Perfusion media development for scalable processes

Patrick Mayrhofer1*, Andreas Castan2, Renate Kunert1
1University of Natural Resources and Life Sciences (BOKU), Vienna, Muthgasse 11, 1190 Vienna, Austria
2GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden. For local office contact information, visit gelifesciences.com/contact
© 2019 General Electric Company.
gelifesciences.com

Abstract
Cell culture perfusion processes are considered optimal for a truly integrated continuous biomanufacturing pipeline. The nutrient-rich but balanced media should be designed to support very low cell-specific perfusion rates (CSPR) that minimize media consumption while maximizing viable cell days and productivities. Optimized processes at low CSPR drastically reduce equipment costs, lab space, and product dilution. Finally, operating at very low CSPR will allow for minimal cell bioprocesses to run as true chemostat cultures in the future.

In this study, we demonstrate a general workflow to develop high-performing perfusion media using small-scale models and transfer the process to a 50 L scale at CSPR of 20 µL/cell/d.

Materials and methods
Cell line: HyClone™ Chinese hamster ovary (CHO) cells (CHO-K1, GE)
Basal media: HyClone CDM4NS0 or HyClone ActiPro™
Feed supplements: see Figure 1
Analytical methods: see Table 1

Design of Experiment (DoE, Steps 1 and 2)

BioProfile™ 100 plus (glucose, lactate, glutamine, glutamate, ammonia, Osmotic G01 (mOsm/kg), Octet™ QK (inter))

Semi-continuous small-scale perfusion models (Steps 2 and 3)

Start VCD: 10 × 10^5 cells/mL in 10 mL (spiked) basal medium

One volume exchange per day (1 reactor volume [RV])/d by centrifugation at 300 g/7 min after bleeding if applicable

220 rpm at 50 mm shaking diameter in a Kuhner shaker instrument at 7% CO2 and 85% humidity at 37°C

Biorreactor verification runs (Step 4)

Optimized CDM4NS0 or ActiPro perfusion medium was applied to perfusion bioreactors using a ReadyProcess WAVe 25 or Xcellerex™ XDR 50 bioreactor; cells seeded at 1 × 10^5 cells/mL in unspiked basal medium, and perfusion initiated on days 2 to 4 at a working volume of 500 mL or 40 L; culture parameters set to control > 30% dissolved oxygen (DO), 37°C, pH 6.8 to 7.0

Results
A generally applicable perfusion medium development workflow was applied to two different HyClone CHO basal media ActiPro and CDM4NS0. In a first screening round (Fig 2, step 1), beneficial effects of Cell Boost supplements 1, 3, 7a, and 7b were identified using a DoE approach in spiked batch cultures. The pre-selected supplements were subsequently applied to a second DoE using 10 µL/shaking cultures in a semi-continuous perfusion mode by daily media exchange (Fig 2, step 2), where the primary objective was to fine-tune the ratio of pre-selected Cell Boost supplements. High VCDs of more than 50 × 10^6 cells/mL in a quasi steady-state were reached. Spiking basal medium with Cell Boost supplements improved viabilities and daily titers, with values up to 3 µL. Subsequent bleeding experiments in semi-perfusion cultures (Fig 2, step 3) revealed higher maintained growth rates at higher bleeding rates, correlating with higher specific productivities. Despite lower steady-state VCDs, increased specific productivity resulted in the titer increasing by 20% when a 30% daily bleed was used. N-glycosylation profiles of antibodies produced in the semi-continuous models showed a decreased galactosylation patterns at later process times.

Two novel perfusion media developed within this project and based on basal CDM4NS0 or ActiPro and Cell Boost 1 and Cell Boost 3 were applied to different bioreactor perfusion verification runs. Using a continuous volumetric perfusion rate, the minimum CSPR of 10 µL/cell/d was determined to generate the highest VCD of more than 200 × 10^6 cells/mL. A similar high VCD was reached with ActiPro + Cell Boost 1/3 by using a constant CSPR of 15 to 30 µL/cell/d, to reduce medium consumption. The novel perfusion media were also applied to bioreactor production runs at a constant VCD of 50 × 10^6 cells/mL at 500 mL or 40 L scale. An increase of galactosylated glycan species was observed over process time, and a good correlation of various bioreactor parameters compared to the 30%-bled small-scale model was identified. Major differences were only fixed for the glutamate/glutamine/hK4 behavior, which might be responsible for the discrepancy of the terminal galactosylation profile.

Conclusion

A DoE-based workflow was developed to leverage established feed supplements for definition of novel, high-performing perfusion media.

Small-scale models in semi-continuous perfusion mode were used to screen different conditions within a single operator. A minimum CSPR of 10 µL/cell/d was determined by constant volumetric perfusion rates in a ReadyProcess WAVe 25 bioreactor to reach 200 × 10^6 cells/mL.

A steady-state production perfusion run was scaled up to an Xcellerex XDR 50 L bioreactor.

Galactosylation increased in galactosylated species in bioreactor perfusion runs but decreased in the semi-continuous models, likely due to higher amounts of ammonia accumulation.

Critical culture parameters were very similar between bled small-scale cultures and the perfusion bioreactor at similar CSPRs.

Download this poster
Use the QR code or link bit.ly/2G2ck0

gelifesciences.com