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Patrick Mayrhofer[1]*, Andreas Castan[2], and Renate Kunert[1]

[1]University of Natural Resources and Life Sciences (BOKU), Vienna, Muthgasse 11, 1190 Vienna, Austria; [2]GE Healthcare Bio-Sciences AB, Björkgatan 30, 75184 Uppsala, Sweden

*patrick.mayrhofer@boku.ac.

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 mammalian 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 pL/cell/d.

Materials and methods

Cell line: HyClone™ Chinese hamster ovary (CHO) cells (CHO-K1, IgG1)

Basal media: HyClone CDM4NS0 or Hyclone ActiPro™

Feed supplements: see Figure 1

Analytical methods: Vi-CELL™ (viable cell density [VCD]), BioProfile™ 100 plus (glucose, lactate, glutamine, glutamate, NH4+), Osmomat 030 (mOsm/kg), Octet™ QK (titer)

Design of Experiment (DoE, Steps 1 and 2)

Three DoE levels (-1, 0, +1)

- Level -1: translates into 0% spike concentration
- Level 0: half-maximum spike concentrations
- Level +1: all Cell Boost™ supplements were mixed according to total amino acid concentration and spiked into basal media to reach 400 mOsm/kg; design of DoE matrix and establishment of final statistical models were performed using MODDE™ software

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

Start VCD: 10 × 10^6 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% CO_2 and 85% humidity at 37°C

Bioreactor verification runs (Step 4)

Optimized CDM4NS0 or ActiPro perfusion medium was applied to perfusion bioreactors using a ReadyToProcess WAVE™ 25 or Xcellerex™ XDR 50 bioreactor; cells seeded at 1 × 10^6 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

Cell Boost supplement	Stock conc. [w/w]	Amino acids	Vitamins	Glucose	Trace elements	Growth factors (peptides)	Hypoxanthine/ Thymidine	ADCF lipids	ADCF cholesterol
1	10%	•	•	•					
2	10%	•		•					
3	5%	•	•	•	•		•		
4	10%	•	•	•	•	•		•	•
5	5%	•	•	•	•	•	•	•	•
6	5%	•	•	•	•	•	•	•	•
7a	18.1%	•	•	•	•				
7b	9.5%	•							

Fig 1. HyClone Cell Boost supplements for development of high-performing perfusion media. ADCF = animal-derived component-free.

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 mL 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 1 g/L. 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 pL/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 pL/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 a 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 found for the glutamate/glutamine/NH4+ behavior, which might be responsible for the discrepancy of the terminal galactosylation profile.

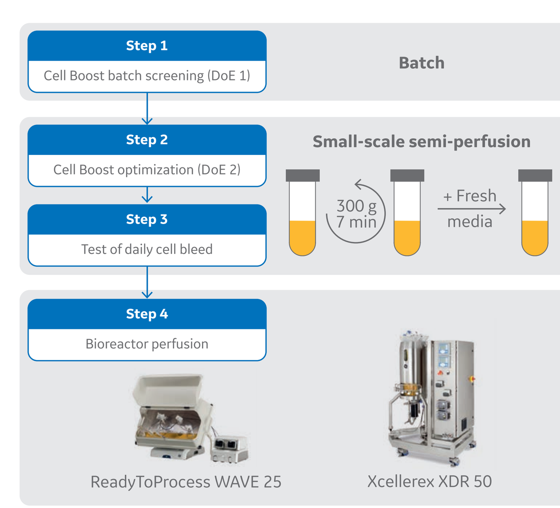

Fig 2. Fast workflow for perfusion media development. After initial definition of optimal basal media, eight different Cell Boost supplements were screened, optimized, and tested in four consecutive steps using DoE and small-scale semi-continuous perfusion models.

