

Investigations into idiosyncratic drug-induced hepatotoxicity and chronic proliferation of cancer cells using a label-free method

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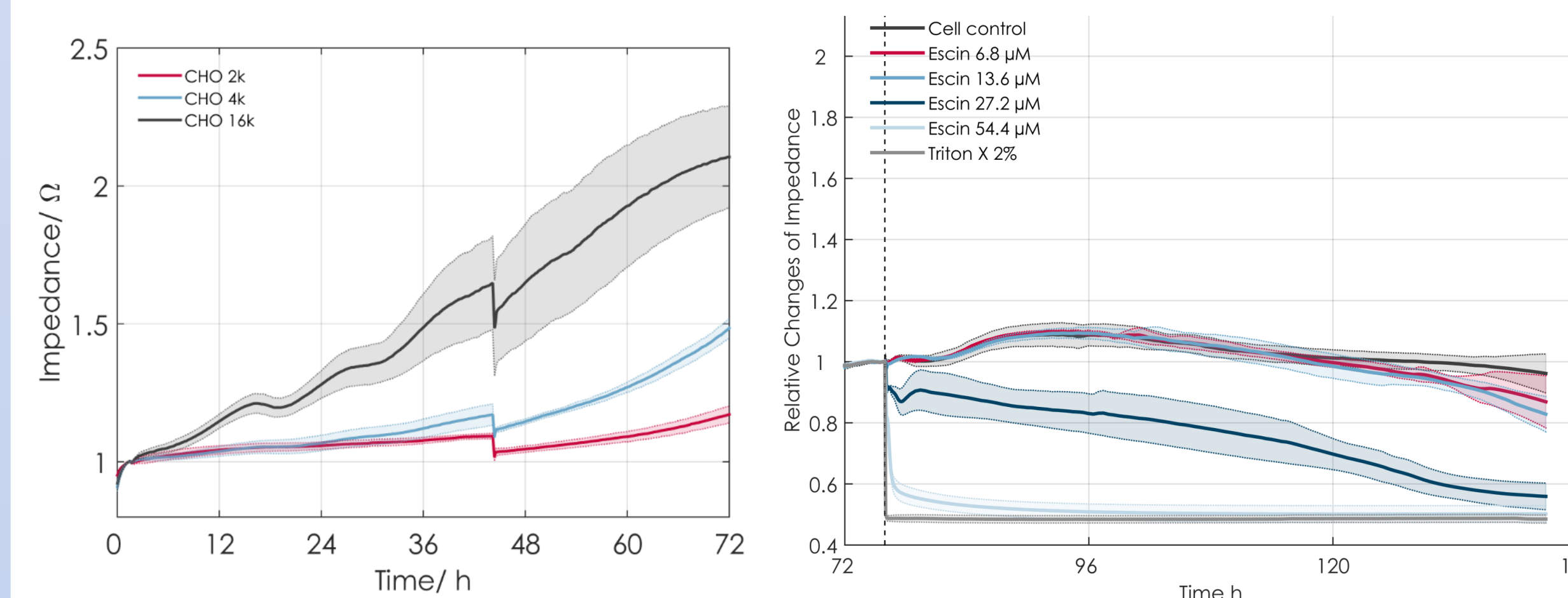
Introduction

Hepatic toxicity has accounted for 15 of the 47 drugs withdrawn from the market in the last two decades. More specifically, 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. DILI is classified as intrinsic (or dose-dependent) or as idiosyncratic. A prominent example of idiosyncrasy is acetaminophen (paracetamol), with a variable time of onset and not directly dependent on dose. We present a non-invasive DILI assay approach based on impedance measurements in monocyte-derived hepatocyte-like (MH) cells from MetaHeps®.

MH cells were used on a 96-well screening system that monitors changes in impedance (CardioExcyte 96). Once the monolayer is exposed to a cytotoxic agent, the impedance changes and measures of toxicity can be quantified long-term. We investigated the hepatotoxic effects of paracetamol on MH cells when exposed for 24 and 48 hours. In agreement with other standard toxicity assays, such as the lactate dehydrogenase release assay, low doses of paracetamol caused transient toxicity and 'adaptation' was observed. At higher doses, hepatotoxic effects of paracetamol could be reversed upon washout after 24 hours, but continued exposure caused increased hepatotoxicity.

