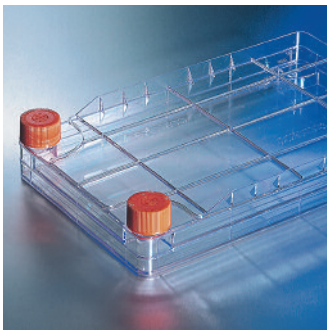
Effects of the Medium Volume Reduction on Cell Growth in Corning® CellSTACK® Vessels

Application Note



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Introduction

Multiple environmental factors need to be optimized to achieve the best results during cell growth. One of the most important considerations for attachment-dependent cells is the volume of medium per cm^2 of growth surface area. The availability and nature of the nutrients, the amount of dissolved gasses, as well as the amount of metabolic waste accumulation are all key factors for optimizing medium volume.

With risings costs, researchers and pharmaceutical companies alike are looking to maximize their available funds. One area that has been given significant consideration is reducing the volume of medium used for cell growth. The cost of maintaining cells in culture can be lowered significantly by decreasing the volume of medium used in large scale culture systems, such as the Corning CellSTACK vessels. Through the years, scientists have developed recommendations for medium usage for anchorage-dependent cell growth. However, the current economic environment dictates the need to find novel ways to reduce costs in the manufacturing processes, the product development, and the research setting. Important considerations when reducing medium volume include changes in pH due to metabolite accumulation, amount of dissolved gasses (O_2 and CO_2), nutrient sources and overall cell viability.

The purpose of this study is to analyze the impact of reducing medium volume on two widely used attachment-dependent mammalian cells lines cultured in Corning CellSTACK vessels. Cell viability, nutrient levels, and metabolic indicators were measured and presented.

Materials and Methods

Cell growth and Viability

Madin Darby Bovine Kidney (MDBK) (ATCC Cat. No. CRL-22) and Vero cells (ATCC Cat. No. CRL-1586) were seeded at 2,500 cells per cm^2 into tissue culture treated 2-layer Corning CellSTACK vessels (Corning Cat No. 3269) using Iscove's Modified Dulbecco's Medium (IMDM) (Mediatech, Inc. Cat. No. 10-016-CM) supplemented with 5% donor calf serum (DCS) (Mediatech, Inc. Cat. No. 35-022-CV). Different volumes of medium per layer were used in three sets of 2 layer vessels (either 100 mL, 150 mL, or 200 mL) and the experiment was repeated three times. Cells were allowed to grow for 96 hours at 37°C and 5% CO_2 in a humidified incubator. Prior to harvest, a sample of medium was taken from each vessel for analysis using the Nova® BioProfile® FLEX analyzer (Nova Biomedical Corporation) to assess the levels of gas, nutrients, and metabolite contents. Cells were harvested using 0.05% (w/v) trypsin (Cambrex Cat. No. 17-160E) and the reaction was stopped by addition of serum-containing medium. To ensure that all cells were collected from each vessel, an additional phosphate buffered saline (PBS) wash was performed and collected. The final volume of the cell suspension was 110 mL. Cell morphology and overall growth characteristics were monitored visually using an Olympus Inverted Microscope (Olympus, Inc).

Kinetic Analysis

MDBK cells were seeded at 5,000 cells per cm^2 into tissue culture treated 10-layer Corning CellSTACK vessels (Corning Cat. No.3271) using IMDM medium supplemented with 5% DCS. Different volumes of medium per layer were used (either 100 mL, 150 mL or 200 mL) as previously described. The experiment was repeated three times. Due to the large number of vessels and their large size, the vessels were pre-gassed with humidified air containing 5% CO_2 for 30 minutes, seeded, closed with solid caps (Corning Cat. No. 3969) and incubated in a warm room at 37°C for 72 hours. Nutrient, metabolite, gas, and electrolyte content of the vessel was assessed daily using the Nova BioProfile FLEX analyzer. Two samples were taken for analysis: one sample was collected from the bottom layer of the CellSTACK vessel and the other after medium from all the layers was thoroughly mixed. Cells were detached with 0.05% trypsin and growth efficiency was calculated by determining the number of cells per cm^2.