DoE run #	Cell Boost 1	Cell Boost 3	Cell Boost 7a	Cell Boost 7b	
	DoE levels				
1	-1	-1	-1	-1	Fractional factorial
2	+1	-1	-1	-1	
3	-1	+1	-1	-1	
4	+1	-1	+1	-1	
5	-1	+1	+1	-1	
6	+1	+1	+1	-1	
7	-1	-1	-1	+1	
8	-1	+1	-1	+1	
9	+1	+1	-1	+1	
10	-1	-1	+1	+1	
11	+1	-1	+1	+1	
12	+1	+1	+1	+1	
13	-1	0	0	0	Star point (face centered)
14	+1	0	0	0	
15	0	-1	0	0	
16	0	+1	0	0	
17	0	0	-1	0	
18	0	0	+1	0	
19	0	0	0	-1	
20	0	0	0	+1	
21	0	0	0	0	Center point
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23	0	0	0	0	

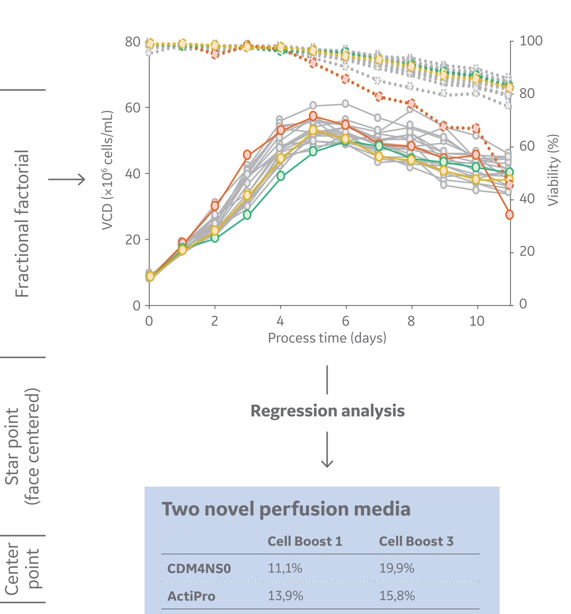

Fig 3. DoE 2 optimization of pre-selected Cell Boost supplements in small-scale semi-continuous perfusion models (Step 2). Regression analysis was performed to define the final perfusion media.

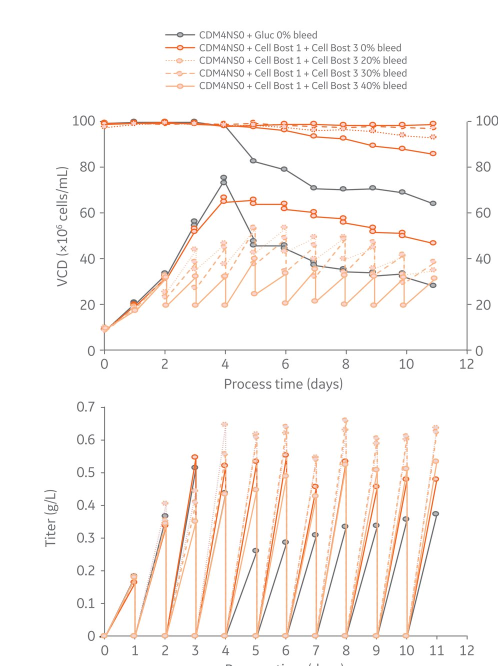

Fig 4. Effect of daily cell bleed (Step 3). Semi-perfusion cultures were bled daily at 20% to 40% before each media exchange.