In addition to hepatotoxicity, another validation of the principle is shown for chronic proliferation of cancer cells. Traditional cell proliferation assays involve labeling cells of interest with compounds that become reduced in the environment of metabolically active cells, or by incubating cells with radioactive labels.

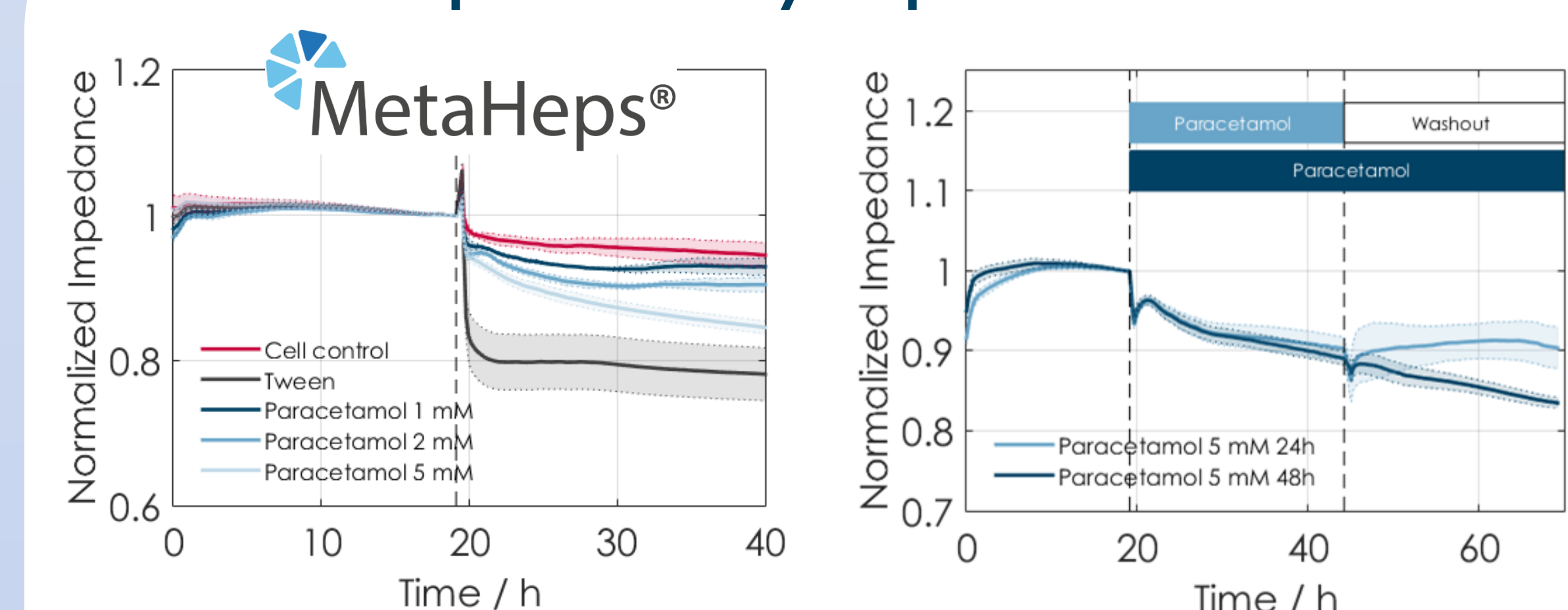
Cell proliferation monitored via impedance



Left: 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.

