INTENSIFICATION OF MVA AND INFLUENZA VIRUS PRODUCTION THROUGH HIGH-CELL-DENSITY CULTIVATION APPROACHES

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Background. Unlike production of recombinant proteins, continuous production of viral vaccines at high cell densities (HCD) is often constrained by a decrease in cell-specific virus yields, early host cell lysis during virus propagation and limited virus recovery from culture broth. Nevertheless, advanced fed-batch [1] and perfusion strategies can be applied to achieve high-yield virus production processes. In this study, the development of a semi-continuous process for the production of the modified vaccinia Ankara virus isolate MVA-CR19 and influenza virus A/PR/8/34 (H1N1) in HCD cultivations of the suspension cell line AGE1.CR.pIX (ProBioGen AG, Berlin) is presented.

Methods. Depending on the required scale, high cell concentrations (~ 50×10^6 cells/mL) were achieved either through medium renewal by periodic centrifugation (semi-perfusion) in 50 mL cultivations or using an alternating tangential flow (ATF) perfusion system for 1 L bioreactors. Process development and optimization comprised three phases: 1) assessment of different fed-batch and medium exchange strategies for the propagation of MVA-CR19 or influenza A/PR/8/34 viruses in 50 mL cultivations; 2) scale-up and process optimization of the selected high-yield process strategy to a 1 L bioreactor with the ATF system, and 3) integration of a one-step purification process using magnetic sulfated cellulose particles (MSCP). For both viruses, conventional batch cultivation (no addition/medium exchange after infection) was compared with processes applying fed-batch, periodic medium exchange and the combination of both during virus propagation.

Results. Perfusion and semi-perfusion at a feeding rate of 0.05 nL/cell×d was suitable to propagate AGE1.CR.pIX cells above 60×10^6 cells/mL with neither limitation nor overload of nutrients. For infections at 50 mL scale, the application of a combined strategy comprising an initial fed-batch phase followed by a periodic virus harvest phase resulted in the highest product yield with a more than 10-fold increase in virus particles concentration compared to the conventional batch processes operated at 4 to 8×10^6 cells/mL [2]. Additionally, a 3-fold increase in both cell-specific yield (virus particles/cell) and volumetric productivity (virus particles/L×d) could be obtained. Comparable yields were observed when up-scaling to a 1 L bioreactor using an ATF-system, even when virus particles were retained within the bioreactor. Further selection of the optimal pore size of the ATF membrane allowed semi-continuous harvesting of the produced viruses and its purification with MSCPs with a recovery from 30 to 50%. In all cases, cell-specific yields and volumetric productivities reached their maxima at 72 h post-infection, indicating that the process should be stopped at that time point.

Conclusion. Compared to conventional batch processes, the developed HCD process offers significantly higher productivities including the option to integrate a one-step purification process in a semi-continuous mode. Overall, the results show that there is a great potential for semi-continuous HCD processes for the production of viral vaccines in larger scales, which could support efforts towards the establishment of continuous vaccine manufacturing.

References.

1. Pohlscheidt, M., et al., Development and optimisation of a procedure for the production of Parapoxvirus ovis by large-scale microcarrier cell culture in a non-animal, non-human and non-plant-derived medium. Vaccine, 2008. 26(12): p. 1552-65.
2. Lohr, V., et al., New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: studies on growth, metabolism and virus propagation. Vaccine, 2009. 27(36): p. 4975-82.