Flexible production of an Fc-fusion protein via transient transfection of mammalian cells in a single-use bioreactor

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INTRODUCTION

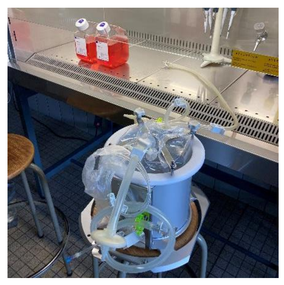
Large-scale production of recombinant therapeutic proteins is usually achieved by stable transfection of mammalian cells in stirred-tank bioreactors.[1,2] This approach requires the complex and lengthy process of stable cell line engineering.[1] In contrast, transient transfection is an attractive alternative for the development of small batches of protein for detailed characterization and pre-clinical studies.[3] However, transiently transfected cells are sensitive to the substantial shear stress caused by the stirring motion in stirred-tank bioreactors. Orbitally shaken bioreactors are a valuable alternative to stirred-tank bioreactors due to their easy scale-up and the low shear forces.[4,5] In addition, single-use technologies such as 3D bags enable very efficient biomanufacturing by reducing cross-contamination risks and avoiding the need for bioreactor cleaning and sterilization.

Here, we demonstrate the production of an Fc-fusion protein via transient transfection of mammalian cells in an orbitally shaken single-use bioreactor.

MATERIALS AND METHODS

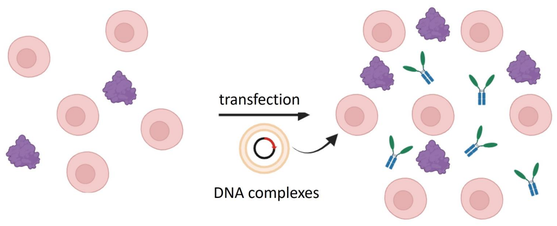
Cell culture

Expi293™ cells (Thermo Fisher) were expanded according to manufacturer's protocol prior to transfer to an SB10-X orbital shaken bioreactor (Adolf Kühner AG) using 3 L single-use bags with built-in optical sensors for pH and dissolved oxygen (DO) measurement. Cells were cultivated using either a controlled pH of 6.8 and a DO of 40% or using constant gas flow rates (21% O_2, 8% CO_2). The cell density was 1.5-2.0 x 10^6 cells/ml in a culture volume of 1.5 or 2 L at the time of transfection.

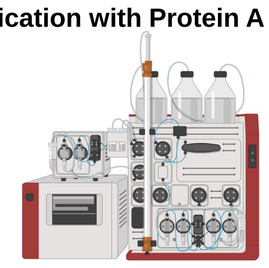


Transient transfection

Proteins were produced by transient transfection using FectoPRO® reagent (1 µl/mL medium) and plasmid DNA (0.5 µg/ml medium). FectoPRO® Booster (0.5 µl/mL medium) was added 2 h post-transfection. Cell concentration and viability were determined with a hemocytometer and trypan blue staining.



Purification with Protein A and SEC



Gel electrophoresis (SDS-PAGE)

The samples were analyzed on SERVAGel™ TG PRiME™ 4 - 20% gels using a Dual Color Protein Standard III from SERVA Electrophoresis GmbH.

Biolayer interferometry (BLI)

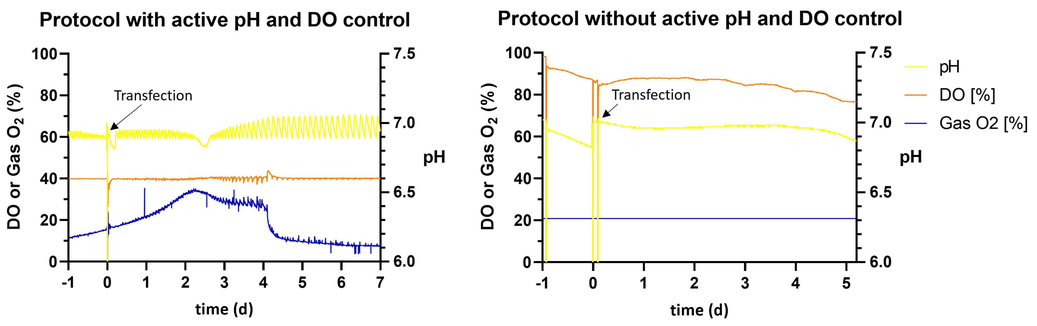
The concentration of Fc-fusion protein in supernatant was determined with Protein A Dip and Read™ Biosensors (Sartorius) using a BLItz system (FortéBio).

