INTEGRATED END-TO-END MVA VIRAL VECTOR PRODUCTION: PERFUSION CULTURE SHOWS ECONOMICAL ADVANTAGE OVER BATCH CULTURE

Gwendal Gränicher, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
graenicher@mpi-magdeburg.mpg.de
Pavel Marichal-Gallardo, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Sven Göbel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Masoud Babakhani, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Ingo Jordan, ProBioGen AG, Germany
Volker Sandig, ProBioGen AG, Germany
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Otto-von-Guericke University Magdeburg, Chair of Bioprocess Engineering, Germany

Key Words: Intensified USP and DSP, perfusion cell culture, continuous viral vector production, economic analysis, process automation

Modified Vaccinia Ankara (MVA) virus is a promising viral vector for gene therapy. Several pre-clinical and clinical trials are currently being conducted with MVA as a live vector vaccine against COVID-19, Ebola disease, influenza or various types of cancers. For most applications, a large amount of the vector will be required (>10^8 infectious virus per dose). High cell concentrations are favorable for developing high-yield MVA vector production systems. Efficient production of MVA in an avian suspension cell line (AGE1.CR.pIX) cultivated in perfusion mode with a membrane-based cell retention system has previously been demonstrated. However, up to now a direct harvest through the membrane for a continuous integrated process was not feasible.

Here, we show a highly efficient perfusion system allowing continuous virus harvesting, using either an acoustic settler or a membrane-based ATF system. For the first time, we performed continuous virus harvesting in perfusion mode directly integrated to semi-continuous chromatography (at 1 L bioreactor scale). Continuous cell clarification was done by depth filtration (with a throughput of at least 250 L/m^2) followed by a novel inline continuous enzymatic DNA digestion. The clarified and endonuclease treated virus harvest was then successfully semi-continuously purified using membrane-based steric exclusion chromatography (70 cm^2). The total infectious virus recovery was 50.5%± 20.2 with less than 10 ng host cell DNA per dose as per regulatory requirements. Compared to an end-to-end integrated batch system (in triplicate), the volumetric yield of purified viral vector (in infectious virus/$L_{bioreactor}$/day) was increased more than 500% with the perfusion.

Using SuperPro Designer, an economic analysis for the production process of MVA-CR19 was calculated. It clearly showed an advantage of perfusion-based virus production over batch. With an assumed 200L bioreactor and 20 chromatography columns with a 1.4 m^2 surface of regenerated cellulose membrane, 2 million doses could be potentially produced within 14 days (from bioreactor cell inoculation to purified virus) for vaccination against Ebola virus disease, influenza or various types of cancers.

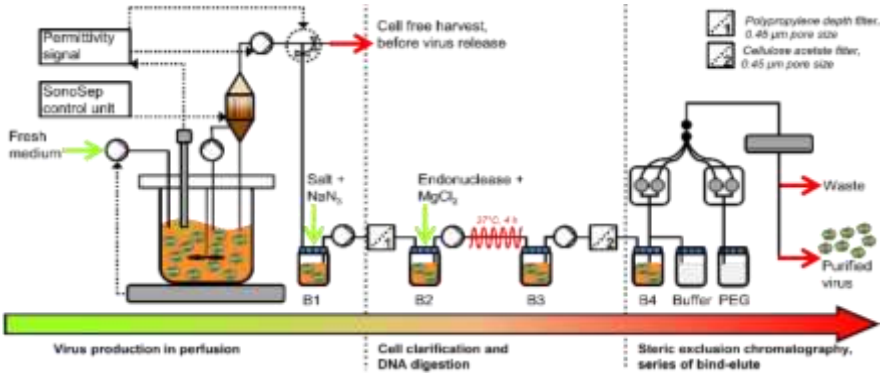

Figure – Illustration of an integrated process for the production of MVA virus in AGE1.CR.pIX cells using an acoustic settler. The process is separated in three main steps: 1) virus production in perfusion mode, 2) cell clarification and enzymatic DNA digestion, and 3) membrane-based steric exclusion chromatography.