Active Gassing Improves Cell Output During Scale Up In Multilayer Culture Vessels

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Overview: **Purpose:** To demonstrate the performance of Corning® CellSTACK™-40 Culture Chambers to similar competitor products under static and actively gassed conditions.

Methods: Forty layer chambers were grown under gassed and non-gassed conditions using two cell lines. Cells were harvested and enumerated. Layers were also stained via crystal violet for determination of cell coverage.

Results: MDBK cells grew to a higher density under both gassed and non-gassed conditions in Corning CellSTACK-40 culture chambers as compared to a similar competitor product. Active gassing of multi-layer culture vessels increased the growth capacity of the lower levels, resulting in equal growth among all cell layers. Finally, a CHO cell line, grown under gassed conditions, showed similar performance in cell yields and production of a secreted product between the Corning CellSTACK-40 Culture Chamber and the competitor product.

Introduction

Background

Historically, scale up of adherent cell cultures requires the use of many individual culture vessels such as large surface area flasks or roller bottles. The risk of contamination and variability from vessel to vessel seen with large numbers of individual culture vessels is problematic for consistent bioproduction, (e.g. protein, viral and cellular therapeutic products). Therefore the development of a large scale culture vessel capable of the scalable growth of adherent cells would enable users to generate quality reproducible bioproducts for end use or as an intermediary to larger bioreactor scale production.

Corning CellSTACK Culture Chambers:

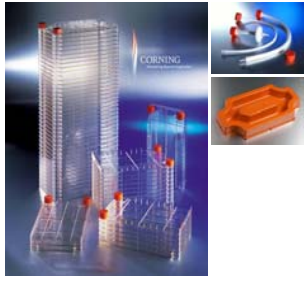
- Multilayered culture system for the scale up of adherent cell types.
- Variety of sizes from 1 to 40 layers

Advantages

- Large access ports for ease of liquid handling
  - Easily accommodates up to 100 ml pipets
  - Pipets reach bottom layer of 1 thru 10 layer stacks
  - Liquids can be pipeted, pumped and poured simply
- Large vented slots for rapid liquid equilibration between layers
- Sturdy construction
- Can withstand up to 1.5 psi which allows air shipment
- Multiple accessories for filling, stacking, and venting

Critical Attributes:

Culture Chamber	Surface area (cm^2)	Media Volume (ml)	T75 or RB equivalent
1 STACK	636	150	8.5 T75s
2 STACK	1272	300	17 T75s
5 STACK	3180	750	4 RBs
10 STACK	6360	1500	8 RBs
40 STACK	25440	6000	340 T75s or 30 RBs



Materials & Methods

Materials

Cells: Madin-Darby Bovine Kidney (MDBK) cells (ATCC # CRL-1573) were maintained in either IMDM or CO_2 independent media with 5% horse serum, 1% penicillin/streptomycin. 5/9 m alpha3-18 Chinese Hamster Ovary (5/9 alpha) cells (ATCC # CRL-10154) were maintained in minimum essential medium (MEM) alpha medium with 10% fetal bovine serum, 1% penicillin/streptomycin.

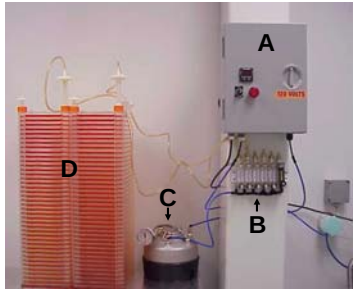
Stack Chambers: Corning CellSTACK-40 Culture Chambers (cat # 3272) and competitor 40 layer standard and active gas chambers were used in these studies. In experiments utilizing active gas, each chamber was vented with high air flow filters to allow for gas exhaust.

Methods

Non-gassed cultures: MDBK cells were seeded into Corning or competitor 40 layer chambers at 15,000 cells/cm^2 in CO_2 independent medium. Cultures were allowed to grow for 72 hr at 37°C. At the end of the culture period, cells were harvested from each vessel, centrifuged at 250x*g* for 20 min, resuspended in PBS and enumerated via hemocytometer counting.

