A Biologically Relevant *In Vitro* Culture System (SciFlow 1000) for DMPK Analysis

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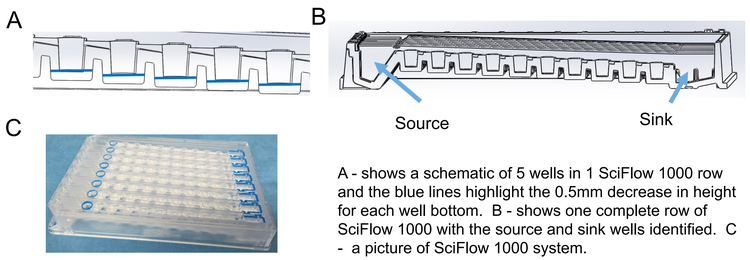
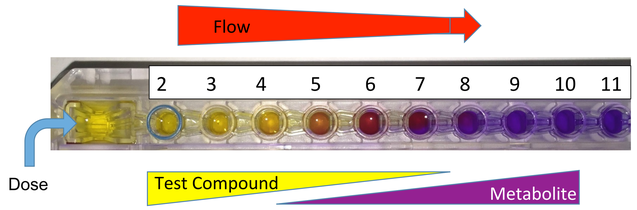
Technology



A - shows a schematic of 5 wells in 1 SciFlow 1000 row and the blue lines highlight the 0.5mm decrease in height for each well bottom. B - shows one complete row of SciFlow 1000 with the source and sink wells identified. C - a picture of SciFlow 1000 system.

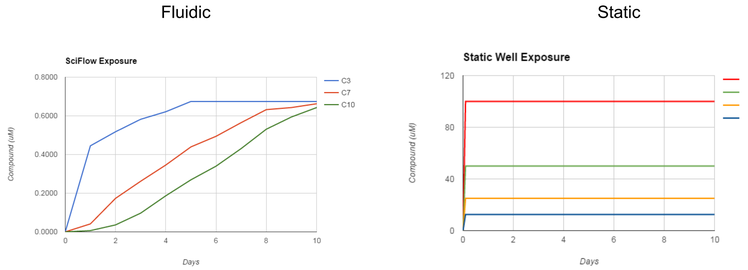
Flow Dynamics



Repeated dosing generates a gradient of decreasing compound concentration across the connected wells of a SciFlow row. Cellular metabolism further decreases the compound concentration within a given well. Fluid flow causes the metabolites and cellular responses to also flow downhill. Downstream wells are exposed to metabolites yet may never see significant concentrations of parent compound.

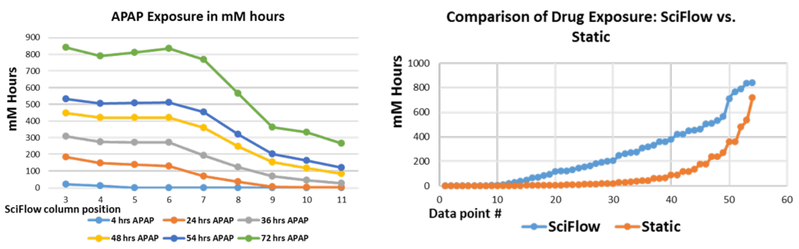
Compound Exposure

A



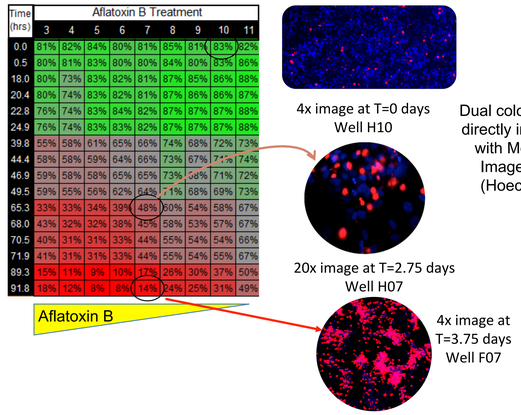
In an acellular scenario (A), SciFlow 1000 (left) dosing creates dynamic gradients of compound concentrations. The concentration of compound increases in each well until the equilibrium concentration is reached. Wells at different distances from the source well have very different concentration exposure profiles. Static plates (right) are at a fixed concentration, very different than an in vivo drug ADME profile.

B



B - Compound exposure in SciFlow 1000. Left panel, 150 µl of media with 20 mM APAP was dosed into the SciFlow 1000 source well six times over 72 hours. Aliquots were taken at specified times and LC/MS was used to determine APAP concentration. Right panel, comparison of SciFlow to static compound exposure. SciFlow data is a much more linear exposure through the entire exposure range, static plates have fewer data points in the intermediate exposure range.

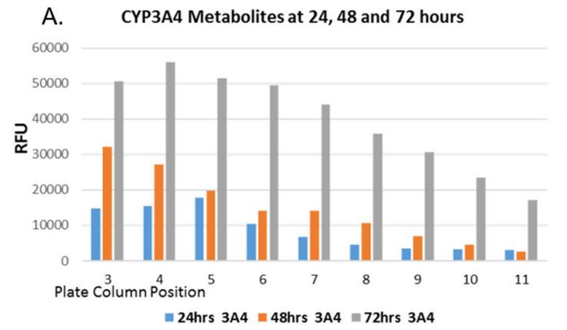
Aflatoxin B Treatment



Dual color images captured directly in the SciFlow1000 with Molecular Devices ImageXpress System (Hoechst and CellTox Green)

HepaRG cells (Biopredic International) were cultured in SciFlow 1000 then exposed to Aflatoxin B for 4 days. CellTox Green (Promega) and Hoechst dye were used for the real-time assessment of cell viability. Cells were visualized at discrete intervals and the percentage of viable cells calculated. The cells exposed to the highest cumulative dose (concentration x time) of Aflatoxin show the lowest percentage of viable cells. Those cells further from the source well, a lower cumulative dose, have higher percentages of viable cells.

