Monitoring drug-induced cytotoxicity and hepatotoxicity using impedance

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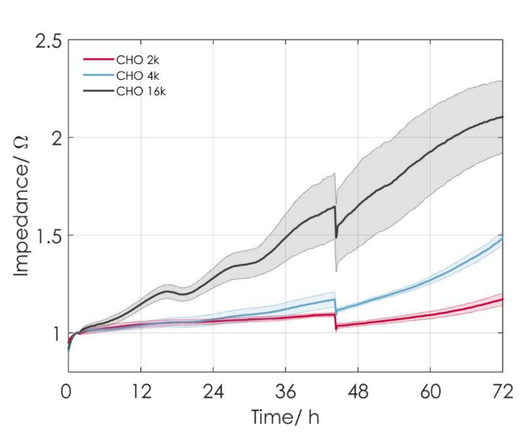
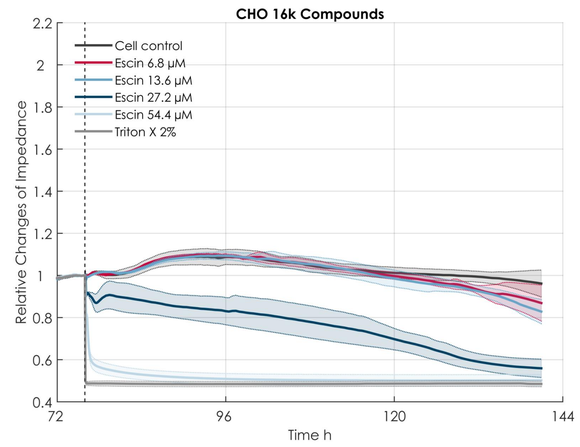
Introduction

A number of different cell-based assays for cytotoxic effects of drugs exist including the lactate dehydrogenase (LDH) leakage assay, the neutral red assay, protein measurement and methyl tetrazolium (MTT) assay. We describe the development and optimization of a cell-based assay for cytotoxicity using impedance measurements. This assay is sensitive and provides reproducible results for safety pharmacology, toxicity screens of adherent, proliferating or non-proliferating cells. Changes in the impedance signal indicate effects on cell contractility, cell morphology and proliferation. One advantage of this technique over standard cytotoxicity assays is that continual monitoring of the development of cytotoxicity is possible. We show the effect of reference compounds on the impedance signal of CHO cells, hepatocyte-like cells and human induced pluripotent stem cell derived cardiomyocytes (hIPSC-CMs). In addition, we show hepatotoxic effects of paracetamol and recovery from hepatotoxicity after short exposure to the drug. This method provides a viable alternative to standard cell-based cytotoxic screening assays for drug induced liver injury (DILI[1, 2,]) as well as cytotoxicity of cells and stem cells.

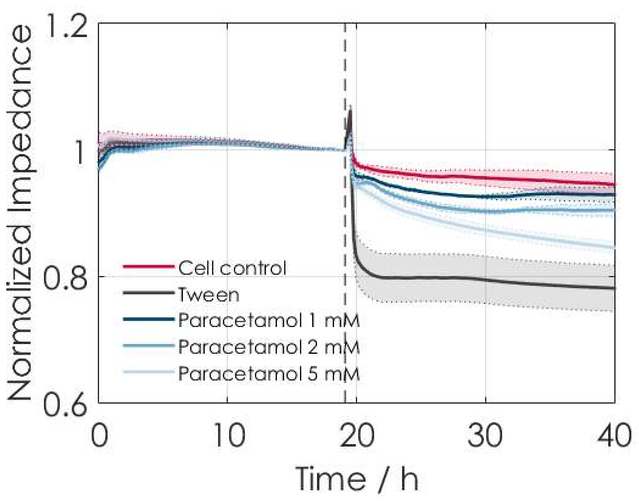
Cell proliferation monitored via impedance