Gassed cultures: MDBK cells were seeded as above in IMDM medium. 5/9 alpha cells were seeded at 15,000 cells/cm^2 in MEM medium. For cultures in Corning chambers, gas containing 5% CO2 and 95% air was pumped into the vessel at 300 ml/min. For cultures in competitor active gas chambers the same gas mixture was pumped into the vessel at 2 L/min, per manufacturer's instructions. Cultures were grown for 72 hr at 37°C and harvested as above.

Gassing setup: Below is the setup used for actively gassing the multilayer culture vessels.



Legend:
A – Timer/Controller
B – Flow regulators
C – Humidity chamber
D – Culture Chambers

Results

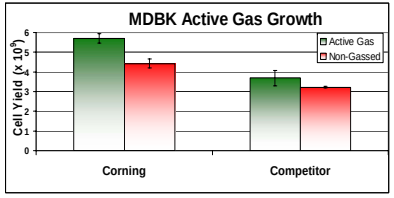


Figure 1: Growth of MDBK cells in Corning CellSTACK-40 Culture chambers and similar competitor products under gassed and non-gassed conditions. Each experiment was performed at least 3 times with at least 2 replicates per condition. Pairwise comparison of cell yields of Corning and competitor product for both gassed and non-gassed conditions indicated a significant difference, p value < 0.004 and 0.002, respectively.

Figure 3: Growth of 5/9 alpha cells under gassed conditions in multilayer culture vessels.

Experiment was performed 3X with at least 2 replicates per condition.

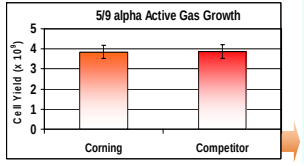
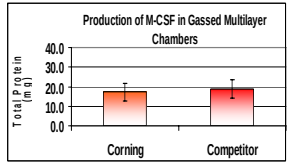


Figure 4: Recombinant protein production in gassed chambers. At the end of the 5/9 alpha cultures, media was equilibrated amongst the layers and a sample taken for ELISA analysis of M-CSF production.



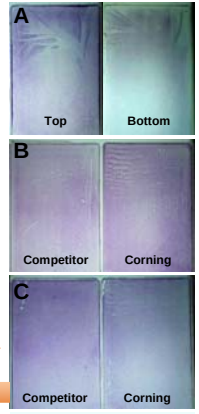


Figure 2: Crystal violet staining of Corning and competitor multilayer chambers. Panel A – Non-Gassed Layers: Top layer (left panel) and bottom layer (right panel) of a non-gassed Corning CellSTACK -40 culture chamber. The bottom layer is substantially less stained than the top layer indicating reduced cell growth in the lower layers. **Panel B – Gassed Top Layers:** competitor chamber (left panel) and Corning chamber (right panel). Each panel is uniformly stained over the entire surface of the layer. **Panel C – Gassed Bottom Layers:** competitor chamber (left panel) and a Corning chamber (right panel). Each panel is uniformly stained over the entire surface of the layer. Additionally, unlike the non-gassed culture chamber (panel A), the top and bottom layers (panels B and C, respectively) are similarly stained indicating similar extents of growth.

Conclusions:

- **On average, active gassing improved cell yields by 29% for Corning CellSTACK-40 Culture Chambers and 14% for competitor chambers.**
- **Active gassing promoted the uniform growth of cells on all 40 layers of the multilayer culture vessels.**
- **For MDBK cells, the Corning CellSTACK-40 culture chamber is capable of producing, on average, 40% more cells than the competitor product.**
- **For 5/9 alpha cells, both multilayer stacks produced the same amount of cells under gassed conditions.**
- **Production of human M-CSF from the engineered 5/9 alpha cell line here was equivalent in both culture vessels.**