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BIOPROCESSING

Tutorial

Scalable Production of Influenza Virus

Biomanufacturing on Adherent Cells Using a Single-Use Stirred-Tank Bioreactor Process

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The future of influenza vaccine production most likely lies with cell culture-based processes. Cell-based systems offer numerous advantages over traditional egg-based production, including speed of production, lack of dependence on egg supply, avoidance of egg allergens, higher initial purity, and ability to combine upstream and downstream into an automated process.

Cell culture is well suited to rapid pandemic response because time-consuming recombination of the virus into an egg-adapted flu strain is not required. Cell-based processes can therefore produce large quantities of vaccine in a relatively short period of time, provided they can scale up efficiently.

Growth of influenza virus in single-use bioreactors and methods for scaling up production are the topics of this article.

Influenza virus is typically grown on adherent cells such as the Madin-Darby canine kidney (MDCK) cell line. These types of cells have traditionally been cultured using 2D tissue culture flasks or roller bottles that are inefficient to scale to industrial volumes. The Mobius® 3L single-use stirred-tank bioreactor is a benchtop vessel suitable for cell-based vaccine process development. This tutorial focuses on the optimization of an MDCK cell-based influenza production process using Cytodex® 3 and Cytodex 1 microcarriers in a Mobius 3L single-use stir tank bioreactor and scale-up of this process to a Mobius 50L bioreactor (*Figure 1*).

Efficient viral production in a stirred-tank bioreactor requires optimization of numerous parameters, including cell attachment to the microcarriers, suspension of the microcarriers, cell growth on the microcarriers, virus infection of the cells, and viral output. Baffled shake flasks (125 mL with 40 mL working volume)

Figure 1.
Mobius® 3 L and 50 L single-use bioreactor systems

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were found to be predictive of stirred-tank performance and were used for preliminary experiments. The parameters tested and the optimal conditions found are shown in *Table 1*.

Cell Attachment

Previous practices for cell attachment to microcarriers recommend using a reduced media volume to increase microcarrier density and intermittent agitation to produce cycles of microcarrier suspension and settling. However, this complex regimen requiring programmed agitation cycling and an additional media feeding was found to be unnecessary to achieve optimal microcarrier attachment. Efficient attachment was achieved by inoculating the Mobius 3 L bioreactor with 4 g/L of Cytodex 3 or Cytodex 1 microcarriers and MDCK cells at a density between 2×10^5 and 3×10^5 cells/mL in the full working volume of 2.0 L media.

The bioreactor was simply agitated continuously at 90 rpm for 24 hours to complete attachment. The media used for both attachment and cell growth was Dulbecco's Modified Eagle's medium (DMEM) with glucose with 10% fetal bovine serum (FBS), 4mM L-glutamine, 1% nonessential amino acids (NEAA) and 1% sodium pyruvate.

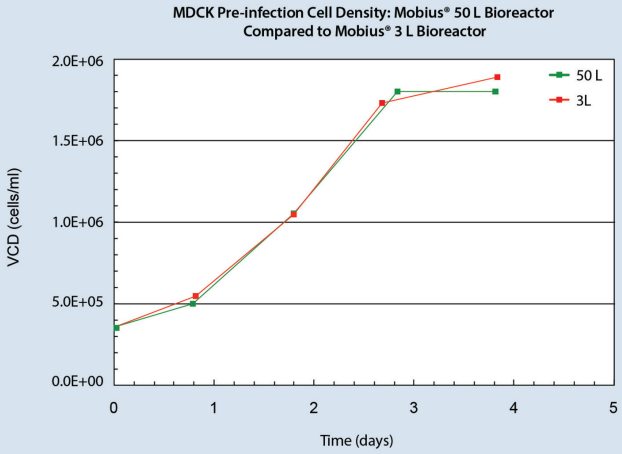

Figure 2. MDCK cell growth at 3 L and 50 L scales. VCD: viable cell density.

