FUJIFILM
Value from Innovation

IrvineScientific

Advanced Approaches for Developing and Optimizing Perfusion Platforms for Bioreactor Scale-up

INTRODUCTION

Monoclonal antibodies have become the most successful class of recombinant proteins in biopharmaceutical therapies, due to their specificity and ability to quickly produce and manufacture at large scales for commercial supply.

Large-scale pharmaceutical manufacturing of recombinant proteins is dominated by the use of Chinese Hamster Ovary (CHO) cells and fed-batch suspension cultures in large bioreactors, ranging from 2,000 to 25,000 Liters. The space and financial investment to run these large-scale fed-batch facilities comes at a significant cost to the manufacturer. Currently, there is a demand for producers to increase production yield while reducing the cost-per-dose, without compromising the safety of the product.

Perfusion platforms provide the advantage of a smaller manufacturing footprint, thus lowering the financial investment and space needed to manufacture a product. This is a key reason pharmaceutical manufacturers are turning to perfusion technologies. It also provides the ability to quickly change production from one product to another for prolonged periods.

In fact, a perfusion process is capable of more than 30 days of production compared to only 14 days in a standard fed-batch process, and longer periods of production are currently being achieved. To address the potential of converting to a perfusion platform for manufacturing, FUJIFILM Irvine Scientific evaluated several perfusion-mimic systems and a small-scale perfusion bioreactor system, along with their components, including perfusion medium, multiple CHO cell lines, and various bioreactor process parameters, aiming to achieve optimal perfusion metrics.

Background Technology

Considerations for Perfusion Platforms

Perfusion platforms provide many advantages in the production of pharmaceutical therapies, including a smaller production footprint, longer production periods, and potential savings.

However, a continuous steady-state perfusion cell culture process requires an elaborate bioreactor system equipped with an alternating tangential flow (ATF) device.

Typical perfusion systems are fully automated and capable of real-time monitoring of bioreactor conditions. The ability to control gassing, temperature, pH, and agitation of the culture are features required for a perfusion bioreactor setup.

However, small-scale or perfusion-mimic systems are required for the evaluation and optimization of cell culture media, and the development of the scale-up process. To fully realize the potential of a perfusion process, an optimal perfusion medium is required.

Commercial continuous bioreactor systems are available but their acquisition and setup remain costly, time-consuming to master, and not practical for companies only considering switching from current processes to continuous cell culture.

Commercial systems are also limited in their ability to evaluate perfusion media due to the limited number of stations available, and the high quantities of cells and medium required for large-scale bioreactors. Suppliers for equipment and cell culture media can act as valuable partners during the evaluation process, offering industry expertise in perfusion platforms.

EVALUATION

To investigate the performance of perfusion media, key perfusion metrics must be considered, including cell specific productivity (qP), volumetric productivity (VP), and cell specific perfusion rate (CSPR). Perfusion is typically performed with a bioreactor system capable of continuous medium exchange and equipped with a filtration unit to continuously harvest the product.

As previously mentioned, their acquisition and setup remain costly, but to overcome this limitation, perfusion-mimic systems can be used to evaluate perfusion media and assist in determining whether perfusion will benefit production.

Here, we describe the evaluation of perfusion media with perfusion-mimic models, including a spin tube model and a microbioreactor system with daily medium exchanges and cell bleeds. We further evaluate media using a 0.1 L perfusion-capable bioreactor.

Small-Scale Perfusion-Mimic Systems – Spin/Shake Tube

A spin tube mimic model, also referred to as a shake tube, is useful for evaluating a large number of media because it allows simultaneous screening under similar conditions, exceeding the capabilities of any tabletop bioreactor.

To begin the process of evaluating perfusion media, a target viable cell density (VCD) should be predetermined based on the knowledge of a cell line, or it can be determined experimentally for multiple media using this small-scale perfusion-mimic system.

As described in **Figure 1**, a spin/shake tube model is used to quickly assess the highest cell density that media can achieve with a cell line **(Figure 1A)**, which we will refer to as the steady-state VCD, under non-optimal control of culture parameters. The mimic model can eliminate media that are not capable of sustaining cell growth, as observed for medium 3 **(Figure 1B)**.

With medium 3 eliminated due to poor growth, this model can also be used to eliminate media that underperforms for titer like media 4 and 5 **(Figure 1D)**, while selecting perfusion medium candidates that promote efficient productivity.

