# Application Note

Cells: MetaHeps® Cells
Tools: CardioExcyte 96

# Investigating DILI using MetaHeps® cells on Nanion's CardioExcyte 96

Nanion Technologies GmbH, Munich.
Hepatocyte-like cells kindly provided by MetaHeps®

MetaHeps®

## Summary

Drug Induced Liver Injury (DILI) is the major cause of acute liver failure in the USA and Europe and is one of the main reasons for regulatory actions and market withdrawals[1]. Indeed, hepatic toxicity has accounted for 15 of the 47 drugs withdrawn from the market in the period 1975 - 2007[2]. DILI is classified as intrinsic or dose-dependent, acetaminophen (paracetamol) being the most important example of this class[3], or idiosyncratic which is unpredictable and not directly dependent on dose[4]. A number of factors contribute to an individual's susceptibility to develop idiosyncratic DILI including age, sex, alcohol consumption, drug interactions and genetic variability[4]. Although improvements have been made to cellular and animal models to predict intrinsic (dose-dependent) DILI, it is almost impossible to predict idiosyncratic DILI. Withdrawal of compounds at a late stage (or postmarketing) due to idiosyncratic DILI is costly and can lead to incorrect withdrawal of potentially useful compounds. Monocyte-derived hepatocyte-like (MH) cells have been developed as a tool to investigate long-term hepatotoxicity, metabolism and drug interactions[5]. Furthermore, patient-derived MH cells could provide a tool for diagnosis or exclusion of idiosyncratic DILI[1,6] and provide the causative agent in polymedicated patients.

In this study, MH cells (MetaHeps®) were used on the CardioExcyte 96 and changes in impedance, and therefore confluency, were used as a measure of toxicity. Intrinsic (dose-dependent) effects of paracetamol could be identified consistent with other methods of liver injury detection. Therefore, the CardioExcyte 96 in combination with patient-specific MH cells provides a novel tool for investigating intrinsic and idiosyncratic DILI.

## Results

The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells and thereby provides a measure of toxicity. MH cells from MetaHeps® were grown on NSP-96 plates and base impedance was monitored over time. Paracetamol showed a dose-dependent decrease in base impedance (Figure 1A). This result is directly comparable with a standard toxicity (lactate dehydrogenase (LDH) release) assay[5,6] (Figure 1B). Importantly, the CardioExcyte 96 allows continuous monitoring of base impedance using a single plate revealing that the maximum effect is reached within the first few hours of exposure. Using the standard toxicity assay, measurements of LDH in the supernatant and lysate are made only at 24 hour intervals or at several time points requiring several plates.

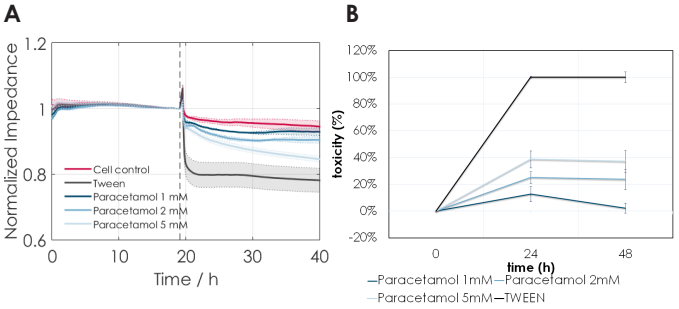


**Figure 1: A** Increasing concentrations of paracetamol induce a decrease in base impedance of MH cells which can be monitored continuously. Tween (2%) induced 100% cell death and was used as a positive control. **B** Toxicity measurement of MH cells using an LDH release assay. Paracetamol showed a dose-dependent effect on toxicity in both assays.



nan][on

# Application Note

In subsequent experiments, the effect of low and intermediate concentrations of paracetamol and washout was investigated. Figure 2A shows the effect on base impedance of MH cells after exposure for 24 or 48 hours of 1 mM paracetamol. The results show that exposure to low doses of paracetamol causes only transient effects on toxicity parameters compatible with cellular "adaptation". This is a phenomenon reported in humans[7] and animals[8], even when subsequent doses of paracetamol would normally induce liver injury[8]. This effect is also consistent with toxicity assays using LDH release experiments (Figure 2B).

