CONTINUOUS MODE OF PRODUCTION FOR TWO CLASSES OF DEFECTIVE INTERFERING INFLUENZA A VIRUS PARTICLES AS ANTIVIRAL CANDIDATES

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Influenza A virus (IAV) is a major human pathogen with a high mutation rate that causes annual epidemics. Defective interfering particles (DIPs) are naturally occurring IAV mutants that are responsible for low influenza virus yields in continuous passaging. Due to that, previous research suggested that DIPs may be utilized as an antiviral agent [1]. In contrast to infectious influenza standard virus (STV), DIPs typically contain a large internal deletion in at least one of the eight genomic viral RNA (vRNA) segments. For such a DIP, named DI244, protection of ferrets against pandemic influenza A virus was shown [1]. Furthermore, we have recently reported on a novel type of IAV-derived DIP, called OP7 virus, which only contains nucleotide substitutions in segment 7 vRNA instead of large internal deletions [2]. Hence, the focus of this work was to evaluate cell-based production in continuous mode for both DI244 and the newly discovered OP7 DIP.
Madine Darby canine kidney (MDCK) cells were grown in Smif8-CDM as a substrate for virus propagation. The bioreactor used was a cascade of continuous stirred tank bioreactors (CSTRs), where cell propagation and virus replication occur in separated vessels [3]. To improve comparability of DIP dynamics, the bioreactor setup was modified to a parallel two-stage continuous process sharing one CSTR for cell growth (Figure 1). It was shown before that in continuous production the propagation of DIPs leads to oscillations in virus titres [3]. DI244 production showed the expected oscillations. Virus titre as high as 2.4 $\log_{10}$ HAU/100 µL were reached with 4×10^9 DI244 copies/mL, sufficient for animal trials. During the production of OP7, instead of oscillations, a low constant virus titre of approximately 1.7 $\log_{10}$ HAU/100 µL was observed from 2 to 6 days post infection before other *de novo* generated DIPs arose. The difference in the dynamics between both DIPS could be caused by a strong self-interference of OP7. To increase OP7 virus titres, a media and MOI screening in 125 mL shake flasks in batch mode was conducted. This screening, together with a newly established interfering assay, showed that the most potent material was produced at an MOI of 1E-2 in chemically defined medium (Xeno-CDM). The remaining STV in the produced DI244 and OP7 material was inactivated by UV light. OP7 was subsequently concentrated by steric exclusion chromatography [4] resulting in highly potent material reducing infectious virus titre by a factor of 7,700 from 7.7E8 to 1.0E5 PFU/mL in the cell culture-based interfering assay. Both DIPs are currently tested in animal trials (C57BL/6JRj mice).

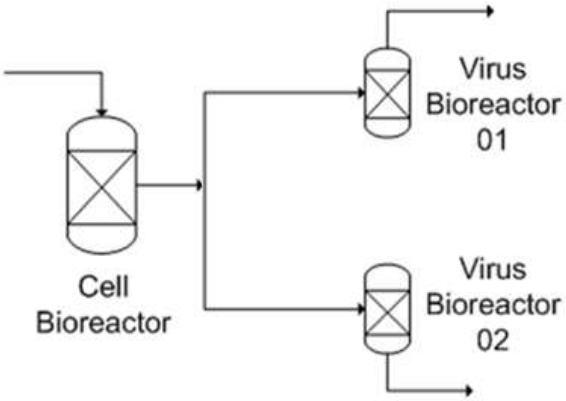

Figure 1 - Diagram of the parallel cascade of CSTRs used for continuous production of defective interfering influenza A viral particles.

In summary, continuous production of two types of DIPs, DI244 and OP7, was successfully established in a parallel cascade of CSTRs. DIP propagation dynamics suggested that continuous production might be a good approach for production of certain DIPs, such as DI244. However, OP7 production might be more efficient in other types of continuous systems. Currently, OP7 production using a continuous alternating flow (ATF) bioreactor system is being explored.

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