Toxicity Assessment of Co-Culture Human Systems during Serial Multi-Well Gradient Exposures

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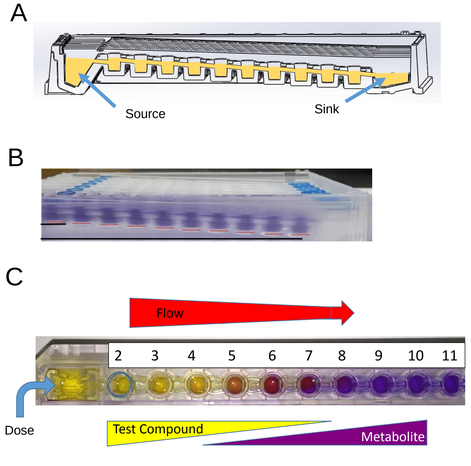
Abstract

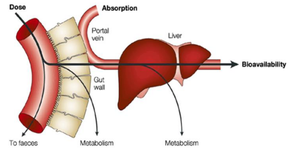
We evaluate the robustness and applicability of a newly designed microfluidic system to mimic passage of biological fluids and materials from one tissue type to another. A goal for applying this technology is to couple liver and breast cancer cell line models through microfluidics for a benchtop bioactivation system. Many chemotherapeutic drugs require bioactivation before they reach full potency. This bioactivation is accomplished through many different mechanisms but is frequently accomplished in the liver before the drug enters circulation. The inadequacy of animal models to predict human biology in the drug development process is becoming increasingly clear due to species differences in uptake and metabolism at both the cellular and organ levels. As a result, there is a need for more human model systems to be incorporated earlier in research and development. Innovative concepts such as "body-on-a-chip" have been introduced, but the complexity and miniaturization of the many of the formats has limited applicability on a commercial scale. SciKon is developing tools that better recapitulate biological systems in benchtop cell culture formats which are amenable to mass manufacturing.
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 (e.g. liver cells) creating metabolites (active drugs) that will mix and interact in downstream wells (e.g. cancer cells) forming a parent-metabolite gradient in a time-resolved and inverse concentration gradient fashion. Such a system enables concentration by time kinetics of toxicity measurements in a more life-like environment.
To demonstrate the value of this system, we have evaluated the effects of 7 chemotherapeutic drugs on human liver cell lines, rat primary liver cells, and a human cancer cell line using the reagent Cell Titer Glo ™. Using a fluorescein tracer molecule we demonstrate that exposures in the fluidic system were non-linear and shaped similar to an expected plasma curve in vivo.

Technology

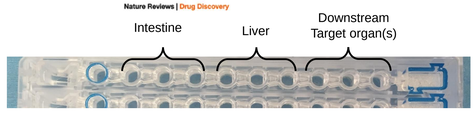


Panel A shows a schematic side view of SciFlow with the source and sink wells identified 0.5mm decrease in height for each well bottom. Panel B is a picture of SciFlow, with purple liquid filling one row to highlight the 0.5mm decrease in Z-height (height of well bottom) across the plate. Panel C is a top down view of one SciFlow row highlighting the dual gradients of decreasing parent compound and increasing metabolite down the row.

Multi-Organ Culture System

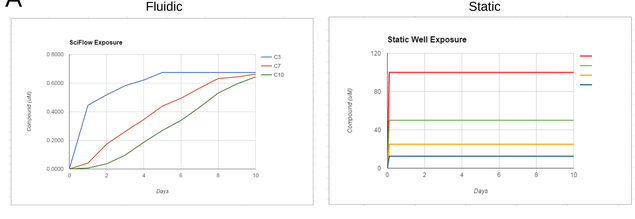


SciFlow enables culturing different cell/tissue types within an interconnected row of wells. Cells can be initially cultured in isolation within a well to establish the cells, and then the media volume increased to establish flow for exposure and signaling studies. Interconnected cell types allow for mimicking a larger organ system and better prediction in vivo effects/outcomes.



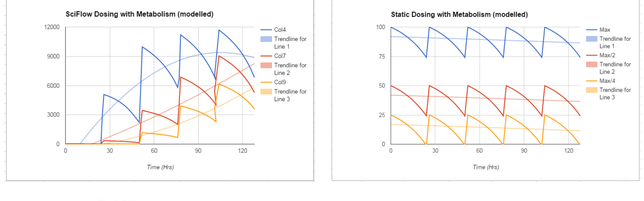
Exposure

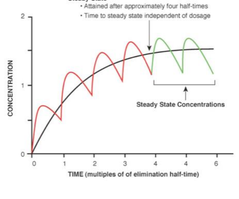
A



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





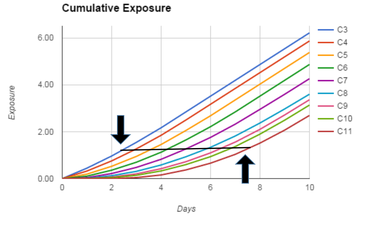
Modelling metabolism onto the SciFlow and static dosing graphs (B) produces the above left and right graphs. The SciFlow dosing has an increasing concentration over time, followed by a equilibrium, which is very similar to the classic in vivo plasma drug concentrations graph shown to the left. This increasing 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