During this assessment for a steady-state VCD, spent media is collected and analyzed, while cells are supplied with fresh media daily. This process mimics media exchange of one vessel volume per day (VVD).

Collected media are measured for product titer. In the study described here, although the benchmark medium had the highest peak VCD, it was not the highest producing culture over the 14-day period for cumulative titer and average titer per day **(Figure 1C and 1D)**. The spin tube model, however, has its limitations in representing the real-time monitoring of culture conditions exhibited by perfusion bioreactors.

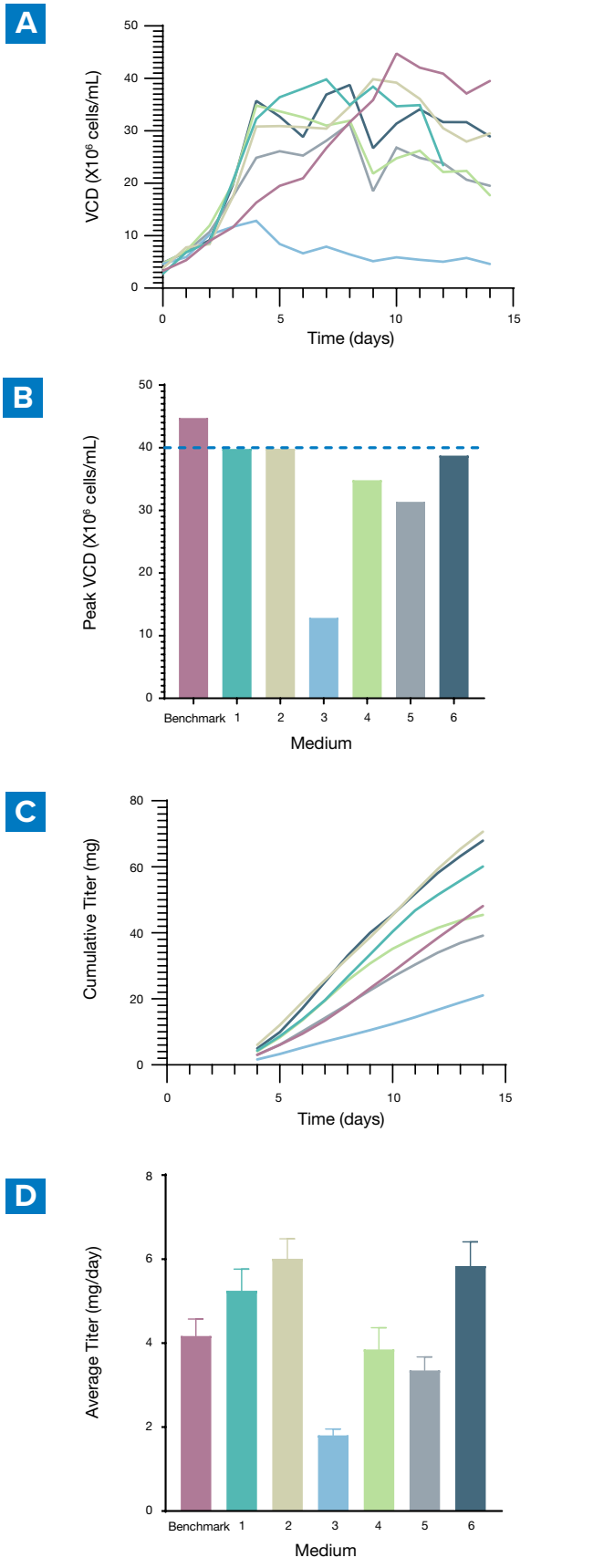

Figure 1. Spin/shake tube. CHO-GS cells were grown in 50 mL spin tubes. Every 24 hours, tubes were sampled for cell counts **(A)**. The remaining cells were collected by centrifugation, spent medium was collected and cells were suspended in fresh medium. Peak VCD for each of the 7 media were plotted **(B)**. Spent media were measured for titer and cumulative titer was plotted **(C)**. Average titer per day was calculated from the cumulative titer and plotted **(D)**. Media 1-6 represent candidates from a screening of over 40 media utilizing the spin-tube model system. Select candidates were used and/or modified for subsequent experiments.

