Controlled Conditions Reduce Critical Edge Effect in 96-Well Plates

Abstract #1438

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Abstract

The Edge Effect causes a substantial loss of useable assay space in pharmaceutical drug discovery assays conducted with 96-well culture plates. While mechanisms for reducing evaporation in edge wells have been employed to reduce variability in cell density, we have preliminary data suggesting that differences in temperature in edge wells while cells are settling may be a major contributing factor. Here we extend those results with careful temperature studies during and after cell plating processes with the premise that maintaining the temperature of all parts of the cell environment including plates, reservoirs, and tips, as well as the liquids at a constant 37°C during cell plating will reduce edge effects. Our null hypothesis is that the Edge Effect cannot be reduced by comprehensive temperature control. We used the Xvivo System to control the environment, including the cell processing chamber floor, to a constant 37°C during cell plating and cell settling, comparing results there to those obtained plating in a room temperature laminar flow hood. In both settings, we used a FLIR ONE thermal imaging camera to record movies of temperature changes in 96-well plates as well as the pipetting reservoir during and after routine cell plating. We used the HoloMonitor M4 microscope to record time lapse images of cells settling in the wells. We also used standard cell density assays to assess variability in plated adherent A549 human lung carcinoma cells. We found that plating cells under controlled, constant temperature conditions did eliminate swings in well temperatures during cell plating and cell settling that were produced by plating cells in uncontrolled room air conditions. Constant conditions also reduced variability in edge well cell density, disproving the null hypothesis. We concluded that constant conditions for cell plating and cell settling could reduce Edge Effect and, by allowing usage of all wells, have a tremendous impact upon the time and resources devoted to all cell-based toxicity assays.

Background

- ***In Vitro* Toxicological Assays in 96-well plates are limited by variability in the edge wells the so-called "edge effect"**
- **Often the edge wells are eliminated from the assay, filled with buffer, causing a 37% reduction in usable assay wells and wasting materials and money**
- **Approaches to avoid edge effect include moat plates and leaving plated cells at room temperature for extended periods of time.[1]**
- **We previously reported that plating cells at constant 37°C can reduce edge effect.[2]**

Objectives

- **Record temperature changes during cell plating via FLIR One thermal imager**
- **Monitor settling patterns of cells in wells via HoloMonitor M4 microscope**
- **Compare variability caused by edge effect to cell density assays of plates filled at constant physiologic conditions**

References

1. Denmark J, Chessum B: Standardization of enzyme-linked immunosorbent assay (ELISA) and the detection of Toxoplasma antibody. Medical laboratory sciences 1978, 35(3):227-232.
2. Lundholt BK, Scudder KM, Pagliaro L: A simple technique for reducing edge effect in cell-based assays. Journal of biomolecular screening 2003, 8(5):566-570.
3. Henn A, Darou S, Yerden R: Reducing plate edge effect by controlling cell handling conditions for in vitro tumor hypoxia assays. In.: AACR; 2018

Conflict of Interest Disclosure

The authors all are employees of BioSpherix, Ltd.

Experimental Design

Figure 1. Experimental Set-Up. Human lung A549 cells (ATCC, USA) or medium (37°C) were plated in standard 96-well culture plates (CellTreat, USA) in a completely enclosed and controlled environment using the Xvivo System (BioSpherix). **(A)** The chamber air and floor could be heated in the Xvivo System to a constant 37°C or left at ambient RT conditions. The plates were filled with warmed medium (37°C) at either traditional Room Temperature (RT) BSC conditions or with all materials at constant 37°C including reservoirs and pipette tips. **(B)** All materials were brought to the appropriate temp before plating. Thermal conditions were recorded with the FLIR ONE thermal imaging camera. **(C)** Cells were imaged during settling with the HoloMonitor M4 (PHI AB, Sweden). Plates were stained with crystal violet after incubation overnight. All image analysis was done with the HoloMonitor Hstudio software (PHI).

Results

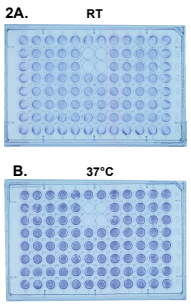

Figure 2. Edge Effect. **(A)** Cells plated at traditional BSC conditions (RT) with warmed medium (37°C) showed a higher cell density at the outer edges of edge wells. **(B)** Cells plated at constant 37°C conditions with all materials at 37°C produced a more even cell distribution.

