Predicting the Performance of Perfusion Media in a Continuous Bioreactor from Small-Scale Perfusion Mimics

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BACKGROUND AND NOVELTY

Bioprocessing continues to face challenges in the scale-up manufacturing of therapeutic proteins from small-scale models during development to large-scale bioreactors, particularly when manufacturers aim to switch from the more common fed-batch process to a continuous perfusion process. Both processing methods provide advantages and disadvantages; however, both have become established methods in the production of biomolecule therapeutics. Here, we describe the evaluation of perfusion media, utilizing small-scale perfusion-mimic models and comparing data from a 0.1 L perfusion-capable bioreactor as a method for evaluation and selection of a steady-state continuous perfusion medium.

MATERIALS AND METHODS

The evaluation of CHO perfusion media was accomplished by utilizing perfusion-mimic models and a perfusion-capable tabletop bioreactor. Both CHO-GS and CHO DG44 cells were used in the evaluation with seeding densities at 0.3X10^6 or 0.5X10^6 cells/mL, respectively. Cell culture mini-bioreactor centrifuge tubes, commonly referred to as spin tubes or shake tubes, were utilized to maximize cell densities and titer production of recombinant antibodies. Once maximized cell densities and titers were achieved, an Ambr15 micro-bioreactor system was utilized in a perfusion-mimic mode, and the evaluation of perfusion media was accomplished in a 0.1L perfusion-capable tabletop bioreactor.

The mini-bioreactors and Ambr15 perfusion-mimic process was accomplished by growing cells and replacing media daily to represent a medium exchange rate of 1 vessel volume per day (VVD), while a 0.1L working volume tabletop perfusion-capable bioreactor was used to continuously feed media to the bioreactor at a similar VVD. Steady-state cell densities in the Ambr15 micro-bioreactor were determined by the maximized cell densities in the mini-bioreactors, while three steady states were targeted on the tabletop bioreactor in a push-to-low strategy by increasing cell density after 3 or more days. In both the micro-bioreactor and tabletop bioreactor systems, cells were manually bled daily after reaching the steady-state (SS) target.

Daily sampling of the bioreactor and harvest media provided cell density, titer and metabolite data. Titer and viable cell density measurements were converted to cell specific productivity (qP), volumetric productivity (VP) and cell specific perfusion rate (CSPR) to compare the productivity values across cell lines and the performance of the media.

