Media and process development for high cell density cryopreservation and N-1 perfusion to intensify seed train operations

BioContinuum™ Platform
Converge, Intensify and Evolve. Together.

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Introduction

A typical seed train operation starts by thawing of a single vial followed by several expansion steps that are time-consuming and impacts flexibility. In addition, manual and open cell culture operations are a major source of process variability and potential contamination resulting in the need for intensified upstream processes.

High cell density cryopreservation (HCDC) is a method of freezing cells in bags instead of vials, at higher cell densities resulting in the ability to decouple expansion and product in both space and time, greatly increasing biomanufacturing plant flexibility. Additionally, using HCDC may significantly reduce cost of goods for mAb manufacturing.

Secondly, we looked into media for the expansion steps. Here, exponential growth needs to be maintained to reach the production stage as soon as possible, and there should be no adaptation required when going into the production medium. During development, we could observe that productivity in the final bioreactor could be impacted by using specific expansion media, both for perfusion and fed-batch in the main stage.

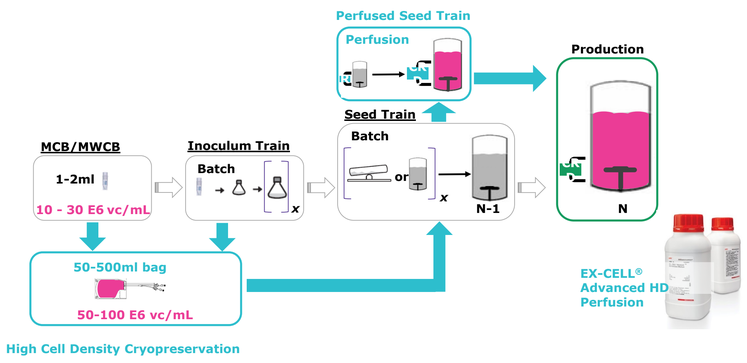

***Fig. 1: Opportunities for upstream intensification*.**

Expansion Medium

For designing a seed train expansion medium, a TPP tube model that represents a typical production campaign was established. We found that a predictive scale-down model must represent the exact sequence of media the cells experience in the seed train and production processes.

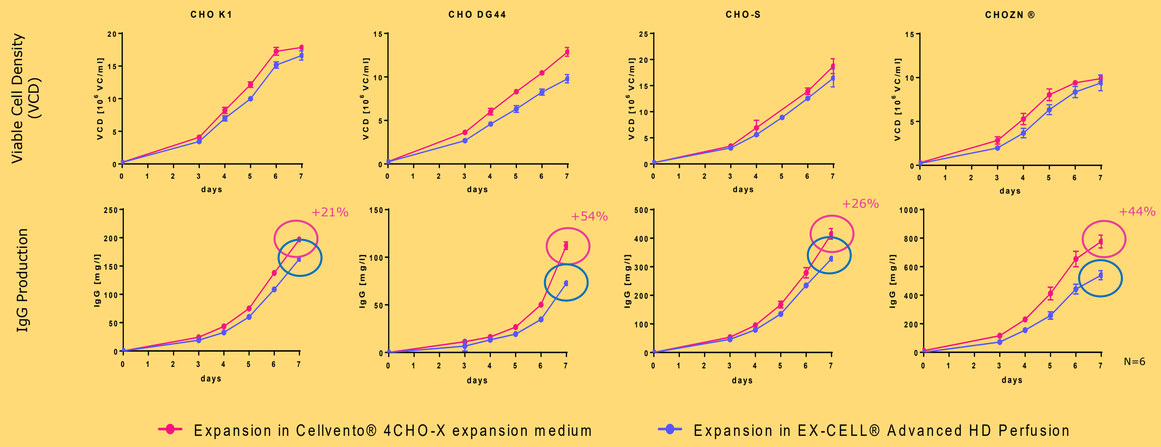

***Fig. 4: Growth and mAb concentrations in batch using* EX-CELL® Advanced HD Perfusion. Expansion prior to batch start was done either in the Cellvento® 4CHO-X expansion medium or in EX-CELL® Advanced HD Perfusion.**

EX-CELL® Advanced HD Perfusion was used as final medium for perfusion, and Cellvento® 4CHO and EX-CELL® Advanced medium were tested as Fed-Batch platforms. Cells were expanded in their recommended passaging media, or in Cellvento® 4CHO-X expansion medium. Our results in Fig 4 and 5 suggest that the selection of the expansion medium has a significant impact on the cellular productivity of the main stage. We found that Cellvento® 4CHO-X expansion medium was able to increase productivity in the main stage between 21 and 54% for several of the investigated CHO cell lines, both in our commercial perfusion as well as our fed-batch media platforms.