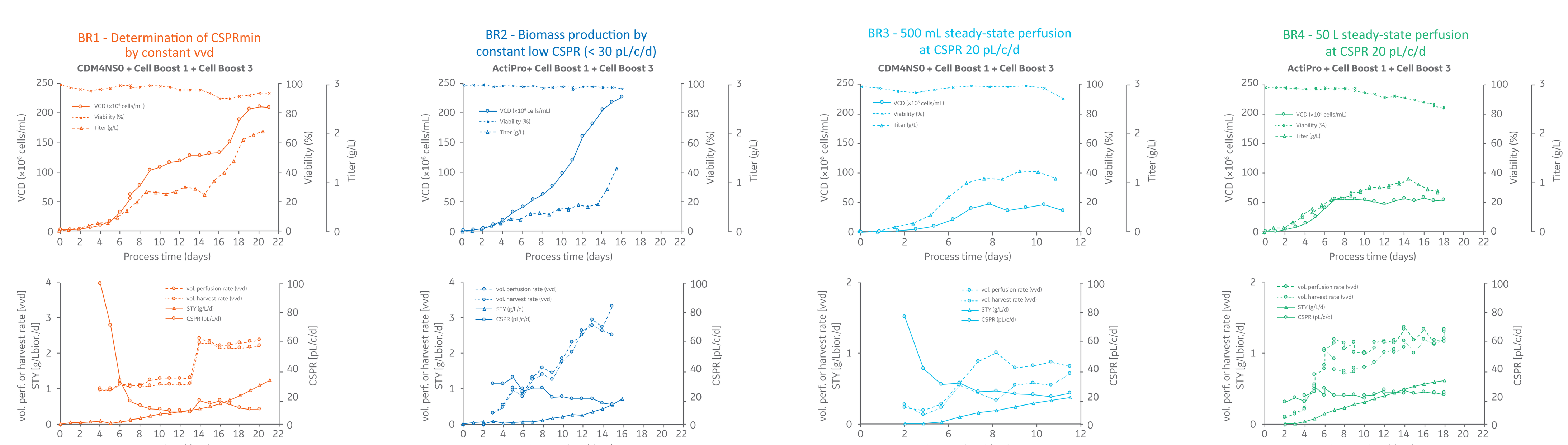

Fig 5. Optimized perfusion media applied to different bioreactor runs (Step 4). CDM4NS0- or ActiPro-based perfusion media were applied to 500 mL ReadyToProcess WAVE 25 or 50 L XDR 50 bioreactor perfusion runs. VCD = viable cell density; vvd = media volume per bioreactor volume per day.

