Nunc Roller Bottles excel with ST, Vero, and MDBK cells

Abstract

A third-party study was conducted to compare the performance of Thermo Scientific™ Nunc™ Roller Bottles (1.2 and 2.5X) and 850 cm^2 roller bottles from another supplier, for the culturing of ST, Vero, and MDBK cells. Cells were seeded at relatively low density into each bottle and allowed to grow to confluence. At confluence, cells were harvested and passaged at the same density into new bottles; this was repeated 4 times, or until the culture failed to produce enough cells to seed. Confluency at 24, 48, 72, 96, and 120 hours was estimated visually. Cell counts per bottle (yield), as well as count per unit of surface area and percent viability, were determined at the end of each passage. Nunc Roller Bottles performed as well as or better than the alternative 850 cm^2 bottles with all cell lines tested.

Introduction

A large proportion of vaccine production is devoted to doses administered to animals, and the majority of these are produced through a large-scale cell culture system. Roller bottles made from polystyrene (PS) or polyethylene terephthalate (PETG) are commonly used in these culture systems as a growth substrate. The perception exists, however, that the cells used for such cell cultures have been conditioned to grow on PS, and as such would either require acclimation to the new surface or fail to grow properly.

Several factors could affect cell growth on different surfaces. Attachment of cells can alter their morphological and phenotypic profile, and requires interactions between cellular proteins, growth media contents, and the surface chemistry of the bottle being used. PS and PETG resins have different chemical properties and therefore can alter interactions. At the same time, surface treatments may also play a role, as PS surfaces are made more reactive through corona or plasma treatments and irradiation, while PETG surfaces are simply irradiated. Because not all cells in a given culture are genetically identical, evolutionary processes may have preselected the cell lines used in production to prefer PS growth surfaces since they were previously grown under these conditions.

There is a lack of quantitative data describing cell growth on PETG versus PS surfaces. To determine how bottle material affects cell growth, third-party testing was conducted using Nunc PETG Roller Bottles and PS roller bottles from another supplier (Supplier A). Cells were grown without an acclimation period in Nunc 1.2X, Nunc 2.5X, and Supplier A's 850 cm^2 roller bottles. ST, Vero, and MDBK cell lines were chosen to represent cells commonly used in animal vaccine production.

Materials

Chemical and reagents	Supplier	Cat. No.	Lot
DMEM	Mediatech	10-013-CM	10013359
L-Glutamine		25-005-CI	25005146
HEPES		25-060-CI	25060070
PBS	HRA	N/A	032504
Trypsin	Thermo Fisher Scientific	SH30042.02	JPD21046
Fetal bovine serum	JRH	12107-500M	2D0070
Gentamicin	Sigma	G1272	24K2388

Methods

Vero, ST, and MDBK cells were derived from previously established cultures growing on PS flasks. Cells were seeded at the concentrations and in the medium specified in Table 1.

Table 1. Plating densities and media

Cell line	Plating density ($cells/cm^2$)	Growth medium	Serum	Supplements
Vero	7×10^3	DMEM	5% FBS	2 mM glutamax supplement 50 µg/mL gentamicin
MDBK	1×10^4			
ST	1×10^3			

Cells were seeded into Nunc 1.2X, Nunc 2.5X, and Supplier A's 850 cm^2 roller bottles and allowed to grow for a total of 120 hours. The percent confluency was estimated visually at 24, 48, 72, 96, and 120 hours post-plating. After 24 hours, plating efficiency was estimated briefly as follows: 100 mL of medium was removed and centrifuged. The cell pellet was resuspended in 1 mL DMEM, and a viable cell count was performed using trypan blue. The cell density was calculated as the viable cell count from 1 mL suspension was multiplied by 100, since the original volume sampled was 100 mL.

The total number of recovered cells was estimated by multiplying the sample cell density by the total volume of medium in the bottle. Plating efficiency was calculated as [1 – (recovered cells/total number of cells plated)] x 100. At 120 hours, cells were trypsinized and counted via trypan blue exclusion.

The total viable cell yield per bottle was thus calculated and used to calculate the coverage per unit of surface area. Growth constants and doubling times were calculated from the data below using the following assumptions and equations. Simple exponential growth is described by the equation $N = N_0 + e^{kt}$, where:

- N = number of cells at a given time
- N_0 = initial number of cells
- k = growth constant
- t = time (hours)

From k, a doubling time can be calculated as follows: Doubling time (hours) = [in(2)]/k. k was determined by curve-fitting using the basic exponential equation in an Excel™ spreadsheet and plugging in the start and end values. Since the plating efficiency was so high for all groups (close to 100%), the number of cells at time 0 was defined as the seeding concentration multiplied by the seeding volume (assumes 100% efficiency) and was defined as the total cell yield. Since the medium was disposed of before cells were trypsinized and counted, the cell yield represents only those cells that were attached, not the total number of cells present in the bottle.

Cells were passaged at confluence (approximately 120 hours) through four passages, or until the culture failed to produce enough cells to seed. Confluency was estimated visually every 24 hours, and cell yield (cell count per bottle), cell count per unit of surface area, and cell viability were calculated at the end of each passage.

