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Characteristics of rVSV-ZEBOV production kinetics in HEK293 and Vero cells

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Characteristics of rVSV-ZEBOV production kinetics in HEK293 and Vero cells

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Introduction

The vesicular stomatitis virus (VSV) can be used as an effective vaccine platform, inducing both cellular and humoral immunity. Since VSV infections of humans are mostly asymptomatic, recombinant VSV (rVSV) can be used as a platform to safely deliver and express foreign antigens. This research study focused on cell culture production of an rVSV expressing the Ebola virus glycoprotein on its surface (rVSV-ZEBOV).

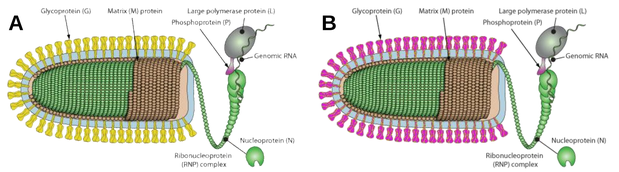


Figure 1: Schematic of VSV-WT (A) expressing the VSV-G glycoprotein (yellow) and rVSV-ZEBOV (B) expressing the Ebola virus glycoprotein (pink).

Limited data is available in the literature about the growth characteristics of this virus during the production process. In our study, we investigated the influence of several process parameters on the viral titer of rVSV-ZEBOV produced in the Vero cell line and in a suspension-adapted HEK293-based cell line without serum.

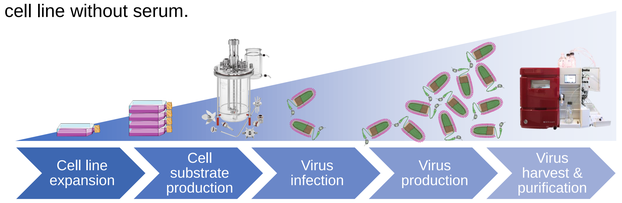
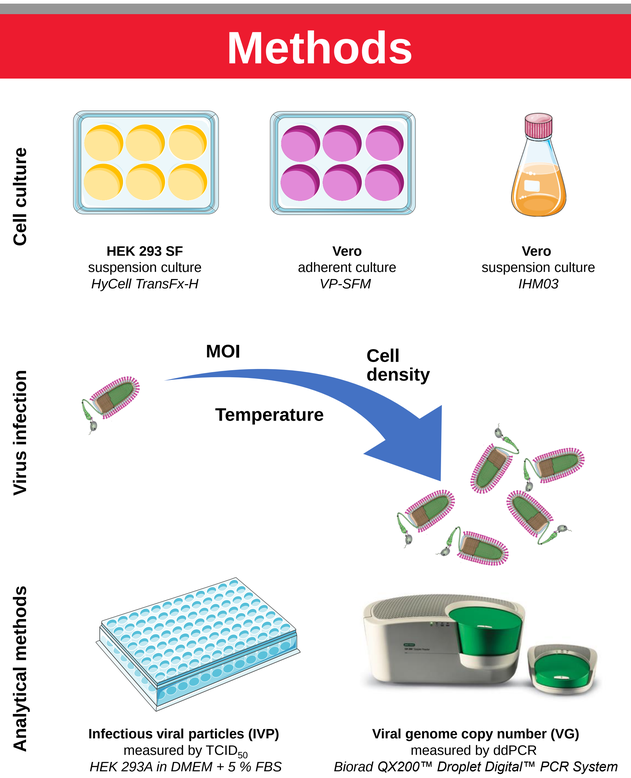


Figure 2: Outline of rVSV bioprocess development.

