Corning® BioCoat™ High Content Imaging Glass Bottom Microplates Provide Enhanced Performance for Cell-based Assays

Application Note

CORNING

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Introduction

Historically, glass bottom microplates have been chosen for high content cell-based assays because of their high optical quality, flatness, and scratch resistance. Conversely, the natural hydrophobicity of glass may cause adherence issues with some cell lines. In those cases, cell lines may benefit greatly from a protein coating to aid in cell attachment, spreading, and/or function. To meet this need, Corning has developed a series of ready-to-use, glass bottom high content imaging microplates that are pre-coated with collagen, fibronectin, or poly-D-lysine.

This study evaluates the benefits of using Corning BioCoat high content imaging glass bottom microplates by assessing cell attachment and retention compared to uncoated glass bottom high content microplates. Additionally, we demonstrate the advantages of these plates by performing a hepatotoxicity assay, which is a commonly used assay in the research phase of drug discovery.

Materials and Methods

Cell Attachment and Spreading

HepG2 cells (ATCC® Cat. No. HB-8065) were seeded into two different 384 well Corning high content imaging glass bottom microplates: BioCoat Collagen I (Cat. No. 4583) and uncoated (Cat. No. 4581) at either 80,000 cells/cm^2, 40,000 cells/cm^2, or 20,000 cells/cm^2 in 40 µL of Corning DMEM (Cat. No. 10-013-CM) supplemented with 5% Corning FBS (Cat. No. 35-010-CV). After an overnight incubation at 37°C and 5% CO_2, the medium was aspirated and the cells were fixed with 4% paraformaldehyde (Boston BioProducts Cat. No. BM-155-250ML). Nuclei were then stained with Hoechst 34580 at 1 µg/mL (Life Technologies® Cat. No. H21486). After a final wash to remove unbound stain, nuclei were enumerated using the Thermo Scientific™ CellInsight™ Personal Cell Imager. This study was performed two independent times.

Cell Retention

HEK-293 cells (ATCC Cat. No. CRL-1573) were seeded into four different 384 well Corning high content imaging glass bottom microplates: uncoated glass, Corning BioCoat poly-D-lysine (PDL) (Cat. No. 4587), Fibronectin (Cat. No. 4585), and Collagen I. The microplates were seeded at 80,000 cells/cm^2 in 40 µL DMEM supplemented with 5% FBS. After overnight incubation at 37°C and 5% CO_2, plates were washed twice with Corning HBSS (Cat. No. 21-023-CM) using Molecular Devices DW4 AquaMax® Plate Washer. After washing, the cells were fixed with 4% paraformaldehyde for 15 minutes at 4°C, washed again, and then stained as described previously. Nuclei were analyzed on the CellInsight Personal Cell Imager. This study was performed on two independent occasions.

Hepatotoxicity Assay

HepG2 cells were seeded into the 384 well Corning BioCoat PDL, Fibronectin, and Collagen I high content imaging microplates at 25,000 cells/cm^2 in Corning Minimum Essential medium (Cat. No. 10-010-CV) supplemented with 1X non-essential amino acids (Cat. No. 25-025-CI), 1 mM sodium pyruvate (Cat. No. 25-000-CI) and 10% FBS. After overnight incubation, the medium was changed to contain various concentrations of valinomycin (MP Biomedicals Cat. No. 105010) and tacrine (Cayman Chemical Cat. No. 70240). The cells were exposed to the compounds for 24 hours and then stained using Cellomics® Multiparameter Cytotoxicity 2 kit (Thermo Scientific Cat. No. 8400002) following the manufacturer's protocol. Cells were analyzed for (1) changes in mitochondrial membrane potential as indicated by a loss in fluorescence intensity due to toxic exposure and (2) an increase in lysosomal mass/pH from increasing toxicity. Changes in fluorescence intensity were quantified using the CellInsight Personal Cell imager.

Results and Discussion

Cell Attachment and Spreading

Despite the excellent optical properties and flatness of glass bottom microplates, their hydrophobicity can adversely impact cell attachment. This can be problematic with some cell-based assays. A protein coating can remedy this difficulty by aiding in cell attachment and spreading. By utilizing HepG2 cells, a cell line commonly used for hepatotoxicity assays, we have shown that Corning BioCoat Collagen I high content imaging microplates can improve cell attachment, and therefore, the accuracy of an assay. Improved attachment and statistically higher cell counts were achieved on the Collagen I high content imaging microplates compared to uncoated microplates at 3 different seeding concentrations ($p<0.001$) (Figure 1). Additionally, the analysis of nuclei was more accurate when examining samples on the BioCoat Collagen I microplates (Figure 2). HepG2 cells cultured on uncoated glass microplates exhibited large clusters and were not evenly distributed compared to those on BioCoat Collagen I high content imaging microplates.

