Increasing Productivity in Influenza Virus Production – Virus Yields in High Cell Density Cultivations

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Mammalian cell culture for manufacturing of influenza vaccines plays an increasing role in the prevention and the control of seasonal but also pandemic outbreaks. Due to the enormous socio-economic impact of influenza virus disease not only the development of new vaccination approaches but also the optimization of cell culture-based manufacturing technologies currently being established by various companies is of crucial importance.

The main goal of our group is to develop integrated concepts to optimize cell culture-derived vaccine production processes covering a wide range of unit operations in upstream and downstream processing. As an example we investigate influenza virus replication in various anchorage-dependent and suspension cell lines in stirred tanks and wave bioreactors.

Here, results are presented for cultivation of adherent Madin Darby canine kidney (MDCK) cells in high cell density microcarrier cultures[1]. Maximum cell numbers of about 10^7 cells/mL were obtained by various medium feeding strategies and optimization of incoulum and microcarrier concentrations. Use of serum free medium avoided washing steps before infection[2]. With a cell specific virus yield similar to low density cultivations, productivity of cultivations was increased at least 4 to 6 fold. Level of contaminating proteins of about 220 µg/mL at time of harvest was proportional to cell concentration while concentration of host cell DNA was in the range 8 – 16 µg/mL depending on virus strain and cell lysis. Glycosylation profiles of influenza A virus hemagglutinin showed no significant differences compared to control cultivations performed at lower cell densities[3]. Therefore, no significant problems due to these process modifications are anticipated for use of conventional downstream processing strategies. Quantitative mathematical models for mammalian cell metabolism[4] clearly indicated that precursor supply (amino acids, nucleotides) or consumption of cellular energy (ATP) and redox equivalents (NAD(P)H) is no bottleneck for intracellular virus replication. In contrast, it seems that virus-induced host cell defense and apoptosis are crucial factors to be considered for further increase in cell specific virus yields.

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