Controlled Temp for Cell Plating Eliminates Room Temp Cell Settling in 96-well plates, Reducing Edge Effect and Improving Cell Conditions

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ABSTRACT

The "edge effect" has always limited the use of edge wells of widely-used 96-well plates, reduced useable assay space by 37%, increased materials, time, and costs for these assays. Methods reported to reduce edge effect include leaving the plates at room temperature (RT) for extended cell settling times (30-120 min). We previously reported that controlling conditions to 37° C during cell plating prevents thermal currents and non-random distribution of cells seen during cell settling in edge wells. Here we report studies with human A549 lung carcinoma cells, used for in vitro viral assays. Our null hypothesis is that there would be no difference in cell density or viability between cells plated/ settled at constant 37° C and extended cell settling times at RT conditions. We plated A549 under controlled conditions in modular cell handling and incubation chambers in the Xvivo System. We pre-incubated plates for 30, 60, 90, and 120 min at RT and compared these with cells handled at constant 37 degrees. We used the HoloMonitor M4 (PHI, Inc) to monitor cell settling and assessed cell density at 24 and 48 hrs later using crystal violet. We found that cell settling at RT resulted in delayed cell attachment, delayed time to first division, and different cell densities as compared with cell handling at 37° C. Cell agitation and thermal variation was still seen between center and edge wells during cell settling with medium warmed to 37 degrees before traditional cell plating at RT. We concluded that full-time control of 37° C during cell plating is necessary to prevent cell density and cell viability edge effect that occurs during routine handling of cells under RT conditions.

BACKGROUND

- Edge Effect has been seen in 96-well plates since 1978[1]
- Another group reported that leaving plates at RT for extended times (30, 60, 90, 120 minutes) before incubation could reduce edge effect[2]
- We previously reported that:
  - Cells roll toward the edges of the plate during plate warming, with more rolling in edge/corner than center wells
  - Inverting temperature changes reversed the roll direction
  - Plating cells with all materials at 37°C reduced rolling and evened cell distribution

A Thermal Model of Edge Effect

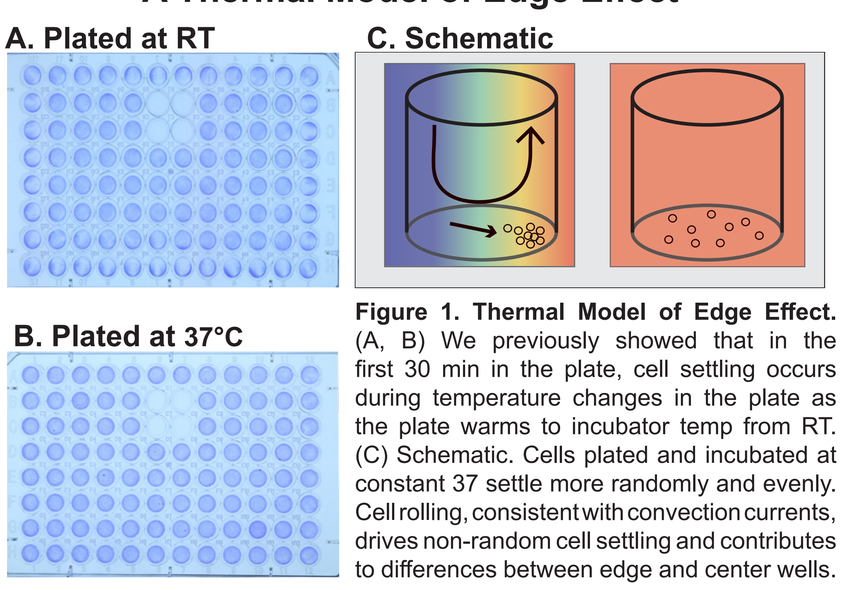

Figure 1. Thermal Model of Edge Effect. (A, B) We previously showed that in the first 30 min in the plate, cell settling occurs during temperature changes in the plate as the plate warms to incubator temp from RT. (C) Schematic. Cells plated and incubated at constant 37 settle more randomly and evenly. Cell rolling, consistent with convection currents, drives non-random cell settling and contributes to differences between edge and center wells.

Here, we examine the same conditions as the previous group to look at effects on cells left at RT for extended periods of time.