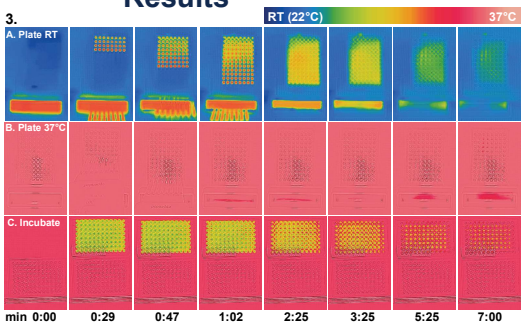

Figure 3. Cells in plates filled at RT before incubation at 37°C experience sub-optimal conditions, warming from the edges of the plate to the center. **(A)** Warmed medium (37°C) cooled immediately when plated using RT chamber and materials (reservoir, tips, plate). **(B)** When all materials were maintained at 37°C before and during plating using the Xvivo System, well temperature more closely matched incubation temp (37°C). **(C)** One plate filled at RT (top) and one plate filled at 37°C (bottom) were placed at 37°C side-by-side. The plate filled at 37°C was at even incubator temperatures while the plate filled at RT warmed slowly from the edges to the center. This is typically the time that cells are settling in the wells.

Results (cont.)

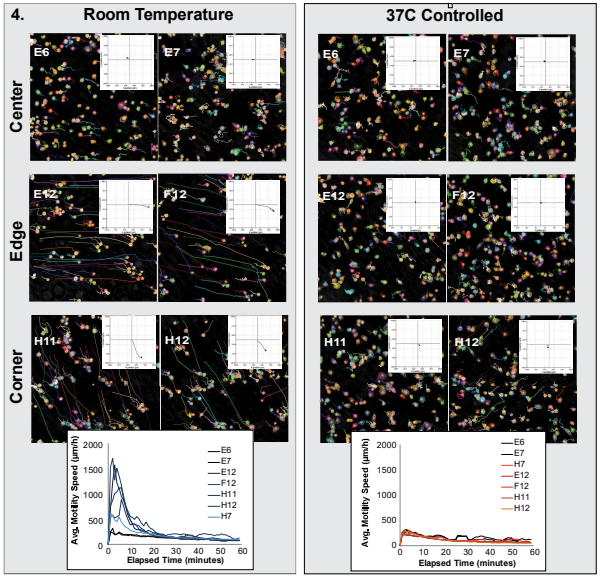

Figure 4. Random cell settling is disrupted in suboptimal RT BSC temperature. A549 cells were plated at RT or 37°C were immediately moved to a 37°C chamber and placed on the HoloMonitor M4 holographic microscope and imaged every 60 seconds for up to 80 minutes. Cells in wells warming to 37°C after plating at RT move directionally toward the wall of the well closest to the edge of the plate. In Hstudio cell tracing analysis showed this non-random directionality. (Insets) Composite migration patterns for each well clearly show that for both plates filled at RT and at 37°C, cell settling in the plate center was largely random, with the cell motions averaging to a vector near the origin. Cells settling in edge wells of plates filled at RT had composite motions toward the warming surfaces of the well wall. (Graphs at Bottom) Plating cells at 37°C Reduces variability in cell motion (average cell motility) as compared with traditional room temp BSC conditions.

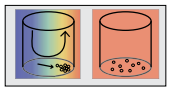

Figure 5. Schematic of thermal model of disruption of cell settling when cells are plated at RT. The findings of our study and others are not inconsistent with a thermal model of cell settling disruption. In this model the difference between the temperatures at the edge and the center of the plate drive thermal currents in each well with different strengths depending upon thermal gradient and well position. Cells in the process of settling during plate temperature changes are rolled toward the outside edges of the wells as they settle. This results in non-random cell settling patterns with cells accumulated at the edge of each well that is warmer immediately after plating. Plating cells at constant temperature eliminates these currents and reduces edge effect.

Conclusions

Cells experience suboptimal thermal conditions during cell plating in a BSC at RT.

Cell motion in edge wells of plates filled at RT is directional, causing uneven settling.

Under controlled thermal conditions, cells settled more randomly, reducing edge effect.