Simplifying Progress

Microcarrier-Based Production of Dengue Virus in an Optimized Animal-Free Virus Production Medium

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Introduction

Microcarriers have been a mainstay production platform for vaccine production for decades. They are used for production of both human and animal vaccines. However, many processes still use animal derived components in their manufacturing processes. The use of undefined components in animal product-free (APF) cell culture media is one source of variability in cell culture processes. Therefore a production medium optimized for use with microcarrier-based production systems would be desirable. In these studies, a hydrolysate-free and APF, Vero cell production medium that is ideal for use in stirred-tank vessels with Sartorius SoloHill® microcarriers was developed. During development, medium formulations were optimized through an iterative process in concert with various microcarrier types to support robust cell growth and virus production in small-scale spinners. Further optimization then occurred in larger bioreactors.

Materials and Methods

Cells, Virus and Media:
Vero African green monkey kidney cells from American Type Culture Collection (ATCC;CCL-81; P124) were expanded directly into SFM for four passages prior to use. Wild-type Dengue viruses (DEN-2 16681) and attenuated (DEN2 IC/VV45R) vaccine strains were received from Center of Disease Control (CDC) and used for inoculation of cultures. Three media were employed in these studies: an optimized SFM formulation from InVitria designated as IM 1, a commercially-available SFM medium; and Gibco Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific: Cat#12100-046) containing 10% HyClone fetal bovine serum (FBS) (GE Healthcare Cat# SH30071.03).

Microcarrier, Spinner and Bioreactors:
Four types of Sartorius SoloHill® microcarriers (Hillex®II, Plastic, Plastic Plus and ProNectin*F) were used at 5 and 10 cm^2/mL microcarrier densities in spinner cultures, and 10 and 13 cm^2/mL microcarrier densities in bioreactors. Corning 250 mL (Corning: # 4500-250) reusable glass spinner flasks were used to assess Vero cell growth and Dengue virus production. Processes were transferred to 2 L stirred-tank bioreactor systems.

Harvest Enzymes:
Cells were harvested with trypsin (Thermo Fisher Scientific, Cat# 15050-065) or recombinant trypsin (Novozymes, Cat# (EC 3.4.21.4).

Spinner Spinner Culture:
Spinner flasks were incubated in humidified incubators at 37 °C, 5% CO_2, and agitation was maintained at 40 rpm and 65 rpm for the non-Hillex® II and Hillex® II microcarriers, respectively. Spinner cultures were carried out without medium exchange (unless specified) but were supplemented with glucose as needed. A GlucCell* glucose meter (CESCO Bioengineering: Cat# CLS-1322-02) was used to measure glucose concentrations and glucose (Sigma Cat# G8769) was supplemented up to 1.5 g/L when the concentration reached ≤1 g/L. Immobilized cell density on microcarriers was assessed via cell lysis and counting of nuclei.

Bioreactor Culture:
Cells expanded in spinner cultures were used to seed 2 L bioreactors. Agitation was initiated at 35 rpm and 60 rpm for non-Hillex® II and Hillex® II microcarriers, respectively. Bioreactor cultures were seeded at 2×10^4 cells/cm^2 or 4×10^4 cells/cm^2. Temperature was maintained at 37 °C. Bioreactors were equipped with pitched-blade impellers for agitation and 40 µm spin filters for medium removal. Bioreactor cultures were carried out without medium exchange but were supplemented with glucose when the concentration reached less than ≤1 g/L.

Virus Infection:
Virus infection was carried out when cells reached ~90% cell confluence (~14×10^4 cells/cm^2), after 2 to 4 days of cell culture. In spinners, pH of the media was daily adjusted to 7.2 to 7.4 with 1 NaOH during the virus production phase. Dissolved oxygen (DO) in the bioreactors was maintained at 50% air saturation and pH at 7.4 via using a hollow fiber membrane external gas exchange method. Glucose was measured daily and supplemented up to 1.5 g/L (in spinners) and 2 g/L (in bioreactors) when concentrations were ≤1 g/L. Daily 5 mL samples were retrieved from spinners and bioreactors to monitor cell density on microcarriers, access cytopathic effect (CPE) after virus inoculation, and for plaque assays to measure virus infectivity (PFU/mL) up to 12 days post-infection.

*ProNectin F is a trademark of Sanyo Chemical Industries ltd.; GlucCell is a trademark of CESCO BioProducts.

Results

The developed medium supports sustained, high-density cell growth on multiple types of APF SoloHill® microcarriers over a seven day expansion cycle without need for medium exchange. Vero cells achieved cell densities of >3 M cells/mL and maintained cell growth for 8-9 days. In mock infections, the medium enables cell densities of up to 6 M cells/mL in bioreactors. Virus production was demonstrated to be equal to or greater than DMEM containing FBS and two different commercially-available serum-free media. The peak of wild type Dengue 2 virus production advanced up to 3 days earlier in microcarrier culture when compared to static conditions, and cumulative titer was increased.