EXPERIMENTAL DESIGN

Figure 2. Experimental Design. All processes were performed in the Xvivo System. This system provides a completely controlled atmosphere from filtered, tanked gases. The atmosphere was continuously HEPA filtered and the temperature of the floor and air of each chamber can be controlled to constant 37°C. The black doors cover cell incubation chambers. Materials are disinfected in the laminar flow hood to the far left and brought into the system through buffer chamber air locks. Any entering room air was replaced with tanked gases before materials were imported. Probable risk surfaces inside the Xvivo and all imported materials were surface disinfected with SporKlenz RTU (Steris).

RESULTS

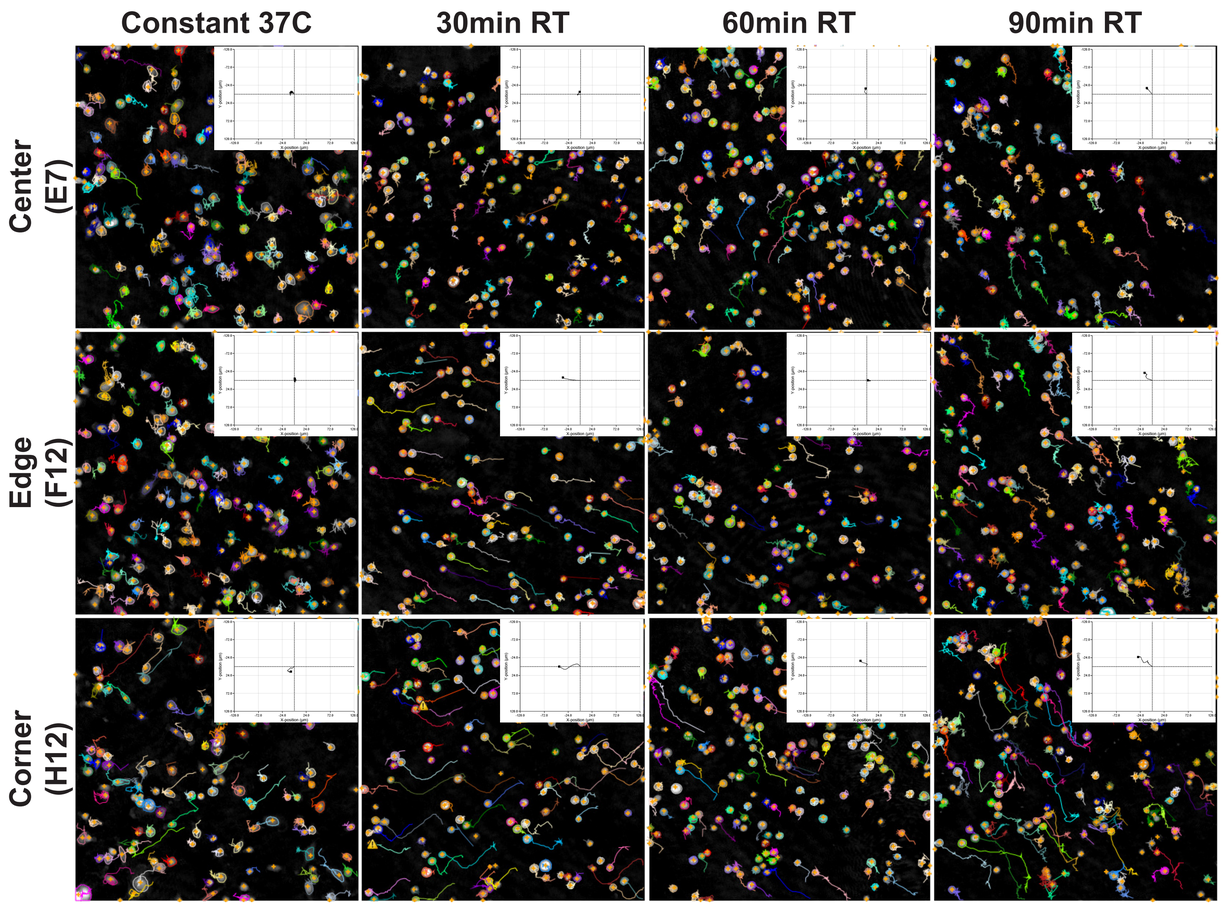

Figure 3. Cell Motion During Temperature Changes Upon Return of the Plates to the Incubator After Pre-Incubation at Room Temperature or 37 Degrees C. The HoloMonitor M4 (PHI AB) was used to record the movement of A549 human lung carcinoma cells (ATCC, St. Louis, USA) during the first 3 hours of incubation. Cell migration analysis software from PHI showed that the cells moved very little after they were completely settled at RT, although some directional motion was seen, particularly in corner wells. These were smaller motions than we had seen previously when cells were subjected to temperature changes during cell settling. The graphs inset into each image show the overall averaged motion of the whole population of cells identified and traced. The cells plated and incubated at 37 degrees C attached quickly and did not migrate far from the origin, whereas cells incubated at RT tended to move farther. They did not seem to attach as quickly or as well as the cells plated at 37 C. n = 3 experiments. Representative images shown.