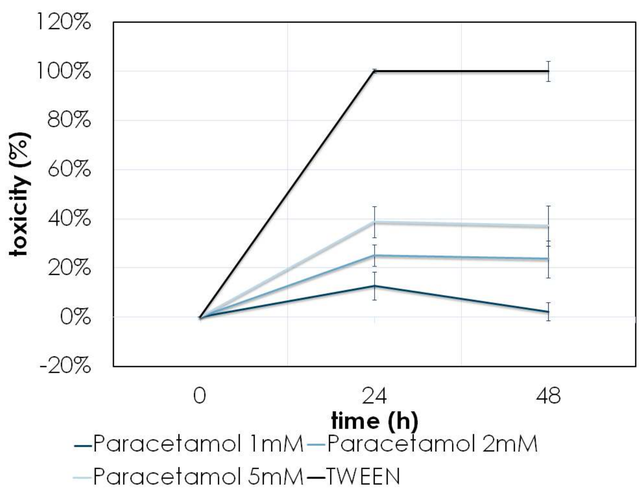




Averaged relative changes of the impedance signal with corresponding standard deviation for CHO cells with differing initial cell density (as indicated) over a cell proliferation time period of 72 h (n = 24; left). Data are normalized to medium reference without cells and cell addition time point. Effect of Escin on the impedance signal of CHO cells seeded with initial cell density of 16 k cells/well in concentrations of 6.8 µM, 13.6 µM, 27.2 µM and 54.4 µM (n = 4; right). Effect of Triton X-100 (2 %) is also shown. Data are normalized to the respective compound reference without cells and the compound addition time point.

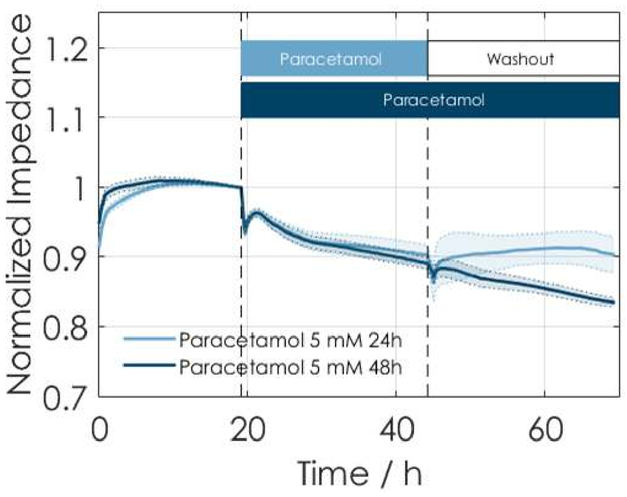
Hepatotoxicity of paracetamol using hepatocyte-like cells

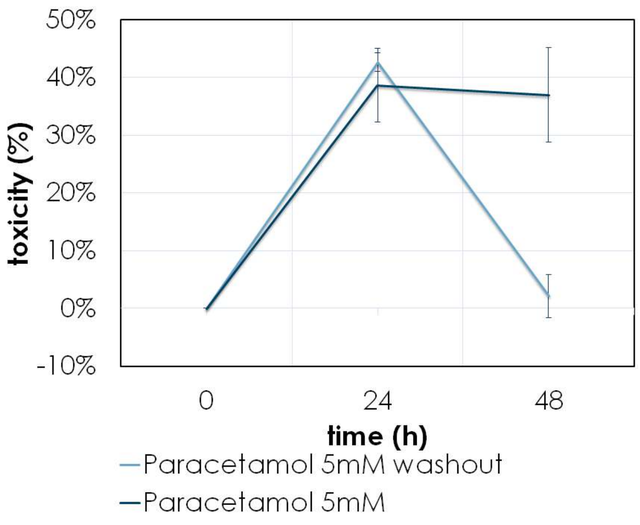




Increasing concentrations of paracetamol induce a decrease in base impedance of monocyte-derived hepatocyte-like (MH) cells which can be monitored continuously (left). Tween (2%) induced 100% cell death and was used as a positive control. The data is compared with the toxicity measurement of MH cells using an LDH release assay (right).

