HIGHLY EFFICIENT INFLUENZA VIRUS PRODUCTION: A MDCK-BASED HIGH-CELL-DENSITY PROCESS

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Seasonal vaccination campaigns for influenza in developed and developing countries create a massive demand for 500 million (2015) vaccine doses every year [1]. Besides egg-based vaccine manufacturing, production platforms based on animal cell culture increasingly contribute to this overall growing market. In order to intensify cell culture-based influenza virus production, high-cell-density (HCD) cultivation of suspension cells can be applied to improve virus titer, process productivity and production costs [2]. For process optimization and evaluation of HCD conditions, cells cultivated using semi-perfusion approaches in small shakers can be used as a scale-down model for bioreactors operating in full perfusion mode [3].

In this study, a previously developed MDCK suspension cell line [4] was adapted to a new serum free medium [5] to facilitate higher growth rate, cell density and virus titer both in batch and in HCD. Therefore, MDCK cells cultivated in Smif-8 medium were slowly adapted to a new cultivation medium (Xeno™) by stepwise increasing the Xeno content. Fully adapted cells were cultivated in shaker flasks to evaluate the performance of influenza A virus production in batch and HCD. Cell densities exceeding $2 \cdot 10^7$ cells/mL were achieved in shakers using semi-perfusion, where cell free medium was manually replaced with fresh medium. Volume and time interval of media replacement were chosen to achieve a constant cell-specific perfusion rate of 2.5 pL/(cell h). Cell cultures were infected with influenza virus (A/PR/8/34 H1N1 RKI) with trypsin addition. Cell count, viability, main metabolites and virus titer (HA-assay & $TCID_{50}$) were monitored pre and post infection.

Medium adaptation resulted in a MDCK suspension cell line with morphological, growth, and metabolic characteristics different from parental cells. Cells fully adapted to Xeno medium were growing to higher cell densities ($1.4 \cdot 10^7$ vs $6 \cdot 10^6$ cells/mL) with higher specific growth rate (μ_{max}: 0.036 vs 0.026 1/h), cells were bigger (15-16 vs 13-14 µm) and grew without aggregate formation. Due to higher cell densities at time of infection, virus titers up to 3.6 $\log_{10}$(HAU/100µL) were reached. In semi-perfusion, adapted MDCK cells were grown up to $6 \cdot 10^7$ cells/mL, however, maximum virus titer and productivity were observed with $4 \cdot 10^7$ cells/mL. In multiple harvests, very high virus titer exceeding 4 $\log_{10}$(HAU/100µL) and up to $9 \cdot 10^9$ virions/mL ($TCID_{50}$) were measured, which corresponded to an accumulated titer of 4.5 $\log_{10}$(HAU/100µL). Cell-specific virus titer was similar or higher compared to the reference batch infections, depending on perfusion and infection strategy.

Overall, results in this semi-perfusion scale-down model for influenza A virus production suggest a highly efficient and productive upstream process for influenza virus production, with an up to six-fold improved space time yield compared to batch mode.

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