Cell Retention

Many cell-based assays require multiple wash steps for high content imaging applications (e.g., hepatotoxicity assays). Cell loss during liquid handling steps can be highly problematic resulting in instrument focusing errors, increased scan times, reduced cells for analysis, and higher coefficients of variance. Utilization of Corning® BioCoat™ Collagen I and Fibronectin high content imaging microplates, resulted in statistically higher HEK-293 cell retention after washing when compared to traditional uncoated glass high content microplates ($p<0.001$) (Figure 3). In addition to higher cell retention, the coefficient of variance was decreased when cells were cultured on BioCoat Fibronectin and BioCoat PDL high content imaging microplates (Figure 4).

Hepatotoxicity Assay

An important component in the research stage of the drug discovery process is the analysis of potential hepatotoxicity. The early identification of hepatotoxic compounds helps to reduce the rate of drug candidate failures.

In this assay, HepG2 cells were cultured on Corning BioCoat high content imaging microplates and analyzed for changes in mitochondrial potential (mmp) and lysosomal mass, which are two early indicators of hepatotoxicity. Valinomycin, a potent antibiotic, was added to the cells to depolarize the mitochondrial membrane, resulting in cellular toxicity[1-2]. As depicted in Figure 5, cells exposed to valinomycin demonstrate a dose-

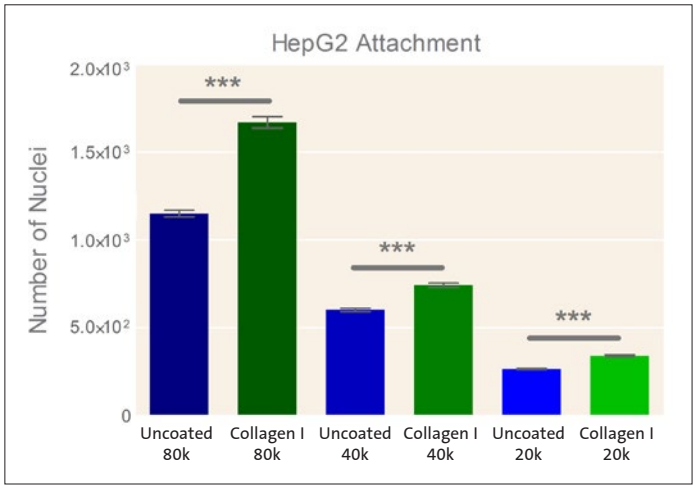

Figure 1. HepG2 cells exhibit improved attachment on Corning BioCoat Collagen I high content imaging glass bottom microplates, as compared to uncoated glass microplates at all three seeding concentrations. Data is shown with standard errors. One-way ANOVA with Newman-Keuls post test ***$p<0.001$. n = 128 from 2 independent studies. 16 fields per well analyzed.

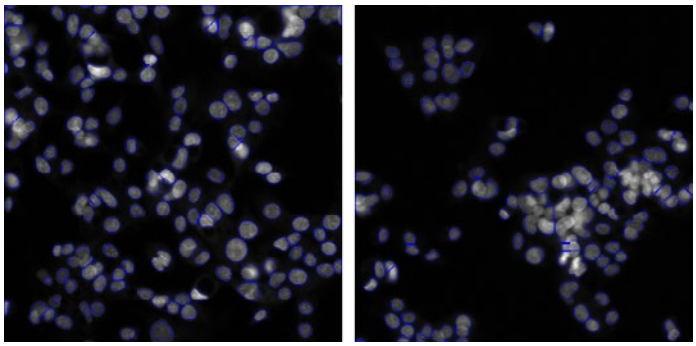

Figure 2. Representative photomicrographs of HepG2 cells on uncoated glass microplates (right) and Corning BioCoat Collagen I (left) high content imaging glass bottom microplates at 80,000 cells/cm^2. HepG2 cells on Collagen I exhibit more even distribution and less clumping compared to uncoated glass microplates. Improved cell distribution on Collagen I enables a more accurate analysis of cell nuclei using high content imaging (note the distribution of nuclei outlined in blue).