Paracetamol showed a dose-dependent effect on toxicity in both assays.





Normalized impedance versus time (in hours) after MH cells were exposed to 5 mM paracetamol for 24 or 48 hours (left). Cells recovered, indicated by the increase in base impedance, when paracetamol was washed out after 24 hours but toxicity continued when 5 mM paracetamol was added again for a further 24 hours. This is consistent with data obtained using the LDH release assay which shows that toxicity is reversed upon washout of paracetamol but continues with a 2nd dose of paracetamol after 24 hours (right).

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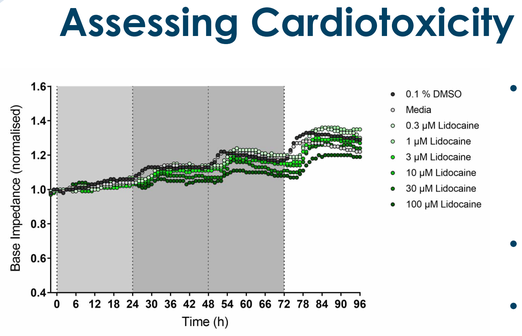
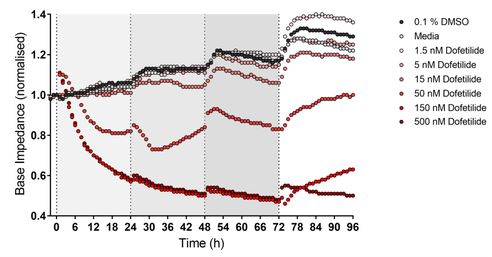
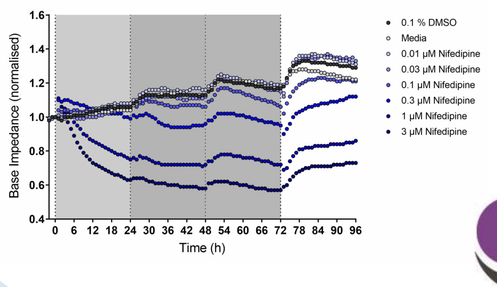
Assessing Cardiotoxicity using iPSC Cardiomyocytes







- Methodology: Axiogenesis vCor.4U iPSC cardiomyocytes were plated at 30,000 cells per well on fibronectin (10 µg/ml). Experiment was performed 5 days post-seeding after spontaneous beating had stabilized.
- Cardiotoxicity was assessed by measuring basal cell impedance over several days.
- Normalized cell impedance is plotted against time (in hours) for wells exposed to media (open symbols) or 0.1% DMSO vehicle (black traces), or increasing concentrations of Lidocaine (green traces), Dofetilide (red traces) or Nifedipine (blue traces). Test solutions were refreshed every 24h.
- Lidocaine had no effect on base impedance, whereas Nifedipine and Dofetilide showed a dose-dependent cardiotoxicity effect.
- Cardiotoxicity effects only partially recovered, indicated by the small increase in base impedance when Nifedipine and Dofetilide were washed out after 72 hours.

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Conclusions

- Cytotoxic effects of Escin and Triton X can be detected using impedance measurements of proliferating cells
- The hepatotoxic effects of paracetamol on hepatocyte-like cells (MetaHeps) are reversible upon removal of paracetamol after 24 hours but remain upon chronic exposure
- Data comparable to LDH studies but with continuous monitoring possible
- Impedance readouts from human iPSC-derived cardiomyocytes offer a flexible cardiotoxicity assay system capable of measuring both fast time-course spontaneous beating, as well as slower changes in cardiac cell health
- The label-free CardioExcyte 96 impedance platform enables acute and chronic assessment of iPSC cardiomyocyte activity and toxicity in a continuous fashion from living cells without the confounding effects of dyes that may affect cell function

References

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2. Stevens, J.L., & Baker, T.K. 2008. Drug Discovery Today. 14(3/4): 162-167