Right: 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

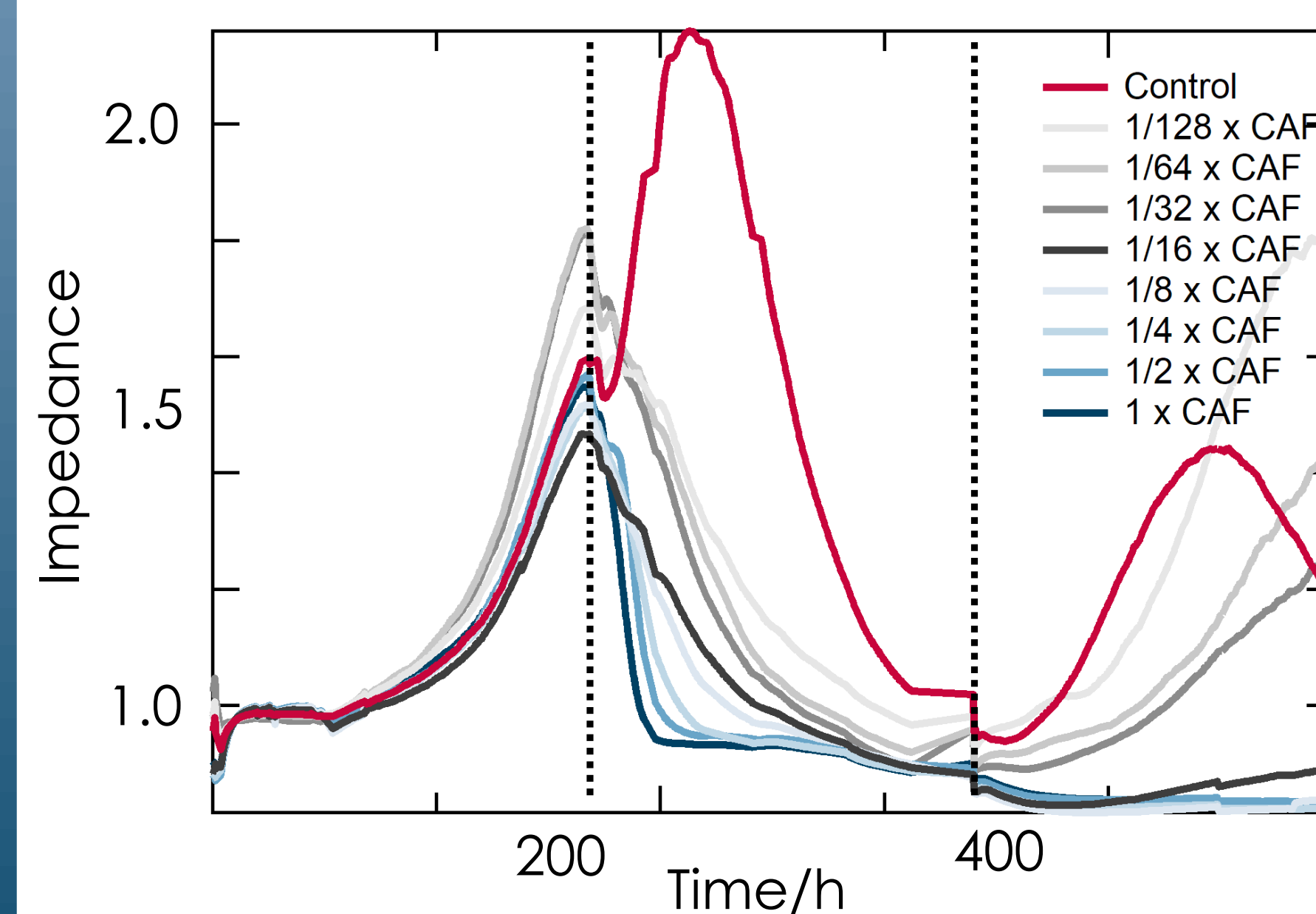


Left: Increasing concentrations of paracetamol induce a decrease in base impedance of monocyte-derived hepatocyte-like (MH) cells which can be monitored continuously (left). Tween (2%) induced 100% cell death and was used as a positive control.

