Development of suspensions adapted Vero cell culture process for production of viruses

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Development of Suspension Adapted Vero Cell Culture Process for Production of Viruses

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Background

- Vero cells are considered as the most widely accepted continuous cell line by the regulatory authorities (such as WHO) for the manufacture of viral vaccines for human use.

- The continuous Vero cell line has been used, after propagation on microcarriers, for the production of rabies, polio, enterovirus 71 and hantaan virus vaccines.

- The Vero cell line has gained worldwide acceptance as cell culture production platform in the vaccine field.

- The Vero cell culture technologies were explored for productions of many more anti-viral vaccines in the last two decades.
The growth of Vero cell is anchorage-dependent. They are labor intensive and limited in scale-up production when the Vero cell is grown in traditional two-dimensional culture methods and in microcarriers.

**Current Vero Cell Culture Technologies**

- **Traditional two-dimensional culture technique**

- **Microcarrier cell culture technology**
The growth of Vero cell is anchorage-dependent. They are labor-intensive and limited in scale-up production when the Vero cell is grown in traditional two-dimensional culture methods and in microcarriers.
Limitations of Anchorage-dependent Cell Culture Technologies in process scale-up

- Cells are dissociated enzymatically or mechanically, labor intensive.
- Growth is limited by surface area, which may limit product yields.

Vaccine manufacturing process using adherent cells in single-use bioreactors (Vero, 200-L scale) Calvosa & Seve. US 20110151506 A1
Suspension Cell Culture

- Subculturing is a simple matter of dilution.
- There is little or no growth lag after splitting a suspension culture.
- Scale-up is straightforward.

High-scale virus production process overview with single-use bioreactor systems using suspension EB66 cells at 200-L scale (Madeline et al. 2015)
Media for anchorage Vero cell culture:

- Serum-containing media: costly, lot-to-lot inconsistency, threat of contamination.

- Serum-free media:
  - VP-SFM (Life technologies)
  - OptiPro SFM (Life technologies)
  - EX-CELL Vero SFM (SAFC Biosciences)
  - ProVero (Lonza)
  - HyQ SFM4MegaVir (Hyclone)
Benchmark of Commercial Serum-free Media for Vero Cell Cultures

Performance Indicators of culture medium

- Maximum cell density in batch culture
- Cell doubling time
- Virus productivity

Comparison of Vero cell growth on Cytodex 1 microcarriers in five different serum-free media (Chen et al. 2011)
Current status of commercial serum-free media for Vero cell

Sum up:

• No commercial serum-containing or serum-free media is available for suspension Vero cell.

• Maximum cell density of adherent Vero cell achieved in microcarriers batch culture is generally lower than $2.5 \times 10^6$ cells/mL.

• Doubling time of adherent Vero cell in microcarrier culture is 45 hours or longer.
Overview of Suspension Adapted Vero Cell

- Advantage and benefit of suspension Vero cell culture process over adherent culture in large-scale manufacturing.

  ✓ A Vero cell line (ATCC CCL-81 origin) has been adapted to grow in suspension in an in-house developed serum-free medium.

  ✓ The adapted cell was found genetically stable by short tandem repeat analysis.

  ➢ Tumorigenicity test of suspension adapted cell is in progress.

Shake flask batch culture of suspension adapted Vero cell in serum-free and animal component free medium
Adaptation of Vero cell to grow in suspension and serum-free media at NRC Montreal

Suspension adapted Vero cell cultured in 125 mL shake flask and observation of culture under a microscope.
Comparison on virus productivity between adherent and suspension adapted Vero cells

- Production of vesicular stomatitis virus (VSV) in adherent (T175) and suspension adapted (SF, 125 mL shake flask) Vero cell cultures with medium replacement before the viral infection.
- The suspension adapted cell showed better productivity than the adherent one.

- Influenza virus was used as another model to investigate the virus productivity of suspension adapted Vero cell.
- The suspension adapted cell seems to have lost some productivity of hemagglutinin (HA).
Production of VSV in a 3L bioreactor perfusion culture

- The cell density in the bioreactor reached 6.8x10^6 cells/mL after 2 days of perfusion at 0.5 vvd and another day at 1 vvd before the virus infection (A).

- The VSV titer in the bioreactor culture was similar to that achieved in the shake flask control cultures infected at 6x10^6 cells/mL and was nearly one log higher than that in the reference culture infected at 1x10^6 cells/mL (B).