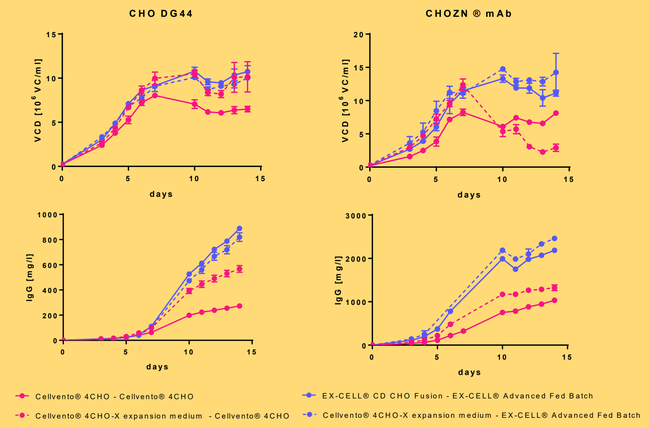

***Fig. 5: Growth and mAb concentrations in fed-batch using either* EX-CELL® Advanced CHO Fed-Batch or Cellvento® 4CHO fed-batch platforms. Expansion prior to batch start was done either in Cellvento® 4CHO-X expansion medium or in the typical companion expansion media.**

HCDC

A single-use bag assembly was developed that supports closed filling and inoculation (Fig. 2). For filling, cryo medium with a concentrated DMSO-concentration was prefilled into the cryobags. Subsequently, cell suspension was added to the cryomedium, leading to a filling volume of 150 mL in total. Afterwards bags were disconnected directly with the NovaSeal™ crimping tool, frozen and stored in a -80 °C freezer.

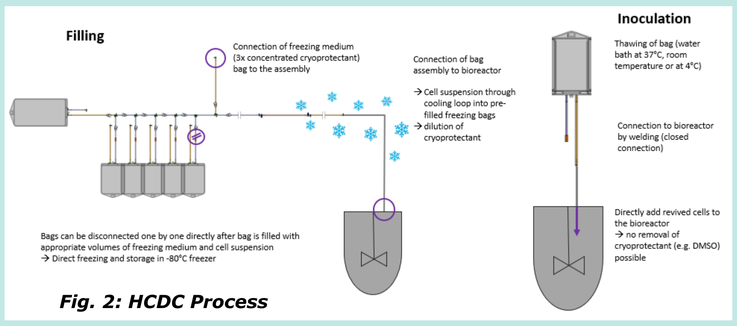

Fig. 2: HCDC Process

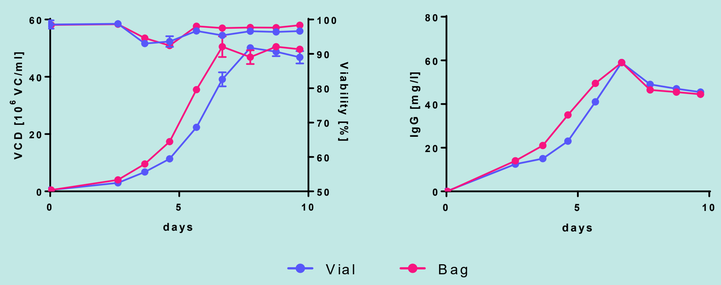

Fig. 3: Confirmation bioreactor run. A bioreactor was inoculated with a HCDC bag. A bioreactor which was inoculated with a standard vial expansion was used as control.

After thaw, the bags were used to simulate a production campaign in lab scale, using a frozen intermediate for inoculation of the perfused N-1 bioreactor, followed by a steady-state perfusion bioreactor step (Fig. 3). A standard expansion starting with thawing of a vial was used as control. Results showed that growth and productivity were comparable.

Summary

We developed two technologies to intensify upstream processes:

- **High Cell Density Cryopreservation (HCDC)** can significantly increase the flexibility of a manufacturing plant of the future.
- **HCDC bag assembly** and **Application Note** available on demand
- **Collaboration offers on HCDC** are welcome
- Furthermore, our results indicate that using our prototype expansion medium in the seed train can **enhance productivity** in final process stage for perfusion and fed-batch.
- Samples of **Cellvento® 4CHO-X expansion medium** are available now