Microfluidic modulation spectroscopy (MMS)

Secondary protein structure was measured with an Aurora (RedShiftBio) using a protein concentration of 1.5 mg/mL in PBS.

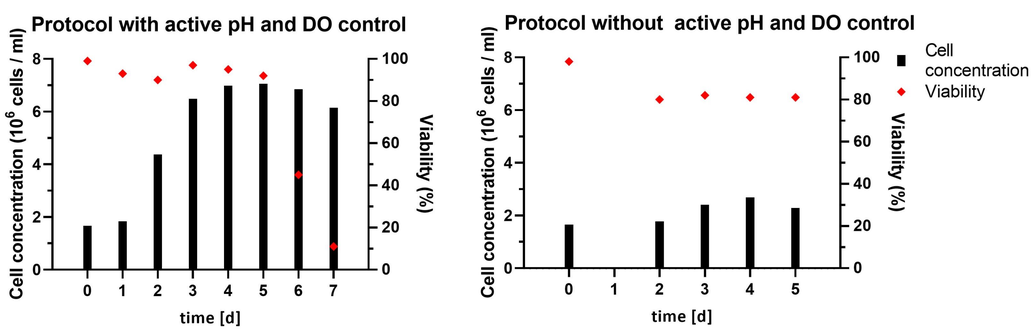
RESULTS

Comparing two production protocols



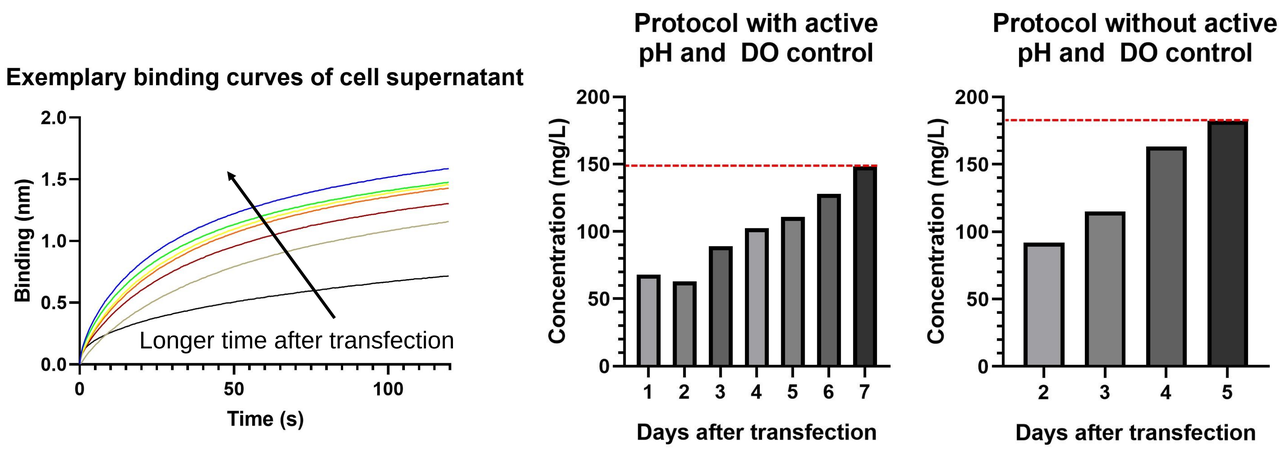
- With active pH and DO control, oxygen demand by the cells increases slowly with a peak around 2 days after transfection and drops after day 4
- Without active control, the DO is higher, and pH also stays constant