Small-Scale Perfusion-Mimic Systems – Microbioreactor

Bioreactors offer the ability to control many of the parameters cells required for optimal growth and productivity, including pH, dissolved oxygen, and agitation.

Figure 2 describes and demonstrates two distinct cell lines utilizing an Ambr15 microbioreactor system as a perfusion-mimic. The cell lines are at target steady-state VCDs of 40 and 50 million cells/mL, respectively, based on the previous spin tube mimic model.

For CHO-GS, media C and D maintain the steady-state VCD throughout the culture period, unlike media A and B that falter after day 10. Additionally, from **Figure 2A**, CHO-GS cell densities can far exceed what was determined in the spin tube model, reaching a cell density of over 60 million cells/mL, which likely is due to the increased control of culture condition parameters in the tabletop microbioreactor.

During the evaluation process in a mimic system, we recommend a steady state that is 10-20% below the actual peak density, which should allow for the most productive cultures during the evaluation. For DG44 cells, we demonstrate that all media can support reaching the steady-state target of 50 million cells per mL, but medium A cannot sustain the culture at the target density over the designated period **(Figure 2B)**.

Titer measurements were analyzed, and based on the cumulative titer produced, medium D is the most productive medium in both cell lines **(Figure 2C and 2D)**. However, viable cell densities and cumulative titer alone cannot determine whether a perfusion medium is suitable for a switch to a perfusion process.

Other key perfusion metrics must be considered, including cell specific productivity (qP), volumetric productivity (VP), and cell specific perfusion rate (CSPR). qP is a measure for the productivity per cell per day, a normalization of the titer and perfusion rate by the number of viable cells.

The VP of a culture is a measure of the grams per liter per day that a bioreactor is capable of producing. It is derived from the qP multiplied by the cell density of the culture. The final key parameter to consider is CSPR for performance of the cell culture media, as it measures the exchange rate in picoliters per cell per day, which is derived from the perfusion rate normalized with the cell density.

