SARTORIUS

Simplifying Progress

Development of Chemically Defined Medium for Vero Cells

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Introduction

Cell culture media, including currently available animal component free media, contain undefined polypeptides. To optimize the cell and virus culture it is desirable to know which nutrients are depleted at the different cultivation phases. However, media containing undefined ingredients make this more challenging. Therefore, a chemically defined medium is needed. In collaboration with Biological Industries the development of a chemically defined (BiCD) medium for the growth of Vero cells, and subsequent virus production, was perused. The developed medium is chemically defined and animal component free, it supports Vero cell growth in static and bioreactor cultures and is able to support propagation of different type of viruses.

Methods and Results

During development Vero cells were cultured in several media formulations and compared with a commercially available animal component free (ACF) culture medium. After each set of experiments the formulations were adjusted. As the media formulations improved the culture methods also advanced, from static T-flask culture to Spinner cultures (Figure 1) and finally bioreactor cultures (Figure 2). Primary parameters used for Vero cell growth evaluation were microcarrier coverage, maximum cell density and growth rate.

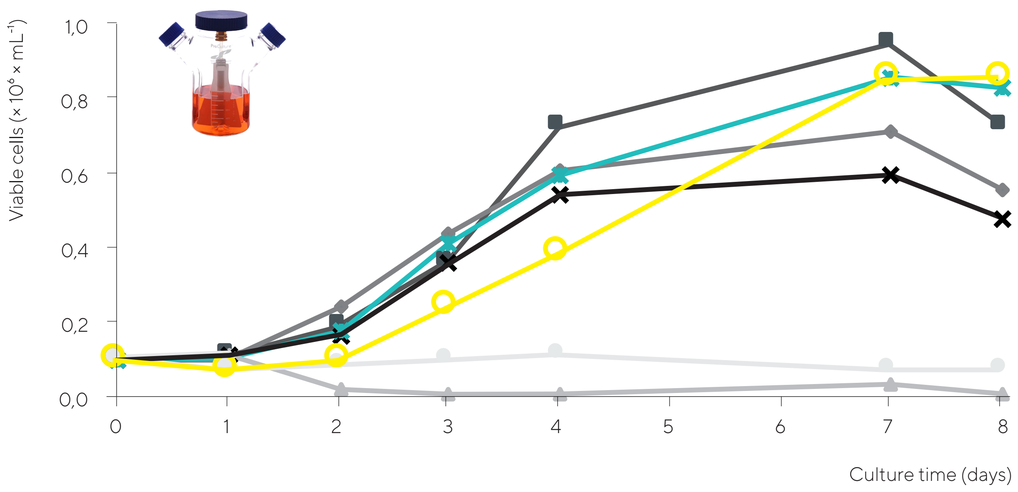

Figure 1: Growth curve of Vero cells on microcarriers cultured in Spinner flasks in 6 different chemically defined media formulations (BiCD-A to F). The line with yellow open circles represents the growth in the commercial ACF media.