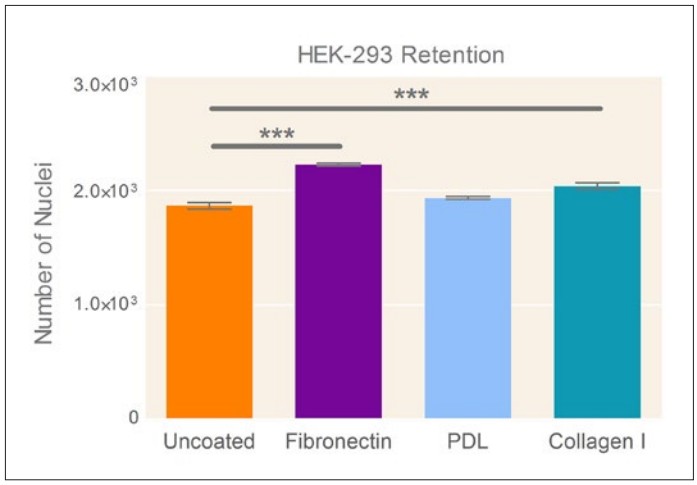

Figure 3. HEK-293 cells exhibit improved retention on Corning BioCoat Fibronectin and Collagen I high content imaging glass bottom microplates compared to uncoated glass microplates. Data shown with standard errors. One-way ANOVA with Newman-Keuls post-test ***$p<0.001$. n = 768 from 2 independent studies. 16 fields per well analyzed.

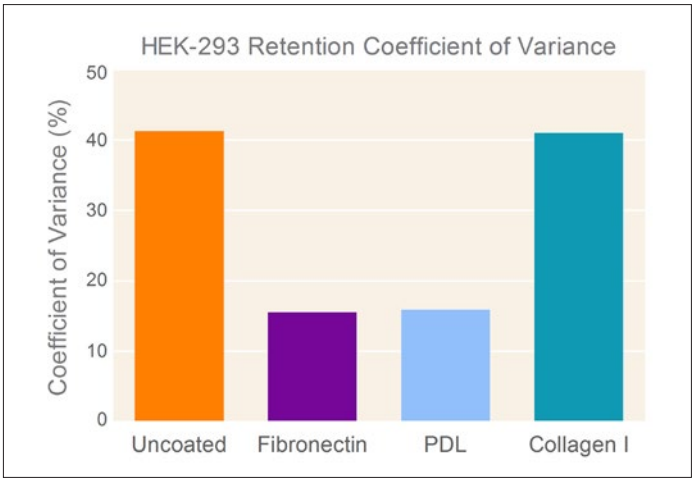

Figure 4. HEK-293 cells cultured on Corning BioCoat PDL and Fibronectin high content imaging glass bottom microplates exhibit lower coefficient of variance (CV) values compared to uncoated glass microplates.

dependent decrease in mmp staining as indicated by a decrease in the intensity of the mitochondrial stain. This decrease in mmp staining as the valinomycin concentration increases is an indication of cytotoxicity. Representative photomicrographs of mmp stained HepG2 cells at 0 µM (left image) and 12.5 µM (right image) concentrations of valinomycin are shown in Figure 6. A second hepatotoxic compound, tacrine, was used in the treatment of Alzheimer's disease prior to discontinuation in the U.S.[3] Tacrine was added to HepG2 cells to induce changes in lysosomal mass, which is an early indicator of cell toxicity[4]. As depicted in Figure 7, cells exposed to tacrine (100 µM) demonstrated an increase in the number of lysosomes, indicating hepatotoxicity. High content analysis confirmed a dose-dependent increase in lysosomal mass (Figure 8) with increasing concentrations of tacrine.

Conclusions

- Corning® BioCoat™ high content imaging glass bottom microplates support improved cell attachment and retention when compared to uncoated glass microplates.
- Cells cultured on Corning BioCoat microplates exhibit improved spreading and even distribution resulting in improved data quality.
- Corning BioCoat high content imaging glass bottom microplates are a useful tool for multistep cell-based assays such as a multiparameter toxicity assay.

