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PRODUCTION OF INFLUENZA A VIRUS DEFECTIVE INTERFERING PARTICLES IN A HIGH CELL DENSITY PERFUSION CULTIVATION WITH CONTINUOUS VIRUS HARVESTING

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Causing annual epidemics and occasional pandemics, influenza A virus (IAV) is a human pathogen that has severe impact on health and society. Defective interfering particles (DIPs) have been discussed as a potential antiviral therapeutic [1]. Most IAV DIPs are naturally occurring viral mutants harboring large internal deletions in at least one of their eight viral RNA (vRNA) segments. Due to these deletions, DIPs can only replicate in the presence of infectious standard virus (STV), compensating for the missing gene function. In a co-infection, the defective vRNA segment interferes with STV genome replication, which is causative for the therapeutic potential of the DIP. In the presented study, we report on the cell culture-based production and the evaluation of the antiviral potency of a DIP with a deletion in segment 1 vRNA, called DI244.

DIP replication usually relies on a co-infection of the desired DIP and STV as a helper virus. However, this results in the necessity to inactive the STV by-product, e.g. by UV-irradiation. Naturally, the UV-irradiation also affects the antiviral potency of the DIP material [2]. Therefore, a production system independent of STV co-infection was developed for DI244. This was achieved by genetic engineering of MDCK suspension cells to express PB2, the “missing” protein encoded by segment 1 vRNA to allow propagation of DI244 in the absence of STV [3]. The production process was first established in shake flasks resulting in a DI244 titer of 1.0E+8 PFU/mL. Next, the process was scaled up to a 1 L-stirred tank bioreactor and process intensification strategies were applied. More specifically, a high cell density perfusion process using an alternating tangential flow filtration system (ATF) for cell retention was established. Here, cell concentrations up to 28.4E+6 cells/mL and DIP titers up to 7.4E+9 PFU/mL were observed [4]. Furthermore, different cell retention membranes (a standard hollow fiber membrane and a novel tubular membrane, called VHU) were compared for their potential for continuous virus harvesting into the permeate. Very comparable cell growth was observed using either membrane. However, during the virus replication phase, only the VHU allowed to collect virus particles via the permeate [4]. Finally, to evaluate the antiviral potency of produced DI244 preparations, a cell culture-based interfering efficacy assay, which quantifies the DIP-induced reduction of released STVs in a co-infection, was utilized. Here, the final DI244 material reduced the released STV titer by approx. three orders of magnitude. In animal experiments with mice, treatment with DI244 alone did not show any negative effects on body weight or survival rate, indicating no apparent toxic effects. Furthermore, all mice infected with a lethal dose of STV and co-treated with DI244 survived the infection [5].
In sum, results clearly demonstrate the potential of cell culture-based produced DIP preparations for use as an antiviral. Further, to our knowledge, this is the first time continuous virus harvesting for a membrane-based perfusion process was shown. This offers interesting options for advanced process integration strategies for next-generation virus particle and virus vector manufacturing.

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