Results (Continued)

Figure 1
Benefits of microcarrier-based vaccine production. For anchorage dependent cell types, microcarriers offer a three-dimensional (3D) surface with a larger surface area to volume ratio. This supports dense systems at a larger scale with a relatively small footprint in the manufacturing suite. Sartorius SoloHill® solid core microcarriers are designed to meet the challenges faced by today's cell and tissue culture researchers. Whether moving an existing process out of a 2D system or scaling up a new process, SoloHill® microcarriers offer solutions to meet your needs.

Flat Surface Versus Microcarrier Culture

Platform	Surface area per unit [cm^2]	Number required	Media [L]
HyperFlask (10 tray)**	1720	60	34
Roller bottle**	1750	59	21
Cell Factory (10 tray)*	6320	16	32
Microcarriers¥		1¥¥	10

Growth area of 103,000 cm^2. *Nunc, **Corning, ¥ 280 g Sartorius SoloHill® standard or 200 Hillex® II microcarriers, ¥¥ PadReactor® Mini bioreactor

Microcarrier Type	Charge	Relative Density	Bead Diameter (microns)	Surface Area [cm^2/gram]
Hillex® II	Yes	1.11	160-180	515
Plastic	No	1.02 & 1.04	125-212	360
Plastic Plus	Yes	1.02 & 1.04	125-212	360
ProNectin F	No	1.02 & 1.04	125-212	360
New! Star-Plus	Yes	1.02	125-212	360

Core material is crosslinked modified polystyrene and selections shown are animal protein free

Figure 2
Cell growth on APF microcarrier types in spinners at 10 cm^2/mL using different SFM formulations. Cell growth in 2 different SFM was evaluated using four Sartorius SoloHill® APF microcarrier types in spinner cultures (Figure 2). Cell densities on all four microcarrier types tested were equivalent to or greater than densities reached in serum-containing medium (data not shown).

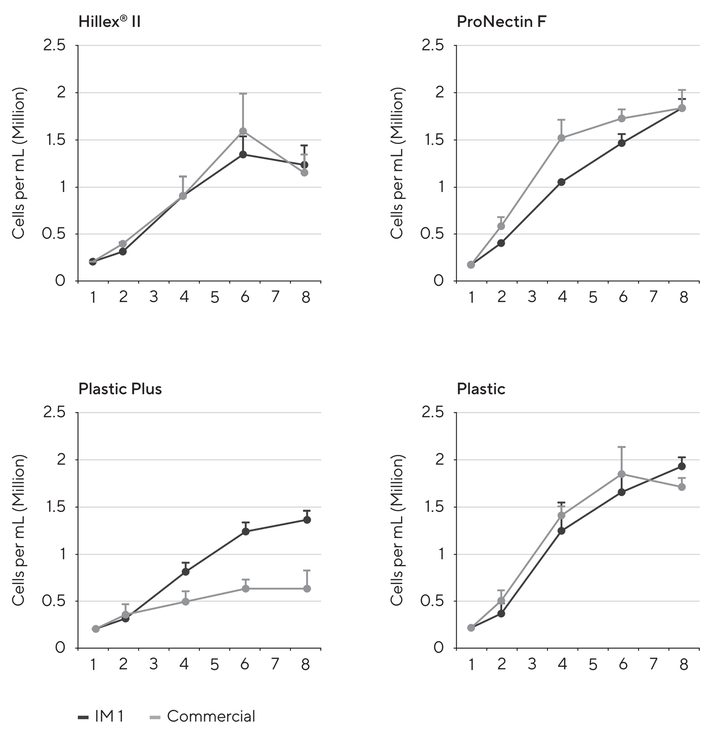
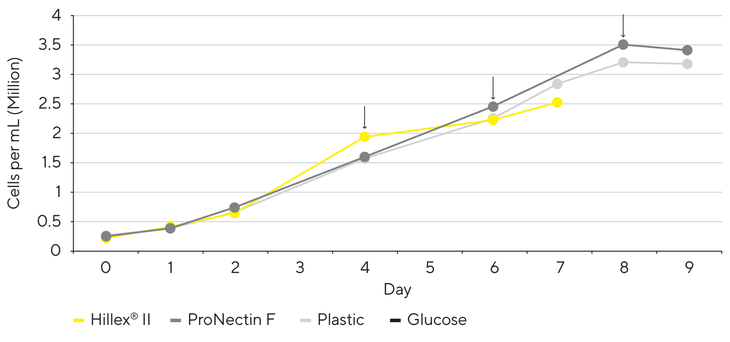


Figure 3
Cell growth on APF microcarrier types in bioreactor cultures containing IM 1 SFM. Black arrows represent glucose feed to the culture. Cell numbers in glucose-supplemented cultures reached 2.5 to 3.5 million cells/mL. Maintenance of glucose above 1 g/L was sufficient to achieve excellent cell growth with these three SFM formulations with all microcarrier types tested.



Results (Continued)

Figure 4
Wild-type dengue virus production (WT DEN2 16681) in IM 1 SFM, at 10 cm^2/mL microcarrier density in spinners and T-flask cultures. Active wild-type Dengue virus was first detected in the medium from microcarrier cultures on day 3 post-infection, and levels peaked on day 4. Viral expression was maintained for 5 additional days but titers were lower at later time points.