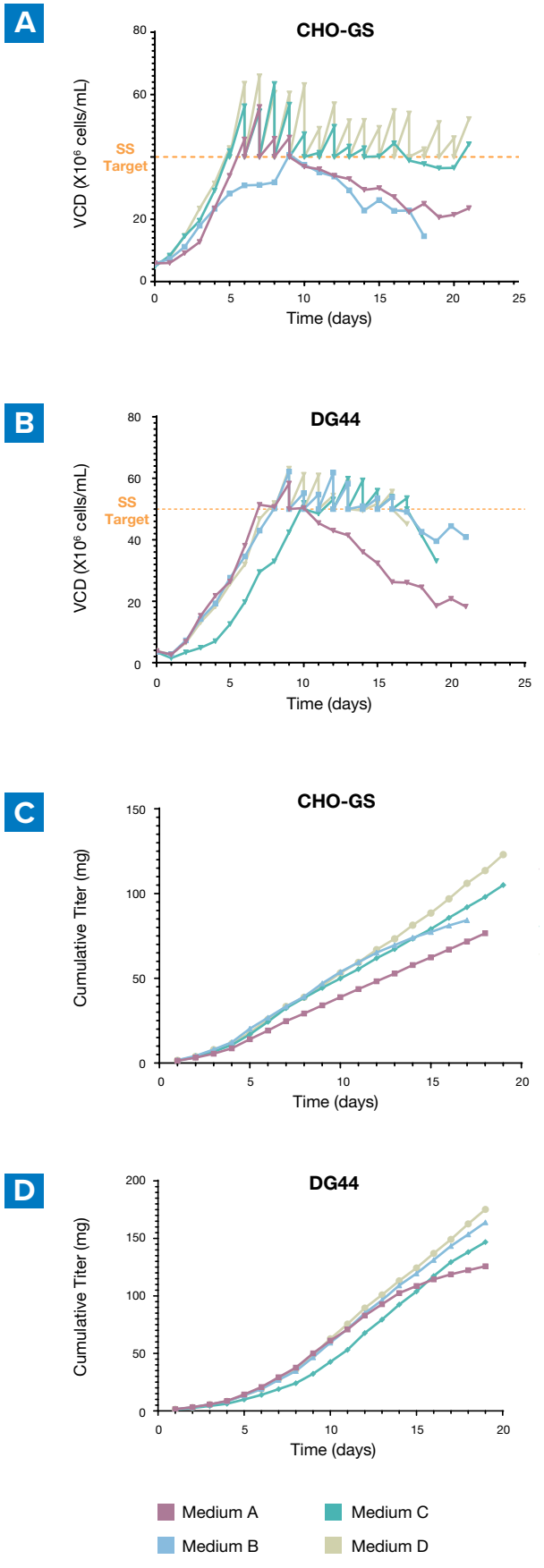

Figure 2. Ambr15 Bioreactor System. CHO-GS and DG44 cells were inoculated into a microbioreactor vessel with a targeted steady state VCD depicted above. Every 24 hours, vessels were sampled for cell counts **(A,B)**. After sampling, cells were collected by centrifugation, spent medium was collected and cells were suspended in fresh medium. Cells were manually bled daily after reaching the SS target. The collected spent media was measured for titer and cumulative titer was plotted **(C,D)**.

MEDIA CONSIDERATIONS

Evaluation of Perfusion Media

The success of a perfusion culture is measured by the ability to maintain high cell densities and sustain high productivity for prolonged culture periods. Viable cell densities and protein production easily characterize these measurable attributes.

In modern bioreactor systems, cell densities greater than 40 million cells/mL are typically seen, with an upward boundary capable of exceeding 100 million cells/mL. Titers between 1-3 g/L are easily achieved and the promise of product titers greater than 3 g/L is achievable, as improvements to cell lines through molecular engineering and perfusion-specific cell culture media take root.

In perfusion, the productivity of a cell is a product of cell densities and cell-specific productivities that are related to titers. However, these densities and titers are cell line dependent. Thus, to consider transitioning to a perfusion process, a medium must be selected that is capable of achieving high cell densities and sustained productivities.

Media Composition

The media composition is vital, as high perfusion rates can be costly over a long perfusion process. The goal in a perfusion process is to target a low or minimum perfusion rate to minimize media consumption and improve the process economy.

An optimal medium should be capable of sustaining high cell densities for prolonged culture periods while being productive during the targeted steady state with a minimum CSPR.

In **Figure 3**, perfusion metrics were calculated for media capable of sustaining the steady-state cell density in the Ambr15 microbioreactor system. Values for qP, VP, and CSPR were calculated from day 6-21 of the culture for CHO-GS cells, and day 7-17 for DG44 cells, corresponding to the steady-state phase of the culture.

The top performing media at steady state for both cell lines were media C and D, with respect to qP and VP, while medium D was comparable to CSPR near 15 pL/cell/day. A lower CSPR is desirable, suggesting that less medium is being used per cell per day at the steady state.