Results and discussion

For all cell lines and surfaces, 24-hour plating efficiency was exceptionally high (>95% in all cases), indicating that all surfaces are sufficiently hydrophilic to facilitate fast initial attachment. Since 24-hour plating efficiency was essentially equal among all groups (i.e., they all start at the same density after 24 hours), differences at later times must be due to the effect of long-term exposure to the surface.

Nunc Roller Bottles outperformed Supplier A roller bottles in estimated confluency levels for all three cell lines tested, and yielded more cells for two cell lines (Vero and ST) while performing at least as well with MDBK cells (Figures 1–6). For both types of Nunc PETG Roller Bottle, the results shown represent the mean yield of two bottles. Supplier A results represent a single roller bottle, as the yield from only one bottle was sufficiently high to make the first passage.

A faster doubling time of attached cells would lead to greater cell yields in a given period of incubation. The doubling time (Table 2) for Vero and ST cells grown in Nunc PETG Roller Bottles was shorter than for the same cell types frown in Supplier A's bottles. Doubling times for MDBK cells were similar in all bottles; however, cells in Supplier A's roller bottles were not grown past passage 2, due to low viable cell yield.

Cell growth is dependent on a number of factors, including seeding density, media formulations, age of the cell line, cell type, and the surface on which they are cultured. In general, higher seeding densities lead to faster, more robust growth, since the cells condition the media with autocrine and paracrine growth factors more quickly when seeded at high density. Because of this, high seeding densities may mask any underlying failures due to differences in surface chemistries. In order to eliminate this masking effect, the roller bottles in this study were seeded at densities far lower than they would be under normal conditions; cell growth would likely be better if they were seeded at normal densities. It is also important to note that the cell growth here represents the growth of cells attached to the bottle surface, as unattached cells were removed during the trypsinization process.

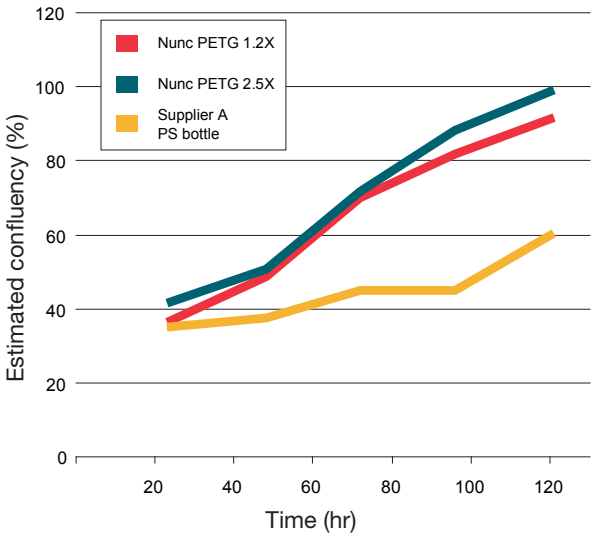

Figure 1. Estimated confluency of Vero cells through 120 hours.

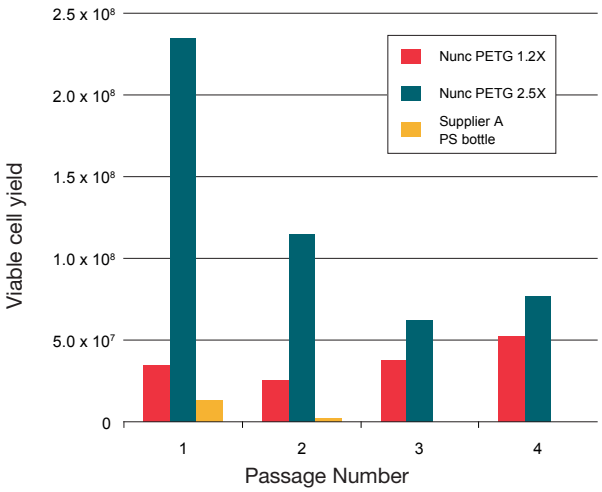

Figure 2. Viable cell yield of Vero line after each passage.

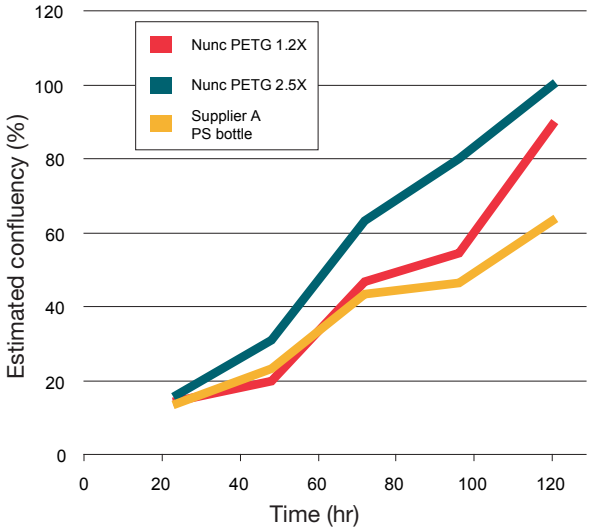

Figure 3. Estimated confluency of ST cells through 120 hours.