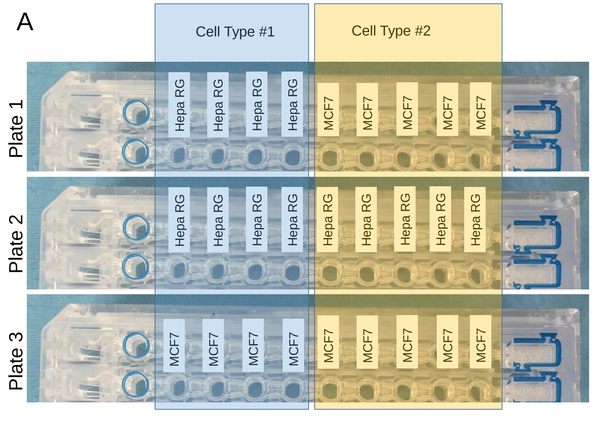
Cumulative Exposure



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.

Bioactivation System (Chemotherapeutic Example)

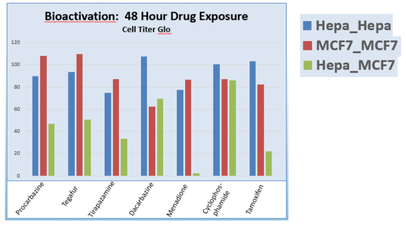
A



Drugs Tested	
DMSO	Dacarbazine
Procarbazine	Menadione
Tegafur	Cyclophosphamide
Tirapazamine	Tamoxifen

To test the effects of passaging 7 different chemotherapeutic drugs through a metabolically competent human liver cell line (HepaRG), we set-up 3 SciFlow plates with unique combinations of cell lines. Plate 1, HepaRG (liver) cells upstream of MCF7 (breast cancer) cells tests the full bioactivation system. Plates 2 and 3 are control plates with only HepaRG and only MCF7 cells respectively.

B



The Cell Titer Glo data in panel B is normalized to DMSO (vehicle) control. The 3 bar graphs for each drug represent the percentage of viable cells remaining in column 7 post 48 hours of drug exposure. The effects of the seven chemotherapeutic drugs on the cells in column 7 is very dependent upon the upstream cells and the ability of these cells to activate the chemotherapeutic compounds. Five of the seven drugs lead to a greater than 50% reduction in viability when first passaged through metabolically competent HepaRG cells before encountering the breast cancer cell line MCF7. Of these five drugs, three drugs (Tirapazamine, Menadione, and Tamoxifen) had modest effects in the absence of exposure to liver cells, but greatly increased efficacy when exposed to the liver cells upstream of the cancer cells. None of the drugs had a major effect on the HepaRG cells alone. Two drugs (Dacarbazine and Cyclophosphamide) always decreased MCF7 cell viability regardless of the upstream cell type indicating that these two drugs do not appear to require bioactivation through the liver to elicit a biological effect.

Bioactivation System – Rat Primary Hepatocytes

Cell Titer Glo –MCF7
(normalized to DMSO control)

	Col8	Col9	Col10	Col11
DMSO	100%	100%	100%	100%
Tamoxifen	89%	93%	100%	89%
Tirapazamine	95%	92%	101%	96%
Menadione	88%	97%	96%	83%

No significant difference in Cell Titer Glo data between DMSO and chemotherapeutic treated MCF7 cells downstream of primary rat hepatocytes. In data not shown, the primary rat hepatocytes are metabolically competent and do possess CYP3A4 activity. This stands in stark contrast to the results when the same MCF7 cells were exposed to chemotherapeutic drugs downstream of the human hepatic cell line HepaRG. The lack of response in this experiment demonstrates the differences between human and rat model systems, highlights the importance of biologically relevant human testing systems, and stresses the pitfalls of relying upon animal testing for drug safety and efficacy studies.

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 here demonstrate the feasibility of creating a multi-organ system capable of testing for the ability of a human liver cell line (HepaRG) to activate a panel of chemotherapeutic drugs. We tested 7 chemotherapeutic drugs for their ability to kill human MCF7 cells both with and without bioactivation by passage through metabolically competent human HepaRG cells. Additionally we tested 3 of the chemotherapeutic drugs in a rat primary hepatocyte/human breast cancer cell line system with the goal of determining if metabolically competent primary rat hepatocytes could activate the chemotherapeutic drugs in an analogous manner to the human cells. Highlighting the importance of human models, the rat primary hepatocytes do not function in the same manner as the human hepatocyte cell line and were unable to activate the chemotherapeutic drugs and kill the downstream cancer cells. 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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