APPLICATION NOTE

Predict cytotoxic effect while generating more *in vivo*-like data

Introduction

The next step in cell-based assays is to reproduce organ and living system complexity for more accurate assessment of adaptive vs. toxic mechanisms of compound treatment. A dynamic exposure scenario allows better prediction of concentration thresholds for response rather than extrapolating from endpoint dose-response curves.

SciFlow™ 1000 Fluidic Culture System is a multi-well microfluidics platform in an SBS-standard format that is compatible with automated liquid handling and imaging systems. The entire SciFlow 1000 System is contained within a 96-well format that includes 8 identical channels that enable fluidic motion and gradient compound exposure to cell-based assays without the need for external pumps or connected tubes (Figure 1). Using the ImageXpress® Micro High-Content Imaging System we were able to dynamically image and measure cell responses across the channels and automatically adjust for the changing z-heights.

To demonstrate the value of this system using high-content imaging, we evaluated the time-course effect of Aflatoxin B on a metabolically competent hepatocyte model using the DNA-binding reagent CellTox™ Green.

Materials used

- SciFlow 1000 Fluidic Culture System (SciKon Cat. No. AA-1-50)
- HepG2 cells (ATCC Cat. No. HB-8605)
- Culture media: DMEM supplemented with 10% FBS, 1X Glutamax, 1X Pen/Strep
- CellTox™ Green Cytotoxicity Assay (Promega Cat. No. G8731)
- Hoechst DNA Dye (Sigma Cat. No. 33342)
- Aflatoxin B (Sigma Cat. No. A6636)
- Fluorescein Salt (Sigma Cat. No. F6377)
- Instrument: ImageXpress Micro XLS High-Content Imaging System (Molecular Devices)

Benefits

- **Automate real-time cytotoxic effects with reproducible and reliable data for 2D or 3D assays**
- **Generate in-focus images with variable well depths**
- **Predict more relevant concentrations of thresholds for response**
- **Create quantifiable data with real-time outcomes of gradient drug exposures**

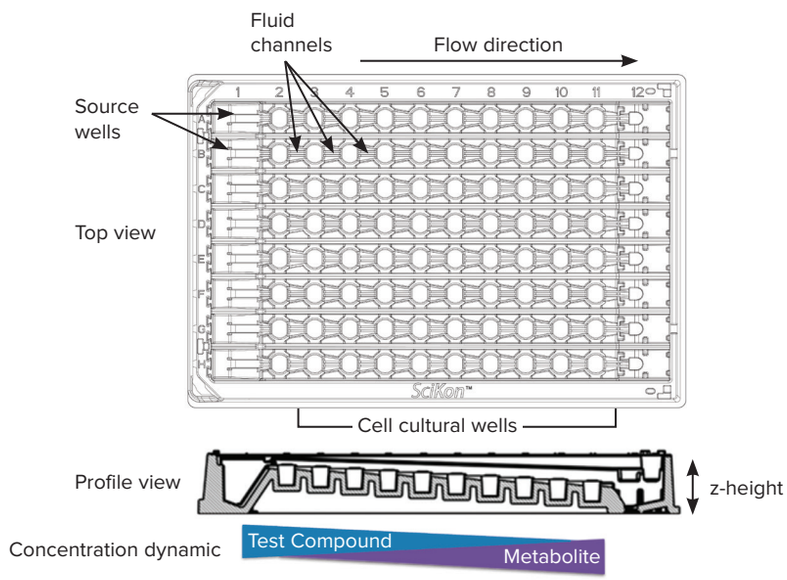

Figure 1. SciFlow 1000 System Features. Top and profile views of SciFlow illustrate fluidics and multiwell major features. Note the difference in z-height of each well in the profile view. By applying a test chemical to the source well (column 1), serial exposure into subsequent wells results in a gradient of decreasing chemical concentration and a concurrent increase in metabolites and cellular responses across the linear array of linked wells.

Method for Z-height focus using the ImageXpress Micro XLS

To optimize z-height for each individual well, HepG2 cells were plated in the SciFlow 1000 System then analyzed via nuclear DNA staining with Hoechst. The z-height adjustments for the SciFlow 1000 System are listed in Table 1, column 7 was the mid-system or zero-reference point used for optimizing autofocus during imaging.