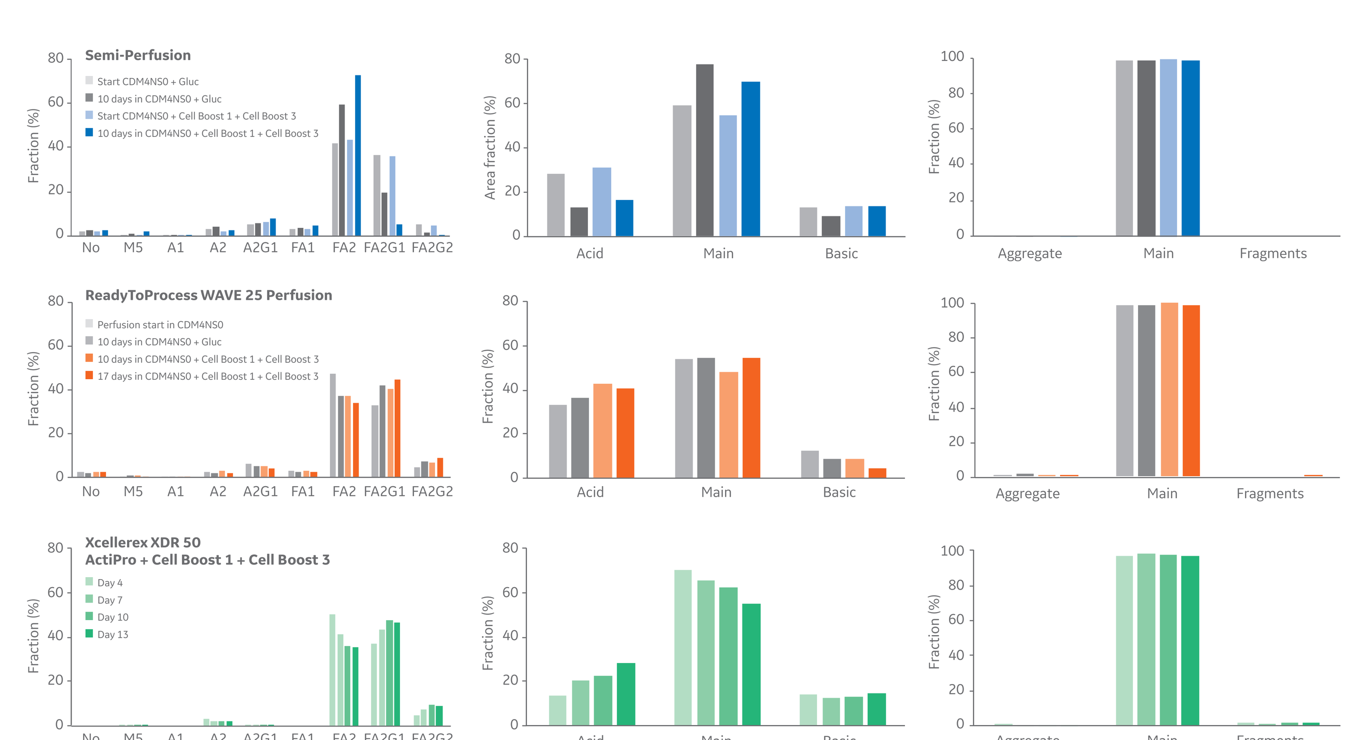

Fig 6. Consistent product qualities between basal and Cell Boost-spiked perfusion media. Quality attributes were analyzed for N-glycosylation, charge distribution, and aggregation/fragmentation.

	Small-scale model (30% bleed)	ReadyToProcess WAVE perfusion (BR 3)	Ratio model/ reactor
Perfusion mode	Semi-perfusion	VCD const	–
Vessel	Tube	ReadyToProcess WAVE 25	–
Basal media	CDM4NS0	CDM4NS0	–
Supplement	Cell Boost 1 + Cell Boost 3	Cell Boost 1 + Cell Boost 3	–
Period	Days 8 to 15	Days 6 to 11	–
pH control	None	Setpoint pH 6.8	–
Perfusion rate (vvd)	1.0	0.9	1.15
Harvest rate (vvd)	0.7	0.5	1.32
Bleed rate (vvd)	0.3	0.3	0.90
Total cell conc. (mio. cell/mL)	40	43	0.93
Viability (%)	98	96	1.02
Titer (mg/L)	1034	1123	0.92
µv (1/d)	0.36	0.48	0.76
qP (pg/cell/d)	27.8	24.9	1.12
CSPR (pL/cell/d)	21.1	21.9	0.96
Glucose (mg/L)	7284	5884	1.24
Lactate (mg/L)	2137	1580	1.35
Glutamate (mg/L)	267	107	2.49
Glutamine (mg/L)	52	0	N/A
NH4+ (mg/L)	84	28	3.02
qGlucose cons. (pg/cell/d)	221	224	0.99
qLactate prod. (pg/cell/d)	51	31	1.65
qGlutamate cons. (pg/cell/d)	9.4	12.0	0.78
qGlutamine prod. (pg/cell/d)	0.5	-0.3	-1.58
qNH4+ prod. (pg/cell/d)	0.6	0.2	2.79
qLactate/qGlucose (mol/mol)	0.4	0.3	1.41
Residual Osm (mOsm/kg)	295	283	1.04

Fig 7. Comparison of small-scale versus bioreactor perfusion cultures. Small-scale perfusion cultures daily bled at 30% was predictive for many parameters of a ReadyToProcess WAVE bioreactor perfusion run.

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 pL/cell/d was determined by constant volumetric perfusion rates in a ReadyToProcess 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.
- Glycoslyation increased in galactoslyated 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.

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