Helpful Hints to Manage Edge Effect of Cultured Cells for High Throughput Screening

Allison Tanner, Ph.D., Corning Incorporated
Advanced Life Science Products Division
Portsmouth, NH 03801

Many routines involved with the culture of cells in the modern laboratory are devised for increasing productivity while maintaining an environment conducive to respected microbiological practices. Although these routines are set up to maximize throughput, problems are often encountered that produce less than satisfactory conditions for conducting cell based assays. One of the problems that can contribute to reduced productivity is unequal distribution of attached cells in microplates. "Edge effect," is a term used to describe a crescent moon pattern of cells adhering to the outer edge of wells on the perimeter of microplates. Edge effect may be found with regularity in some laboratories and yet the cause and the cure remain irritatingly illusive.

Adhesion of cells in culture, to the substratum and to each other, is a complex process involving the intricate interplay of many factors (1). The extracellular matrix (ECM) is composed of a variety of proteins secreted by cells that organize into a structural network to which cell surface adhesion receptors bind (2). Adhesion to tissue culture treated plasticware is facilitated by ECM proteins that are normal components of serum containing cell culture media (1,3). The proteins comprising the ECM differ between cell types, and can influence cell structure and function (2-4). Although these factors are cell-type specific, many elements affecting the adherence of cells in culture arise from the procedures used routinely in the cell culture laboratory. The following is a compendium of techniques, collected from Corning Cell Culture facilities and customers, to reduce the occurrence of irregular patterns of cell adhesion or "edge effect" in microplates.

1. Avoid bubble formation in the media when seeding cells. Bubbles may appear to float on the media surface, but also lie below, and may reach to the polymer surface restricting the deposition of proteins and cells (5). Proper pipetting techniques or robotic dispensors with controls for adjusting the speed of dispensing will help avoid bubbles and foam.

2. Use cell harvesting methods that foster adequate mixing of the cells in the media and complete dissociation of the monolayer into single cells. Develop a system to maintain adequate mixing of cells in media being dispensed robotically.Seeding cells in clumps can lead to uneven distribution and uneven attachment of cells in microplates.

3. Seed cells at a density that will support attachment and growth. Seeding 50,000 CHO K1 cells/well in a 96 well plate provides a confluent monolayer in 24 hours. Certain ECM proteins are secreted based on the seeding density and are necessary for the growth of some cells (6,7).

4. Allow cell attachment to occur in media of the proper formulation for the particular cell type. Many media formulations of the same name vary in composition and cell growth tests may be necessary to validate media and serum quality.

5. Dispense cells in an appropriate volume of media. Reducing the seeding volume for 50,000 cells/well from 200 to 100 µL in 96 well plates has been reported to reduce edge effect. Meniscus effects can result in a halo of cell adherence around the edge of the microplate because this region is deeper and may contain more cells and attachment factors (5). The media volume can also affect the cell settling time, where larger volumes

increase the time taken for cells to reach the microplate surface as well as reducing gaseous diffusion. The recommended media depth is 2 to 5 mm based on the oxygen requirements of the cells in culture (8).

6. Follow strict aseptic techniques according to respected microbiological practices (9). Ideally the cell culture area should be in a separate room, or away from high volume foot traffic and drafts that can increase the risk of contamination. Use of laminar flow hoods is recommended. Asepsis of robotic handling equipment should be maintained.

7. Cultures should be routinely monitored for mycoplasmal contamination. Mycoplasma is microscopically invisible and can be an insidious detriment to cell adhesion and growth.

8. Minimize entrance to incubators to help reduce fluctuations in the internal environment. Consistent temperatures and humidity levels are necessary to avoid evaporation and the formation of condensation. Local convection currents of heat and gas coupled with the location of microplates within the incubator are among the most influencial factors involved with patterns of cell adhesion (5).

9. Allow cells to settle in the microplates while the microplates are on the benchtop. 15 to 120 minutes of settling time before microplates are placed in an incubator is reported to reduce edge effect. Levelness of the incubator and shelving, vibrations within the incubator, or from heavy foot traffic, equipment operation or construction can also contribute to aberrant cell attachment.

10. Culture cells in an environment that reduces the formation of static electrical charges. Avoid rubbing microplates against one another and against packaging materials. Increasing relative humidity and using a static discharge pad can also reduce static (5,10).

Contributors of information for this article include: Rose McBreairty and Judy Gibbs of Corning in Kennebunk, ME, Marie Bryhan of Corning at Sullivan Park, NY, John Ryan of Corning in Acton, MA, Catherine Ennis of Corning in Portsmouth, NH, and Martin Kirk of Molecular Devices in Sunnyvale, CA.

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For further information, please contact Technical Support, Corning Incorporated, Science Products Division, Advanced Life Science Products (USA), Tel: 1-800-492-1110, Fax: 1-978-635-2479, e-mail: ccc.webmail@corning.com

Corning Incorporated

Science Products Division
Advanced Life Science Products
45 Nagog Park, Acton, MA 01720
Tel: 1-800-492-1110, Fax: 1-978-635-2476
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