SciFlow 1000 System z-height adjustments									
Column	3	4	5	6	7	8	9	10	11
microns	+ 2000	+ 1500	+ 1000	+ 500	0	- 500	- 1000	- 1500	- 2000

Table 1. Z- Heights in the SciFlow 1000 System using ImageXpress Micro XLS.

Experiment

Our process workflow (Figure 2) outlines how to run the experimental design (Figure 3) of Aflatoxin B treatment of HepG2 cells. HepG2 cells were plated in 50 µL media. Following attachment, media was removed and we added 100 µL/well fresh media containing 1:2000 dilution of CellTox Green and 0.5 µg/ml Hoechst. Then, 400 µL of media containing CellTox Green was applied to the source wells. The plate was incubated for 30 minutes to allow for connection of the fluidic channels. Once fully connected, 100 µL of test compound, vehicle control, or 1 µM fluorescein (fluid tracer control) was added to the source wells. To maintain fluid flow over multiple days, 100 µL was removed manually from wells in column 11 and the new drug, vehicle, or fluorescein standard was added to the source well three times per day. This results in a complete turnover of media over four days while providing a constant infusion of increasing concentrations of chemical in each group. Hoechst signal (all cells) and CellTox Green signals (dead cells) were monitored on the ImageXpress Micro XLS.

Fluorescein as a fluid tracer

Fluorescein is used as a standard fluid tracer, allowing the concentration of test compounds to be determined at any time. This is done by monitoring fluorescein tracer fluorescence and calculating concentration based on a predetermined standard curve (0.001 µM to 1 µM). The standard curve was created from individual wells with fluorescein dissolved in culture media and FITC imaged using the ImageXpress Micro XLS. During flow experiments, the source well (column 1) start concentration of fluorescein was 1 µM and AFb was 10 µM (5 µM is considered a 100% lethal dose). To extrapolate AFb concentrations, the amount of fluorescein

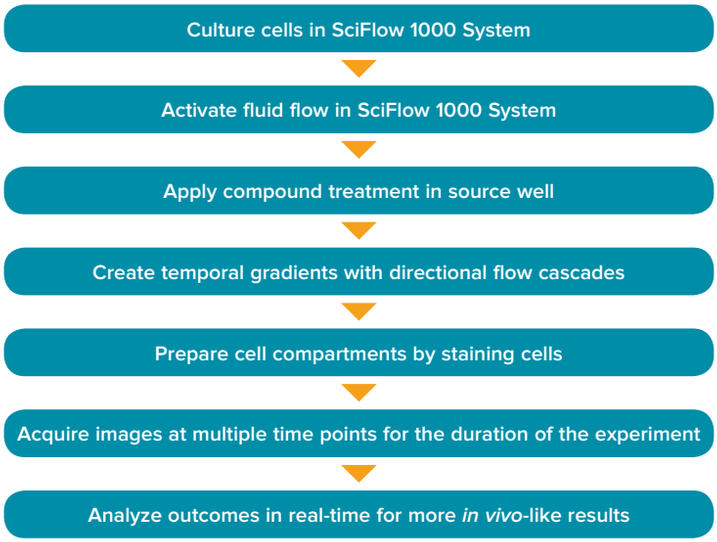

Figure 2. Method workflow.

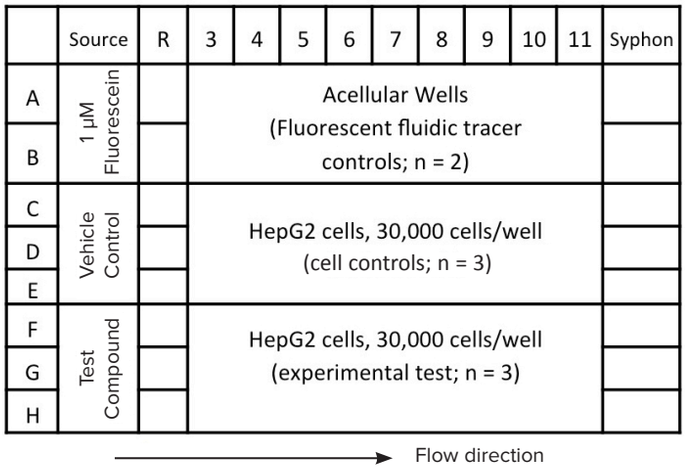

Figure 3. Example of a single SciFlow 1000 System with the experimental layout. Cell seeding and attachment (50 µL) is done in columns 3 - 11. Then, well volume is increased to 100 µL allowing fluid connection between wells. Lastly, the test compound is added to the source well in column 1.

in each well was multiplied by the starting AFb concentration to obtain the corresponding concentration of vehicle or test compound over time (Figure 4).

