EVALUATION OF PRODUCER CELL LINES FOR YELLOW FEVER VIRUS PRODUCTION IN UP TO 1 L BIOREACTOR SCALE

Alexander Nikolay, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
nikolay@mpi-magdeburg.mpg.de
Katharina Hermann, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto-von-Guercke University, Chair of Bioprocess Engineering, Germany

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Yellow fever virus (YFV) vaccine is currently produced in embryonated chicken eggs. Following recent outbreaks of flavivirus-related diseases, such as Zika fever, significant efforts are needed towards fast establishment of cell culture-based production processes for attenuated or inactivated virus vaccines.

To support the development of such processes, we have screened various cell lines, including adherent and suspension cells, for permissiveness and productivity of YFV. In particular, the parental adherent Vero cell line possesses a reasonable cell-specific productivity of about 13 PFU/cell. However, surface-depended scale-up restricts production processes to roller bottles, microcarrier-based or fixed-bed bioreactors with limited monitoring and excessive efforts for large-scale production. A preferential alternative is the cultivation of single-cells in stirred-tank bioreactors, which can be operated in perfusion mode to achieve higher cell-densities. Towards this process intensification, we have adapted the parental WHO Vero cell line to grow in suspension. However, infection studies of Vero suspension cells with YFV in spinner flasks using chemically defined medium showed a reduced cell-specific titer (2 PFU/cell).

Another option might be the use of BHK-21 cells reaching cell-densities above 5×10^6 cells/mL in shake flasks. Infection studies with YFV in small-scale have resulted in a cell-specific productivity of 10 PFU/cell. Thus, infection parameters (time of infection, MOI = ratio of virus to cell) were optimized and subsequently transferred into 1 L bioreactors. Final titer of 5×10^7 PFU/mL could be reached. As a reference, adherent Vero cells were cultivated on Cytodex-1 microcarriers in 1 L scale resulting in a final titer of 2×10^7 PFU/mL. In both cultivations, cell-specific yields were comparable but due to the adjusted MOI of 10^{-4} in the BHK-21 cultivation, the overall virus production was 50 × higher than for the Vero cultivation on microcarriers.

Although BHK-21 cells and their application for human vaccines are controversial with respect to tumorigenicity and oncogenicity, our results show that it may be worth to reconsider this cell line for future production processes.