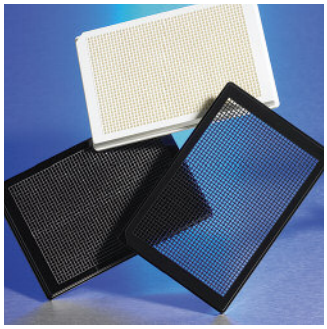
Comparative Cell-Based Analysis of Various 1536 Well Microplate Surfaces



SnAPPShots

A brief technical report from the Corning Applications Group

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Introduction

As appreciation and understanding of the importance of microplates in assay development and high throughput screens grow, so does our need for further characterization of the microplate itself. This article reviews the work presented at the 2009 Society for Biomolecular Sciences Conference comparing and contrasting various 1536 well microplate surfaces, with emphasis on the new Corning® 1536 Well Low Base Black Clear Bottom Microplate, designed for compatibility with the MDS Analytic technologies FLIPR^{Tetra} and a number of High Content Screening Instruments. The 1536 well microplate offers scientists improved throughput as well as cost savings. The 1536 well microplate density presents several unique challenges, including cell culture volumes, evaporation and the microenvironment in which cells are expected to function. We examined two different cell lines on various 1536 well microplate surfaces and tested for adherence, growth and physiologic response. Cell adherence and proliferation were tested using Promega's CellTiter Glo® Luminescent Cell Viability Assay Kit, which measures the amount of adenosine-5'-triphosphate (ATP) present in viable cells. Physiologic response was assessed using Promega's CytoTox-ONE™ Homogeneous Membrane Integrity Assay Kit. This kit measures the relative number of live/dead cells in a given population. By understanding and optimizing how cells perform in a 1536 well microplate, one can benefit from the advantages of this format, while taking advantage of cost savings associated with 1536 well microplate miniaturization.

Methods and Results

To compare and contrast the flatness (Figure 1) of the new Corning 1536 Well Low Base Black Clear Bottom Tissue Culture Treated (TCT) Microplate (Cat. No. 3893) to an equivalent competitor microplate, consistency of signal was tested. Five microliters of Promega's CellTiter-Glo Luminescent Cell Viability Assay along with 50 pM ATP (Sigma) were dispensed in multiple microplates using the Thermo Fisher Multidrop Combi Liquid Dispenser. The plates were read on a PerkinElmer® ViewLux™ ultraHTS Microplate Imager. Data presented is an average of five microplates of each type, and shows that both microplates have equivalent flatness, with the peaks at the right side of both images being attributed to an artifact of liquid handling.

To assess cell attachment and imaging capabilities of the new 1536 well low base microplates (Figure 2), as well as compare the different surfaces, HeLa cells (ATCC CCL-2) were cultured and transfected in the Corning HYPER*Flask*® Cell Culture Vessel using $CaPO_4$. Transfections were done using 0.25 µg/cm^2 pWIZGFP-1 (GENEWIZ®) plasmid DNA. After 48 hours at 37°C with 5% CO_2 post-transfection, the cells were harvested and seeded at a density of 5,000 cells/well in Corning 1536 Well Low Base Black Clear Bottom Microplates treated with Corning CellBIND® Surface (Cat. No. 3832), TCT Microplates (Cat. No. 3893), and Competitor 1536 well TCT and poly-D-lysine (PDL)-coated microplates (in triplicate). The cells were fixed with 4% paraformaldehyde containing 0.1 µg/mL of Hoechst nuclear