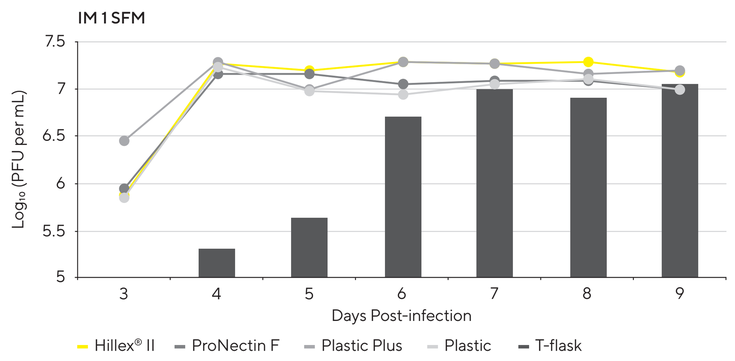


Figure 5
Wild-type Dengue virus (DEN2 16681) production on different microcarrier types (top) and in different medium formulations (bottom) in IM 1 SFM. Expression was evaluated in 13 cm^2/mL bioreactor cultures. All three microcarrier types tested performed equally well when virus expression in IM 1 medium was quantified. Measurable virus expression was detected as early as day 1 post-infection and levels increased to 6.5 to 7.5 Log_{10} PFU/mL per day at later time points (day 4 through 10).

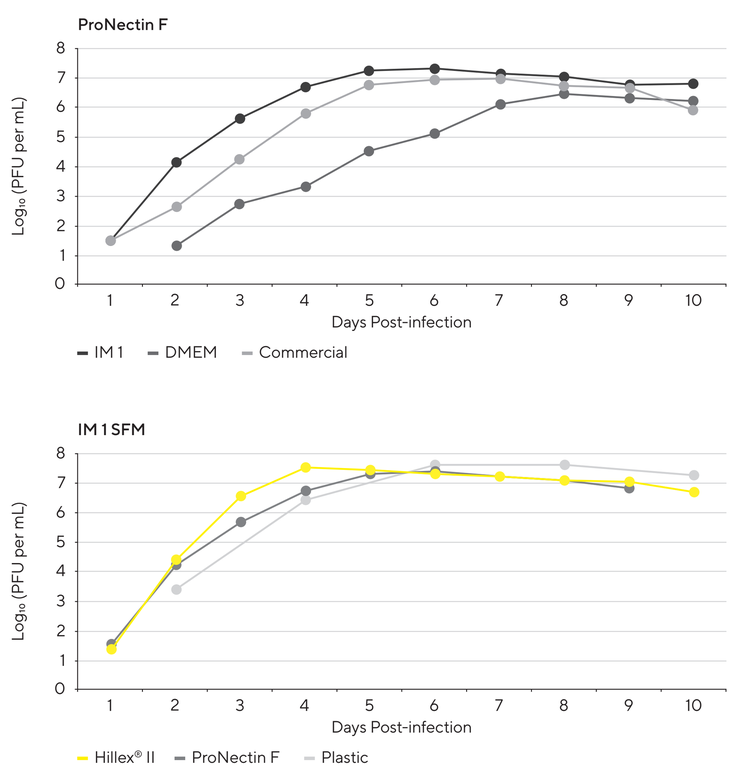
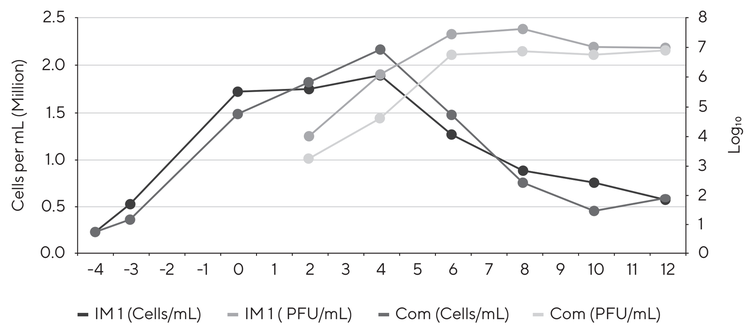


Figure 6
Attenuated Dengue virus (D2/IC-VV45R) production in SFM in 10 cm^2/mL ProNectin F microcarrier spinner cultures. Com; commercial SFM. Virus expression was detectable in both media on day 2 post-infection; expression peaked on day 6 and was maintained at or near peak levels for the remainder of the culture period, demonstrating that ProNectin F microcarriers paired with SFM formulations support high viral expression over a 12 day period.



Conclusions

- Microcarriers provide an economical and robust platform for vaccine production using adherent cell types.
- The microcarrier-based animal component free platform described here is ready to be implemented for large scale vaccine production reactors.
- All SFM media support excellent viral production with SoloHill® microcarriers in stirred-tank cultures.
- Wild-type and attenuated dengue viruses were expressed three days earlier and with higher overall titers in microcarrier cultures when compare to flatware.
- Vero cells achieve densities of >3 to 6 million cells/mL without a medium exchange in batch culture, and maintained cell growth for 8 to 9 days.