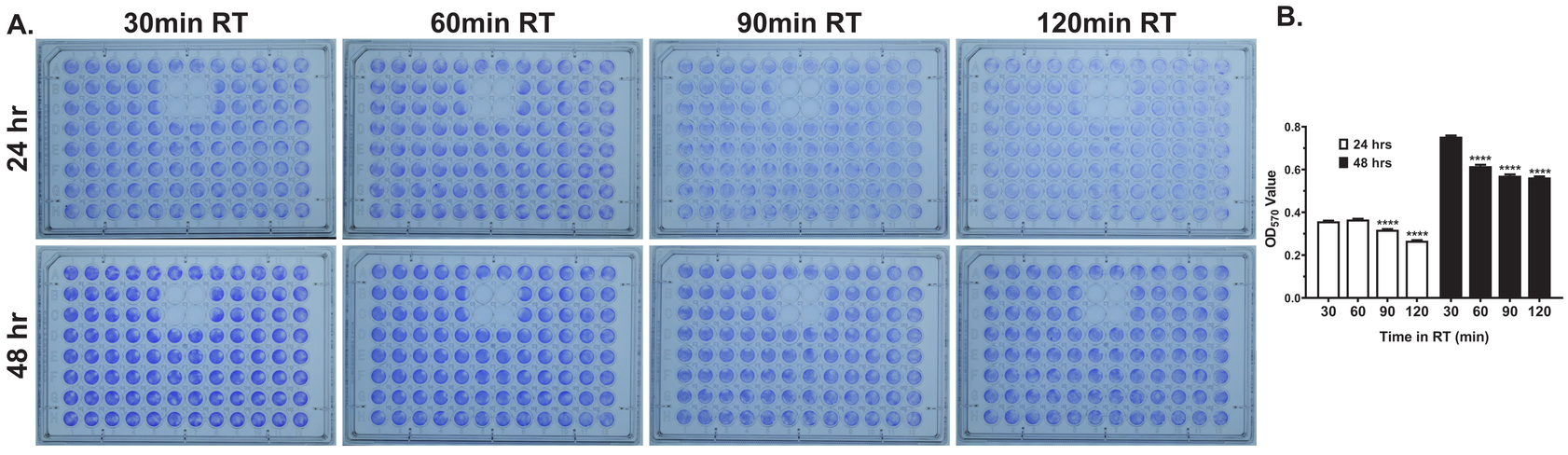

Figure 4. Pre-Incubation at RT Lead to a Time-Dependent Decrease in Cell Density. (A) A549 cells were plated in all but four control wells near the center of each of duplicate plates. Plates were incubated for the indicated times and then transfered to a 37 degree chamber. At 24 and 48 hours, the plates were fixed with ice-cold methanol and stained with standard Crystal Violet methods. The plates were photographed before the dye was dissolved and read on a plate reader at 570nm. **(B)** The average OD after background subtraction was significantly lower at 60, 90, and 120min of RT as compared to 30 min (Two-way ANOVA with Tukey's multiple comparisons test (GraphPad Prism v. 8.1.0 software). We concluded that the longer the cells were pre-incubated at RT before transfer to 37 degrees C, the fewer cells were present after 24 and 48 hours of culture.

CONCLUSIONS

Incubating A549 cells at room temperature lead to a pre-incubation time-dependent decrease in cell density

Full-time control of temperature is necessary to both reduce edge effect and protect cell viabiity

REFERENCES

1. Denmark J, Chessum B. Standardization of enzyme-linked immunosorbent assay (ELISA) and the detection of Toxoplasma antibody. Med lab sciences 1978, 35(3):227-232.
2. Lundholt RK, Scudder KM, Pagliaro I. A simple technique for reducing edge effect in cell-based assays. J biomolecular screening. 2003, 8(5):566-570.
3. S, Darou S, Henn AD, Frank AM, Alm K, Yerden R. Eliminating Edge Effect in 96-Well Plates by Controlling Thermal Conditions during Plating. AAACR Annual Meeting Apr-May, 2019.