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AUTOMATED SINGLE-CELL CLONING IN CHEMICALLY DEFINED MEDIUM FOR NEW SUSPENSION MDCK CELL LINES AND SCALE-DOWN OF INFLUENZA A VIRUS PRODUCTION INTO AMBR®15 MICROBIOREACTORS

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Vaccines for known and emerging diseases as well as new therapeutic applications of viruses will require multiple doses and high virus input per dose or treatment. Therefore, there is a clear need for innovation in vaccine manufacturing. Yet, process optimization and intensification are cost- and labor-intensive challenges and thus not always considered as economically reasonable. To tackle this problem and compete against outdated cost calculations, this work aims to transfer knowledge and techniques that were acquired within a decade of protein production process development into the field of viral vaccine manufacturing. This comprises the development of monoclonal suspension MDCK cell lines using an automated single-cell cloning strategy in chemically defined medium. Subsequently, a scale-down into the fully automated single-use Ambr®15 microbioreactor system (Sartorius, Germany) was established for influenza A virus (IAV) production. This may help to accelerate process development and optimization by parallelized screening of multiple parameters in a high-throughput manner.

Adherent MDCK cells (NBL-2 from ATCC) were adapted to suspension growth in the chemically defined MDXK medium (Xell, Germany). Once adapted, a single-cell cloning strategy was performed on the heterogeneous population using the CellCelector™ (ALS, Germany). More precisely, cells were seeded into a nanowell-plate and subsequently monitored daily by the integrated imaging system to follow-up cell growth. This resulted in five monoclonal suspension MDCK cell cultures, with a top performing clone reaching a cell concentration of 8.5×10^6 cells/mL in batch (shake flasks). Subsequently, infection with influenza A/PR/8/34 (H1N1) at a multiplicity of infection (MOI) of 10^{-3} resulted in HA titers of up to 2.61 log10(HAU/100µL). In parallel, two existing non-commercial suspension MDCK cell lines [1, 2] were adapted to MDXK medium to set a benchmark for growth and IAV infection in the Ambr®15 system and shake flasks, respectively. Here, batch cultures reached a maximum at 13.8×10^6 cells/mL with virus production of up to 3.41 log10(HAU/100µL). Finally, scale-down into an Ambr®15 microbioreactor system was successfully implemented. Here, a maximum cell concentration of 12.9×10^6 cells/mL and HA titers up to 3.25 log10(HAU/100µL) were achieved.
By evaluating this approach for a reference IAV process, we aim at delivering a GMP-qualified, monoclonal suspension MDCK cell line that grows in chemically defined medium to high cell concentrations and produces IAV to yields that outcompete existing processes. It relies on the combination of a single-cell cloning strategy combined with high-throughput parallelized candidate evaluation and process parameter screening in an Ambr®15 system. If successful, this approach could be equally taken for other existing or novel virus production processes.

[1] Wu, Y. et al., High cell density perfusion process for high yield of influenza A virus production using MDCK suspension cells. Appl Microbiol Biotechnol, 2021. 105(4).
[2] Bissinger, T., et al., Semi-perfusion cultures of suspension MDCK cells enable high cell concentrations and efficient influenza A virus production. Vaccine, 2019. 37(47).