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PRODUCING VIRUSES IN ORBIT: CURRENT DEVELOPMENTS FOR ORBITAL SHAKEN VIRAL VACCINE MANUFACTURING

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Preculture of suspension cells is successfully performed in shake flasks. Especially newly developed designer cells are passaged up to 100 times in shake flasks at high shaking frequency and are then perfectly adapted to growth in a CO₂ incubator with pH control and maximum oxygen supply (typically above 80% pO₂). When they are subsequently transferred to stirred tank bioreactors for scaling up, specific cell growth rates are often lower and cells become sensitive to pH control via acid/base addition and shear stress due to submers gassing (bubbles). This was also seen for avian AGE1.CR.pIX and human HEK 293 cells. To avoid these problems, scale up in shaken mode was evaluated.

Here we present the latest developments of the SB10-X OSB bioreactor with regard to bag design and improvement of the control unit. A new control strategy was introduced leading to a faster and more precise pH and DO control. Furthermore, the perfusion bag was optimized, so that on TFF or two ATF systems can be easily connected. Both developments have led to a more robust SB10-X system that allows to easily perform batch, fed batch or perfusion runs.

Avian AGE1.CR.pIX cells (ProBioGen AG, Germany) were cultivated in the chemically defined medium CD-U3 (Biochrom-Merck, Germany) in 10 L single-use standard bags at 70 rpm shaking frequency. For perfusion, an alternating tangential flow system (ATF2, Repligen, 500 kDa cut-off) was used. After infection with influenza virus A/PR/8/34 (H1N1) at an MOI of 0.001, the working volume was increased from 5 to 9 L while perfusion was maintained. Cell concentrations of 25 and 50 x 10⁶ cells/mL were evaluated with different filling volumes to understand the impact of head space aeration. Overall, very high cell specific virus yields of 3500 virions/cell could be obtained, leading to a production of HA titers of up to 3.7 log₁₀(HA units/100 µL) and infectious titers of up to 8.8 x 10⁹ TCID₅₀/mL.

Recombinant AAV based vectors are not only suitable vehicles for gene therapeutic purposes, but are also capable of inducing a strong, primarily cellular immune response against various antigens. So far, AAV production has been mainly done using transiently transfected adherent human HEK 293 cells (e.g. in cell stacks) representing a major challenge for large scale AAV production. Here, we tested HEK 293 cells adapted in-house to suspension growth for their capacity to produce AAV9 in a process that allows for simple scale up. Therefore, HEK 293 suspension cells were cultivated in 5 L of chemically defined, serum-free medium at a cell density of 1 x 10⁶ cells/ml using the SB10-X OSB bioreactor at a shaking frequency of 65 rpm. Polyethylenimine (PEI) mediated triple transfection (including a GFP reporter) was performed 24 h later at a shaking frequency of 70 rpm. Finally, 48 h post transfection, cells and supernatant were harvested for AAV isolation and the amount of DNase I-resistant vector particles (DRP) was determined in the lysate. Due to the efficient transfection (>90% transfection rate based on the GFP reporter) and the good performance of the whole batch process in the SB10-X system, a manufacturing-relevant AAV titer in the range of 1.4 x 10¹² DRP/ml or 7 x 10¹⁵ DRP/batch (5 L) was reached.

Taken together, producing viruses in orbit could be an attractive alternative for innovative vaccine manufacturing.