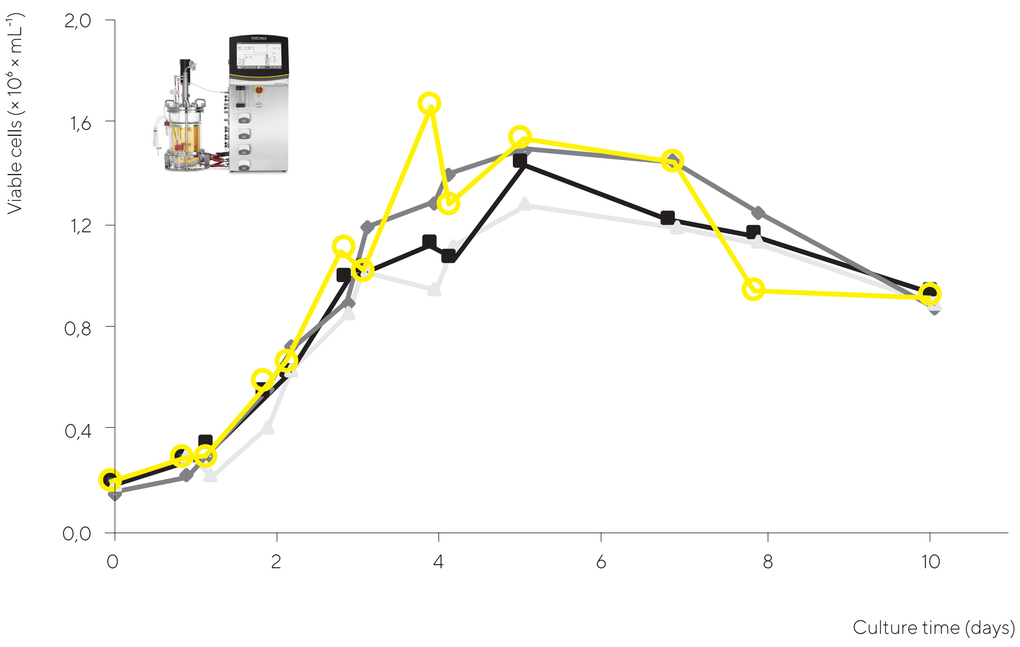

Figure 2: Growth curve of Vero cells on microcarriers cultured in bioreactors in 3 different chemically defined media formulations (BiCD-J, Q, R). The line with yellow open circles represents the growth in the commercial ACF media.

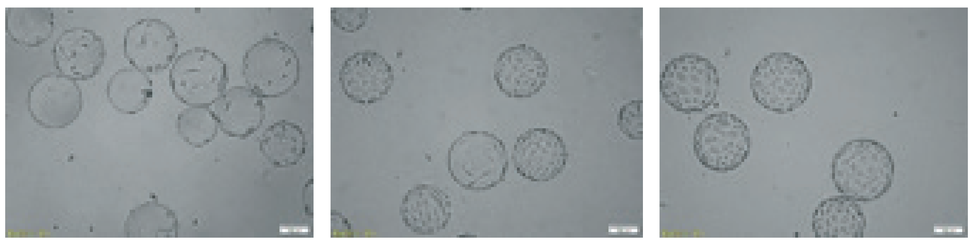

Figure 3: Progression of Vero cell growth on microcarriers in a bioreactor with a chemically defined medium (BiCD-J). From left to right, after 1, 3 and 5 days of culture.

Virus Propagation

Two different media formulations (BiCD-J and BiCD-K) were selected to evaluate the capability to support virus propagation. Cells were infected with Measles, Enterovirus 71-C4 and Sabin type 1 poliovirus.

The differences in titers observed using the various culture media indicate that the nutricial needs may differ between viruses.

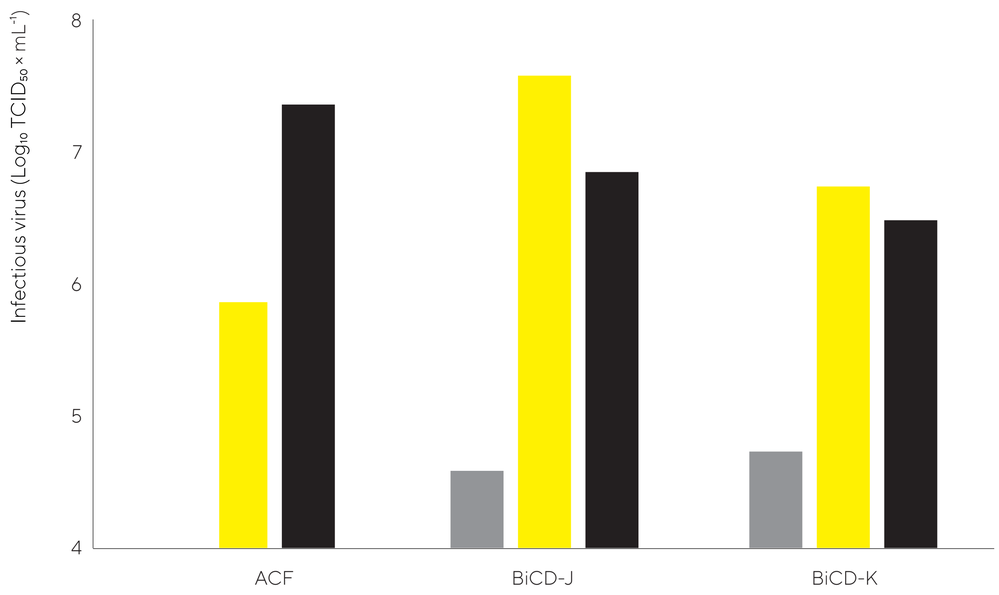

Figure 4: Infectious virus titers calculated for Measles (grey), Enterovirus 71-C4 (yellow) and Sabin type 1 poliovirus (black) replicated on Vero cells growth in different culture media formulations. The infectious virus titer for Measles on ACF was below the limit of quantification.