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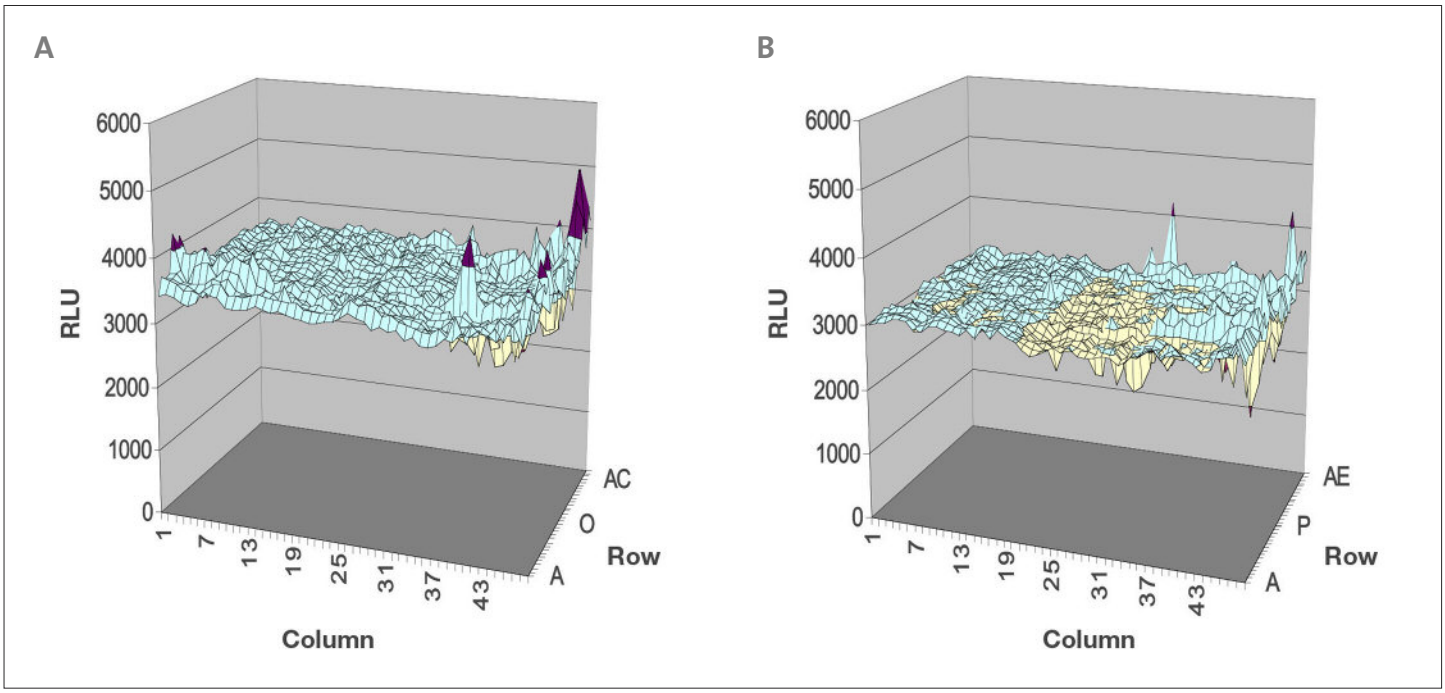


Figure 1. Surface maps of luminescent signal across Corning® 1536 Well Low Base TCT Microplate (A) and Competitor low base TCT microplate (B). Comparable plate flatness and CVs for both A (%CV = 6.4) and B (%CV = 7.2) were observed. The apparent right edge variation in signal is an artifact of the liquid handling instrumentation. Data represent n = 5 microplates.

stain (Invitrogen Cat. No. 34580) and examined under 10x magnification using the Thermo Fisher Cellomics ArrayScan HCS reader. All liquid handling was done using the Thermo Fisher Multidrop Combi Liquid Dispenser. Data collected and presented in Figure 2A and 2B show that the Corning TCT 1536 Well Microplates performed as well as the competitor TCT brand and Corning CellBIND® Surface (Figure 2C) performed comparably to the competitor's PDL microplate (Figure 2D).

To examine microplate performance in both a luminescence and fluorescence based assay, 5/9 M 13/8 Alpha (ATCC CRL-10154™) and MDCKII/MDR1 (Dr. Piet Borst Netherlands Cancer Institute) cells were seeded at a density of 3,000 cells/well the evening prior to performing the assays into Corning (Corning CellBIND Surface and TCT) and competitor microplates. Micrographs were taken using an Olympus inverted microscope with a 10x objective. To compare overall cell growth on the different 1536 well surfaces, 5 µL of Promega's CellTiter-Glo (G7571) reagent were added to the appropriate wells +/- cells and incubated in the dark for 10 minutes at room temperature. The experiment was done in triplicate. To measure cell function on the different microplate surfaces, a live/dead assay was performed using the CytoTox-ONE Assay Kit from Promega (G7891). One microliter of 0.1% Triton X-100 (Sigma) was added to wells +/- cells and incubated for 10 minutes at room temperature, after which 4 µL of the CytoTox One Reagent were added and incubated for another 10 minutes under the same conditions in the dark. Both assays were read using the Perkin Elmer® ViewLux™ ultraHTS Microplate Imager. Data presented in Figure 3A demonstrates

