

Virus Production in Vero Cells Using a Serum-Free Medium

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Abstract

The manufacture of viral vaccines has historically been accomplished using animal products such as chicken eggs, or cell cultures using fetal bovine serum. To reduce regulatory concerns in vaccine production, serum-free cell culture processes are being embraced by the vaccine industry. The Vero cell line initiated from the African green monkey is an excellent cell line for the production of animal and human prophylactic viral vaccines. We have developed a serum-free (SF) and animal-component free (ACF) medium for the production of viral vaccines using the Vero cell line. This medium supports growth of Vero cells on microcarriers in a controlled bioreactor environment and virus production equivalent to serum-containing cultures. These characteristics make this an ideal medium for vaccine production using the Vero cell line.

Introduction

The Vero cell line, isolated from the kidney of a normal adult African Green monkey (*Cercopithecus aethiops*), has been well characterized and is instrumental in the biotechnology sector for virus replication studies, viral plaque assays, TCID₅₀ determinations and production of viral vaccines.

JRH Biosciences (Lenexa, Kansas USA) has developed a serum-free medium specifically for the Vero cell line. EX-CELL™ Vero is serum-free and free of animal-derived components. The medium contains a plant-derived hydrolysate and low levels of recombinant proteins, but does not contain phenol red or Pluronic® F68. In these studies, we show that EX-CELL™ Vero supports high-density cell growth in both stationary flasks and on microcarriers in bioreactor culture.

Materials and Methods

Cells
 Vero, *Cercopithecus aethiops* (monkey, African green), American Type Culture Collection, ATCC Number CCL-81

Virus
 Herpes Simplex Virus 2 (HSV-2), American Type Culture Collection, ATCC, Number VR-540

JRH Media and Supplements

- EX-CELL™ Vero Serum-Free Medium for Vero Cells, JRH Catalog No. 14585
- Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High), JRH Catalog No. 51444
- Fetal Bovine Serum (FBS), JRH Catalog No. 12103
- L-Glutamine Solution 200 mM, JRH Catalog No. 59202
- Trypsin-EDTA Solution 1X (0.25% trypsin, 0.1% EDTA, trypsin gamma irradiated by SER-TAIN™ Process), JRH Catalog No. 59429
- Dulbecco's Phosphate Buffered Saline (DPBS Modified), JRH Catalog No. 59321
- Pluronic® F68, JRH Catalog No. 59915

Other Media and Supplements

- Trypsin inhibitor from Glycine max (soybean) (STI), Sigma-Aldrich Co. (St. Louis, Missouri USA), Product No. T6522

Bioreactor Supplies

- 3 L stirred tank bioreactor, Applikon, Inc. (Foster City, California USA)
- Cytodex™ 1 microcarriers, Amersham Biosciences (Piscataway, New Jersey USA), Catalog No. 17-0448
- Crystal Violet, Sigma-Aldrich Co., Product No. C3886

Results and Discussion

Vero cells maintained in DMEM/High with 5% FBS were seeded in triplicate 75 cm² T-flasks at 5 x 10⁶ cells/cm² in a total volume of 20 mL EX-CELL™ Vero (supplemented with 4 mM L-glutamine). The flasks were subcultured every three days for five passages. The seeding density was then reduced to 2 x 10⁶ cells/cm² (maintenance seeding density) and the flasks were passaged every four days. The figure illustrates the growth of Vero cells in EX-CELL™ Vero for six passages during adaptation from serum-containing basal medium. Viability remained 95% or greater during adaptation (Figure 1).

Vero cells were seeded in triplicate 25cm² T-flasks at 2 x 10⁶ cells/cm². Cells were harvested from flasks and viable cells were counted daily for seven days. Culture viabilities remained above 95% in all media during the study (Figure 2).

Vero cells were inoculated in Applikon stirred tank bioreactors at a 1 L working volume. Cytodex™ 1 microcarriers were hydrated, sterilized and acclimatized to each medium per the manufacturer's directions. Cells were seeded at 20 cells per microcarrier (approximately 4 x 10⁶ cells/mL). The total concentration of microcarriers was 3 g/L. Figure displays cell attachment at Day 0 and Day 4 (Figure 3).