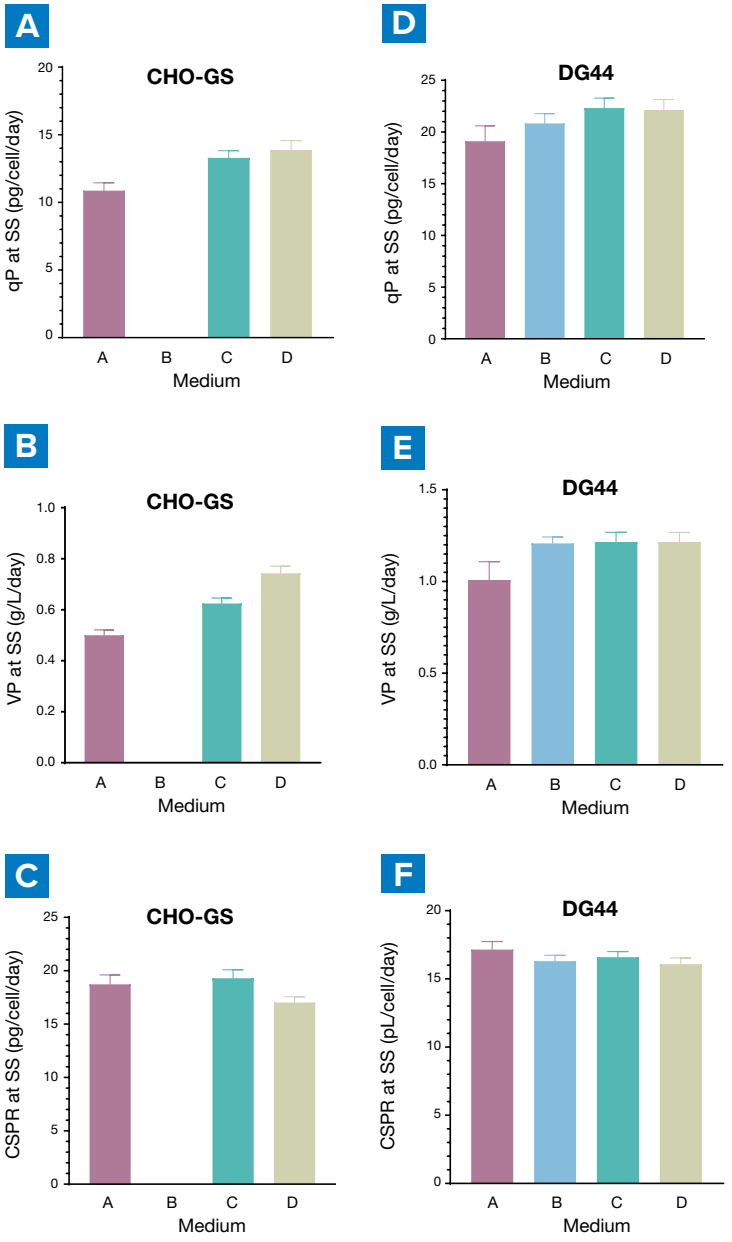

Figure 3. Perfusion Metrics. Titer and viable cell density measurements were converted to cell specific productivity (qP), volumetric productivity (VP) and cell specific perfusion rate (CSPR) to compare productivity values across cell lines and the performance of the media. Medium B was not calculated for CHO-GS as it failed to reach and maintain the steady state target density.

Perfusion Verification

To verify the performance of perfusion medium candidates, three media candidates were tested in a 0.1 L perfusion capable bioreactor with an alternating tangential flow filtration (ATF) device.

As described in **Figure 4A**, the media were 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 (medium E) 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 spin tube model.

The increased capacity for cell density likely resulted from the use of the bioreactor capable of continuous flow of media into the bioreactor and more stringent controls of environmental parameters that cannot be controlled in the small-scale perfusion mimics.