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Functional Analysis

CHO 5/9 M 13/8 Alpha cells were grown for 96 hours in a 2-layer CellSTACK® vessels containing different volumes of medium per layer as described above. Growth medium was collected and analyzed for MCSF production using human MCSF ELISA kit from CellSciences® according to the manufacturer's protocol (Cat. No. CKH166). The data were quantified using PerkinElmer® EnVision® microplate reader.

Results

Cell Growth and Viability

Traditionally, it is recommended to use 0.2 to 0.3 mL medium per cm^2 of growth surface area for attachment dependent cells because this amount allows for optimum growth environment for most cell types. This is why Corning recommends using between 130 and 200 mL of medium per layer in the Corning® CellSTACK vessels (surface area is 636 cm^2 per layer). In this study, multiple components were analyzed to assess the feasibility of reducing the medium volume in these vessels using MDBK and Vero cell lines. Particular attention was paid to the leveling of CellSTACK vessels, especially in 100 mL per layer vessels. Ability to level the CellSTACK vessels contributed to defining the minimum volume used in this study.

Initially, we investigated the impact of medium volume on cell proliferation and viability. MDBK and Vero cells were seeded at 2,500 cells/cm^2 to allow the cells to remain in the growth phase throughout the 96-hour study. As indicated in Fig. 1A, MDBK cells achieved equivalent yields when grown in either 150 or 200 mL of medium per layer ($p > 0.05$ in two-tail t-test). However, when cells were grown in 100 mL volume per layer, there was a statistically significant reduction in cell density per mL as compared to those grown in the 150 and 200 mL volumes ($p = 0.02$ and $p = 0.01$ respectively in a two-tailed t-test). No visible differences in cellular morphology or growth patterns were observed (Fig. 1B). Upon further analysis of the medium, the MBDK cells cultured in the 100 mL per layer volume had the highest concentration of lactic acid and the lowest concentration of glucose (Fig. 2A). Additionally, these samples on average had higher concentrations of dissolved oxygen compared to

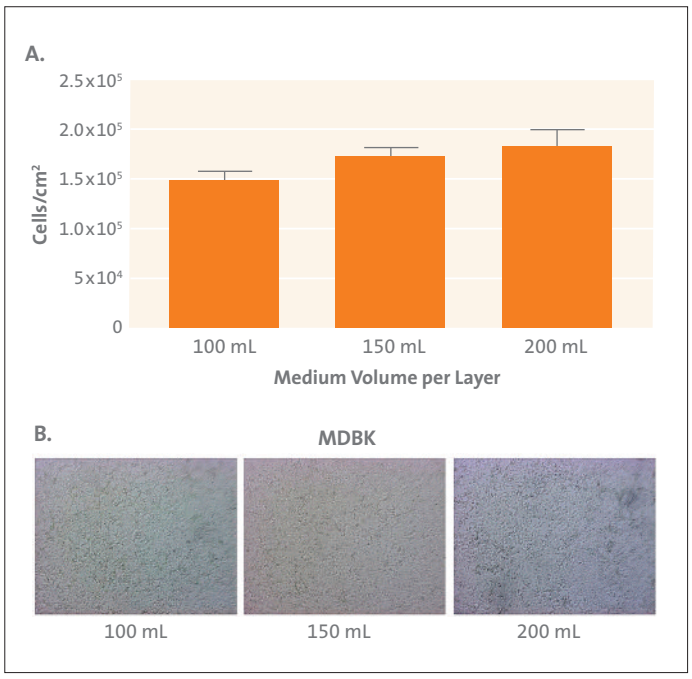

Figure 1. Cell growth assessment of MDBK cell line. (A) Graph shows cell yield after 96 hours of incubation. Data shown are the average of three independent experiments (n = 9). Cellular viability as determined by trypan exclusion method was 98%. Error bars represent ± S.D. (B) Representative cell morphology and confluence in the three growing conditions at 40X magnification before harvesting.