Cell count and viability during production



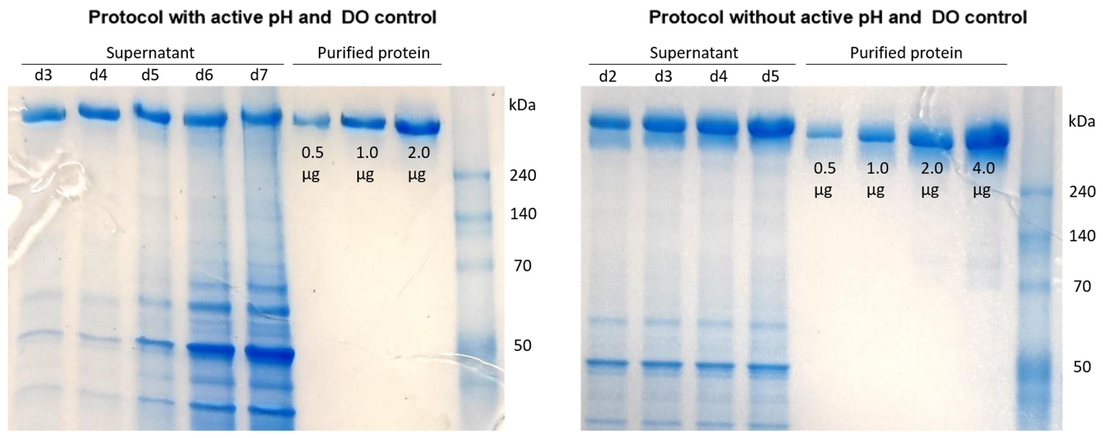
- pH / DO control results in fast cell proliferation with sharp viability drop at day 6
- No pH and DO control leads to slower cell proliferation

Protein secretion and production yields



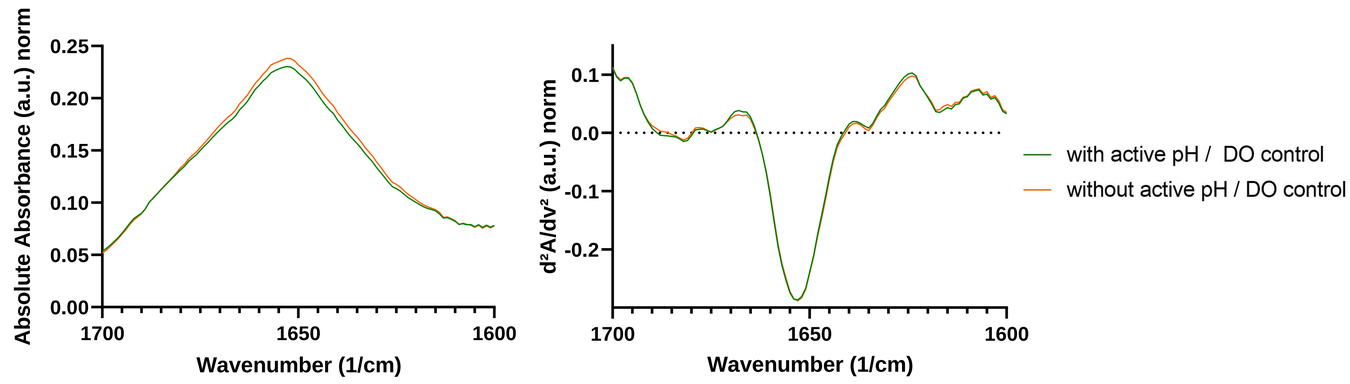
- High titers of secreted Fc-fusion protein (150-180 mg/L) from both protocols as detected by biolayer interferometry measurements of cell supernatants
- Steady increase in secretion yield until day of harvest in both protocols

Protein purity



- Expected molar mass of purified protein from both protocols
- Less host-cell impurities in protocol without active pH and DO control at time of harvest

Protein structure



- Proteins produced from both protocols have a nearly identical secondary structure indicated by MMS

CONCLUSIONS

- Fc-fusion protein can be efficiently produced by transient transfection in a single-use bioreactor
- Slightly higher protein yields were achieved using a protocol without active pH and DO control
- Time of harvest is critical to avoid high levels of host-cell impurities

ACKNOWLEDGEMENTS

We want to thank the Research Foundation – Flanders (FWO) for a travel grant to support the participation in this conference and Adolf Kühner AG for providing the bioreactor.

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14th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, 18 - 21 March 2024, Vienna, Austria