Technical Bulletin

SCIKON INNOVATION INC

SciFlow™ System: Multiwell Cascading Fluidics Cell Culture

Complex cell culture systems are emerging as key tools to improve physiological relevance of in vitro assay systems. There has been two main ways in which investigators try to better mimic physiological conditions in cell and tissue culture. The first is to develop more complex model systems where two or more cell types are co-cultured in a 3D structure either separated by membranes or in spheroids[1]. The second is to incorporate fluidic flow where the motion of the media itself has been shown to improve metabolic function and lifespan [2,3]. Despite the successes of better recapitulating function at the cellular level using these two approaches, neither of these approaches addresses the issue of the non-linear nature of the drug or toxicant exposure as is observed in an in vivo system [4]. As a result, the capacity to accurately predict in vivo pharmacokinetics and pharmacodynamics still falls short reaching at best 60-70% accuracy [5,6].

Features and Benefits of the SciFlow™ System

The **SciFlow™** dynamic culture system enables incorporation of physiological fluid flow in tissue culture assay systems for improving the physiological relevance of cell culture assays. The SciFlow plate is manufactured from polystyrene in an SBS standard microplate configuration modified to accommodate capillary channels that connect together 10 wells in each row (A through H). Controlled fluid flow improves the physiological relevance of existing cell-based assays and allows for new cell-based assays to be developed within a connected environmental system.

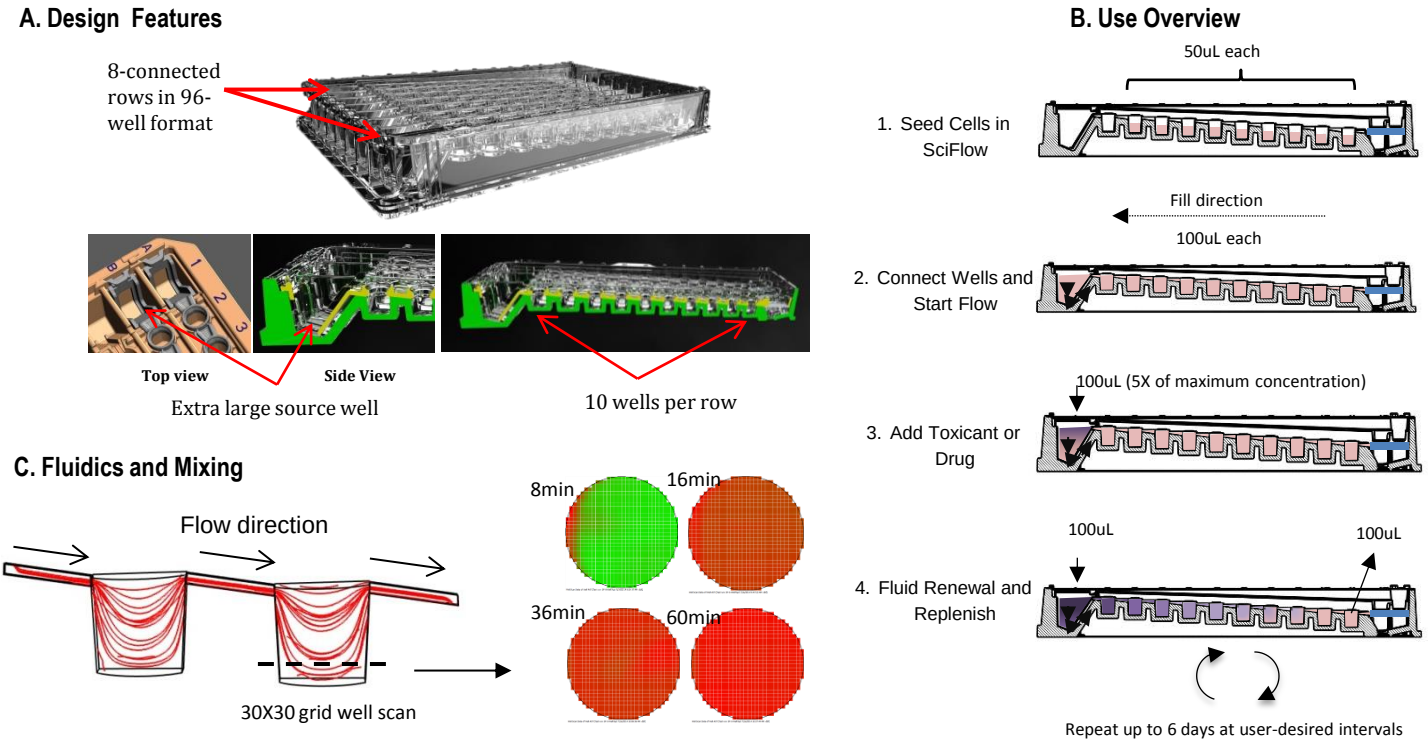
The **SciFlow™** plate allows users to:

