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Sven Göbel

Marie Dorn

Karim Jaén Chavez

Ingo Jordan

Volker Sandig

See next page for additional authors

Authors

Sven Göbel, Marie Dorn, Karim Jaén Chavez, Ingo Jordan, Volker Sandig, Jennifer Altomente, Yvonne Genzel, and Udo Reichl

PRODUCTION OF A FUSOGENIC ONCOLYTIC rVSV-NDV VIRUS IN PERFUSION PROCESSES

Sven Göbel, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
goebel@mpi-magdeburg.mpg.de
Marie Dorn, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg
Karim Jaén Chavez, Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universität München, Germany
Ingo Jordan, ProBioGen AG, Berlin, Germany
Volker Sandig, ProBioGen AG, Berlin, Germany
Jennifer Altomonte, Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universität München, Germany
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto-von-Guericke University, Germany

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Oncolytic viruses (OVs), as a therapeutic vaccine, offer an elegant approach to cancer therapy. On the one side they have the ability to cause direct tumor cell lysis, on the other side they can stimulate immune responses directed against the tumor. By expressing endogenous or heterologous fusion glycoproteins, an enhanced intratumoral spread via syncytia formation can be achieved. Rapid and efficient fusion of infected cells may result in large multinucleated syncytia, in which cells quickly die before high titers are reached [1]. Prospective treatment with OVs will require manufacturing processes that enable the production of a very high number of doses with high titers. As a first step towards this goal, suspension cell substrates were identified to develop a highly efficient and scalable production process of a novel hyper-fusogenic hybrid of vesicular stomatitis virus and Newcastle disease virus (rVSV-NDV).

Here, we present process intensification methods established as a second step that involve the production of rVSV-NDV in two different suspension cell lines (AGE1.CR and BHK-21). Cells were grown to high cell density (HCD), either in shake flasks using semi-perfusion or perfusion with an acoustic settler system coupled to a 0.6 L working volume (wv) stirred tank bioreactor system. Cell growth, metabolism and virus production was characterized. Moreover, performance of the cells was evaluated based on infectious virus titers in the supernatant and cell-specific virus yields (CSVY). Shake flask cultivations using semi-perfusion mode allowed growth of AGE1.CR cells up to $42x10^6$ cells/mL. However, only relatively low infectious virus titers ($2x10^6$ $TCID_{50}$/mL) and CSVY (<0.1 TCID50/cell) were obtained. Further optimizations allowed an over 10-fold increase in infectious virus titer up to $3x10^7$ $TCID_{50}$/mL. For BHK-21 cells, high titers up to $2x10^8$ $TCID_{50}$/mL and CSVY (84 $TCID_{50}$/cell) were already achieved at low cell concentrations of $3x10^6$ cells/mL in batch mode and directly transferred to perfusion cultures. Final evaluation of both cell lines in high cell density perfusion culture using an acoustic settler system with a manually-adapted perfusion rate demonstrated mixed performance: Utilizing a low cell-specific perfusion rate (CSPR) of 0.05 nL/cell/day, AGE1.CR cells grew to cell concentrations of up to $35x10^6$ cells/mL. Accordingly, no improvement compared to optimized semi-perfusion in shake flasks could be obtained. Nevertheless, virus titers of $2x10^7$ $TCID_{50}$/mL with CSVY of 1 $TCID_{50}$/cell were reached again. Switching to BHK-21 cells and utilizing a higher CSPR of 0.11 and 0.16 nL/cell/day, maximum cell concentrations of $28x10^6$ cells/mL and $31x10^6$ cells/mL were achieved. Here, rVSV-NDV titers peaked after two days post infection with $2x10^9$ $TCID_{50}$/mL and $4x10^9$ $TCID_{50}$/mL, respectively. The CSVYs were 113 and 126 $TCID_{50}$/cell, slightly higher compared to the batch experiments. Moreover, for the first time, clear syncytia formation was observed in BHK-21 suspension cell culture starting 12 hours post infection. Interestingly, the performance of the acoustic settler was not affected by syncytia formation: viable cell retention above 97.0% and the harvest of virus into the retentate was possible until the end of the cultivations.

Taken together, both AGE1.CR and BHK-21 cell lines were successfully grown to HCD in perfusion cultures for rVSV-NDV production. Process optimization using AGE1.CR cells allowed to increase virus titers only about 10 fold (maximum titer $2x10^7$ $TCID_{50}$/mL). Using BHK-21 cells in HCD in perfusion cultures, maximum titers of $4x10^9$ $TCID_{50}$/mL were achieved. Therefore, BHK-21 cells seem to be the substrate of choice for large-scale manufacturing. Overall, rVSV-NDV production in a 0.6 L STR using perfusion generated virus material equivalent to about 30,000 doses (8 log infectious units per dose) in an operation time of less than two weeks.

1. Krabbe, T. and J. Altomonte, *Fusogenic Viruses in Oncolytic Immunotherapy.* Cancers (Basel), 2018. 10(7).