Results

After initiating flow, images were acquired over four days and analyzed for live and dead cell counts. Figure 5A shows the percentage of viable control cells over time. Figure 5B shows cell viability for the Aflatoxin B experiment. One channel (columns 3-11) was evaluated at 16 different time points between 0.0 to 91.8 hours. Each well value is actually the average of three replicates. The percentages within the table represent the change in cell viability.

Figure 5B shows the decreasing percentage of viable cells over time which is also visually identified by the heat map.

Conclusion

ImageXpress Micro High Content Imaging System can effectively monitor real-time fluid flow, direct cell culture responses, and dynamic compartment-to-compartment signaling of cellular responses using the SciFlow 1000 Fluidic Culture System. Coupled with robust MetaXpress software, we were able to quickly acquire and analyze the real-time outcomes of gradient drug exposures for faster prediction of concentration thresholds and to produce quantifiable data reflective of *in vivo* models.

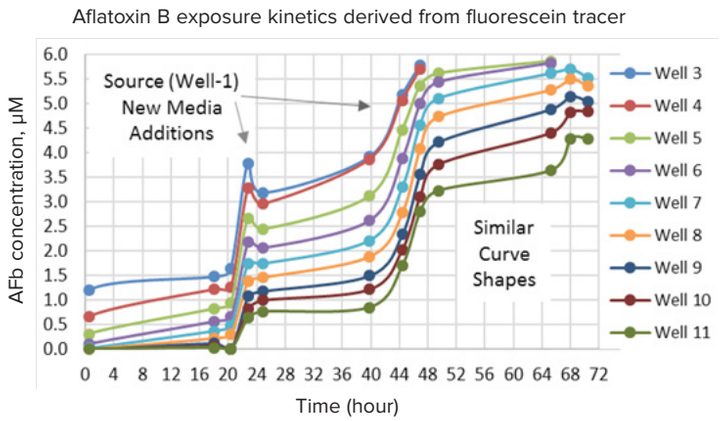

Figure 4. This graph depicts the time course concentration gradients of compound/toxicant exposures in each SciFlow 1000 System column over a 72 hour period. AFb concentration data are extrapolated from fluorescein fluid tracer. Values are the average of 3 replicates.

A Vehicle controls

Hours of exposure/experiment	3	4	5	6	7	8	9	10	11
0.0	80%	80%	80%	77%	81%	80%	86%	86%	90%
0.5	78%	78%	79%	77%	80%	79%	85%	85%	93%
18.0	79%	84%	83%	82%	82%	83%	88%	90%	90%
20.4	81%	85%	85%	85%	83%	84%	89%	89%	88%
22.8	81%	85%	86%	85%	84%	85%	90%	90%	88%
24.9	82%	86%	86%	86%	84%	87%	90%	91%	90%
39.8	79%	82%	84%	86%	85%	86%	85%	84%	87%
44.4	77%	82%	84%	87%	86%	88%	85%	85%	87%
46.9	79%	82%	84%	87%	86%	88%	85%	85%	85%
49.5	77%	82%	84%	87%	87%	88%	85%	85%	84%
65.3	76%	81%	82%	85%	85%	87%	85%	88%	89%
68.0	80%	76%	83%	86%	84%	87%	85%	89%	89%
70.5	79%	79%	83%	86%	85%	88%	85%	88%	90%
71.9	78%	78%	83%	86%	85%	88%	85%	88%	91%
89.3	73%	75%	78%	80%	82%	87%	86%	89%	91%
91.8	73%	75%	79%	81%	83%	86%	84%	88%	91%

B Aflatoxin B

Hours of exposure/experiment	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%

Figure 5. Mapping cytotoxicity by percentage of viable cells. Table data show cell viability across SciFlow 1000 System rows. Table columns represent corresponding columns (3-11) in SciFlow 1000 System. Table rows are time points over which the experiment was measured. Values were generated from 4X images. **(A)** This table shows the percentage of viable control cells over time. The values are an average of C, D, and E rows. **(B)** This table shows cell viability for Aflatoxin B. The values are an average of F, G, and H rows. Images: blue represents Hoechst nuclear staining of all cells. Red are non-viable cells stained with CellTox Green.

For additional information on the Scikon 1000 System, please contact:

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