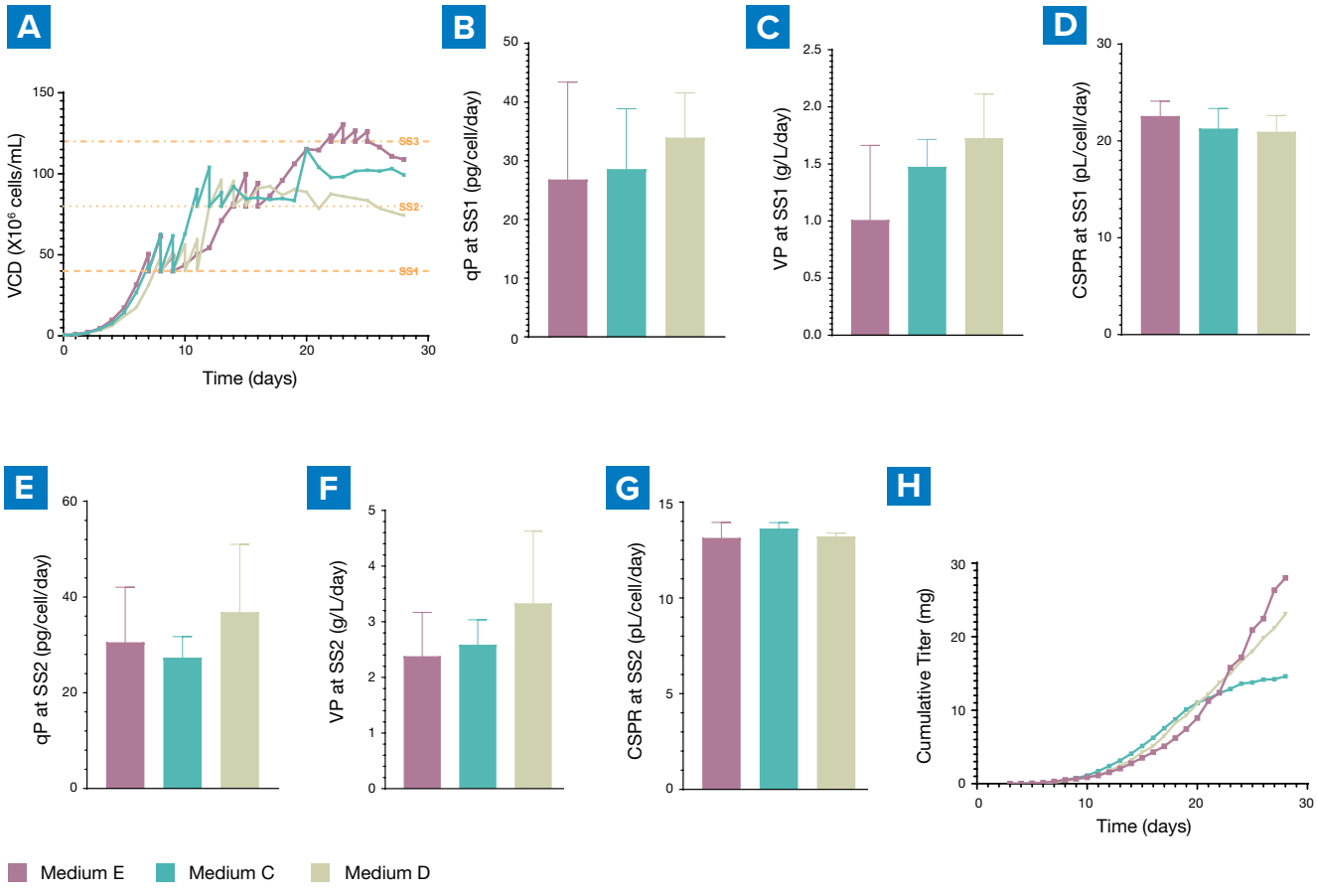

Figure 4. Tabletop Perfusion Capable Bioreactor. DG44 cells expressing a mAb were grown in a 100 mL bioreactor in perfusion mode with manual cell bleeds. Three steady states were targeted as shown above in a push-to-low strategy by increasing cell density after 3 or more days at the targeted steady states. Daily sampling of the bioreactor and harvest media provided cell density and titer data, respectively, and VCD **(A)** and cell culture metrics were calculated for all 3 steady states (data not shown for SS3), including qP **(B,E)**, VP **(C,F)**, and CSPR **(D,G)**. Cumulative titer was also calculated from the data **(H)**.

RESULTS

Analysis of the key perfusion parameters, including qP, VP, and CSPR at each steady state, suggests that medium D is a suitable perfusion medium for the DG44 cell line. VP values are nearly doubled from steady-state 1 (SS1) to steady-state 2 (SS2) for all media, reaching a VP of 3.2 g/L/day for medium D. At 120 million cells/mL, medium E provides a VP of 4 g/L/day (data not shown).

However, at this high cell density, we observed a decline in the VCD during the final three days of the continuous process. This suggests that it may be difficult to sustain the cells at that density with the current perfusion rate of 1 VVD, whereas medium D can be sustained at 80 million cells/mL while being equally productive over a 28-day continuous perfusion culture.

An optimal perfusion medium candidate is capable of sustaining high cell densities and productivity at a low perfusion rate for prolonged culture periods. We demonstrate here that perfusion-mimic models sustained high cell densities in small-scale cultures, while replacing medium daily, corresponding to a perfusion rate of 1 VVD, while a perfusion-capable bioreactor system with a filtration device was used to maximize cell densities and volumetric productivities by utilizing a push-to-low strategy by increasing the biomass set point.

The performance of a perfusion process is highly dependent on the medium and cell line, as observed in the perfusion metrics for CHO-GS and DG44 cell lines **(Figure 3)**. Indeed, volumetric productivities 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 supports 80 million cells/mL (data not shown). Yet by operating at a low perfusion flowrate of 1 VVD, CSPR, a key metric for medium performance, is minimized in both cell lines achieving CSPRs below 20 pL/cell/day **(Figure 3)** in an Ambr15 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 when a steady-state cell density of 120 million cells/mL was achieved (data not shown). This suggest that CSPR can be optimized from perfusion-mimic systems by increasing densities in a bioreactor when a medium is capable of sustaining the increased biomass.

