Microfluidics and High-Content Imaging for *In-Vitro* to *In-Vivo* Safety and Efficacy Assessments

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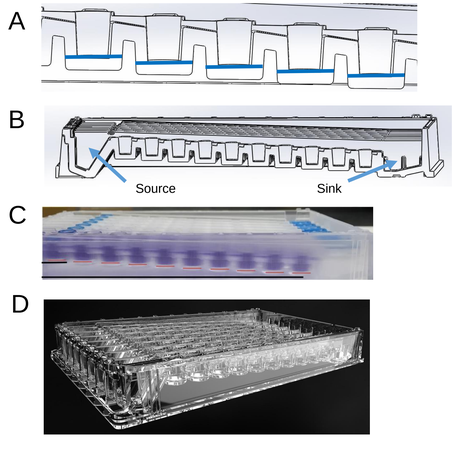
Abstract

We evaluate the robustness and applicability of a newly designed microfluidic system (SciFlow™ 1000) to support high-content imaging via automated, fixed, and adaptable Z-height focal points. In order to enable efficient flow of fluids across integrated cellular substrates, the microfluidics system is designed with descending well-heights from left-to-right so each subsequent well is 0.5mm lower than the previous well (i.e. Z-height changes). Both hydrodynamic and capillary actions allow for cascading flow of fluids across the system without the need for external pumps or tubes. A goal for applying this technology is to visually image "real-time" cellular responses during the movement of biological fluids, chemical compounds, and materials. Time-resolved dynamic exposure scenarios afforded by such a system is more in vivo-like and could enable more accurate assessment of adaptive vs toxic mechanisms. Moreover, the dynamic exposure scenario allows better safety predictions of concentration thresholds for response rather than extrapolating from endpoint dose-response curves.

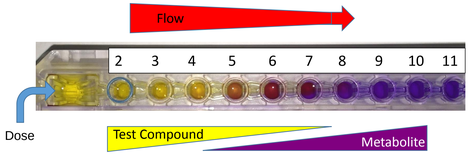
Standard 2-dimensional static cell culture systems are limited tools for efficacy evaluations in vitro. Cells grown in static culture wells are subjected to increasing amounts of waste products and decreasing amounts of dissolved oxygen leading to potential stress responses and toxicity not related to drug/toxicant exposures. To overcome these limitations, researchers are turning to 3D scaffolds, spheroids, hydrogels, and fluidics systems to improve the physiological relevance of the environment and enhance safety assessments. We show here the evaluation of a fluidics system engineered to connect 10-wells of each row in a 96-well plate together with a microfluidic channel. This system sequentially links wells together to form a cascade of cell chambers through which drugs or toxicants can be applied. Toxicants interact with cells in the upstream compartments creating metabolites that will mix and interact in downstream wells forming a parent-metabolite gradient in a time-resolved fashion. Such a system, termed SciFlow, enables concentration by time kinetics of toxicity measurements in a more life-like environment. To demonstrate the value of this system using high-content imaging, we evaluated the cytotoxic effects of Aflatoxin b and acetaminophen on various metabolically competent hepatocyte models in comparison to static conditions using the reagents CellTox Green and Cell Titer Glo (Promega). Using a fluorescein tracer molecule we could demonstrate that exposures in the fluidic system were non-linear and shaped similar to an expected plasma curve in vivo. Furthermore, observation of shifts in the dose-response curves as a function of distance from source well provides an indicator for the role for metabolic activity in safety and efficacy assessments of these chemicals.

Technology



Panel A shows a schematic of 5 wells in 1 row of SciFlow and the blue lines highlight the 0.5mm decrease in height for each well bottom. B shows one complete row of SciFlow with the source and sink wells identified. Panel C is a picture of SciFlow, with purple liquid filling one row to highlight the decreasing Z-axis across the plate. Panel D shows the whole SciFlow system, SBS complaint 96-well plate injection molded in tissue culture treated polystyrene.

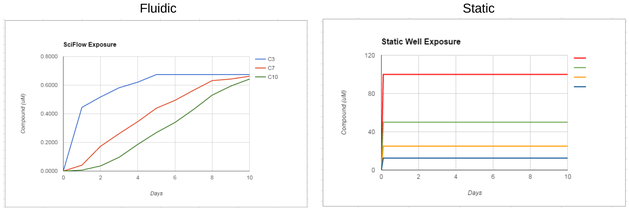
Flow Dynamics



Repeated dosing generates a gradient of decreasing compound concentration across the connected rows of a well. 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.