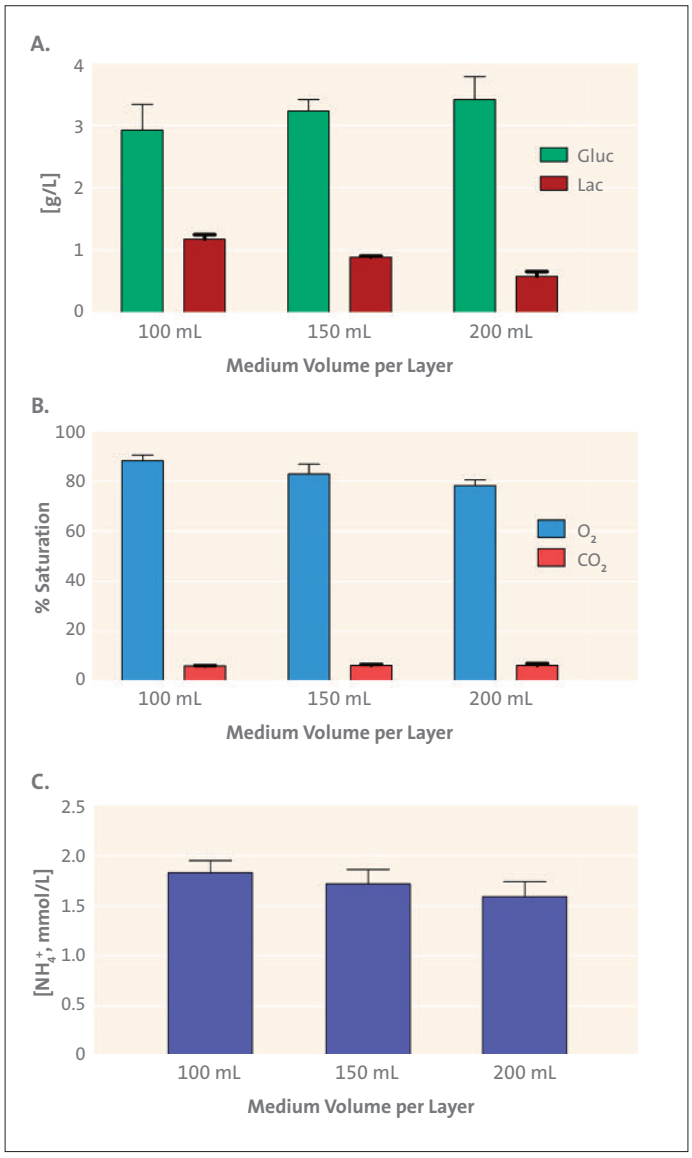

Figure 2. Metabolic assessment of MDBK growth environment. (A) Comparison of Glucose and Lactate levels in the medium after 96 hours of incubation. (B) Oxygen and carbon dioxide saturation in the medium after 96 hours of incubation. (C) NH_4^+ concentration among the indicated medium volumes per layer taken after 96 hours of incubation. Data shown are the average of three independent experiments ± S.D. (n = 9). All data were obtained using the Nova® BioProfile® FLEX analyzer.

the 150 or 200 mL samples, while dissolved CO_2 remained unchanged (Fig. 2B). The same trend was observed with glutamate and glutamine as well (data not shown), both important indicators of cell metabolism and overall cellular health. On the contrary, the concentration of the products of cellular metabolism, such as ammonia, increased with decreasing volumes of medium (Fig. 2C). The values for pH remained constant throughout all the samples in this study due to the buffering capacity of the medium (data not shown). Among other factors that were examined, but showed no observable change were Na^+ and K^+ concentrations (data not shown). Thus we assume that reduced cell density in 100 mL per layer samples could be attributed to the increased concentration of dissolved oxygen, the depletion of nutrients, and the accumulation of metabolic waste.

Vero cells showed no statistical difference in cell density among all the volumes tested ($p > 0.05$ in paired t-test) and no morphological or growth pattern differences were observed (Fig. 3A and 3B). Gasses and metabolite assessment showed similar trends of reduced nutrient concentration and increased metabolites with reduced medium volume per layer as was observed with the MDBK cell line (Fig. 4). The difference in the cell growth between the two cell lines could be attributed either to the difference in the level of metabolism between the cell lines, the susceptibility of each line to changes in their environment, or both.