- Form reagent gradients over time
- Connect multiple different tissues in series
- Perform constant fluid renewal over multiple day experiments
- Monitor experiments in real-time
- Harvest material during or at the end of experiments through accessible well configurations

Value

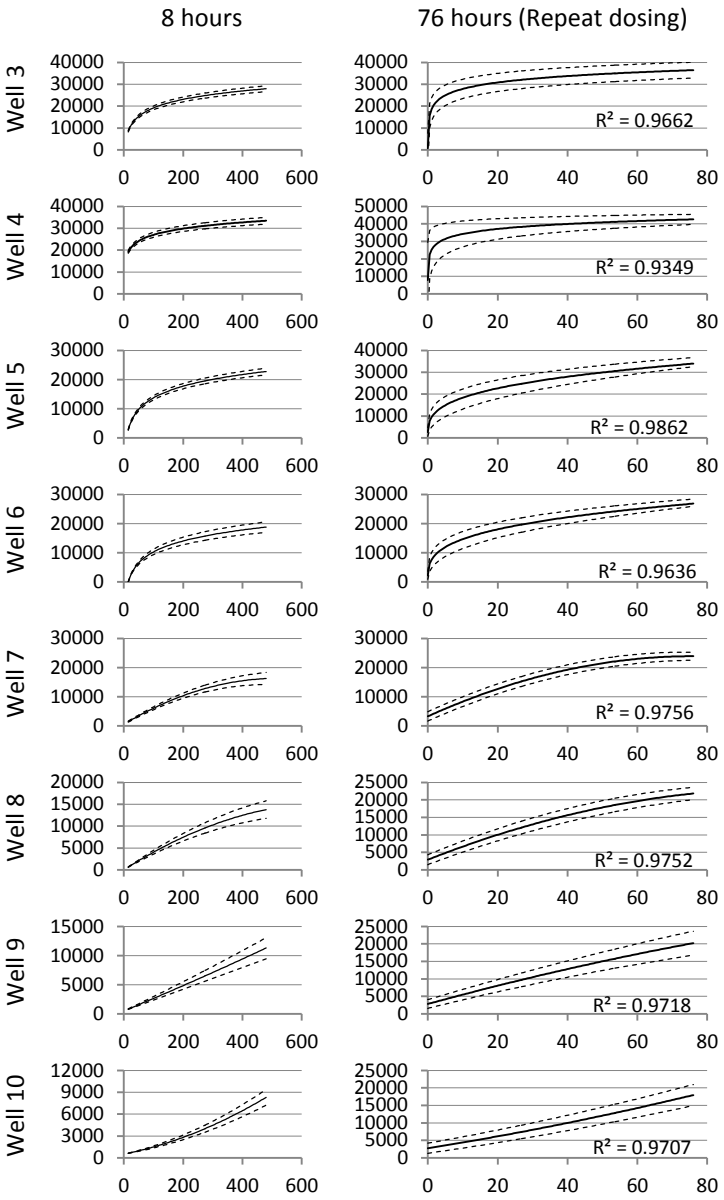
- Enables more life-like drug exposures
- Allows you to monitor experiments in real-time
- Provides you the ability to culture multiple tissues in series
- 100% tissue culture treated polystyrene materials provide better assay performance
- The open well design allows access to samples so that you can add matrices and 3D scaffolds

Figure 1. Overview of the Features and Use of the SciFlow system.