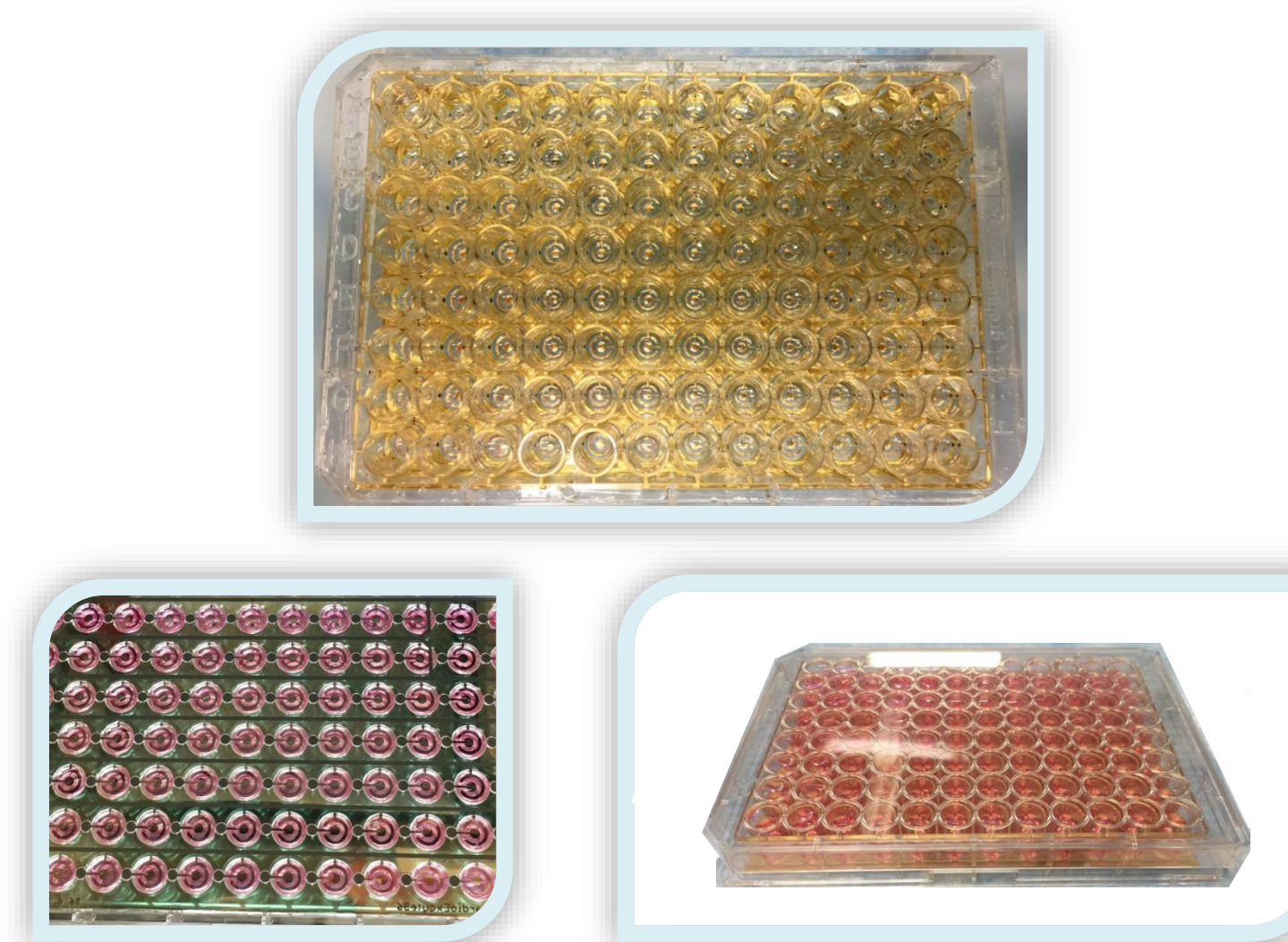
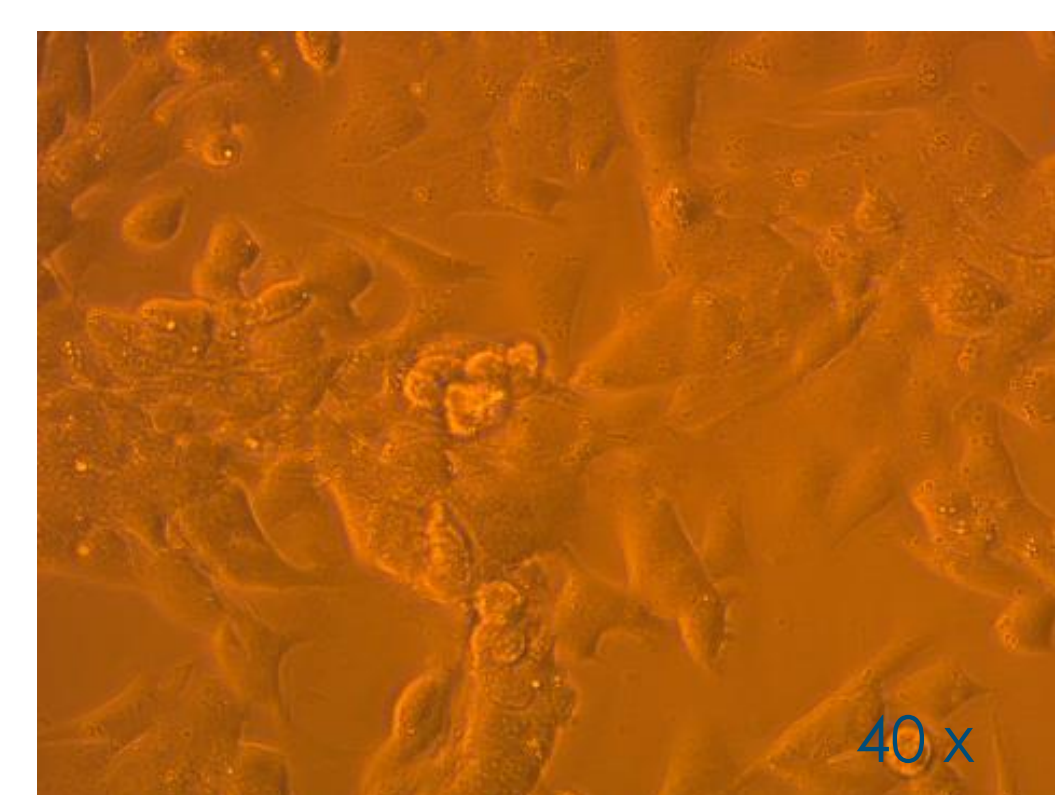
Right: Normalized impedance versus time (in hours) after MH cells were exposed to 5 mM paracetamol for 24 or 48 hours. Cells recovered, indicated by the increase in base impedance, when paracetamol was washed out after 24 hours but toxicity continued when 5 mM paracetamol was added again for a further 24 hours. 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 (data not shown).

Investigating therapy resistance of cancer cells *in vitro*

In this study, murine mammary carcinoma cells (H8N8 and H8N8 T3.2) were used on the CardioExcyte 96 and monitored over a time period of 500 h. Murine mammary carcinoma cells (H8N8 and H8N8 T3.2) were used and changes in impedance, and therefore confluency, were used as a measure of toxicity. Intrinsic (dose-dependent) effects of the standard clinical treatment regimen cyclophosphamide, doxorubicin and 5-fluoracil could be identified consistent with other methods of live cell analysis systems.



Left: Increasing CAF* concentrations showed a dose dependent effect on the viability of H8N8 cancer cells. Re-growth of cells after washout of the standard clinical treatment regimen was dependent on the previously applied dose. Middle: H8N8 cancer cells at 40 x magnification. Right: Transparent CardioExcyte 96 sensor plates allow imaging and optical visualization of seeded cells.



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
- The 96-well impedance system CardioExcyte 96 in combination with murine mammary carcinoma cells provides a novel tool for investigating therapy resistance of cancer cells *in vitro*.
- The label-free CardioExcyte 96 impedance platform enables acute and chronic assessment of toxicity in a continuous fashion from living cells without the confounding effects of dyes that may affect cell function