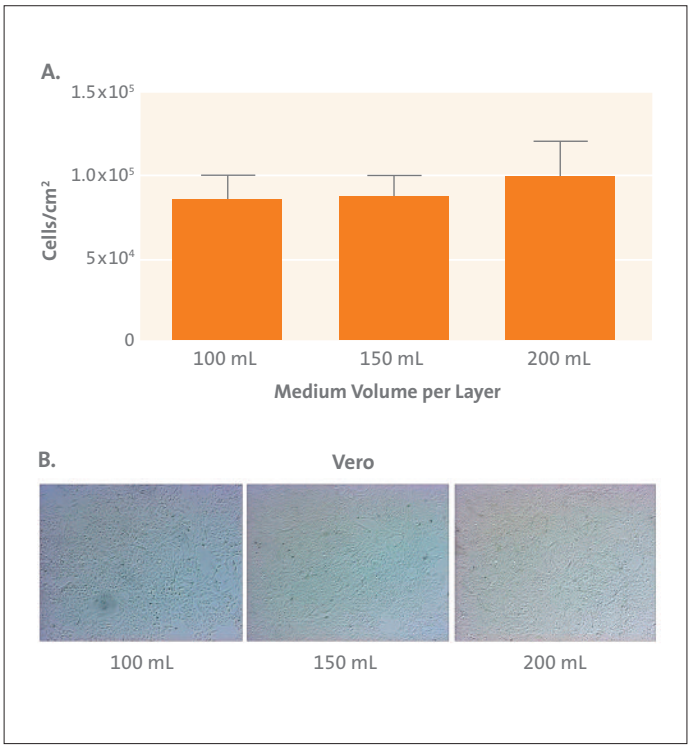

Figure 3. Cell growth assessment of Vero cell line. (A) Graph shows cell yield after 96 hours of incubation. Data shown are the average of three independent experiments (n = 9). Cellular viability as determined by trypan exclusion method was 96%. Error bars represent ± S.D. (B) Representative cell morphology and confluence in the three growing conditions at 40X magnification before harvesting.

Kinetic Analysis

In order to further understand the impact of the reduced medium volume on MDBK cell growth and viability, we sampled the medium daily for three days using the Nova® BioProfile® FLEX. At the end of the study, cells were detached from the surface with trypsin and their density per cm^2 as well as viability were determined. There was no statistically significant difference between the cell densities under these conditions (Fig. 5) with $p > 0.05$ in a two-tail t-test. The difference from the data above, where we observed reduced cell growth at lower volumes of medium per layer could be attributed to the shorter duration of the experiment that did not allow sufficient consumption of nutrients and accumulation of metabolic waste.