CONCLUSIONS

Today, perfusion cell culture technology has improved since its introduction into large-scale manufacturing decades ago, yet fed-batch processes still remain the go-to-standard for the production of biologics, likely resulting from tradition, existing equipment, existing infrastructure, and experience of personnel. The many advantages of the perfusion process over fed-batch cultures, however, may compel manufacturers to break with tradition.

In comparison to fed-batch cultures, the perfusion process confers numerous improvements to both upstream and downstream processes, including increased efficiency of engineered cells, purification methods, and advances in cell culture medium formulations. Additional benefits of a perfusion process for large-scale manufacturing over fed-batch cultures include:

- **Reducing capital equipment footprint.** A 1,000 L perfusion system can be as productive as a 10,000 L fed-batch culture under optimal conditions, reducing the required capital for equipment and cost of generating a large seed train.
- **Delivering more consistent products** and supporting increased manufacturing of large batches of therapeutic products over extended periods.
- **Minimizing media consumption** by optimizing cell culture performance, potentially reducing cost compared to fed-batch.
- **Achieving high productivity** for more than 28 days compared to a typical 14-day fed-batch culture.

In contrast to a batch/fed-batch process, a steady-state perfusion cell culture requires the continuous addition of fresh medium into a bioreactor, with spent medium and the product perfused and harvested through a membrane. In turn, cells are retained within the bioreactor while maintaining a steady-state cell density through a cell bleed process.

Because of this process, and unlike a fed-batch culture, the volume of the culture and the cell density do not change. This provides not only large quantities of product, but also high quality of the product, as the cells and product are not exposed to high concentrations of deleterious metabolites that could produce products with poor stability.

Prior to moving to a perfusion process or proceeding with media optimization for perfusion, it is crucial to understand the requirements of individual cell lines in scale-down models. The two vital components of the perfusion process, the medium and the cell line, must complement each other to obtain the best yields from a manufacturing run.

Perfusion metrics support increasing viable cell density and productivity and benefit 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 periods for production of a therapeutic molecule.

In summary, we have outlined a method towards evaluating a perfusion medium utilizing a small-scale method and scaling it up into a mimic microbioreactor and a true perfusion tabletop bioreactor. Perfusion-mimic systems, while limited in production capacities, are effective performance indicators for the evaluation of perfusion media. They can uncover an appropriate medium capable of scale-up into larger bioreactors.

Perfusion-mimic systems, either small-scale or a microbioreactor, are able to achieve high cell densities and product productivities, which can be improved in a true perfusion system. Whether establishing new platforms or optimizing existing processes, identifying an appropriate medium should be considered a key activity to assure that scale-up and manufacturing needs are met.

With you at every step

From development through to large-scale production, success is highly dependent on providing the optimal culture conditions. The BalanCD platform of cell-specific growth media and feeds have been developed to avoid many of the challenges faced during cell line development through process optimization and into commercial production. These serum-free, chemically defined media and their supplements provide the optimal balance between growth and production to maximize productivity at any scale.

Media optimization and customization

Beginning with the most suitable BalanCD medium reduces development and optimization times necessary to achieve the required yield and quality of end-product. For those requiring customization to meet a specific need, experienced FUJIFILM Irvine Scientific professionals can provide efficient, cost-effective and time-saving assistance.

> To help you further optimize your media development processes, please consult the FUJIFILM Irvine Scientific Process Development Group.

Rapid prototyping

Normally provided within 10 working days, a rapid prototyping service offers flexible, small-scale, non-GMP media production of liquid and powder formulations. By using the same raw materials sourced and quality-controlled as for our large-scale GMP manufacturing, this approach greatly facilitates the step from research to process development activities.

A smooth transition into commercial production

FUJIFILM Irvine Scientific products and services are developed according to the highest medical standards. Every BalanCD product is subject to a stringent Quality System unrivalled in the industry. To fulfill quality and reliability requirements, proprietary and customized media are manufactured under fully cGMP-compliant conditions in dedicated animal component-free zones, state-of-the-art facilities replicated in California, Japan, and Europe. Comprehensive documentation, including information from Supply Chain Management through to Drug Master Files, is available to help minimize the regulatory burden.

- FDA-regulated
- cGMP compliant manufacture
- ISO13485,

  EN 13485:2016 certified
- Drug Master Files*

  *upon request

FUJIFILM
Value from Innovation

IrvineScientific

www.irvinesci.com