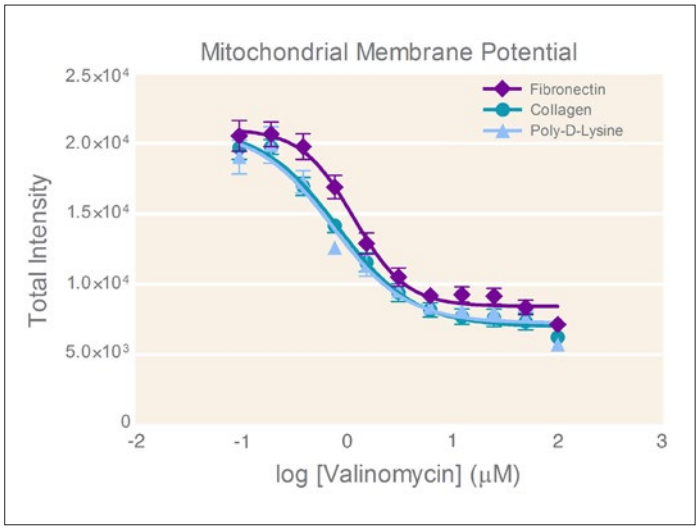

Figure 5. HepG2 cells exhibit reduced mitochondrial membrane potential when exposed to increasing concentrations of valinomycin. n = 12 from 2 independent studies. 16 fields per well analyzed.

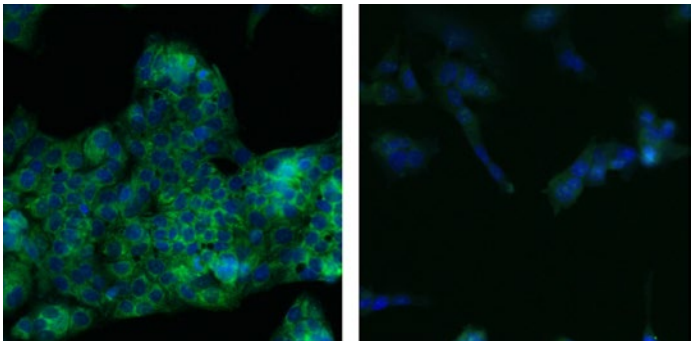

Figure 6. Representative photomicrographs of HepG2 cells exposed to 0 µM valinomycin (left) and 12.5 µM valinomycin (right). Green staining represents mitochondrial membrane potential and blue staining indicates nuclei. Images taken using 20X objective.

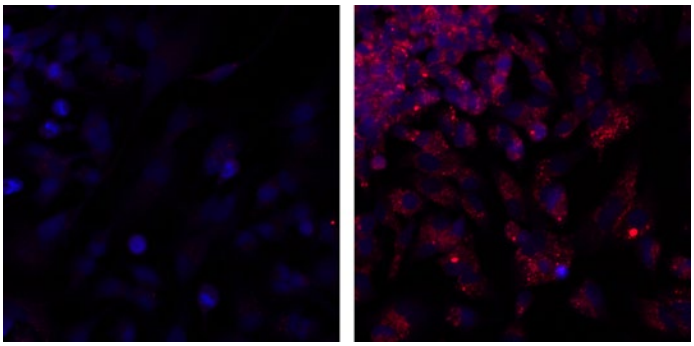

Figure 7. Representative photomicrographs of HepG2 cells exposed to 0 µM tacrine (left) and 100 µM tacrine (right). Cells were stained with a marker to identify lysosomes, red, and the nuclei were stained with Hoechst, blue. Images taken using 20X objective.

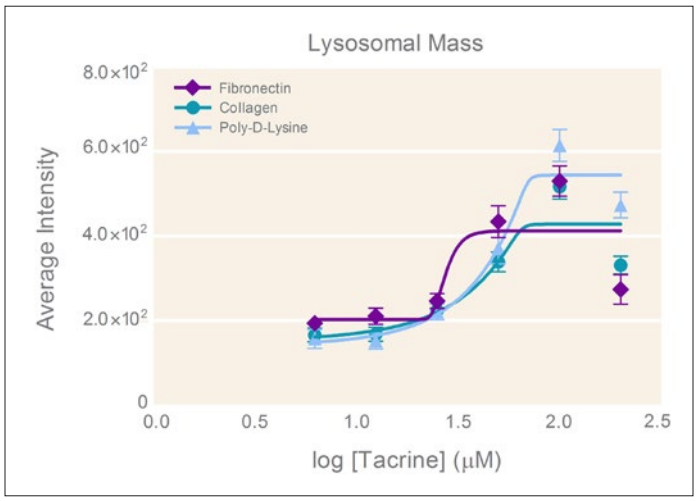

Figure 8. HepG2 cells exhibit increased lysosomal mass when exposed to increasing concentrations of tacrine. n = 12 from 2 independent studies. 16 fields per well analyzed.

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