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TOWARDS INTEGRATED CONTINUOUS VIRAL VACCINES PRODUCTION USING TWO-STAGE BIOREACTOR SYSTEMS

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Typically, many cell culture-derived viral vaccines are being produced in batch processes. However, with increasing demands of a growing world population for potent, safe and affordable vaccines, manufacturing technologies need to be further optimized. In particular, the establishment of two-stage bioreactors appears as an interesting option for continuous production of viral vaccines. In these systems cell growth and virus propagation are being performed in separated vessels [1], which make them suitable for production of lytic viruses, such as influenza and Modified Vaccinia Ankara virus (MVA). MVA virus has received much clinical attention, because it is as a promising vector candidate in gene therapy, and can also be used as a platform for expression of recombinant viral antigens including influenza and other viruses [2]. Both influenza and MVA viruses have been successfully propagated at high yields in batch culture using non-aggregated avian suspension cells [3,4]. To further optimize the productivity in vaccine manufacturing, semi-continuous or ever continuous production systems could be considered. However, a serious drawback for influenza virus production (and many other viruses) is the generation of defective interfering particles (DIPs), known as “von Magnus effect”. This causes oscillations in virus levels and results in low yields [1]. For MVA production, a large DNA virus, this effect has not yet been described. Therefore, in this work, first a scale-down approach of the two-stage bioreactor system using semi-continuous cultivations will be addressed and compared with previous influenza virus experiments [1]. Secondly, the production of MVA virus in two-stage semi-continuous and continuous bioreactors is explored. Finally, the compatibility of the continuous harvests with requirements in downstream processing will be discussed for a direct integration of the continuous mode into the overall production process.

A small scale semi-continuous two-stage cultivation system (100 mL-scale, two shaker flasks, SSC) was established as an approximation to a real continuous bioreactor (1 L-scale, two-stage stirred tank bioreactor, TSB) to facilitate process screening [5]. The SSC system was used to produce influenza virus strain A/PR/8/34 (RKI) [1], the virus strains MVA-CR19, MVA-CR19.GFP, and the duck cell line AGE1.CR.pIX (the latter three from ProBioGen, Berlin). Furthermore, one continuous cultivation with the TSB set-up for production of MVA-CR19 virus was carried out.

Production in the SSC system resulted in steady-state concentrations of cells with influenza virus titers that reflected the oscillating dynamics obtained previously with the TSB system [1]. In contrast, the scale-down of MVA virus production resulted in stable titers of MVA-CR19 virus for over 18 days. PCR analysis of the MVA-CR19.GFP virus showed stable maintenance of the recombinant transgene without deletion of the GFP insertion cassette after 14 days of culture. The continuous experiment with the TSB system and the MVA-CR19 virus showed similar virus titers to those observed in the SSC system and suggested the absence of the “von Magnus effect” over 18 days.

Overall, small scale semi-continuous cultivation was successfully established as a fast and efficient tool for screening purposes. MVA can be produced in continuous two-stage bioreactor systems for at least 18 days without reduction of virus yield due to the absence of DIP formation. Therefore, in the future, this system may be interesting for continuous production of recombinant MVA-based vaccines and gene therapy vectors.

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