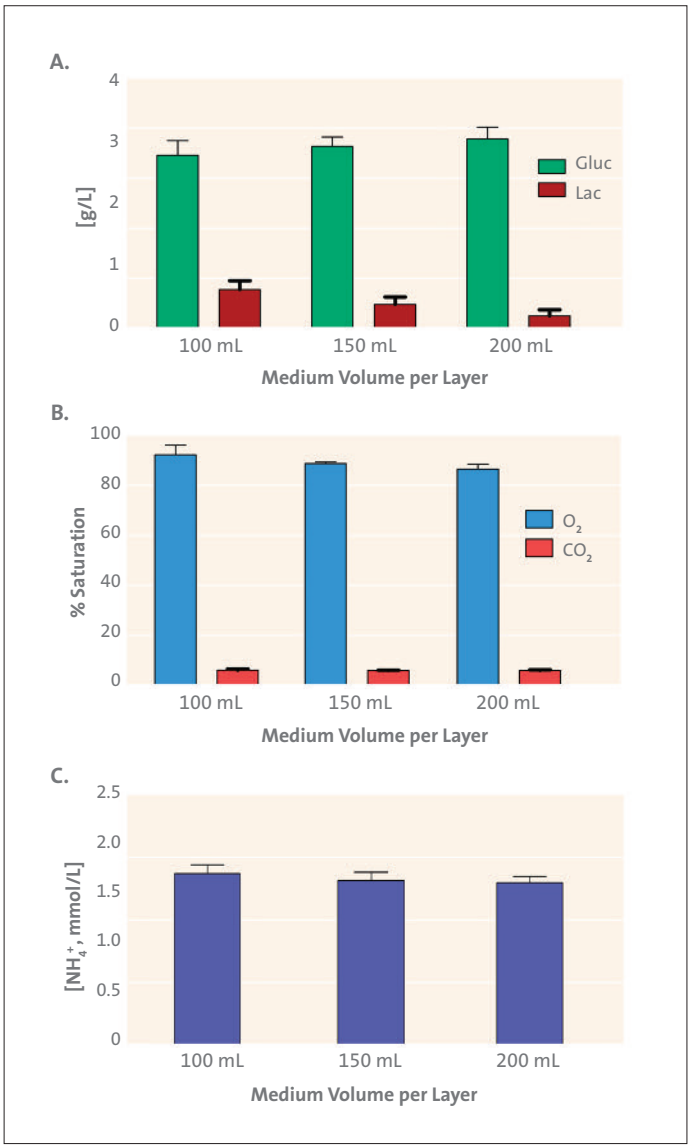

Figure 4. Metabolic assessment of Vero growth environment. (A) Comparison of Glucose and Lactate levels in the medium after 96 hours of incubation. (B) Oxygen and carbon dioxide saturation in the medium after 96 hours of incubation. (C) NH_4^+ concentration among the indicated medium volumes per layer taken after 96 hours of incubation. All data were obtained using the Nova BioProfile FLEX analyzer. Data shown are the average of three independent experiments ± S.D. (n = 9).

Two samples were collected at each time point and analyzed for levels of dissolved gasses and nutrient/metabolite composition: one from the bottom layer and the other one after medium from all the layers was thoroughly mixed. This was done to show that the growth conditions in all the layers of a CellSTACK® were similar. As evident in Fig. 6, there was no significant difference between these two samples in any of the factors analyzed.

As expected, kinetic analysis showed gradual decrease in nutrients in all medium volumes (Fig. 6A and data not shown), while metabolic waste was accumulating during the course of the experiment (Fig. 6B and data not shown). The observed decrease in nutrient concentration was highest at 100 mL and lowest at 200 mL (Fig. 6A). This effect could be attributed to the depletion of nutrients due to cellular activity that could become limiting with a decreased volume of medium. As expected, the trend is reversed for metabolite concentration (Fig. 6B). Since the number of cells remains equivalent in different conditions, the effects of cellular metabolism are more pronounced in the smaller volume of medium.

Analysis of the dissolved gasses allowed us to monitor the rate of gas equilibration inside the CellSTACK vessel. Figures 6C and 6D indicate that by Day 1 the equilibration was complete and the changes observed were probably due to the metabolic activity of the MDBK cells. The same trend was observed for pH (Fig. 6E). This observation could be very useful when deciding on whether medium equilibration is necessary prior to seeding of cells or when troubleshooting poor cell growth.

Functional Assessment of Cell Productivity

In order to evaluate the molecular integrity of cells grown in different media volumes per layer, CHO 5/9 M 13/8 Alpha cells were evaluated for secretion of human MCSF into medium. The protein production was analyzed by

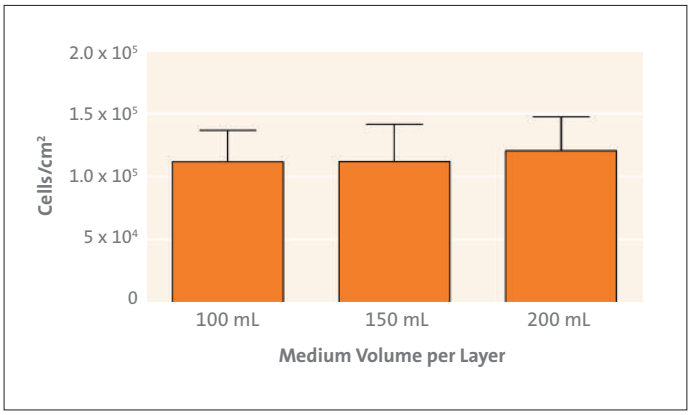

Figure 5. Cell growth of MDBK cell line in different volumes of medium per layer. Graph shows the cell density after 72 hours of incubation. All data were obtained using the Nova® BioProfile® FLEX analyzer. Average cell viability by trypan blue exclusion method was 98%. Data shown are the average of three independent experiments (n = 9). Error bars represent ± S.D.