Methods



rVSV-ZEBOV production in Vero

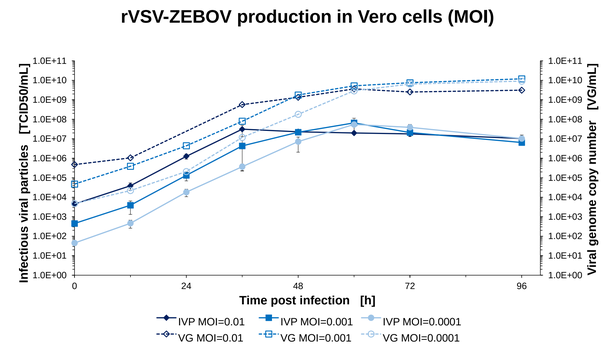


Figure 3: Production yields of infections with rVSV-ZEBOV at MOI 0.01, 0.001 and 0.0001 of adherent Vero cells in 6-well plates at 37°C. Samples were harvested at indicated timepoints and titers were measured by $TCID_{50}$ and ddPCR. Infectious titers were determined in triplicates. The highest titer (6.54×10^7 $TCID_{50}$/mL) was observed after 60 hours post infection at MOI 0.001 with a ratio of 238 VG/IVP.

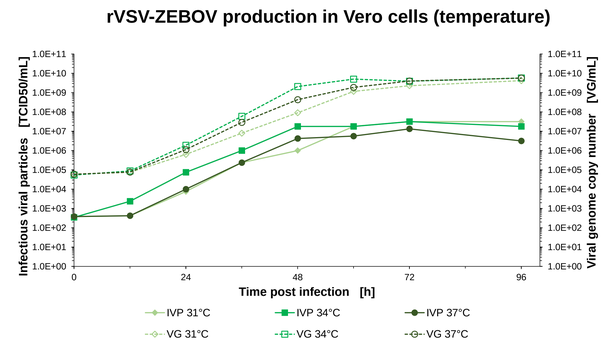


Figure 4: Production yields of infections with rVSV-ZEBOV at MOI 0.001 of adherent Vero cells in 6-well plates at 3 different temperatures (31°C, 34°C and 37°C). Samples were harvested at indicated timepoints and titers were measured by $TCID_{50}$ and ddPCR. The highest titer (3.16×10^7 $TCID_{50}$/mL) was observed after 72 hours post infection at 34°C with a ratio of 179 VG/IVP.

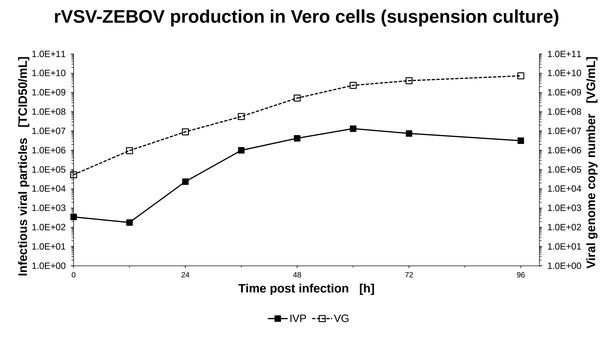


Figure 5: Production yields of rVSV-ZEBOV infection at MOI 0.001 of Vero cells cultivated in suspension culture in 125 mL shake flask and 30 mL working volume at 37°C. Cells were cultivated in IHM03 medium. Samples were harvested at indicated timepoints and titers were measured by $TCID_{50}$ and ddPCR. The highest infectious titer (1.33×10^7 $TCID_{50}$/mL) was observed after 60 hours post infection with a ratio of 258 VG/IVP.

rVSV-ZEBOV stability

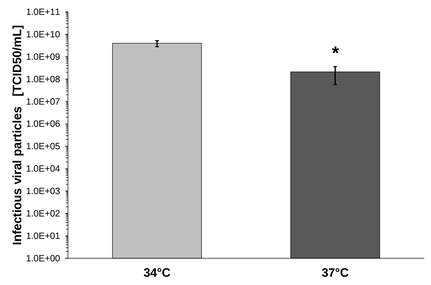


Figure 6: Production yields of rVSV-ZEBOV infection at MOI 0.001 of HEK293 at two different temperatures (34°C and 37°C) in a 6 well plate with 1×10^6 cells/mL in 2 mL per well in suspension culture. Titers were measured by $TCID_{50}$. Bars represent the mean of triplicate production studies ± standard deviation. There was a statistically significant difference (p = 0.0103) between the two group means as determined by unpaired two-tailed t test indicating that production at 34°C was higher than at 37°C.