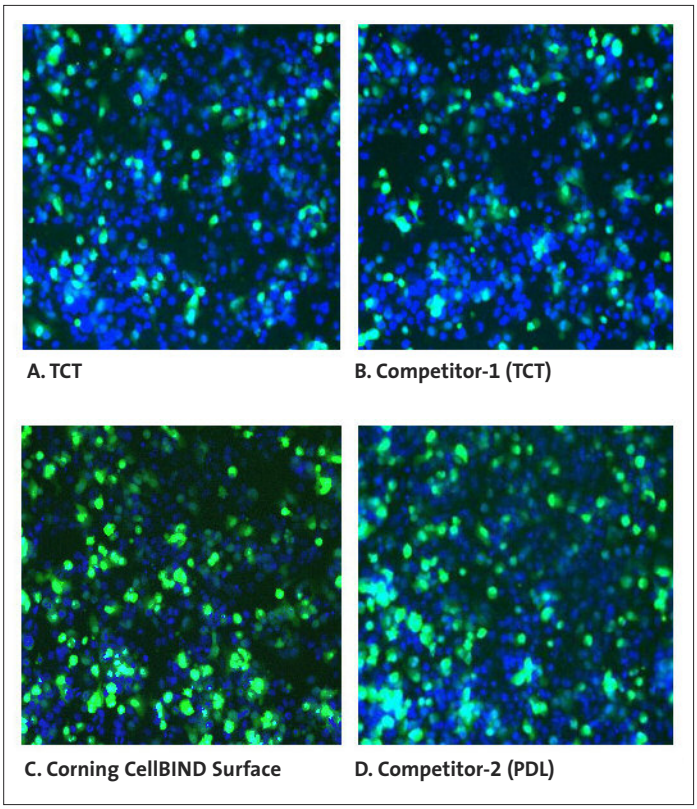
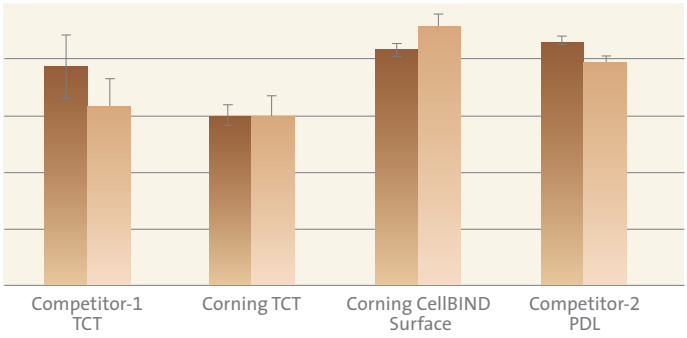


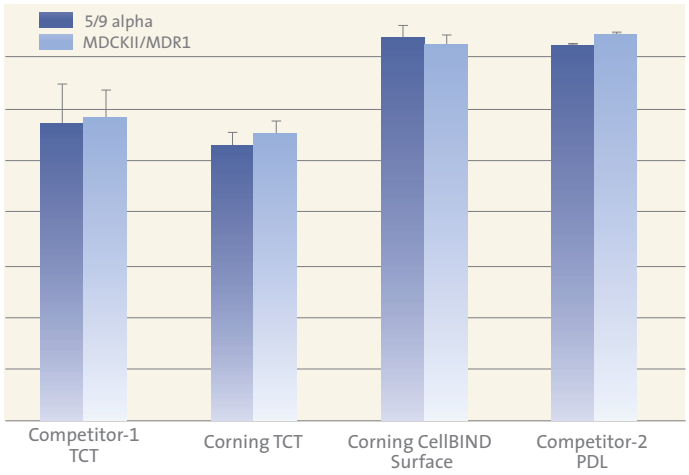
Figure 2. pWIZGFP-transfected HeLa cells were seeding at a density of 5,000 cells/well into: (A) Corning 1536 Low Base TCT Microplate, (B) Competitor-1 low base TCT microplate, (C) Corning CellBIND Surface 1536 Well Low Base Black Clear Microplate, and (D) Competitor-2 1536 PDL microplates and stained with Hoechst nuclear stain. 10x images were captured on the ArrayScan and Hoechst nuclear stained cells (blue) were superimposed with GFP fluorescing cells (green).