EXPERIMENTS AND RESULTS

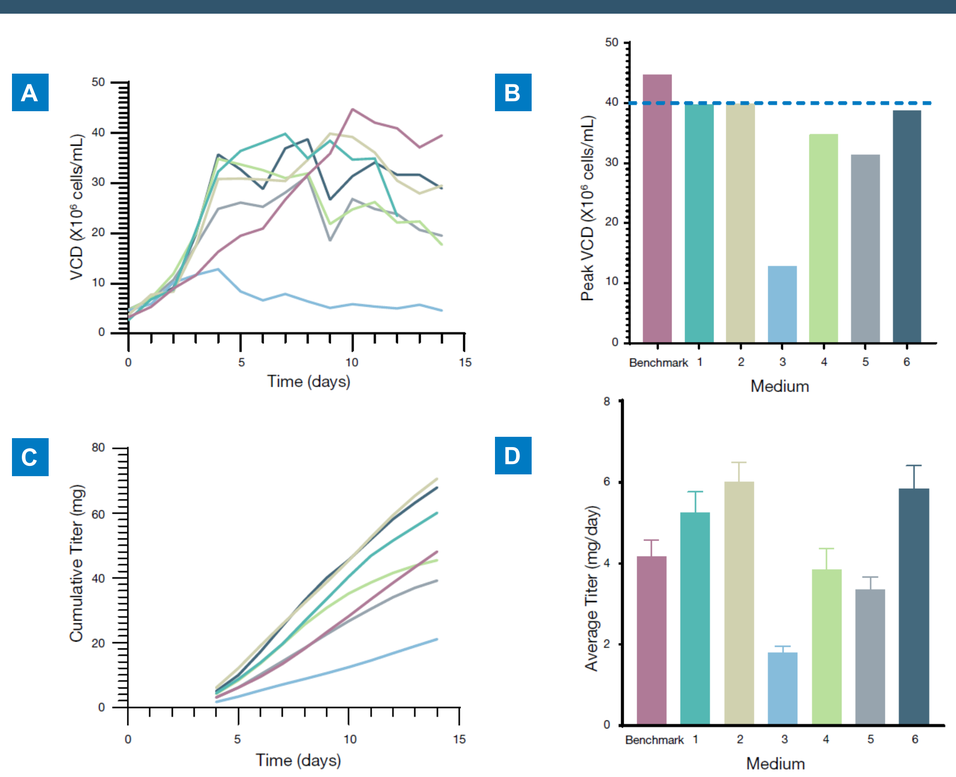

Figure 1. Growth of CHO-GS cells in 50 mL spin tubes under perfusion-mimic conditions. Forty-two distinct cell culture media were designed using DoE to maximize growth and titer, assessing the highest cell density that each medium can achieve with each cell line. DG44 CHO cells were also tested in this manner (data not shown). (A) Seven example media are demonstrated, displaying the variability in growth due to the variability in the media components. (B) The peak VCD was plotted for each medium, demonstrating that the mimic model can be used to eliminate media that are not capable of sustaining cell growth under basic growing conditions. (C and D) Cumulative titer and daily average titer were calculated for each culture, demonstrating that the top growth producing medium was not the most productive medium, as media 2 and 6 outperformed the benchmark medium.

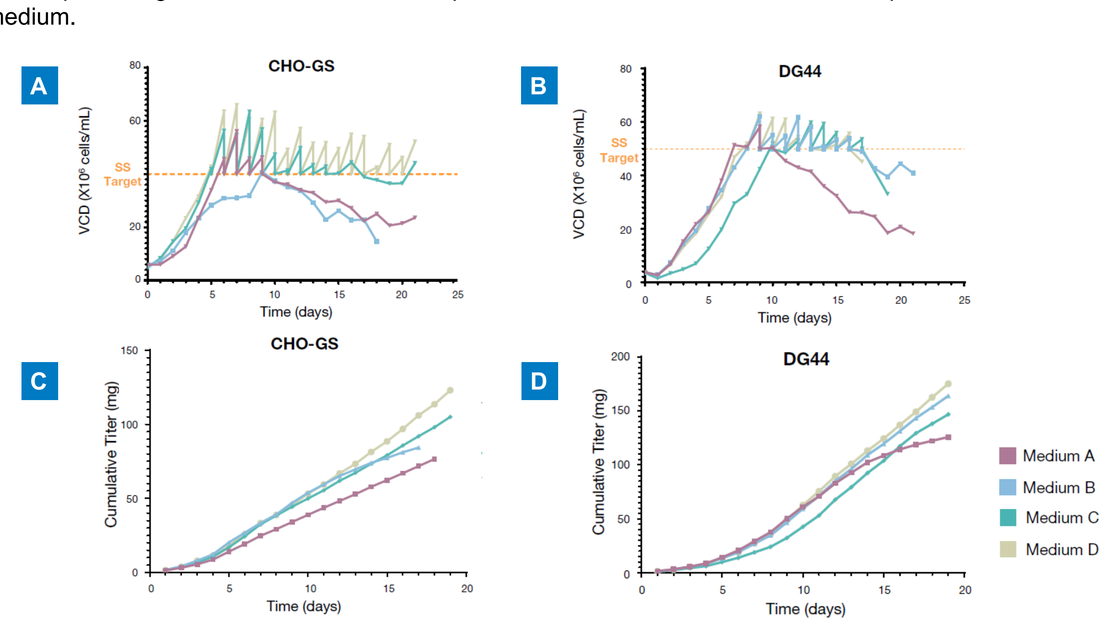

Figure 2. Growth of CHO-GS and DG44 CHO cells in an Ambr15 micro-bioreactor system. Peak cell densities from the mini-bioreactor study, Figure 1, were used to determine a steady state for each cell line. (A and B) Growth profiles with daily manual bleeds are demonstrated, showing the capabilities of medium C and D to sustain the target cell densities. (C and D) Cumulative titer was calculated, demonstrating medium D promoted the highest yield in both cell lines.