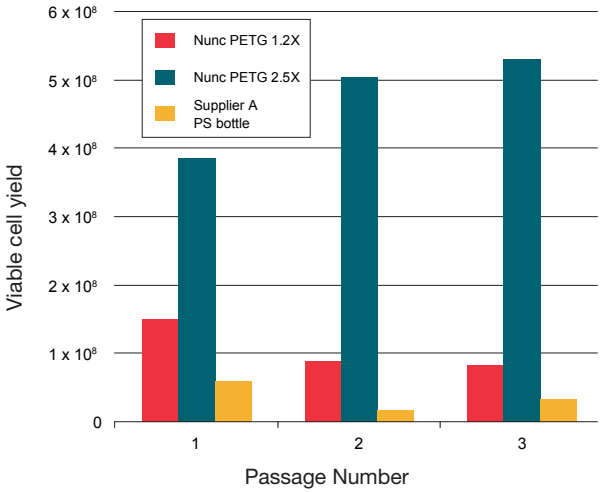

Figure 4. Viable cell yield of ST line after each passage.

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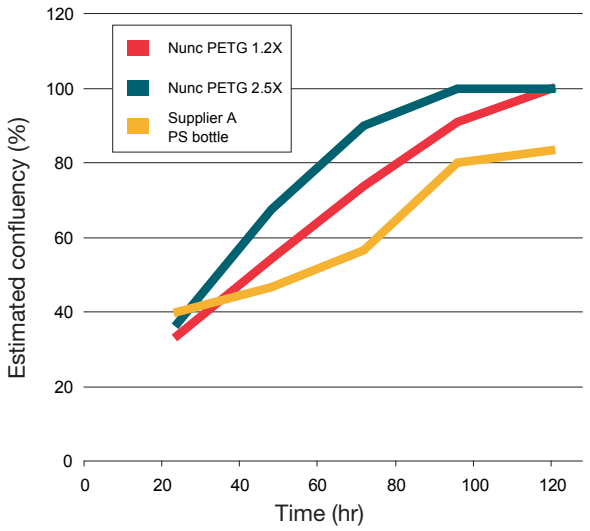

Figure 5. Estimated confluency of MDBK cells through 120 hours.

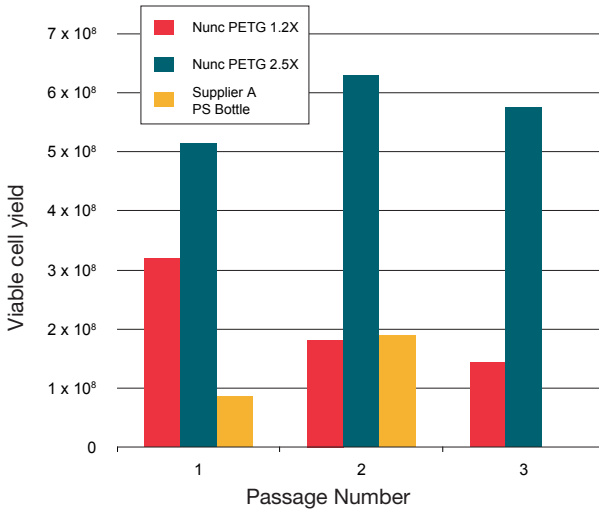

Figure 6. MDBK viable cell yield after each passage.

In cell culture systems for vaccine production, growing more cells in less time and less space may result in lower production costs for the vaccine. Our results indicate that Nunc Roller Bottles will produce higher yields than Supplier A's bottles in systems utilizing Vero and ST cells, and equivalent or possibly better yields where MDBK cells are used.

Table 2. Doubling times calculated from growth constants (k).

Passage	1	2	3	4
Vero doubling time (hr)				
1.2X, lot 521519	70	70	68	33
1.2X, lot 522973	34	44	44	34
2.5X, lot 517943	24	32	48	37
2.5X, lot 517945	25	33	45	45
Supplier A	79	79	No data	No data
ST doubling time (hr)				
1.2X, lot 521519	25	32	53	No data
1.2X, lot 522973	25	29	26	No data
2.5X, lot 517943	23	20	20	No data
2.5X, lot 517945	22	21	21	No data
Supplier A	34	96	49	No data
MDBK doubling time (hr)				
1.2X, lot 521519	18	20	24	No data
1.2X, lot 522973	20	31	26	No data
2.5X, lot 517943	22	19	20	No data
2.5X, lot 517945	20	20	20	No data
Supplier A	29	21	No data	No data

Conclusions

- Cell lines commonly used in animal vaccine production do not need an acclimation period when being switched to PETG surfaces from PS.
- Better cell yields can be expected in Nunc PETG Roller Bottles than in Supplier A PS roller bottles when using the Vero and ST cell lines.
- MDBK cell yields in Nunc PETG Roller Bottles should be equivalent to or better than yields in Supplier A's PS roller bottles.

Find out more at **thermofisher.com/rollerbottles**

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