Table 1. Optimization of Cell-Based Flu Production at 3 L Scale

Parameter	Condition Tested	Optimal Condition
In Baffled Shake-Flask Format		
Inoculation density (cells/mL)	$1x10^5$, $2x10^5$, $4x10^5$, $8x10^5$	$2x10^5 – 3x10^5$
Microcarrier density (g/L)	2, 4, 6	4
Multiplicity of infection (pfu/cell)	1×10^{-4} , 5×10^{-4}, 1×10^{-3}	5×10^{-4}
Time of infection (days after cell inoculation)	2, 3, 4	4 (or cell density > 2×10^6 vc/mL)
In Mobius® 3 L Bioreactor		
Agitation speed (rpm)	75, 90, 150	90
Starting volume (L)	1.0, 1.6, 2.0	2.0
Sparging	O_2 open pipe, O_2 microsparger, Air/O_2 microsparger	O_2 open pipe

Cell Growth

Agitation speed is a critical parameter for cell growth on microcarriers. As cells grow, the microcarriers will gain mass and tend to settle if undersuspended. However, overly vigorous agitation can cause deleterious sheer stress on the cells. Experimentation revealed that 90 rpm was the optimal agitation speed for cell growth. This speed equates to an impeller tip speed of 35.9 cm/s and a power input of 1.3 W/m^2 in the Mobius 3L bioreactor.

Sparging strategy is another important consideration. Sparging needs to supply enough oxygen to support viable cell growth, but excessive sparging will impart shear stress on culture. The Mobius 3L bioreactor can be used with either a microsparger or open O_2 pipe. The latter was found to be optimal, avoiding the foaming and sheer stress on the cells caused by the microsparger.

Cells were grown for four days at 37°C to a density of 2×10^6 viable cells (vc)/mL. The culture was automatically controlled to maintain dissolved O_2 (DO) at 50% ± 10% and pH at 7.0 ± 0.2 throughout the cell growth phase and the subsequent infection phase.

Virus Infection

Correctly timing the infection of the culture is important for maximizing virus production. The optimal time for infection was found to be at ~4 days of culture growth, when the cells had reached a density of between 2×10^6 and 3×10^6 vc/mL. At this time, agitation was turned off so that the microcarriers could settle, and the reactor was drained to 20% volume using the upper harvest line.

The reactor was then re-fed with virus production media to full 2.0 L volume using a top feed line. The production media (DMEM w/glucose + 4 mM L-glutamine, 1% NEAA, and 1% sodium pyruvate) contained influenza virus (strain A/WS/33) at a multiplicity of infection (MOI) of 5×10^{-4} pfu/cell. Agitation was then resumed at 90 rpm and the temperature reset to 33°C (the optimal growth temperature for this viral strain).

After 72 hours, the virus was har-

vested. Typical viral harvest titers were between 2.0 x 10^4 to 4.5 × 10^4 hemagglutination units (HAU)/mL.

Large-Scale Cell Attachment

The 3L protocol was scaled up for MDCK cell growth in a Mobius 50L bioreactor using Cytodex 1 microcarriers. Some adjustments for the larger scale were empirically determined. Optimal cell attachment in the reactor was performed at 25 L working volume with an impeller speed of 70 rpm (~40 cm/s).

An additional consideration was that the Mobius 50L employs an external pump to circulate the culture through the SensorReady® loop to perform in-line sampling of pH and DO for feedback control. During the inoculation step the SensorReady pump speed was set to 350 rpm to allow for cell attachment and feedback control of pH and DO. Once cells were observed to be fully attached, the SensorReady pump speed was increased to 700 rpm to prevent settling of microcarriers and cells during the growth phase.

Large-Scale Cell Growth

For cell growth in the 50L (25L working volume) bioreactor, agitation at 70 rpm was optimal for suspension of the microcarriers. Sparging in the 50L was performed using the microsparger with both air and oxygen, which kept foam accumulation to a minimum during the growth phase without the use of antifoam. Cells were grown for four days at 37°C to a density of 1.8 × 10^6 vc/mL. DO and pH control were the same as 3L.

These methods produced very similar cell growth kinetics between the 3L and 50L scales (*Figure 2*).

This study demonstrated that Mobius bioreactors are a suitable platform for scalable growth of adherent cells and viral vaccine production. The Mobius single-use bioreactor line also includes 200L and 2000L sizes, and future work will extend these protocols to these larger scales.

Also underway are studies to establish methods for using the smaller bioreactors to seed the larger, avoiding the need to use large quantities of 2D flasks to generate the cell seed. GEN