

An Examination of Nunc Cell Factory System for Consistent Reliable Production of Large Quantities of Cells

Key Words

Cell culture, bioproduction, culture density, multi-layer, Cell Factory system, MDCK, MDBK, VERO, CHO

Introduction

Thermo Scientific™ Nunc™ Cell Factory™ systems are solutions designed for adherent cell culture. These systems consist of multiple polystyrene growth surfaces, assembled together to maximize the surface area available for cell growth while minimizing the incubator volume required for maintaining cells. Nunc Cell Factory systems are popular in fields where large quantities of cells are required and are a reliable tool for the production of vaccines, recombinant proteins, and for the generation of cell mass.

Unlike single-layer systems, where the entire culture is easily visualized under a microscope, the middle layers of a multi-layer system are obscured from visualization by the surrounding layers. As a result of this design, it can be difficult to monitor the culture conditions and cell growth on these layers, or verify that cultures grow consistently between layers. Here we examine the growth of several commonly used cell lines within Nunc Cell Factory 10-layer systems by physically separating and photographing each layer after a typical period of cell growth. This protocol enabled the verification of consistent cell growth throughout all Cell Factory system layers.

Materials

Nunc Cell Factory – 10-layer system: Cat. No. 170009

Chinese hamster ovary cells (CHO): ATCC CCL-61

Bovine kidney cells (MDBK): ATCC CCL-22

Canine kidney cells (MDCK): ATCC CCL-34

Monkey kidney cells (VERO): ATCC CCL-81

MEM/EBSS: Hyclone™ Cat. No. SH30024.02

Non-essential amino acids (NEAA):
Sigma Cat. No. M7145

Sodium pyruvate: Hyclone Cat. No. SH30239.01

Antibiotic antimycotic solution (AAS):
Sigma Cat. No. 5955



Fetal bovine serum (FBS): Hyclone Cat. No. SH30910.03 or SH30396.03

Cells were harvested from a source culture using a standard Trypsin™ harvesting procedure and cell density was determined using an automated cell counter. Cells were then diluted in prepared media to 30,000 cells/mL (CHO, MDBK, VERO) or 45,000 cells/mL (MDCK). 2000 mL of diluted cell suspension was seeded into each Cell Factory system. All Cell Factory systems were closed and incubated in a warm room at 37°C for 4 days. The warm room did not contain a CO₂ controlled environment.

Once incubation was complete, all media was removed from the Cell Factory system and cells were stained using crystal violet. After staining, the layers were separated using a hot knife and each individual layer was photographed to examine cell growth on the entire layer. Photomicrographs were also taken of stained cells at three positions on each layer.

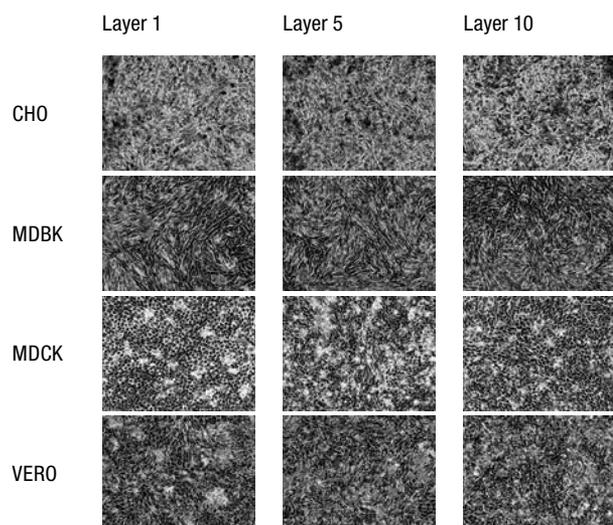


Figure 1. Photomicrographs showing similar cell density and morphology in the top, middle, and bottom layers of Cell Factory systems.

Observation of the stain density over the entire surface of each Cell Factory system layer also shows very consistent growth between all layers for all cell types. Figure 2 shows images of the entire growth surface for each layer depicted. Layers 1 and 5 show similar growth characteristics in all cell types. The cell density was similar in all layers except on layer 10 for all cell types (images not shown). While the bottom layers of the multi-layer culture vessels show cell densities that would produce cell yields similar to all other layers, in most

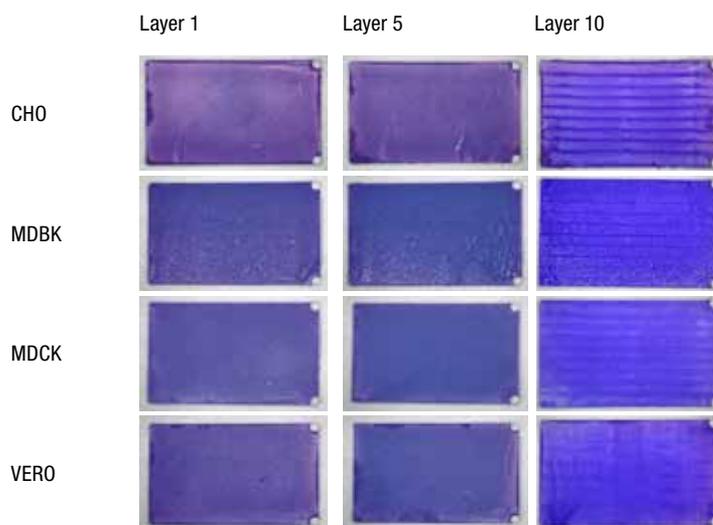


Figure 2. Photographs showing cell density in the entire growth surface of the top, middle, and bottom layers of the Cell Factory systems. Note the growth pattern within the bottom layer due to vibrational effects during incubation; however, this is not detrimental to cell growth.

cases a pattern emerges of variable cell density that resembles a wave pattern. This pattern is typically seen in cell culture, and is found specifically within this layer due to its contact with the incubator shelf. Vibrations within the incubator, as well as exterior sources, can create this pattern due to the waveforms transmitted into the liquid growth media. While these are not necessarily detrimental to cell growth, they can be minimized by reducing vibration as much as possible from the culture conditions.

Results and discussion

Overall cell density in each layer was observed qualitatively through staining of cell layers, making any deficiencies in cell growth visible. Photomicrographs of cells in all cell types show similar cell density and morphology throughout all layers of the 10-layer Cell Factory systems. Figure 1 shows images from the top (layer 1), middle (layer 5) and bottom (layer 10) with each cell type tested. All other layers showed similar results (images not shown).

Conclusion

The photographs and photomicrograph images of the individual layers of a 10-layer Cell Factory system provide visual confirmation that the cell culture conditions are consistent from layer to layer and conclude that there are no significant differences in:

- cell attachment
- growth
- density
- morphology

The multi-layered Nunc Cell Factory system is an ideal solution for consistent reliable production of large quantities of cells.

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