PROPAGATION OF INFLUENZA AND MVA VIRUS IN CASCADES OF CONTINUOUS STIRRED TANK BIOREACTORS: CHALLENGING THE "VON MAGNUS EFFECT"

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Moving from batch to fully continuously operated upstream processes is one of the big challenges for the coming decades in cell culture-based viral vaccine manufacturing. Continuous processes are known to be more efficient than batch systems for production of large volumes of product, and can therefore be an interesting option for production of highly demanded viral vaccines. One example is the seasonal influenza virus that causes annual epidemics in human populations worldwide and is currently produced in batch processes. Another virus of clinical interest is Modified Vaccinia Ankara (MVA) virus which is a potential platform for recombinant vaccines and can be used as a vector in gene therapy [1]. Continuous propagation of MVA virus seems to be feasible using a new MVA virus strain that can propagate at high yields in non-aggregated avian suspension cells [2]. Because both influenza and MVA are lytic viruses a continuous production strategy was employed that involves cascades of two stirred tank bioreactors, where cell growth and virus propagation occur in separated vessels [3]. However, a possible drawback for continuous virus production is the presence of defective interfering particles among the virus population that cause oscillations in virus levels and low production yields [3], known as Von Magnus effect.

In this work, a small scale two-stage cultivation system (two 100 mL shaker flasks; semi-continuous; SSC) was established as screening tool for influenza and MVA virus propagation before scaling to a 1 L continuous two-stage bioreactor system (two 1 L stirred tank bioreactors; TSB). The MVA virus strains MVA-CR19 and MVA-CR19.GFP were used, and propagated 14 days in the duck cell line AGE1.CR.pIX (all three from ProBioGen, Berlin) using the SSC system. Similarly, the influenza virus strain A/PR/8/34 H1N1 (RKI) was propagated 14 days using two different cell lines (MDCK.SUS2 and AGE1.CR.pIX) in the SSC system. From the best screening result, scale-up to the 1 liter TSB was performed with successful virus production in continuous mode for three weeks. PCR analysis was used to monitor the stability of the viruses in continuous culture.

The SSC system resulted in stable production of cells, and influenza virus titers that approached the oscillatory behavior observed in previous experiments [3]. Interestingly, MVA virus cultivated in the SSC system did not show oscillations in the virus titer. Additional cultivations of MVA virus in the SSC system showed that different residence times in the virus bioreactor could influence virus titers. Subsequently, production of MVA-CR19 was scaled to the TSB system and maintained for 18 days in continuous mode. MVA virus titers showed 7 days of a transient phase, followed by stable titers that confirmed the absence of a Von Magnus effect over 18 days. A yield comparison between an eight days batch-cycle process and the TSB showed that the space-time yield of the TSB cultivation approached that of two parallel batches at 11 days of virus production. PCR analysis indicated that the reporter gene in MVA-CR19.GFP was maintained stably for the complete cultivation period.

Overall, it was demonstrated that production of influenza and MVA viruses in a SSC system is feasible and can be used as a fast and cost-efficient tool for optimizing continuous virus production. Finally, MVA virus is a very promising candidate for production of viral vaccines in cascades of continuous stirred tank bioreactors.

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[2] Jordan et al. 2013, Viruses 5(1):321–39.
[3] Frensing et al. 2013, PLOS ONE 8(9):e72288.
[4] Westgate and Emery 1990, Biotech & Bioeng 35(5):437-53.