PERFUSION MICROBIOREACTOR WITH INTEGRATED CELL RETENTION DEVICE

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Introduction

Cell culture media development requires high-throughput in order to generate robust statistical models capable of identifying the critical components that need to be optimized. Currently, there is no commercially available cell retention device that can be used with high-throughput systems (<100mL) in perfusion mode. In an effort to increase capability for perfusion media development we evaluated the Erbi perfusion microbioreactor system, a 2mL working volume system with an embedded filtration based cell retention device. With Erbi Biosystems' expertise in microfluidics and our expertise in perfusion animal cell culture, we have made significant improvements to the system. At this time, we are able to achieve a steady state of at 50x10^{6}vc/mL with a CHOZN®GS cell line producing a fusion protein and a CHOS cell line producing a mAb. It has comparable profiles to bench-top perfusion bioreactors and the results have been reproduced over multiple runs.

Methods

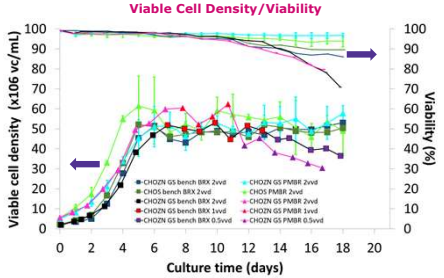
For this work, experiments were performed using a CHOZN®GS cell line producing a fusion protein (n=3) and a CHOS cell line producing a mAb (n=2) cultured in EX-CELL® Advanced HD Perfusion Medium in Erbi Biosystems perfusion microbioreactors (Erbi Biosystems, Massachusetts). Cells were centrifuged and concentrated to inoculate at 5x10^{6}vc/mL with 15% carry over. Perfusion was initiated on day 0 at a constant perfusion rate of 2vvd (minimum CSPR 40pL/cell/day). Dissolved oxygen, pH and target cell density were set at 40%, 6.9+/-0.05 for the CHOZN®GS cell line, 7.1 6.9+/-0.05 for the CHOS cell line and 50*10^{6}vc/mL respectively. Erbi perfusion microbioreactor data is compared to a 3L Applikon® glass bioreactor (Applikon®, Netherlands) with 2L working volume, using the same cell line and medium. Cells were inoculated at 2x10^6 vc/mL with 50% carry over. Perfusion was initiated on day 2 with a constant CSPR of 40 pL/cell/day (perfusion rate range 0.18-2vvd). Dissolved oxygen, pH and target cell density had the same settings used for the Erbi perfusion microbioreactor experiments.

EX-CELL® Advanced HD Perfusion Medium was tested for minimum CSPR using the CHOZN®GS cell line in both the 3L Applikon glass bioreactor and Erbi perfusion microbioreactor. The same protocol was used for both systems. Once the cultures reached 50x10^{6}vc/mL, the perfusion flow rate was changed to 1vvd (20pL/cell/day) for 5 days, and then changed to 0.5 vvd (10pL/cell/day) thereafter.

Cell density and viability were measured using Vi-Cell®XR (Beckman-Coulter, California). Metabolites were analyzed using Nova®FLEX2 (Nova Biomedical, Massachusetts). Protein concentration was measured using HPLC.

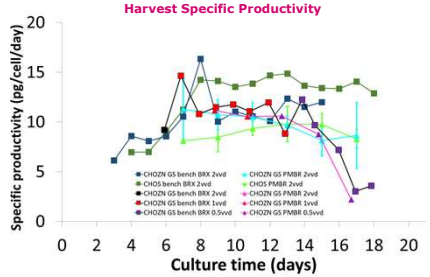
Results

Viable Cell Density/Viability



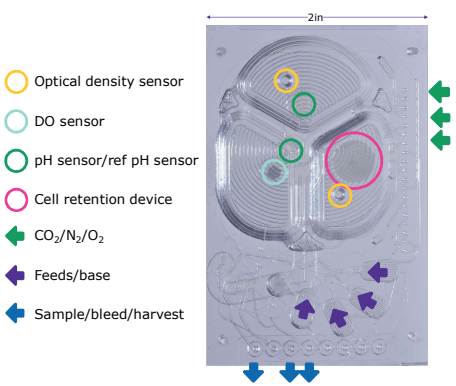
- Target cell density in the Erbi perfusion microbioreactor is maintained through a control loop that regulates the valve on the bleed line in response to the viable cell density measured by the optical sensors; in comparison, bench-top bioreactor's control loop uses capacitance probes to measure viable cell density and regulates cell bleeding pumps.
- Steady state at 50x10^{6}vc/mL was achieved for both cell lines.
- Cell viability in the Erbi perfusion microbioreactor cultures was higher compared to the bench-top bioreactor.