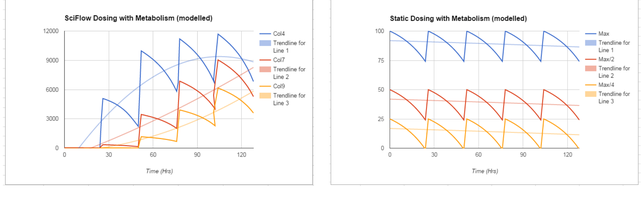
Exposure

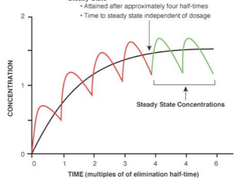
A



In an acellular scenario (A), SciFlow (left) dosing creates gradients of compound concentrations over time. The concentration of compound increases in each well until the equilibrium concentration is reached, and then that concentration is maintained for the duration of the experiment. Wells at different distances from the source well have very different concentration exposure profiles. Static plates (right) are at a fixed concentration, which only varies due to cellular metabolism.

B





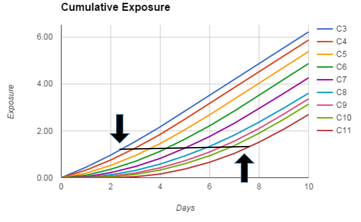
Modelling metabolism onto the SciFlow and static dosing graphs (B) produces the above left and right graphs. The SciFlow dosing has an increasing concentration over time, followed by a equilibrium, which is very similar to the classic in vivo plasma drug concentrations graph shown to the left. This increasing concentration over time allows the cells to respond/adapt to the drug before maximal concentrations are reached, which can result in a very different cellular response than an instantaneous rise in drug concentration from zero to maximum concentration.

C

Dose	3	4	5	6	7	8	9	10	11
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.445	0.322	0.190	0.104	0.041	0.017	0.006	0.007	0.000
2	0.518	0.432	0.334	0.253	0.173	0.106	0.060	0.036	0.000
3	0.582	0.513	0.422	0.340	0.262	0.185	0.134	0.097	0.042
4	0.621	0.578	0.507	0.427	0.346	0.271	0.222	0.187	0.111
5	0.674	0.650	0.597	0.521	0.439	0.350	0.297	0.269	0.214
6	0.674	0.674	0.631	0.568	0.494	0.405	0.378	0.340	0.289
7	0.674	0.674	0.674	0.630	0.565	0.481	0.461	0.431	0.376
8	0.674	0.674	0.674	0.674	0.632	0.555	0.544	0.531	0.480
9	0.674	0.674	0.674	0.674	0.643	0.600	0.613	0.594	0.554
10	0.674	0.674	0.674	0.674	0.663	0.627	0.648	0.643	0.624
	6.21	5.87	5.38	4.87	4.26	3.60	3.36	3.13	2.69

Snapshot Concentrations

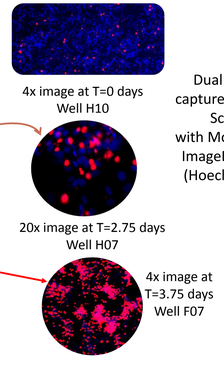
Cumulative Exposure



Treating SciFlow exposure as a cumulative radiation exposure model (C), where the total exposure over time is calculated, allows us to quantify and compare the exposure to traditional methods. While the final equilibrium drug concentration is very similar across the entire plate, the total (cumulative) dosage is very different in each well.

Aflatoxin B Treatment

Time (hrs)	Aflatoxin B Treatment 3	4	5	6	7	8	9	10	11
0.0	81%	82%	84%	80%	81%	85%	81%	83%	82%
0.5	80%	81%	83%	80%	80%	84%	80%	83%	86%
18.0	80%	73%	83%	82%	81%	87%	85%	86%	88%
20.4	80%	74%	83%	82%	81%	87%	86%	86%	87%
22.8	76%	74%	83%	84%	82%	87%	87%	87%	88%
24.9	76%	74%	83%	83%	82%	87%	87%	87%	88%
39.8	55%	58%	61%	65%	66%	74%	68%	72%	73%
44.4	58%	58%	59%	64%	66%	73%	67%	71%	74%
46.9	59%	58%	58%	65%	65%	73%	68%	71%	72%
49.5	59%	55%	56%	62%	64%	71%	68%	69%	73%
65.3	33%	33%	34%	39%	48%	60%	54%	58%	67%
68.0	43%	32%	32%	38%	45%	58%	53%	57%	67%
70.5	40%	31%	31%	33%	44%	55%	54%	54%	66%
71.9	41%	31%	31%	33%	44%	55%	54%	55%	67%
89.3	15%	11%	9%	10%	17%	26%	30%	37%	50%
91.8	18%	12%	8%	8%	14%	24%	25%	31%	49%

