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Medium development beyond production media: Chemically defined media for transfection and single cell cultivation

In this work, the current progress in designing special application media for transfection and single cell growth is presented. In the context of chemically defined medium development, these techniques pose specific challenges which differ from production media. However, interest in such specialized products is high. On one side, clone selection is a key factor for robust and effective processes. On the other side, applications for transient transfection range from R&D to the production of pre-clinical material. In terms of upscaling, efficient transient gene expression processes require a bifunctional medium solution supporting both cell growth and transfection.

RESULTS

A. Medium development for single cell cloning

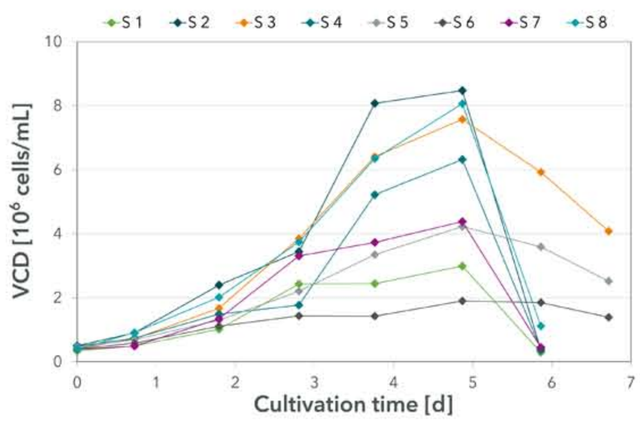

FIG. 1: Single cell clones were isolated from a cell pool by limited dilution using a current development stage of the cloning medium. After subcultivating single cells from 96-well plates to shake flask scale (see scheme in FIG. 2), batch growth curves in spin tubes were performed. Viable cell density (VCD) is shown for eight exemplary subclones.

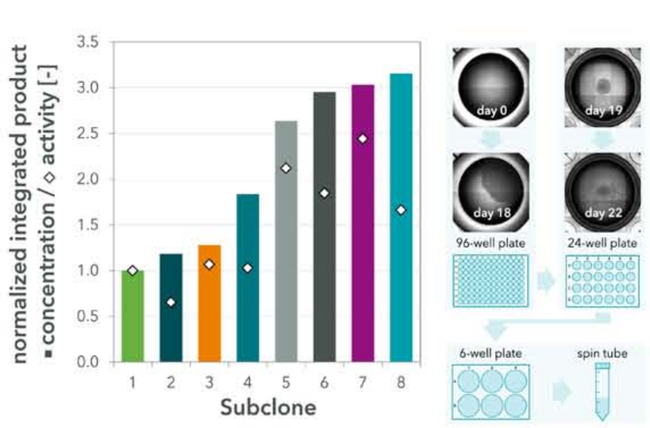

FIG. 2: Production of a labile recombinant protein was measured on day four and five for eight exemplary clones (FIG. 1). Integrated product concentration and activity were normalized to subclone 1. These results show potential heterogeneity of cell pools and illustrate general feasibility of the developed medium to isolate single cell clones.

CONCLUSIONS

- Performed proof of concept already illustrates the capability of the current medium stage to generate subpopulations out of a clone pool.
- To allow easy transfer to the final production process, the use of animal component-free medium is advantageous, even though cloning efficiency is still higher with serum addition.
- Successful utilization of our chemically defined, animal component-free cloning medium for expanding single cells shows that there is no general need for serum supplementation.
- As soon as stable colony growth is reached, upscaling of cells from well-plates to shake flask scale can easily be performed by simply adding increasing volumes of growth medium.

RESULTS

B. Medium development for transient transfection

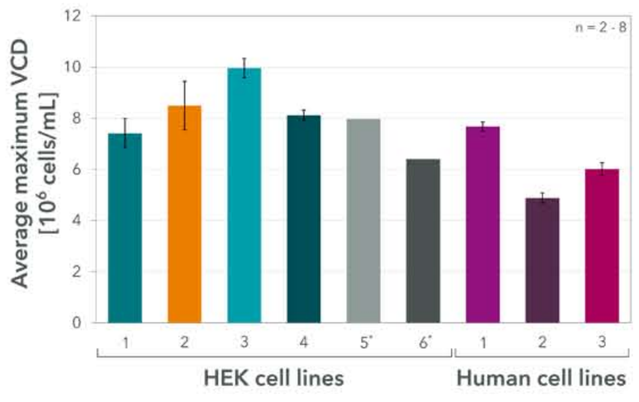

FIG. 3: For a broad variety of six HEK and three additional human cell lines, the maximum viable cell density was determined for batch shaker cultivations in the developed medium. Although mainly developed with HEK cell lines, the current medium also supports cell growth for divergent human cells. (* data provided by external partners)