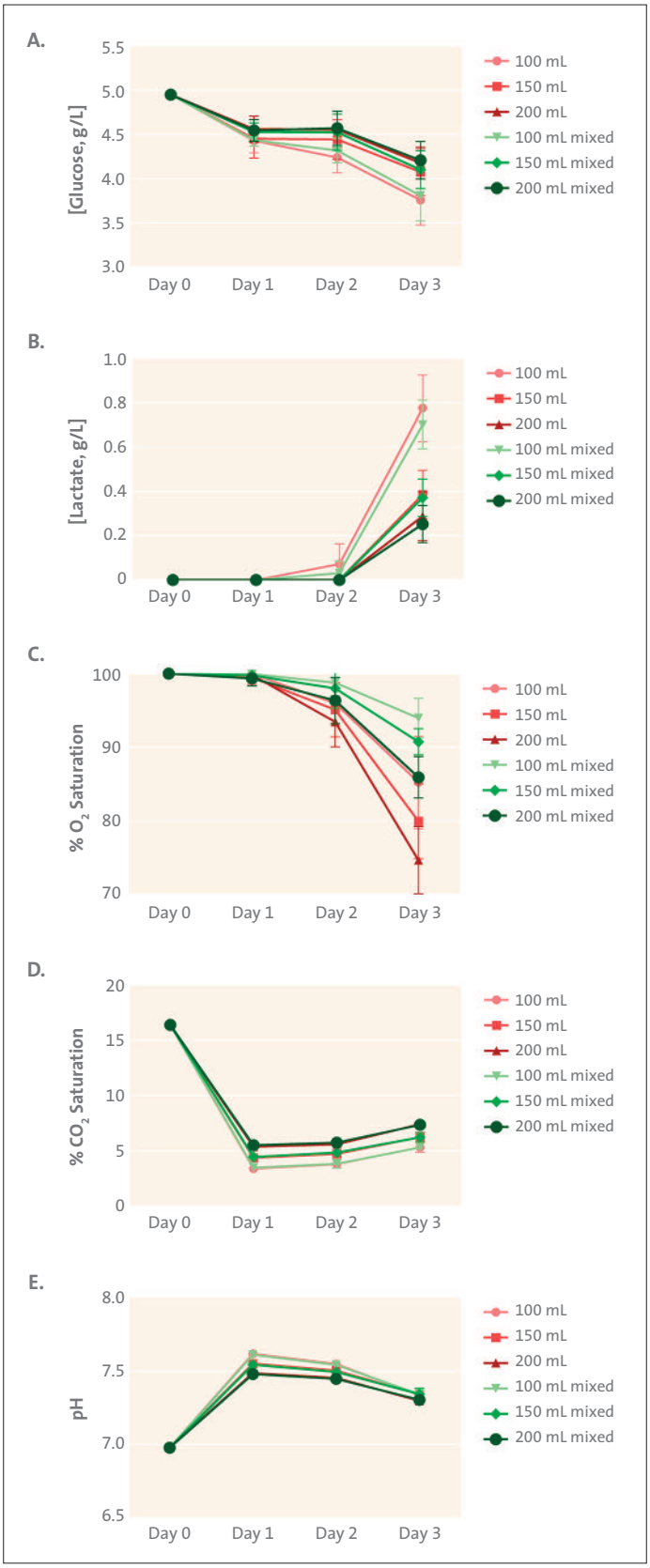

Figure 6. Kinetic profile of metabolic parameters of MDBK growth during 72 hours of incubation. (A) Concentration of glucose. (B) Concentration of lactate. (C) Oxygen saturation. (D) Carbon dioxide saturation. (E) pH levels. All data were obtained using the Nova BioProfile FLEX analyzer. Two samples were analyzed for each vessel: one from the bottom layer, and the other after medium from all the layers were thoroughly mixed. Data shown are the average of three independent experiments (n = 9). Error bars represent ± S.D.

ELISA assay using anti-MCSF antibody supplied with CellSciences® kit. A standard curve was generated using titrated amounts of human MCSF as a control (Fig. 7A). No statistical changes were observed among the samples in the levels of protein production (Fig. 7B). Since the total volume of medium is different in the cell culture vessels according to the experimental setup and the ELISA assay utilized equal volumes of medium per well, the data indicate that cells in 200 mL of medium per layer had the highest protein production and lowest in 100 mL of medium. Therefore, the data were converted to per cell yield (Fig. 7C). The graph and data analyses indicate statistically lower yield in cells grown in 100 mL medium volume per layer.