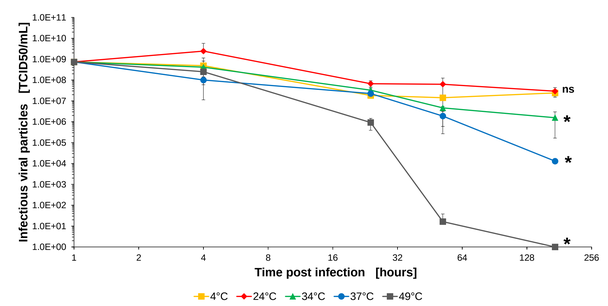


Figure 7: : Titers of rVSV-ZEBOV exposed to different temperatures for increasing amounts of time. Titers were measured by $TCID_{50}$. Bars represent the mean of triplicate production studies ± standard deviation. At 1 week incubation, there were statistically significant differences between group means as determined by one-way ANOVA ($F(4,10) = 8.214$, $p = 0.0033$) followed by Dunnett's post-test for lower titers for incubations at temperatures 34°C and above when compared to incubation at 4°C.

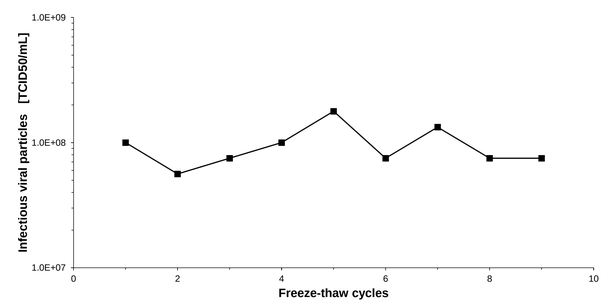


Figure 8: Titer of rVSV-ZEBOV exposed to increasing numbers of freeze-thaw cycles. Titers were measured by $TCID_{50}$. To assess this, nine tubes of rVSV-ZEBOV previously generated in HEK 293SF were each exposed to a different number of freeze-thaw cycles by incubating them at 37°C for 3-5 minutes (until all visible ice had melted) and then at -80°C for 10-15 minutes (until the whole tube had frozen over).

Conclusions

- MOI affects rVSV-ZEBOV production kinetics in both Vero and HEK 293 (data not shown) with a lower MOI resulting in a delayed peak of virus production.
- Typically, during the course of production, the infectious titer reaches a plateau after which it starts to decline. In contrast, the number of viral genomes continues to increase. This effect can be attributed to a loss of viral infectivity over time whereas the total viral particles count increases.
- Production of rVSV-ZEBOV at 34°C results in the highest infectious titers for both cell lines. This might result from the weak rVSV-ZEBOV thermostability at higher temperatures as shown in figure 7. Consequently, rVSV-ZEBOV production should be carried out at 34°C to achieve higher infectious titers and to reduce the observed loss of infectivity. In contrast, freeze-thaw cycles seem to have a lesser impact on viral stability.
- Operation at higher MOIs might contribute to improve the ratio of infectious to total particles, by reducing the process production timelines and enabling earlier virus harvest.
- In addition, this study showed the proof of principle for the production of rVSV-ZEBOV in serum-free suspension cultures of HEK 293 and Vero cells to enable more streamlined process development and scale-up and take advantage of most advanced cell culture technologies.
- Overall, the results indicate significant potential to improve manufacturing of the VSV-vectored Ebola vaccine in order to meet global health needs.

Acknowledgement

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Source of plasticware images: Servier Medical Art (smart.servier.com)

Source of VSV schematics: ViralZone, SIB Swiss Institute of Bioinformatics (www.expasy.org/viralzone)