Chemically defined BiCD-J showed promising results in bioreactor cultures (N=3). Microcarriers were fully and equally covered after 5 days of culture and maximum cell concentration (C_{max}) was 1.5×10^6 cells × mL^{-1} with a specific growth rate (μ) of $0.026h^{-1}$. These results are similar as the maximum cell concentration and a specific growth rate in commercial ACF medium were C_{max} was 1.4×10^6 cells × mL^{-1} and $\mu=0.025h^{-1}$.

The BiCD-J formulation also showed good results as a propagation medium for different viruses, three cultures in bioreactor vessels were performed where after 3 days the culture was inculcated with Enterovirus 71-C4. Infectious virus titers calculated from these cultures, 74 hours post infection, were 6.8 ± 0.1 $log_{10}TCID_{50} \times mL^{-1}$ for EV71-C4 cultured on cells grown in BiCD-J media. In comparison, cells grown in commercial ACF medium supported a titer of 6.7 $log_{10}TCID_{50} \times mL^{-1}$ for EV71-C4.

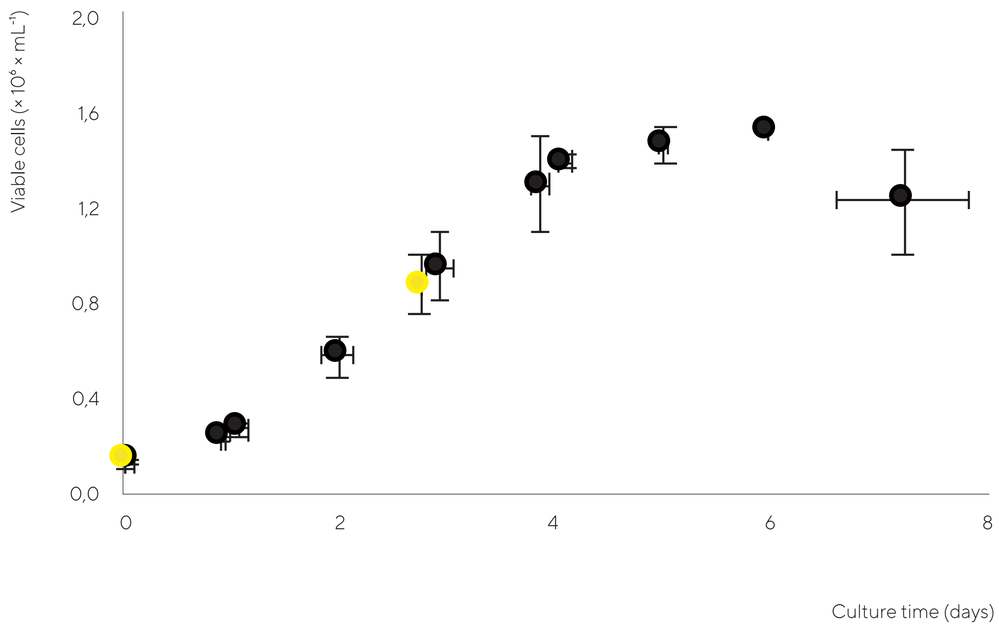

Figure 5: Growth curve of Vero cells cultured in bioreactors with chemically defined media formulation BiCD-J (Black, N=3). Yellow circles represent Vero cells cultured in bioreactors with BiCD-J media to which EV71-C4 was added after 3 days.

Conclusion

BiCD-J is a new developed, fully chemically defined and animal component free culture medium which supports Vero cell growth in static and in bioreactor cultures and is able to support propagation of different types of viruses.

In addition, yields for Vero cell growth and infectious virus particles are equal to a commercial available ACF medium containing less well-defined plant extracts. Biological Industries will refer to this medium as NutriVero™ Flex 10.