Conclusions

Based on our analysis of Vero, MDBK, and CHO 5/9 M 13/8 Alpha cell lines grown in varying medium volumes per layer in the Corning® CellSTACK® vessels, the following conclusions can be made:

- It is possible to successfully grow certain cell lines in the reduced volume of medium per layer in Corning CellSTACK vessels to reduce the cost of medium.
- Pilot experiments need to be conducted to determine the minimum allowable volume of medium for a particular cell line to avoid detrimental effects of nutrient depletion and metabolic waste accumulation.
- The rate of equilibration in the 10-layer Corning CellSTACK vessel is rapid, usually occurring within a 24-hour period.
- There is no detectable difference between the layers in the Corning CellSTACK 10-layer vessel in terms of dissolved gasses, nutrient and metabolite content.
- Care should be taken to evaluate the effects of medium reduction on metabolic and protein production activity of the cells.

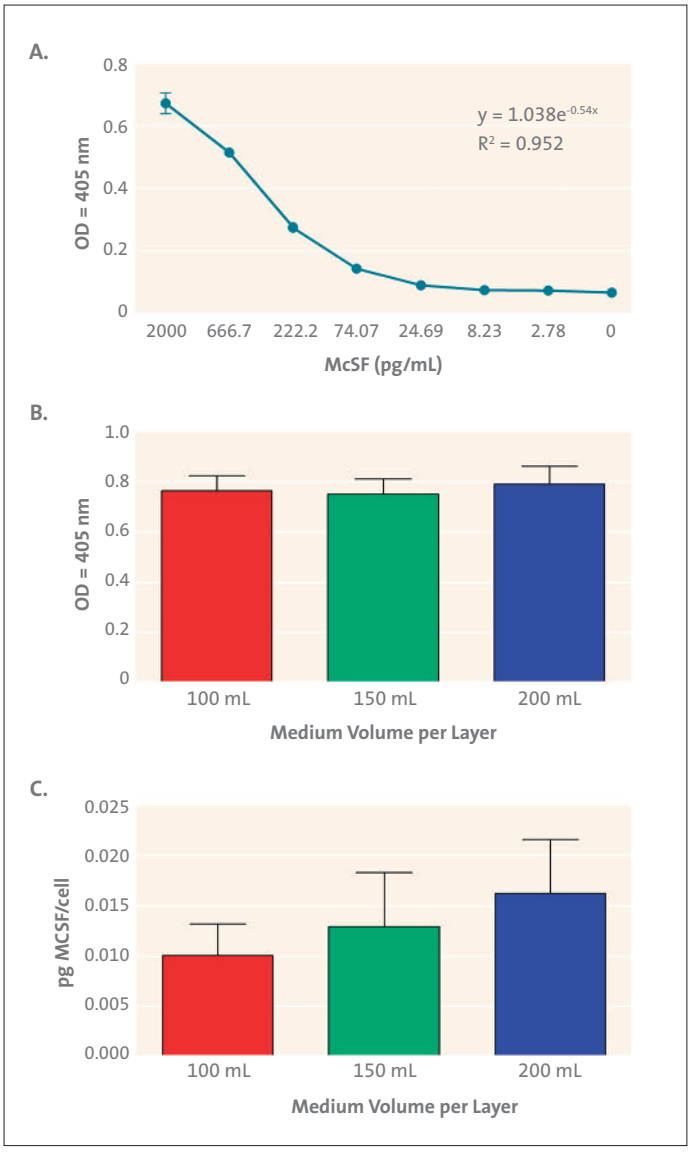

Figure 7. Comparison of MCSF production by CHO 5/9 M 13/8 Alpha cells after 96 hours growth. (A) Standard curve of MCSF production quantification using EnVision® microplate reader. (B) Optical density reading of MCSF production using ELISA reading. (C) Per cell yield of MCSF. Statistical analysis indicates difference between 100 mL and 150 mL ($p = 0.02$) and 100 mL and 200 mL ($p = 0.01$) samples in two-tailed paired t-test, but not between 150 mL and 200 mL ($p = 0.14$) samples. Data shown are the average of three independent experiments ($n = 9$). Error bars represent ± S.D.

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