Harvest Specific Productivity



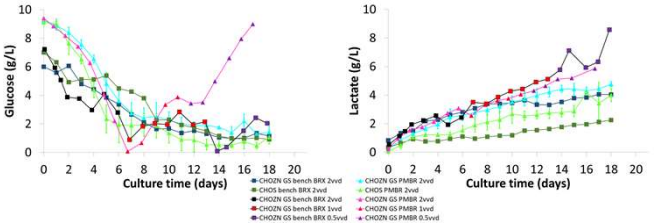
- Harvest specific protein production for CHOZN®GS cell show comparable trends.
- Differences in specific productivity observed for CHOS cell line. Limitations in daily off-line pH correction in the Erbi perfusion microbioreactor may contribute to differences.
- Harvest specific productivity show a decrease after day 13. This correlates with higher protein retention observed in the Erbi perfusion microbioreactor cell retentions device (data not shown).
- Minimum CSPR test with CHOZN®GS cell in Erbi perfusion microbioreactor compare well with benchtop bioreactor data.

Erbi Biosystems 2mL Perfusion Microbioreactor



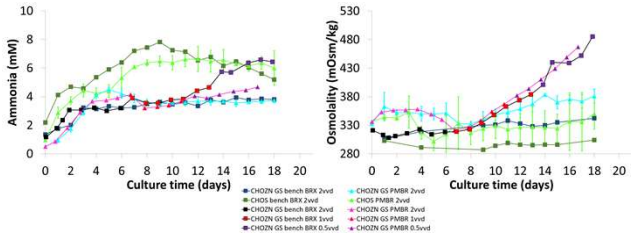
- The 2mL Erbi perfusion microbioreactor contains optical sensors for cell density, dissolved oxygen and pH.
- The growth chamber comprises three interconnected sub-chambers with a silicone membrane that can be inflated to displace the fluid in each sub-chamber.
- By sequentially inflating the three sub-chambers, the culture is efficiently mixed and gasses for cell culture are efficiently transferred to the liquid phase by silicone membrane diffusion.
- Fluid flow is controlled through integrated pumps and valves.
- Fresh media can be added continuously to the culture while continuous harvest is being filtered through the cell retention device and allowing 100% cell retention in the micro-bioreactor.

Glucose/Lactate



- Glucose concentrations for both cell lines show comparable results between the Erbi microbioreactor and benchtop bioreactor at 2vvd.
- Lactate concentrations were comparable for CHOZN ®GS cell line at 0.5-2vvd, but differences were observed with the CHOS cell line.
- Glucose concentrations at 0.5vvd in the CHOZN ®GS cell line show significant differences and lactate concentrations show comparability across all flow rates.

Ammonia/Osmolality



- Ammonia concentrations for both cell lines show comparable results between the Erbi microbioreactor and benchtop bioreactor at 2vvd.
- The CHOZN ®GS cell line show lower concentrations in the Erbi perfusion microbioreactor at 0.5vvd compared to the benchtop bioreactor condition.
- Differences in osmolality were observed between the Erbi perfusion microbioreactor and bench-top bioreactor with the Erbi perfusion microbioreactor showing consistently higher levels.
- Nova® FLEX2 metabolite differences between Erbi perfusion microbioreactor and bench-top bioreactor do not fully explain the differences observed in osmolality.
- Total amino acid concentration in spent media was 20mM higher (data not shown) in the Erbi perfusion microbioreactor compared to the bench-top bioreactor, which better explains the differences in osmolality.

Summary

Erbi Biosystems perfusion microbioreactor shows high potential as a small-scale model for perfusion processes. The critical characteristics that distinguish the micro-bioreactor from other small-scale models evaluated are the cell retention device, continuous fluid flow, automated cell bleeding, pH/DO control and high-throughput potential. Culture profiles were comparable to bench-top perfusion bioreactors and the results have been reproduced over multiple runs. Two critical improvements have been identified for the Erbi perfusion microbioreactor to fulfill the needs of an accurate small-scale model for continuous perfusion processes: increased working volume and an improved cell retention device.

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