Vero cells were inoculated in Applikon stirred tank bioreactors at a 1 L working volume. Reactor temperature was set to 37 °C, the agitation speed was 70 - 85 rpm, dissolved O₂ was maintained at 50% and pH was maintained at 7.0 - 7.3 with CO₂. Reactors were monitored and samples obtained daily for seven days; cell growth was determined by counting released nuclei using a crystal violet staining procedure. Cells grew to confluence achieving maximum densities of approximately 1.9 x 10⁶ cells/mL (~1.5 x 10⁶ cells/cm²) in EX-CELL™ Vero and 1.4 x 10⁶ cells/mL (~1.1 x 10⁶ cells/cm²) in competitor's medium (Figure 4).

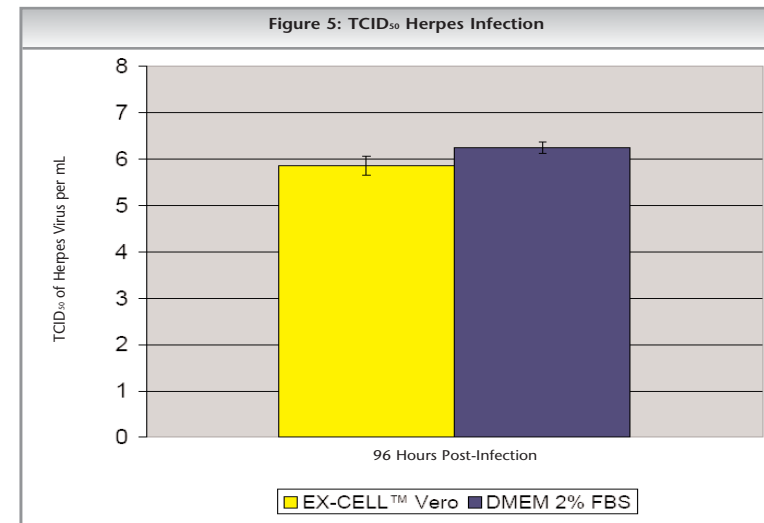
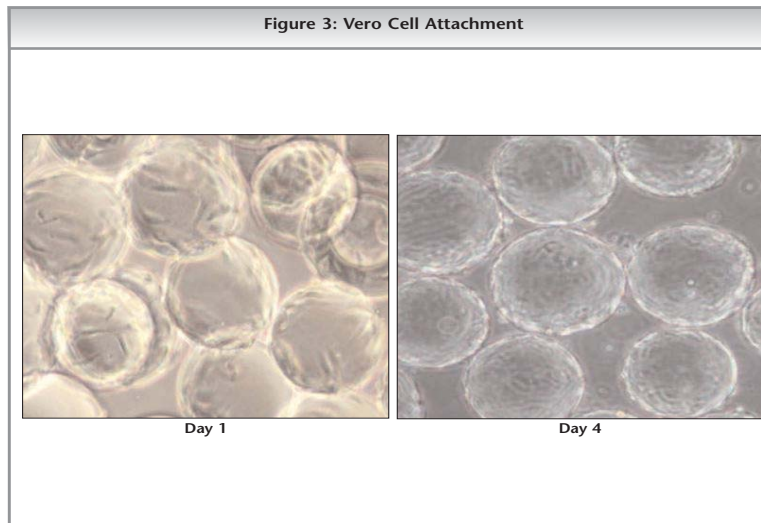
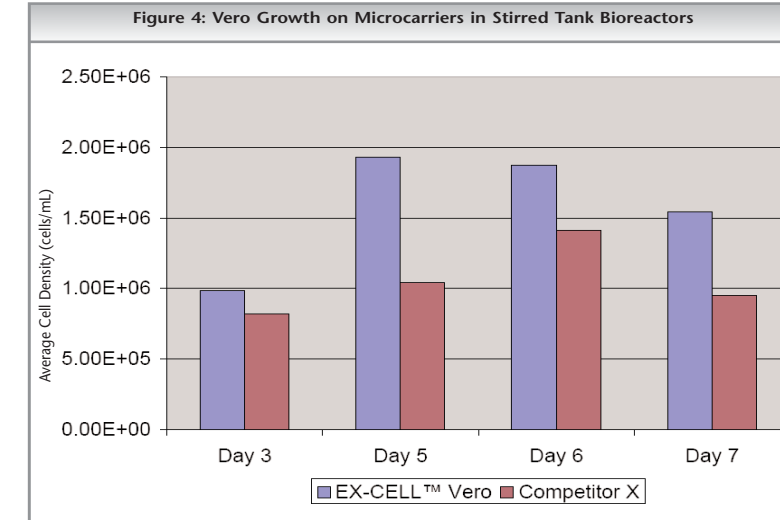
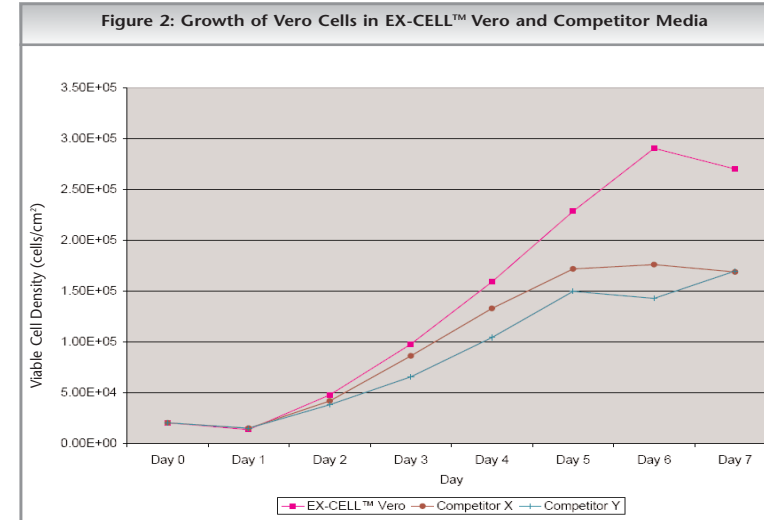
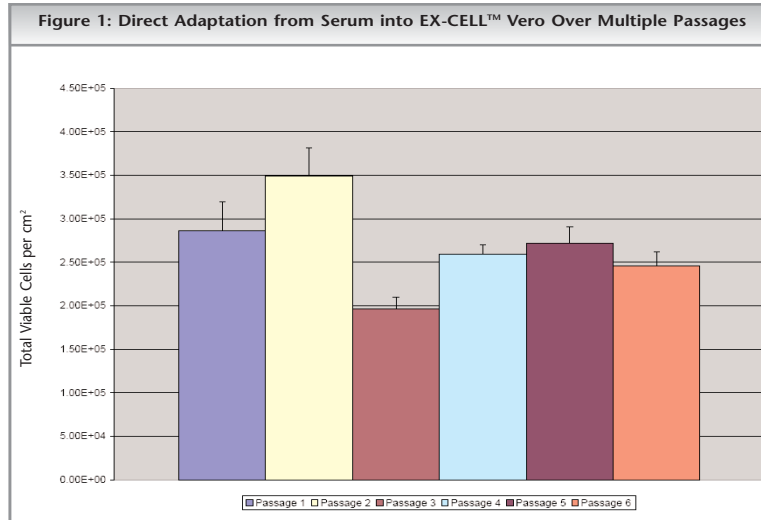
Vero cells were infected with HSV-2 96 hours post-seeding at a multiplicity of infection (MOI) of 0.01. Flasks displayed greater than 75% cytopathic effect (CPE) 96 hours post-infection. Flasks were harvested and results represent TCID₅₀/mL at harvest point (Figure 5).

Conclusion

EX-CELL™ Vero is a serum-free medium designed and optimized for high-density Vero cell growth in adherent-stationary and adherent-suspension conditions. EX-CELL™ Vero is a regulatory-compliant medium, free from all animal-derived components and contains only recombinant proteins. These studies indicate that adaptation to EX-CELL™ Vero from basal medium containing serum can be easily accomplished and EX-CELL™ Vero supports high-density Vero cell growth, superior to competitor formulations.

EX-CELL™ Vero supported HSV-2 production in Vero cells. The cells exhibited classic CPE in culture and produced HSV-2 titers in the range of 10⁶ TCID₅₀/mL, comparable to serum-supplemented cultures.

For further information regarding EX-CELL™ Vero, please call our Technical Services department or e-mail us at technicalservices@jrhbio.com.



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