EXPERIMENTS AND RESULTS (CONT'D)

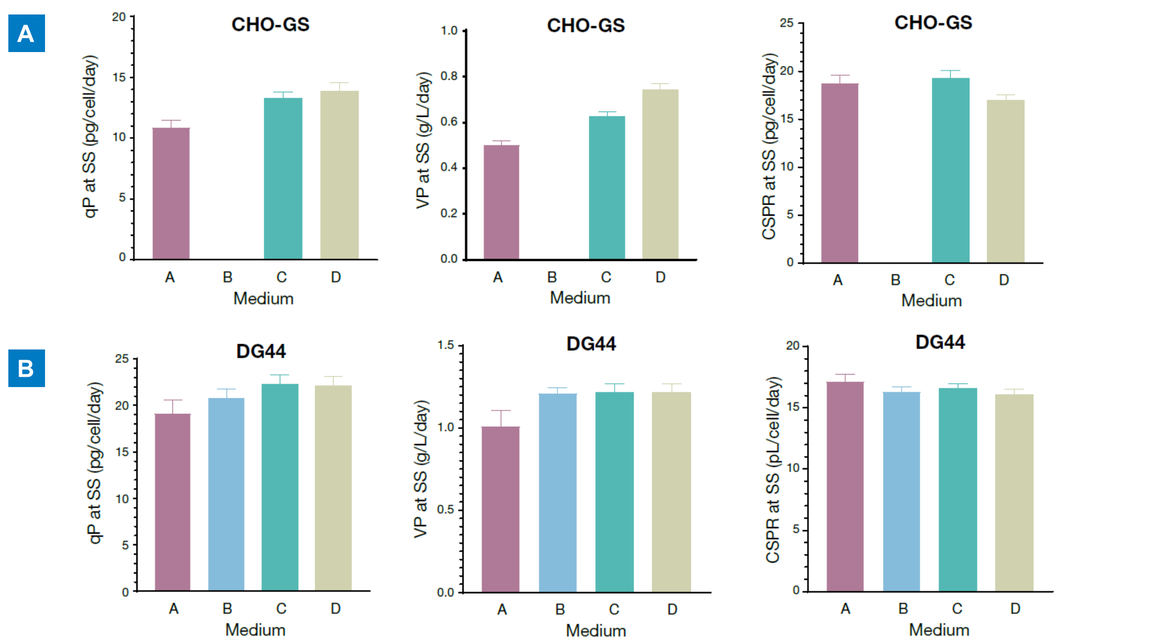

Figure 3. Perfusion metrics including qP, VP, and CSPR were calculated for steady-state growth from growth in the Ambr15 micro-bioreactor for CHO-GS (A) and DG44 (B) cell lines. Media C and D were the top performing media at steady state for both cell lines with respect to qP and VP, while medium D was comparable with regard to CSPR near 15 pL/cell/day.

