PROPAGATION OF BRAZILIAN ZIKA VIRUS STRAINS IN STATIC, MICROCARRIER-BASED AND SUSPENSION CULTURES USING BHK AND VERO CELLS

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The spread of Zika virus (ZIKV) in the Americas results in an urgent need for the development of a ZIKV vaccine. Current strategies for ZIKV propagation in animal cells rely mainly on adherent Vero and C6/36 cells. This work focused on understanding ZIKV replication in animal cell culture to develop an inactivated or live-attenuated ZIKV vaccine in microcarrier culture or, preferably, in suspension cells, so that low cell-specific yields can be overcome by the establishment of high-cell density processes.

First, adherent cells (Vero and BHK-21) were infected with different Brazilian ZIKV isolates. Comparing both cell lines, maximum infectious titers and cell-specific yields (1–48 PFU/cell) of respective virus strains were similar, whereas process yields across different strains strongly varied by two log-scales.

Scale-up of Vero cells in bioreactors using 6 g/L Cytodex 1 resulted in maximum cell concentrations of 5.3×10^6 cells/mL. However, low cell-specific yields of 0.0002 PFU/cell indicated poor virus replication. Using suspension-adapted BHK-21 cells grown in a chemically-defined medium, higher virus titers were achieved when infections were initiated at the mid/late exponential growth phase at MOI 0.001. Nevertheless, cell-specific yields did not exceed 0.0002 PFU/cell. Subsequent RT-qPCR data indicated a poor virus release as intracellular viral RNA levels were 20-fold higher than extracellular levels.

At small-scale, centrifugal spinoculation was evaluated to enhance ZIKV infection in suspension BHK-21 cells, with no significant improvements. In a further investigation with these cells in a perfusion bioreactor using an ATF-2 filtration system, a maximum cell concentration of 14×10^6 cells/mL was achieved with a final titer of 4.6×10^6 PFU/mL and an increased cell-specific yield of 0.09 PFU/cell.

Overall, the present results demonstrate that ZIKV propagation in microcarrier- and suspension-based systems is challenging regarding virus yields. Future investigations will focus on improving cell-specific yields by adapting Zika virus isolates to suspension cell lines, and on increasing maximum titers by process intensification.