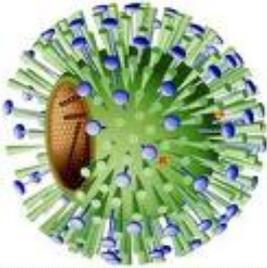


Large pilot scale cultivation process study of adherent MDBK cells for porcine Influenza A virus propagation using a novel disposable stirred-tank bioreactor

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Introduction

Influenza is one of the major viral diseases and has tremendous importance for human and animal health worldwide. Pigs are of special importance as they are susceptible for a number of Influenza A viruses from different species (human, avian, porcine); in the case of multiple infections of swine new and dangerous virus strains can be assembled by reassortment of genes (antigenic shift). Influenza vaccines are often still produced in embryonated hens eggs, although substrate cell lines are available, e. g. VERO or MDCK cells. Egg based processes are limited in terms of capacity whereas cell culture based processes are more flexible and easy to scale up when facing pandemic situations (e. g. emergence of H5N1 in 2005 and H1N1 pandemic virus in 2009). Currently, adherent Madin-Darby bovine kidney (MDBK) cells are used for the production of porcine Influenza vaccines at commercial scale (IDT Biologika GmbH). This process is performed in roller bottles so far using a biphasic process scheme (cell growth phase and virus propagation phase) and is thus limited in capacity, yields and process control. The main goal of this study was to explore options for direct transfer from roller bottles into a novel disposable stirred-tank bioreactor (BIOSTAT CultiBag STR 50, Sartorius Stedim Biotech GmbH) using microcarriers for attachment of MDBK cells. The advantage of this system is its scalability, better process control and presumably high virus yields. Later implementation into routine GMP-production surrounding (multi-purpose facility) is intended.

Materials and Methods

Biologicals

MDBK cells (WCS, IDT Biologika GmbH) were cultivated in roller bottles for preculture. Porcine Influenza A virus/H1N1/ strain 2617 (WSV, IDT Biologika GmbH) was used as model virus in this study.

Cultivation Parameter & Media

Cells were seeded with 2.0×10^5 cells/ml using 2 g/L Cytodex™ 1 microcarrier in a BIOSTAT CultiBag STR 50 with 35 l wv. For cell growth phase (0-118 h) a minimal essential medium (MEM) with 5 % y-irr. FBS and 1 % L-Gln was used. For virus propagation phase (118-192 h) the culture was washed twice with PBS and MEM + 1 % L-Gln was used as infection medium. Cell culture was infected with strain 2617 at a moi^4 and a trypsin concentration of 40 BAEE units/ml. The culture was kept at 37 °C and 50 rpm stirrer speed, pH was controlled at 7.4 with 1 M NaOH, pO_2 was kept > 40 % saturation by gassing with sterile air.



Figure 1: Disposable Stirred-Tank Bioreactor BIOSTAT CultiBag STR50 (Sartorius Stedim Biotech GmbH) for controlled cultivation of animal cells in suspension

Analytics

Cells were analyzed in the supernatant and after trypsinization with a CASY modell TT (Roche Diagnostics GmbH) for cell number and viability. Further cell analytics was performed with a Nexin assay using the Guava easycyte Flow Cytometer (Merck Millipore GmbH). Online data for air flow, pO_2 , pH, temperature and stirrer speed were recorded via the MFCS D/A software (Sartorius Stedim Biotech GmbH). Hemagglutination titre and concentration of infectious virus as TCID₅₀ were determined according to internal protocols (IDT Biologika GmbH).

