Vero cell growth and EV71_C4 replication capacity in small-scale customizable single-use bioreactors

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Abstract

Single-use bioreactors have been increasingly used for animal cell culture in the biopharmaceutical industry. The interest for these systems lays in the considerable reduction of cross-contamination risk, the elimination of cleaning-in-place and sterilization-in-place, no need for cleaning validation, the decrease in production turnaround times and a reduction in validation time which shortens time to market. In the present work, a Vero cell line used to produce viral vaccines was used by Intravacc to perform the cell and virus cultivations in Applikon's newly developed small-scale customizable single-use bioreactors. The growth curves of Vero cells, were compared with the growth curves of Vero cells growing in conventional autoclavable glass bioreactors under the same conditions and in the same culture volume. Subsequently, a virus for which vaccines are needed, EV71_C4, was grown on Vero cells. The single-use bioreactors are suitable for Vero cell culture and EV71_C4 virus propagation because there was no difference with respect to Vero cell culture and EV71_C4 virus culture between the glass bioreactor and disposable bioreactor. Thus the small-scale customizable single-use bioreactor holds promise for future production of viral vaccines.

Figure 1 | Single-use and autoclavable glass mini-bioreactor (Applikon Biotechnology).

Materials and Methods

- A single-use and autoclavable glass 500 mL mini-bioreactors, presented in Figure 1 (Applikon Biotechnology, Delft - The Netherlands), were used to grow Vero cells, under the same culture conditions.
- The mini-bioreactors configuration consisted of 1 marine impeller, 2 overlay inlets used for gas inlet and outlet (pipe for gas outlet had a wider diameter than the inlet), a sampling pipe, an addition pipe and a temperature pocket. The autoclavable pH and dO_2 sensors were used in both systems. In the autoclavable glass mini-bioreactor the sensors were autoclaved together with the bioreactor, whereas in the single-use, the sensors were autoclaved separately, and assembled afterwards on the mini-bioreactor inside the laminar flow cabinet.
- As with the autoclavable mini-bioreactor, the single-use unit is a cylindrical vessel with a hemispherical base reported by previous authors to provide shorter mixing times due to better circulation patterns of the liquid, than a cylindrical vessel with a flat base.

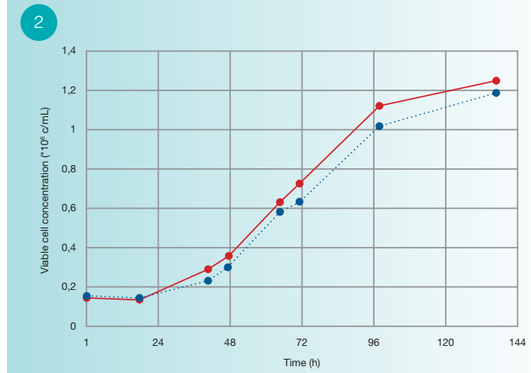

Figure 2 | Growth curves of Vero cells in the single-use bioreactor ● and in the autoclavable glass bioreactor ●

- Vero cells were cultured on microcarriers at 37 °C in VP-SFM medium supplemented with 2 mM glutamine in a culture volume of 250 mL. The initial cell concentration was $0.15*10^6$ Vero cells/mL for both bioreactors.
- Samples were taken daily to analyze the cell concentration, morphology and distribution of the Vero cells on the microcarriers.
- The specific growth rate (μ) of the cells was calculated using a standard exponential fit through the growth curve.
- For the virus culture, Vero cells were infected with the EV71-C4 virus at an MOI of 0.001 when cell concentration reached $0.8*10^6$ – $1.0*10^6$ cells/mL.
- Additional samples were taken to determine the virus titer using CCID50 assay and to monitor CPE with microscopy.

Results and discussion

Vero cell growth and morphology

The growth of Vero cell culture in the single-use and the autoclavable glass bioreactor was monitored for 137 hours, as shown in Figure 2.

The maximal viable cell concentration and growth rate results were comparable in both bioreactors as can be seen in Table 1.

- The distribution and proliferation of the Vero cells on the microcarriers in the single-use and the autoclavable glass bioreactors was observed by light microscopy. Pictures are shown in Figures 3 and 4.
- Figures 3 and 4 show that after the day 1 of culture, cells were attached to the microcarriers and most of the microcarriers contained cells. At day 4, the microcarriers were fully covered by Vero cells and little cell debris were observed. These pictures show there is no difference in distribution and proliferation of Vero cells growing on microcarriers in the two different types of bioreactors.

Table 1 | Yield and specific growth rate for Vero cell culture obtained in the two different bioreactors.