A. The Microtiter plate format was re-designed to feature an extra large source well capable of holding up to 600uL of media. Each subsequent well of each row is connected together by a microchannel running across the top surface of the plate. A row cover serves to create a closed channel system with open wells to allow full access to both the media and cells in the system. A porous wick at well 12 serves as a sink for excess fluid. **B.** Overview of how SciFlow is used to seed cells and tissues, initiate flow, and renew and replenish for long term culturing. **C**. Fluid vector lines showing the direction of fluid moving throughout an individual well. Images acquired using the Wellscan feature of the Clariostar Multimode Reader from BMG LabTech. A 30X30 2D grid was measured for fluorescence intensity at indicated times to measure mixing kinetics. By 60 minutes, the %CV across the grid was less than 2% indicating nearly complete mixing.

Figure 2. Reproducible Concentration Gradients over Time



Left panel. Fluorescein concentrations measured over 470 minutes (approximately 8 hours) following single application of 1uM Fluorescein. Y-axis is presented as Relative Fluorescent Units(RFU) with minutes on the X axis. Solid lines are fitted representations of the data and dotted lines represent 2 standard deviations of the mean (2SD). **Right Panel**. Fluorescein concentrations measured over 76 hours where 1uM Fluorescein was freshly applied to the source well 2X per day. Solid lines are fitted representations of the data and dotted lines represent 2SD. R-squared values of the curve fits are noted.

Time resolved dynamic gradients

For solid tumor treatment, chemotherapeutics form a gradient where the tumor surface is exposed to more drug than the center. This gradient is both a function of diffusion through interstitial fluid as well as cellular metabolism and defense mechanisms of the tumor. This presents a challenge in creating dosing regimens that enable full penetration of the tumor without serious toxic side effects. SciFlow™ enables application of time resolved gradients in vitro. Figure 2 shows the result of application of a 1uM solution of Fluorescien salt to the SciFlow source well. In this experiment, each well of SciFlow was filled with 100uL of complete media and allowed to fill the capillary channels. 1uM Fluorscein salt was then added to the source well. Measurements of the fluorescence at Ex/Em 485/525nm were acquired using a Tecan M1000 Infinite Pro instrument .

Figure 2 left panel shows results from measurements every 30 minutes for approximately 470 minutes (8 hours). To extend the gradient over multiple days, 100uL media was removed from well 11 and 100ul fresh 1uM Fluorescein was applied to the source well. This was performed 2X per day for 3 days (76 hours) (Figure 2 right panel). The ability to form gradients over time from repeat dosing enables modeling 3D tumor biology in a 2D format.

Frequently Asked Questions

1. Are SciFlow wells in the same location as a standard 96 well plate?

-"X" and "Y" well centroids are maintained for wells 2 – 11 according to ANSI standards.

-SciFlow well bottoms diffes in Z-heights with each well offset 0.5mm from the one next to it.

2. What is the surface area of each culture well? The surface area is 16.7 mm^2 or approximately ½ the area of a standard 96-well plate

3. Can I use SciFlow in my platereader? To see whether your plate reader might be compatible, examine the software and hardware specifications for two key features:

-The ability to adjust and optimize z-height focus

-The capacity of the reader to accept 24-well or 6-well plates, or "deep-well" 96-well plates, or plate heights greater than 18mm.

4. Can I do imaging experiments with SciFlow? If you can adjust focal heights either manually or by automation through a 6mm range, then you will be able to image each well of the SciFlow. Narrower focal ranges with capture partial plates

5. Do you make SciFlow with black side-walls for preventing fluorescence cross-talk? SciFlow was designed with thick and impervious sidewalls and a small air-gap between the adjacent well walls. These features render the need for black side walls unnecessary.

References

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6.Yoon, M., et al., Crit Rev Toxicol, 2012. **42**(8): p. 633-52.

For ordering:

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Web Product Page: http://scikoninnovation.com/shop/category/96-well-waterfall-culture-plate/

Product Number	Description
AA-1-50	SciFlow™ 1000 (Pk of 5)

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