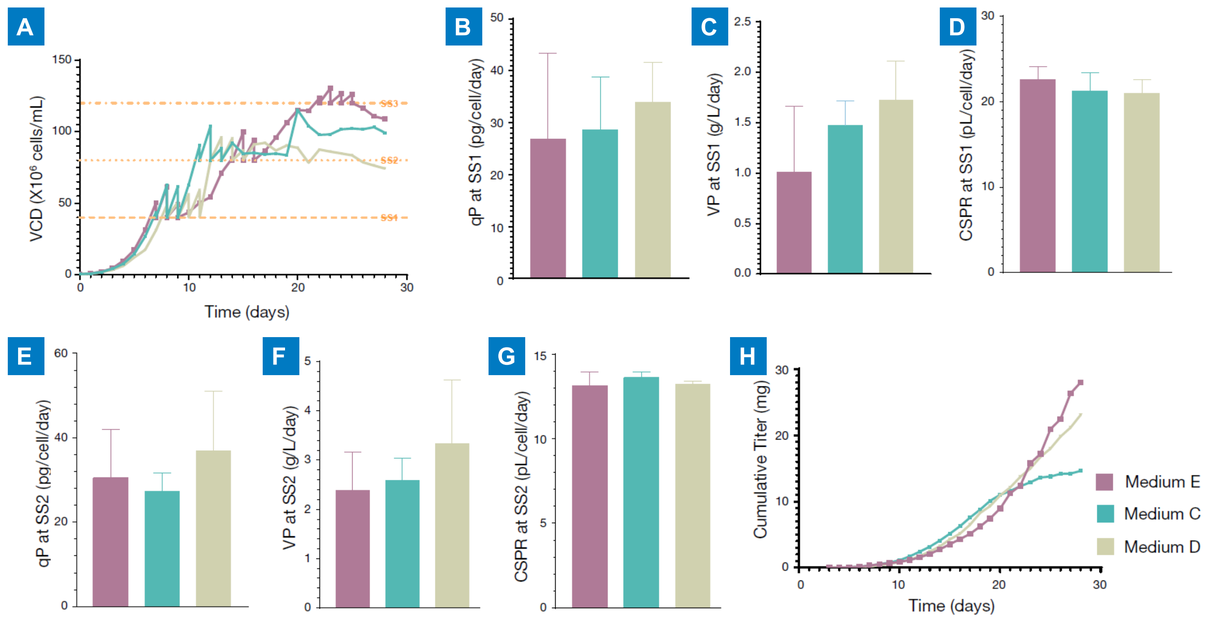

Figure 4. Three perfusion media were evaluated in a 0.1L perfusion-capable bioreactor with an alternating tangential flow filtration (ATF) device. (A) The media was challenged at three different steady states of 40 M, 80 M and 120 M viable cells/mL in a push-to-low strategy. Only one medium was capable of reaching 120 million viable cells/mL, while all media were capable of maintaining high cell densities of 80 million cells/mL throughout the 28-day experiment, double the expected peak cell density from the mimic mini-bioreactor (spin tube) and the Ambr15 models. (B through D) Perfusion metrics at SS1 demonstrate qP, VP, and CSPR values suitable for a perfusion process at low cell densities. (E through G) Perfusion metrics at SS2 demonstrate VP and CSPR can be improved by pushing to higher cell densities. (H) The cumulative titer, or yield, collected through the permeate was calculated for the entire 28-day process.

DISCUSSION

The performance of a perfusion process is highly dependent on the medium and the cell line to be used in the process, as observed in the perfusion metrics between CHO-GS and DG44 cell lines (Figure 3). Indeed, volumetric productivities, VP, of CHO-GS in the perfusion-capable bioreactor were below 1 g/L/day for all media, suggesting a limitation of the cell line as only one medium supported 80 million cells/mL (data not shown).

Here, we have outlined a method towards evaluating perfusion media by utilizing a small-scale model and scaling up into a mimic micro-bioreactor, and further evaluating perfusion media in a true perfusion tabletop bioreactor. We begin the evaluation by understanding the capacity of the cell line and screening media to identify media that can achieve high cell densities and high productivities, thus eliminating media formulations with poor growth or titer production. We demonstrate improvements in viable cell density, productivity, and perfusion metrics as we scale-up into larger cell culture platforms, at a medium exchange rate of 1 VVD, which minimizes cost on cell culture medium. Yet by operating at this low perfusion exchange rate, CSPR, a key metric for medium performance, achieves a value below 20 pL/cell/day (Figure 3) in a micro-bioreactor system. In fact, CSPR for DG44 cells was reduced further in the perfusion-capable bioreactor to under 15 pL/cell/day, and a CSPR under 10 pL/cell/day was achieved in the perfusion-capable bioreactor at a steady-state cell density of 120 million cells/mL (data not shown). This supports that CSPR can be optimized from perfusion-mimic systems like the Ambr15 by increasing densities in a bioreactor when a medium is capable of sustaining the increased biomass.

Two vital components of the perfusion process are the medium and the cell line. The two must complement each other to obtain the best yields from a manufacturing run while maintaining a close eye on bioreactor parameters. It is crucial to understand the requirements of individual cell lines in scale-down models prior to moving to a perfusion process or proceeding with media optimization for perfusion in a large scale system. This benefits the selection of an appropriate perfusion medium, as cells used in bioproduction demonstrate genetic heterogeneity because of the cell line development process. By utilizing an optimized perfusion medium, high cell densities and productivities can be achieved over extended productions of a therapeutic molecule.

SUMMARY

- Demonstrated utility for media screening evaluation, utilizing a small-scale model to identify perfusion media candidates capable of promoting high cell densities and titer
- Perfusion-mimic systems
  - Are good performance indicators for the evaluation of perfusion medium, regardless of production capacity limitations
  - Uncover an appropriate medium capable to scale-up into larger bioreactors
  - Achieve high cell densities and productivities
- Successfully scaled up process from a mini-bioreactor into a micro-bioreactor and perfusion-capable tabletop bioreactor

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