Vero cell culture	Maximal viable cell concetration (cells/mL)	μ (h^{-1})
Glass bioreactor	$1.02*10^6$	0.026
Single-use	$1.12*10^6$	0.027

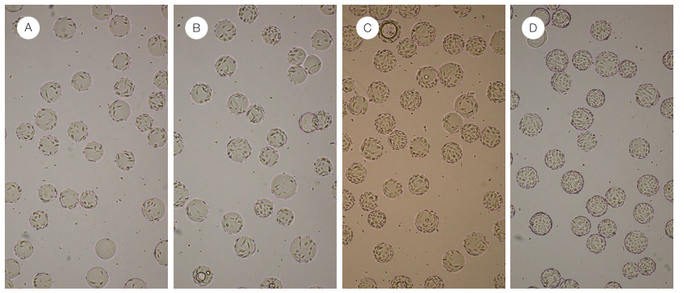

Figure 3 | Vero cells growing on microcarriers as observed with light microscopy during cell culture in the single-use bioreactor. Pictures were taken at day 1 (A), day 2 (B), day 3 (C) and day 4 (D).

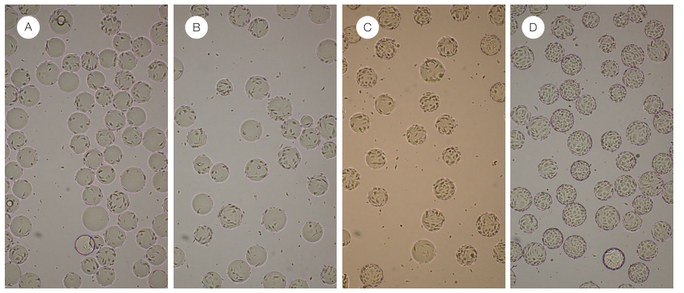

Figure 4 | Vero cells growing on microcarriers as observed with light microscopy during cell culture in the autoclavable glass bioreactor. Pictures were taken at day 1 (A), day 2 (B), day 3 (C) and day 4 (D).

EV71_C4 infection: morphology and virus titer

In a follow-up experiment, Vero cells were cultured for 74 h to a cell concentration of $0.88*10^6$ cells/mL ($\mu = 0.031\ h^{-1}$) in the single-use and $0.93*10^6$ cells/mL ($0.034\ h^{-1}$) in the autoclavable glass bioreactor.

After 74 h, cells were inoculated with the virus EV71-C4 at an MOI of 0.001 and samples were taken after 3, 4 and 5 days post infection to determine the amount of infectious virus in the culture medium (Table 2).

Figures 5 and 6 show the evolution of the cytopathic effect caused by the virus in the single-use bioreactors and autoclavable glass bioreactors respectively. For both bioreactors the observed cytopathic effects are comparable.

The maximum virus titers from both bioreactors, presented in Table 2, are within the standard deviation of the in-process virus titration assay method of 0.5 LOG10, which means that the titers for both reactors are comparable. Also, no significant differences in virus titer at the different time points were observed in both types of bioreactor.

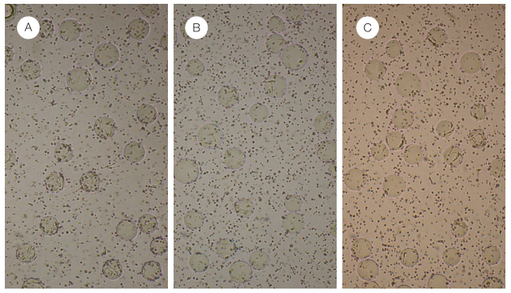

Figure 5 | Pictures of Vero cells during EV71_C4 culture in the single-use bioreactor on day 3 (A), day 4 (B) and day 5 (C) post infection.

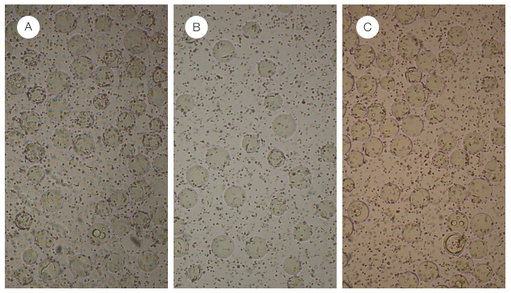

Figure 6 | Pictures of Vero cells during EV71_C4 culture in the autoclavable glass bioreactor on day 3 (A), day 4 (B) and day 5 (C) post infection.

Table 2 | EV71_C4 infectious virus titer in the single-use and the autoclavable bioreactors at days 3, 4 and 5 post infection.

EV71_C4 titer (LOG_{10} $CCID_{50}$ / mL)		
Day	**Single-use**	**Autoclavable**
3	7.10	7.60
4	7.30	7.10
5	7.20	6.80

Conclusions

- A new single-use bioreactor has been developed by Applikon Biotechnology and tested by Intravacc. The results showed that the single-use bioreactor is suitable for Vero cell culture and EV71-C4 virus culture.
- No differences were observed between the autoclavable glass bioreactor and single-use bioreactor when comparing the growth of Vero cells on microcarriers and the yield of EV71-C4 virus in both bioreactors.

References

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