 days and recordings were performed during a time-period of 15 to 21 days, as depicted in experimental layout (Methods).

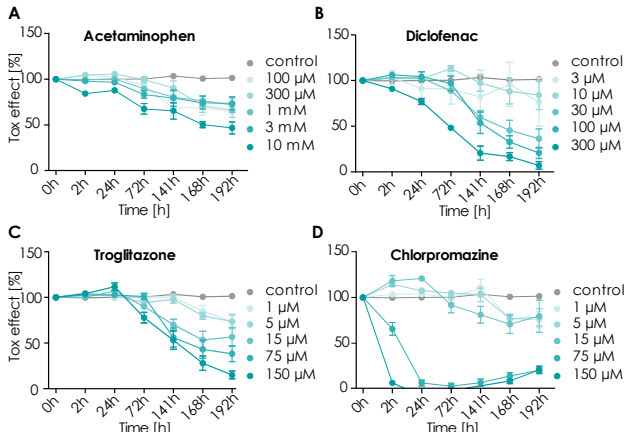


Figure 1: *Real-time impedance monitoring of iCell® Hepatocyte 2.0 at acute and prolonged exposure times to 5 concentration additions of acetaminophen* **A**, *diclofenac* **B**, *troglitazone* **C** *and chlorpromazine* **D**. *0h marks the time when compounds were added. Values represent mean ± SEM from duplicate values.*

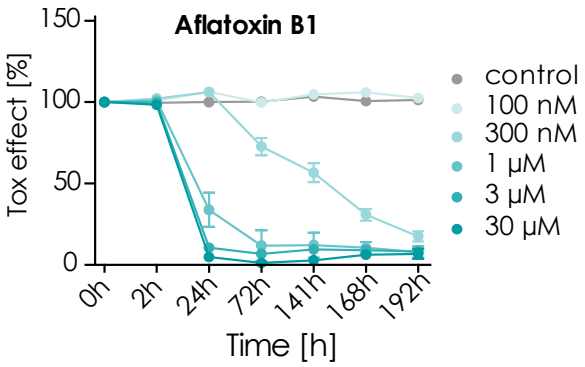


Figure 2: *Cell® Hepatocytes 2.0, cultured in 2D, showed hepatotoxicity of Aflatoxin B1, known to cause such effects in metabolically active cells. Thereby the use of impedance in resolving toxic compound effects on hepatocytes does not always need a 3D culture setting.*

Impedance data showed comparable results to other cell viability and tox assays, rendering impedance as a reliable but non-invasive tool. We were able to show dose- and time-dependent effects for all 5 compounds on iCell® Hepatocytes 2.0. Figure 1 summarizes the impedance change over time, after addition of acetaminophen, diclofenac, troglitazone, and chlorpromazine. Acetaminophen showed expected dose- and time-dependent toxic effects. Diclofenac and troglitazone showed more pronounced effects after 72h, which was consistent for all compound concentrations. Low doses of chlorpromazine had a smaller effect over time than higher doses, which showed full tox effects already after 2h. Additionally, effects of aflatoxin B1 were tested (Figure 2). The importance of this compound in hepatotoxicity assay development lies in the fact that this extremely toxic compound must first be metabolized into its reactive electrophilic form, aflatoxin B1-8,9-exo-epoxide by cytochrome p4503. The acute and chronic effects of Aflatoxin B1 were observed in standard 2D conditions, indicating that the hepatocytes were metabolically active and functional when using this impedance-based, non-invasive assay. The lowest concentration tested did not show any effect, however after only 24h, aflatoxin B1 showed full tox-effect starting with 1µM concentration.

In summary, iCell® Hepatocytes 2.0 in combination with Nanion´s impedance systems demonstrated results comparable to standardized optical readouts such as luminescent cell viability assays, with an important advantage as a non-invasive, time-resolved experimental approach. iCell® Hepatocytes 2.0 are a reliable source of hepatocytes with consistent lot-to-lot performance that maintain hepatic function over long culture periods. Taken together, iPSC-derived hepatocytes paired with impedance time-resolved recordings, give a reliable and accurate hepatotoxicity readout in 96-well format. Additionally, this assay demonstrated that with the use of impedance in resolving toxic compound effects on hepatocytes in a more complex 3D culture setting is not always necessary.

References

1. Onakpova I.J., et al. (2016) BMC Medicine 14: 10
2. Olson H., et al. (2000) Regul. Toxicol. Pharmacol. 32(1): 56-67
3. Kew, M.C. (2013). J. Gastroint. Liver Dis. 22 (3): 305–310. PMID 24078988

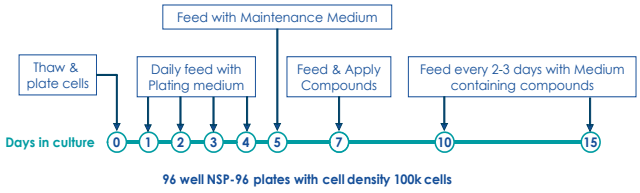
Methods

Cells

We thank FCDI for providing commercially available iCell® Hepatocytes 2.0. We thank Dr. Katherine Czysz, Ouissame Mnie-Filali and Dr. Kirk Twaroski for their help in experimental design and assistance with writing the document.

Impedance and contractility measurements

Impedance measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96 or AtlaZ. iCell Hepatocytes 2.0 were plated on NSP-96 plates and monitored according to protocol described below.



To obtain 2D monolayers in 96-well plates, cells were seeded with density of 300k cells/cm2, and recordings were performed starting day 15 through day 21. Five concentrations per compound were applied in the external media, in technical duplicates. Two hours before drug application the medium was removed from the wells and 200 µl fresh medium was added. Data was analyzed using respective Nanion´s software are presented in scatterplots with mean ± SD.

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