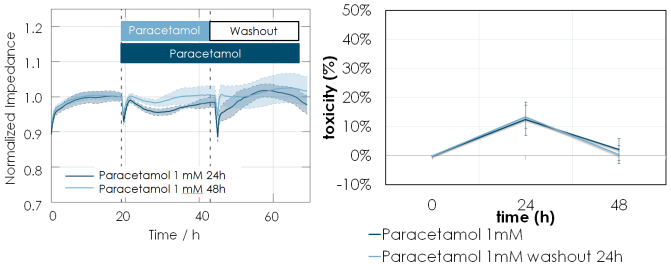


**Figure 2: A** Normalized impedance versus time (in hours) after MH cells were exposed to 1 mM paracetamol for 24 or 48 hours. Repeat exposure to paracetamol causes adaptation and only transient toxicity is observed. **B** Toxicity measurement using LDH release assay also shows repeated exposure to low doses of paracetamol cause transient toxicity of hepatocytes.

Figure 3 shows exposure of MH cells to an intermediate dose (5 mM) of paracetamol on the CardioExcyte 96 (A) and LDH release assay (B). In this case, washout of paracetamol after 24 hours resulted in recovery from

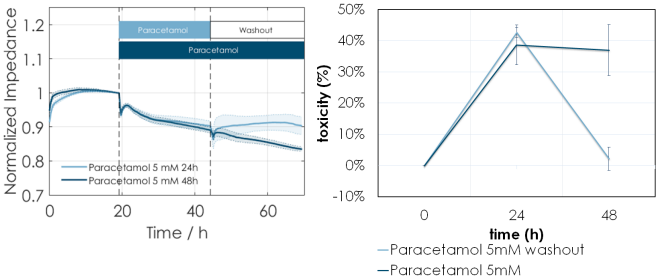


**Figure 3: A** Normalized impedance versus time (in hours) after MH cells were exposed to 5 mM paracetamol for 24 or 48 hours. Cells recovered, indicated by the increase in base impedance, when paracetamol was washed out after 24 hours but toxicity continued when 5 mM paracetamol was added again for a further 24 hours. **B** Toxicity measurement using LDH release assay also shows that toxicity is reversed upon washout of paracetamol but continues with a 2nd dose of paracetamol after 24 hours.

toxicity whereas continued exposure lead to increased hepatoxicity. This is important in order to identify compounds with the potential to cause liver damage despite discontinuation of treatment.

In summary, the CardioExcyte 96 has the capability to record multiple samples of hepatocyte-like cells simultaneously in real-time, a critical feature in monitoring cytotoxicity. The data recorded on the CardioExcyte 96 is comparable with results derived from the standard LDH release assay and thus strenghtens the scientific relevance of impedance-based toxicity assays. A reliable assay for the detection of DILI is urgently needed to prevent the inappropriate withdrawal of potentially life-saving compounds.

## References


1. Benesic, A., & Gerbes, A.L. 2015. Dig. Dis. 33: 486-491
2. Stevens, J.L., & Baker, T.K. 2008. Drug Discovery Today. 14(3/4): 162-167
3. McGill, M.R., & Jaeschke, H. 2013. Pharm Res. 30(9): 2174-2187
4. Chalasani, N., & Björnsson, E. 2010. Gastroenterology. 38: 2246–2259
5. Benesic, A., *et al.*, 2012. Laboratory Investigation. 92: 926-936
6. Benesic, A., *et al.*, 2016. Gut. 65: 1555-1563
7. Watkins, P.B. *et al.*, 2006. JAMA. 296(1): 87-93
8. Shayiq, R.M. *et al.*, 1999. Hepatol. 29(2): 451-463


## Methods

### Cells

Monocyte-derived hepatocyte-like cells from MetaHeps® were used. MH cells were derived from healthy donors and cryopreserved after generation.

### Impedance measurements

Impedance measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. MH cells were thawed, seeded on the Sensor Plate at an appropriate density to provide the desired confluency and cultured under propriety conditions for 24 hours prior to exposure to paracetamol. About 2 hours before drug application the medium was completely removed from the wells and 200 µl fresh medium was added. All signals were normalized to a group of control measurements (n=5-11) on the same plate.


Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de