CYP Activity - Metabolites



Primary Human Hepatocytes in SciFlow 1000. A) Accumulation of fluorogenic CYP3A4 metabolites over a 72 hour incubation. Hepatocytes generate fluorescent CYP3A4 metabolites , and those metabolites begin to flow downstream. B) Accumulation of fluorogenic CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP3A4 metabolites over a 72 hour incubation. Minimal 2B6 and 2D6 activity. Detectable levels of 1A2, 2C9, and 3A4 at 72 hours.

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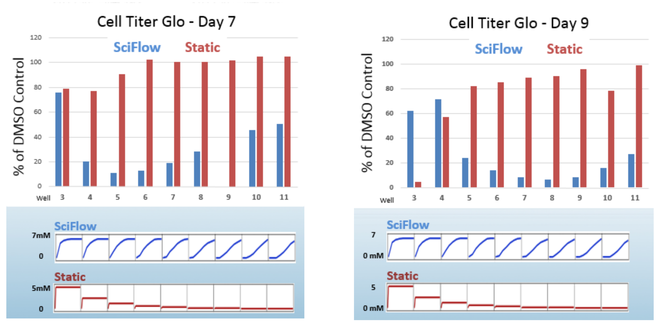
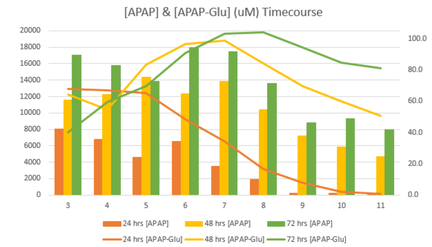
APAP Treatment: Toxic Metabolite Effects

A



A - Comparison of static and SciFlow 1000 cultured HepaRG cells treated with APAP for 7 and 9 days. CellTiter-Glo (Promega) was used to measure viable cells. Day 7: Toxicity of APAP in static plates (red) is marginally detectable only at highest concentration. SciFlow 1000 (blue) shows a downstream cell death effect which is characteristic of a toxic/reactive metabolite. Day 9: Obvious cell death in static plate at the highest APAP concentration (5mM), and marginal effect at 2.5mM. Pronounced downstream metabolite effect in SciFlow 1000. SciFlow 1000 provides insights into mechanisms of toxic drug response.

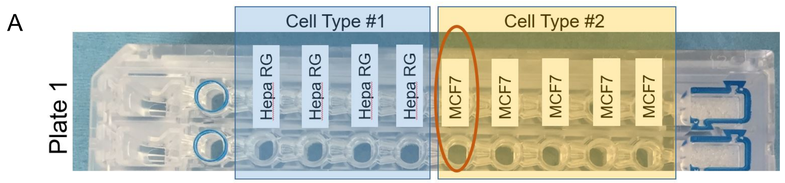
B



B - LC/MS to measure the flow of APAP and its major metabolites across the plate. APAP concentrates upstream and the metabolites flow downstream. Correlates well with the CellTiter-Glo results.

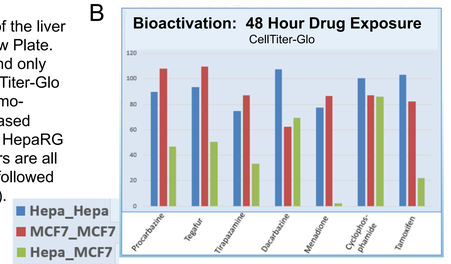
Bioactivation System - Chemotherapeutic Example

A



A) Plate map highlighting the positions of the liver and breast cancer cells within the SciFlow Plate. Two control plates contained only liver and only breast cancer cells (not shown). B) CellTiter-Glo data analyzing bioactivation of the 7 chemo-therapeutic drugs. 5/7 drugs show increased efficacy when first passaged through the HepaRG cells. Blue bars are all liver cells, red bars are all breast cancer cells, green bars are liver followed by breast cancer (bioactivation condition).

B



Conclusions

The SciFlow™ 1000 Fluidic Culture System exhibits more biologically relevant (*in vivo*-like) compound exposures. SciFlow 1000 integrates the generation of parent compound and metabolite gradients across a unidirectional fluidic row. These parent compound gradients are demonstrated with the toxic effects of Aflatoxin B on HepaRG cells in a time resolved manner using high content imaging. Additionally, an APAP study demonstrates the ability to distinguish between parent compound and toxic metabolite effects. APAP treatment shows a more potent downstream effect upon cells which is a hallmark of a metabolite mediated effect, not a direct effect of the parent compound (drug). Additionally, the ability to interconnect different cell types allows for bioactivation of a panel of chemotherapeutic agents in liver cells and detection of increased potency on downstream breast cancer cells (MCF7). The SciFlow 1000 is a versatile fluidic culture system which is compatible with plate readers, high content imagers, and commercially available biochemical assay kits.