CORNING

Integration of an Automated Workstation for Cell-Based Assays Cultured on Synthetic Nanofiber Surfaces For Use With Human Hepatocytes

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Introduction

Automated technologies have become a main stay in today's HTS laboratory. We wanted to test this statement in two different assays and determine if a new three-dimensional synthetic matrix can be used by an established liquid handling system. The human hepatoma derived cell line, HepG2 were seeded on the following 96 well microplates; Ultra-Web™ and Ultra-Web™ polyamine, Tissue Culture Treated (TCT) and BD BioCoat™ collagen I microplates. Two assays were performed, the first examined differences in cell growth and the second compared HepG2 response to troglitazone challenge. Cells were seeded as a titer and assayed using Promega's CellTiter-Glo® Luminescent Viability Assay. All liquid handling was performed using Caliper's Zephyr® liquid handling instrument. HepG2 cells were then seeded in the same microplates as above, and challenged overnight with various doses of troglitazone and cytotoxicity measured using Promega's Multitox-Glo Multiplex Cytotoxicity Assay. Cell seeding, drug dosing and reagent addition were done either manually or by using the Zephyr. Our results demonstrate that first, the Ultra-Web surface can be used by liquid handling automation without impacting the assay and finally complex, multifaceted cell based assays can be automated to yield highly reproducible data.

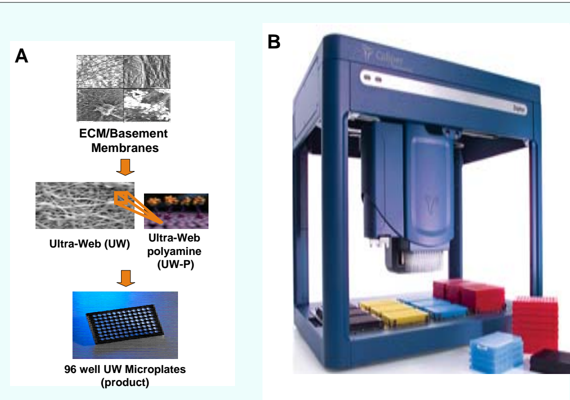
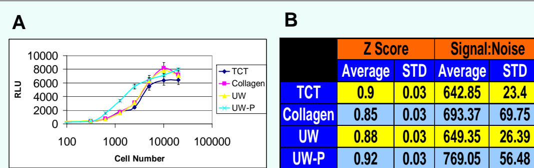


Figure 1: (A) Corning Labware with Ultra-Web nanofiber surface; (B) Caliper's Zephyr® liquid handling instrument.

Materials & Methods

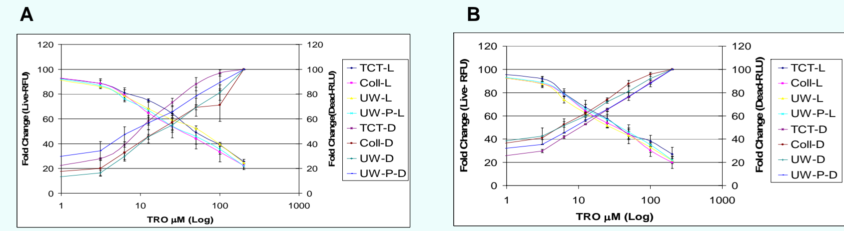
HepG2 Cells: HepG2 cells (ATCC #HB-8065) were cultured in IMDM (MediaTech) and supplemented with 10% fetal bovine serum (Mediatech). Cells were grown in humidity controlled incubators set to 37º and 5% CO_2.
Cell Proliferation Assay: HepG2 cells were seeded at cell densities ranging from 0 to 20,000 cells/well in 96 well black TCT plates (Cat. #3603), 96 well Ultra-Web™ (Cat. #3872xx1) and Ultra-Web™ polyamine (Cat. #3873xx1) and finally BD BioCoat™ 96 well black Collagen 1 coated microplates (BD Bioscience) using the Zephyr. The cells were incubated for 24 hours and cell proliferation assessed using Promega's CellTiter-Glo® Luminescent Cell Viability Assay. Briefly, the CellTiter Glo reagent was added to the cells using the Zephyr and incubated for 10 minutes. The plates were then read using a Perkin Elmer ViewLux™ CCD microplate Imager.
Cytotoxicity Assay: To compare and contrast HepG2 cell response to troglitazone challenge when grown on various surfaces cells were seeded at a density of 20,000 cells/well in the same microplates as previously described. After 18-24 hours of incubation varying concentrations of troglitazone (Sigma Chemicals), diluted in DMSO (Sigma) were added to appropriate wells and incubated for 20 hours as previously described. Cytotoxicity was measured using the MultiTox-Glo Multiplex Cytotoxicity Assay (Promega). Following the manufacturer's protocol, 50µl of live reagent was added to each well and the plates incubated for 30 minutes at 37°C. The microplates were read on the ViewLux using appropriate fluorescence filters. Fifty microliters of the dead reagent were then added to the same wells and the microplates incubated for 10 minutes. The microplates were then read for luminescence on the ViewLux. Cells, drugs and kit reagents were added either manually or using the Zephyr.

Results



	Z Score		Signal:Noise	
	Average	STD	Average	STD
TCT	0.9	0.03	642.85	23.4
Collagen	0.85	0.03	693.37	69.75
UW	0.88	0.03	649.35	26.39
UW-P	0.92	0.03	769.05	56.48

Figure 2: Comparison of HepG2 Cell Growth On Four Different Growth Surfaces Using The Zephyr Liquid Handler and Promega's CellTiter-Glo Assay. (A) HepG2 cell titer curves comparing the four microplate types. (B) Assay statistics. Data shown are the average ± S.D from one representative experiment.



C

		Live Assay		Dead Assay	
		Average	STD	Average	STD
TCT	Manual	0.62	0.06	0.45	0.19
	Zephyr	0.84	0.01	0.84	0.05
Collagen	Manual	0.71	0.14	0.74	0.03
	Zephyr	0.74	0.045	0.83	0.1
UW	Manual	0.8	0.06	0.66	0.05
	Zephyr	0.91	0.04	0.95	0.02
UW-P	Manual	0.689	0.09	0.75	0.07
	Zephyr	0.85	0.04	0.79	0.07

Figure 3: Compare and Contrast Cytotoxic Effects of Troglitazone (TRO) On HepG2 Cells Grown On Four Different Growth Surfaces Using Promega's MultiTox-Glo Multiplex Cytotoxicity Assay. Cell seeding and all liquid handling were done manually (A) or by using the Zephyr (B). The cytotoxic effects of Troglitazone on HepG2 viability were assessed by measuring the amount of viable (L) and dead (D) cells in the same well. Figure C shows the Z Scores for the live portion (left) and dead portion (right) of the assay. Data shown are the average ± S.D from one representative experiment.

Summary & Conclusions

- **Complex cell based assays can be fully automated with Caliper's Zephyr liquid handling instrument allowing for lower plate to plate variation and improved assay performance as compared to manual handling.**
- **Ultra-Web provides a synthetic nanofibrillar surface for cell culture and cell based assays. The Ultra-Web product can be used with liquid handling instrumentation without any special handling.**
- **Ultra-Web and Ultra-Web polyamine nanofiber surfaces perform as well as collagen and out performs TCT in multiple assay formats.**
- **We are interested in your thoughts and comments. Please contact Mark Rothenberg at rothenbem@corning.com**

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