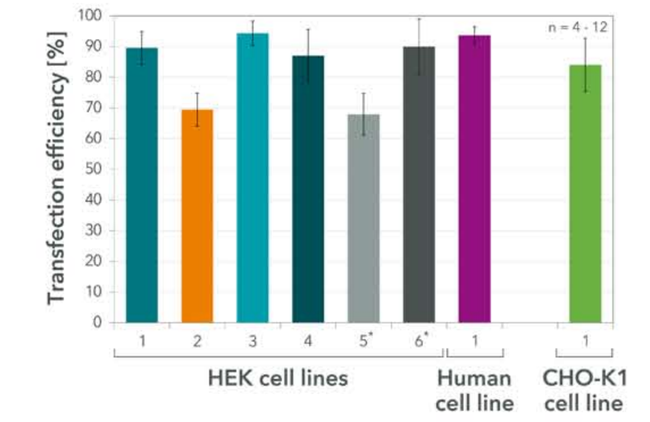

FIG. 4: Transfection efficiencies were determined by GFP expression 48 hours post transfection using flow cytometry analysis. In addition to HEK/ human cell lines, the developed Xell transfection medium was tested for a CHO-K1 cell line as well. For that, transfection efficiencies above 80% were achieved. (* data provided by external partners)

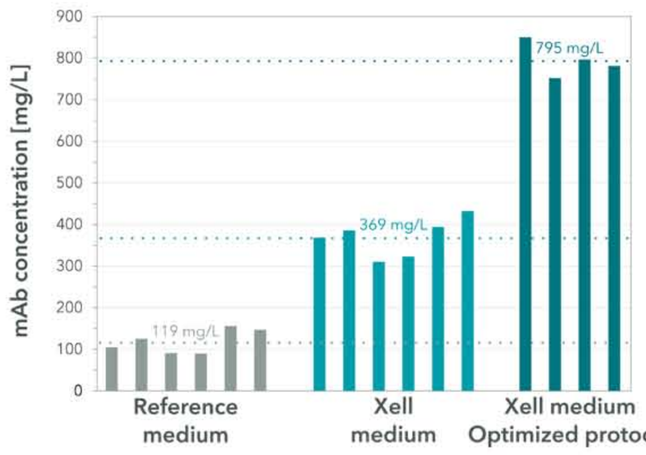

FIG. 5: The yields of transient monoclonal antibody expression with HEK cell line 3 (FIG. 1 & 2) were measured in several independent experiments using the developed Xell transfection medium. Titers were determined six to seven days post transfection and compared to results obtained with a commercially available reference medium recommended for growth and transfection (mean values are depicted by dotted lines).

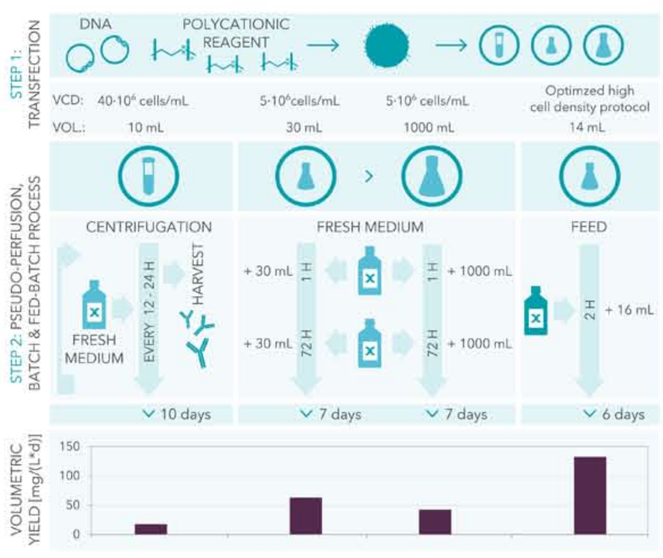

FIG. 6: Transient expression of a monoclonal antibody was evaluated in several independent experiments with differing setups and scales using HEK cell line 3 (FIG. 1 - 3) and the developed transfection medium.

CONCLUSIONS

- The developed HEK transfection medium supports stable cell growth in seed train as well as high cell densities and viability in batch cultivation.
- Transfection efficiencies between 68% to 94%, depending on cell line, were achieved in Xell transfection medium. The medium was also successfully applied for a CHO-K1 suspension cell line.
- Targeted development as well as optimization of protocols have enabled successful transient transfections in liter scale.
- The successful pseudo-perfusion shows the feasibility of this process strategy for transient gene expression and is simply scaled-up to bioreactor scale. Such perfusion processes are highly interesting for labile products.
- While a titer of 119 mg/L was measured in a relevant reference medium, Xell's developed medium led to an increased antibody production of 369 mg/L.
- By further optimizing the transfection protocol and adding a specialized feed supplement post transfection, protein yields are factor eight higher than in the commercial reference. This way, titers of up to 850 mg/L were achieved with HEK cells after six days of cultivation.

METHODS

Cultivation All cell lines were cultivated in indicated culture systems using standard conditions in either chemically defined Xell medium or relevant reference media.
Analytics Viable cell density and viability were measured using a Cedex automated cell counter. Single cell growth was analyzed using a Cellavista. Labile recombinant protein was quantified by ELISA and an activity assay. Antibody yield was measured by protein A HPLC.

Transfection The setup for transfection varied for several approaches. In general, cells were transfected at cell densities of $3 \cdot 10^6$cells/mL or as indicated in Xell medium with a polycationic transfection reagent. 2 pg DNA/cell were used in transfections with plasmids harbouring genes for the expression of GFP or a monoclonal antibody. Culture volumes during transfection ranged from 4 mL to 1000 mL.

PARTNERS & ACKNOWLEDGEMENTS

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