A



	S:N	Max – Min
Corning TCT	113	2995
Competitor-1 (TCT)	128	3800
Corning® CellBIND® Surface	181	4547
Competitor-2 (PDL)	140	4280

B



	S:N	Max – Min
Corning TCT	25	2648
Competitor-1 (TCT)	25	2793
Corning CellBIND Surface	26	3477
Competitor-2 (PDL)	30	3586

C

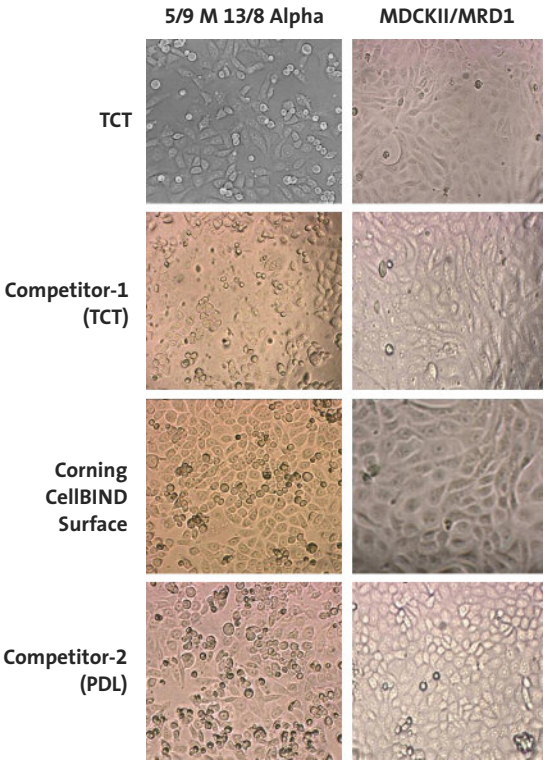


Figure 3. 5/9 M 13/8 Alpha and MDCKII/MDR1 cells were seeded at 3,000 cells/well and analyzed using Promega's CellTiter Glo Luminescent Kit (A) and Promega's CytoTox One fluorescent assay after addition of 0.1% Triton X-100 (B). Data represented an n = 3 microplates. Figure 3C shows 10x micrographs of both cell lines on the different microplate surfaces.

that Corning® TCT and Corning CellBIND® surface treatments show comparable cell concentrations, signal:noise and assay window (maximum signal – minimum signal) as the equivalent competitor microplates. Similarly Figure 3B, using a fluorescent-based assay, measured overall cell response to a cellular lytic agent. Again, as shown in Figure 3A, both the 1536 well TCT and Corning CellBIND surface treated microplates showed equivalent results to their respective competitors with respect to signal:noise and assay window.

Summary

- Corning 1536 well low base microplates are comparable to competitor plates in cell attachment and overall flatness.
- Corning 1536 well low base black clear bottom microplates treated with Corning CellBIND Surface gave equivalent responses in both cellular assays compared to the competitive poly-D-lysine coated plates.
- When comparing signal:noise and assay window (maximum signal – minimum signal) in two different assay formats, the Corning low base TCT microplates were equivalent to the competitor TCT microplates examined.
- Moreover, microplates treated with Corning CellBIND surface were equivalent to the competitor PDL microplates in all matrices studied. The benefits of using Corning microplates treated with Corning CellBIND Surface include the use of a non-biological treatment, no special storage conditions, no lot-to-lot variation and a noticeable cost savings.

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