Results

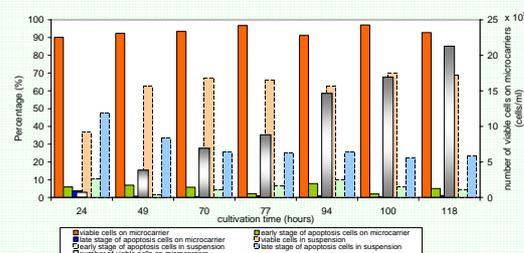


Figure 2: MDBK cell number during cell growth phase (cells/ml) and data from Nexin assay (%)

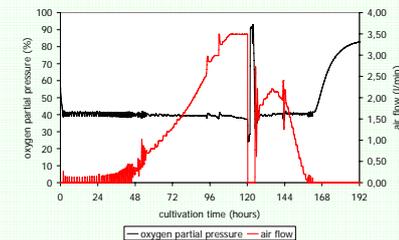


Figure 4: Online data (MFCS D/A software of the BIOSTAT CultiBag STR 50) for oxygen partial pressure and air flow for the complete cultivation process (cell growth and virus propagation)

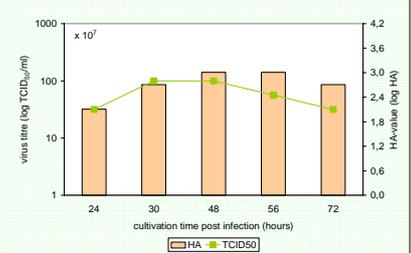


Figure 3: Influenza A H1N1 strain 2617 virus yield as HA and TCID₅₀

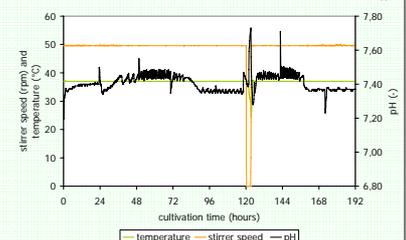


Figure 5: Online data (MFCS D/A software of the BIOSTAT CultiBag STR 50) for temperature, stirrer speed and pH-value for the complete cultivation process (cell growth and virus propagation)

Cell growth and quality

- good cell attachment after seeding, maximum cell number of 2.1×10^6 cells/ml achieved after 118 h
- high cell viability of cells on microcarriers in contrast to cells in supernatant (remarkable part of apoptotic cells)

Virus yields

- typical signs of Influenza related cytopathic effect (CPE) after 24 h p. i.
- maximum HA obtained 48 h p. i. with $\log 3.0$ HA units, infectious titre highest with $10^{8.00}$ TCID₅₀/ml 24 h p. i. with slight decrease until harvest while HA was stable

Process control

- good control of oxygen level, pattern for air flow and pO_2 reflects ongoing of cell growth and virus propagation
- stirrer speed and temperature control OK, pH stable at 7.4 ± 0.1 with few outliers (starting point for media or controller optimization)

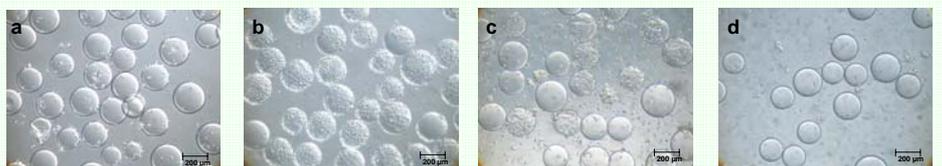


Figure 6: Microscopic pictures for MDBK cell morphology during cell growth and virus propagation phase, (a) 5 h after seeding, (b) 96 h after seeding, (c) 24 h p. i. and (d) 72 h p. i., 200 x magnified

Conclusions and Outlook

The performance of the system in terms of supporting MDBK cell growth and porcine Influenza A virus propagation in a disposable and agitated surrounding in pilot scale was superior to other systems we used so far. The cell distribution was very good and high volumetric cell yields were obtained despite usage of a medium which was not optimized for STR processes. The high level of cell quality is also reflected by the flow cytometer data from a nexin assay. The handling within infection procedure (media change etc) demanded skill, surely in later steps optimization can be done by using customized bags. The virus yields we obtained were at least on a level that was seen for the existing roller bottles based process. Future improvements seem to be feasible especially if one notes that this test run was a direct transfer from a roller bottle process under non-optimized conditions. The possibilities for process control are convincing, even if more sophisticated process schemes need to be applied under scale up conditions. The controller performance was very good especially for the most critical parameters pH and oxygen. From a process point of view, we do not expect any obstacles for implementation of such systems in routine GMP production even under scale up conditions or transfer to other cell/virus systems.