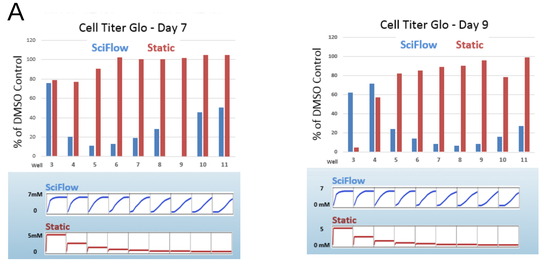


Dual color images captured directly in the SciFlow1000 with Molecular Devices ImageExpress System (Hoechst and CellTox Green)

HepaRG cells (Biopredic International) were cultured in SciFlow plates according to manufacturer's protocols. Cells were exposed to Aflaxtoxin B for 4 days with Cell Tox Green (Promega) and Hoechst dye present in the media. The constant presence of Hoechst and Cell Tox Green allowed for the real-time assessment of cell viability, without needing to interrupt the time course of the study. Cells were visualized on Molecular Devices ImageExpress System, multiple times/days and the percentage of viable cells was calculated. The cells which were exposed to the highest concentrations of Aflatoxin for the longest period of time show the lowest percentage of viable cells, and those cells farther away from the source well, exposed to lower drug concentrations have higher percentages of viable cells. The gradient exposure of toxin across the plate does produce a measurable effect on the cells.

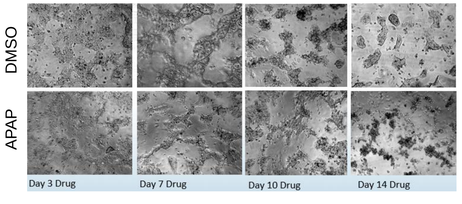
APAP Treatment

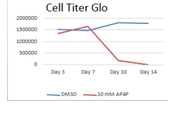
A



9 day treatment of HepaRG cells with APAP (acetaminophen). Comparison of static and SciFlow cultured cells. Cell Titer Glo (Promega) was used as the endpoint for viability/cell death. Above graphs are normalized to DMSO control treated cells. Day 7 – marginally detectable effect in static plates of APAP treatment, and only at highest concentration. SciFlow shows a downstream cell death effect which is characteristic of a toxic/reactive metabolite/cellular response which is produced in the upstream cells and flows downstream. Day 9 – obvious cell death in static plate at the highest APAP concentration, and marginal effect at second highest concentration. More pronounced downstream metabolite effect in SciFlow plate. SciFlow provides insights into mechanisms of toxic drug response. GSH was also quantified in SciFlow plate and depletion of GSH mirrors the downstream decrease in cell viability (data not show).

B





HepaRG cells visualized in SciFlow plate with standard inversion microscope. Comparison of DMSO (vehicle) and APAP treated cells. Corresponding Cell Titer Glo results highlighting the concordance between visual and biochemical data generated in SciFlow. Obvious cellular stress and death observed in days 10 and 14 APAP treated cells with very normal and healthy looking (microscopy and Cell Titer Glo) DMSO treated cells.

Conclusions

The SciFlow 1000 fluidic culture system has gone through multiple rounds of design and is now a well characterized and commercially available product. The fluidic flow and concentration gradients have been studied in multiple model systems. Data presented in this poster highlight the ability to generate parent compound gradients across a plate, and the differential effects on HepaRG cells based upon these time resolved concentration gradients. Additionally, an APAP treatment study demonstrates the ability to distinguish between parent compound and metabolite/cellular response effects on cells. 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). The SciFlow 1000 system is a versatile fluidic culture system which is compatible with plate readers, high content imagers, and commercially available biochemical assay kits.

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