GE Healthcare

An intensified perfusion one-step seed process with high-density cell banks

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Abstract and introduction

This work describes how the use of perfusion in the seed culture expansion process, in combination with the use of high-density cell banks, drastically can reduce processing time, simplify operations, and maximize equipment utilization (Fig 1). A high-density cell bank was created using a method that does not require centrifugation. One vial from this cell bank was used to inoculate a bioreactor culture that, when operated in perfusion mode, enabled a one-step seed culture process that could be used to seed a 2000 L production bioreactor.

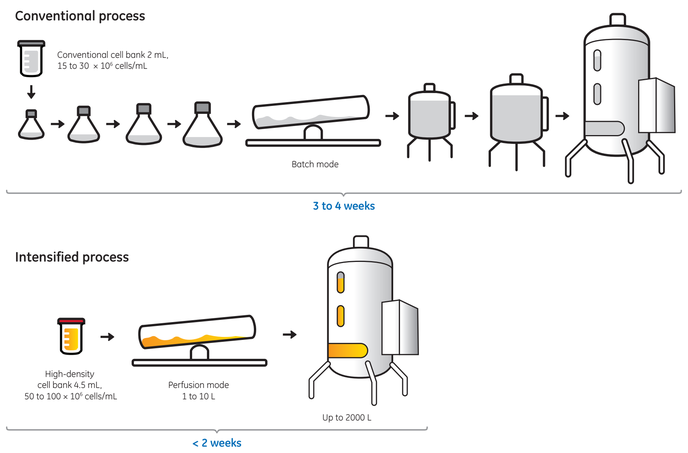

Fig 1. A high-density cell bank can be used for direct seeding of a small bioreactor culture, eliminating the need for shake flask operations. The use of perfusion in seed culturing allows cells to stay in exponential growth phase throughout the entire culture, enabling higher cell densities for a one-step bioreactor seed culture.

Materials and methods

High-density cell banks were created by growing Chinese hamster ovary (CHO) DG44 cells (licensed from Cellca GmbH) in 2 L ActiCHO™ P medium. Culturing was performed in perfusion mode in a disposable 10 L Cellbag™ bioreactor with a floating internal filter using the ReadyToProcess™ WAVE 25 system (Fig 2). For cryopreservation, chilled, fresh medium with DMSO was added (1:1 ratio) to the bioreactor, whereupon the culture was concentrated back to 50 × 10^6 cells/mL before harvested, dispensed, and frozen in 4.5 mL cryovials. Cells from one vial of the cryopreserved cell bank were recovered and directly transferred to a new 20 L Cellbag bioreactor at 1 L. The volume was stepwise expanded to a final working volume of 10 L, whereupon the culture was continued in perfusion mode.

Target criteria:

- Cell bank generation (2 L): > 50 × 10^6 viable cells/mL (> 95% viability)
- Viability at revival from cryopreservation: > 90% viability
- Seed culture (10 L): > 50 × 10^6 viable cells/mL (> 95% viability)

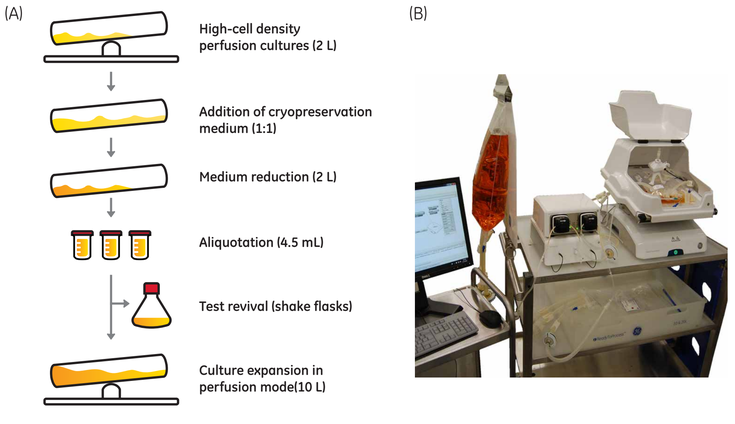

Fig 2. (A) Overall experimental setup. (B) System setup.

Results

The described method allowed for the generation of ~ 400 vials of 4.5 mL cryopreserved high-density cell bank at 50 × 10^6 cells/mL (> 95% viability) from a 2 L culture.

Cells were successfully revived from cryopreservation at a viability of > 90%, which is similar to the performance of the originator conventional cell bank. One vial of the high-density cell bank was used to inoculate a 20 L Cellbag perfusion culture chamber at a starting volume of 1 L. The culture volume was further expanded to 10 L, after which perfusion was started. A final density of > 50 × 10^6 viable cells/mL (> 95% viability), with cells in full exponential growth phase, was achieved.

Results are displayed in Figures 3 to 8.

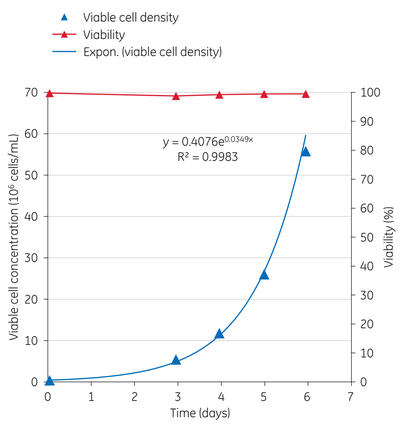

Fig 3. High-density cell bank generation. Cells were maintained in exponential growth phase throughout the culture. Average specific growth rate was 0.838 day^{-1} (doubling time ~ 19.9 h), cell-specific perfusion rate 137.5 pL/cell/d, and culture duration was six days. At harvest: 55.8 × 10^6 viable cells/mL, cell viability > 95%.

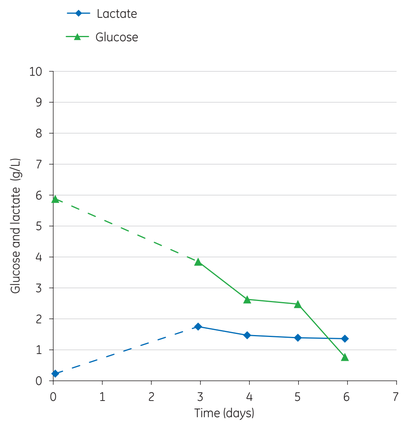

Fig 4. Glucose and lactate concentrations. Glucose concentration decreased from 6 g/L (start) to ~ 1 g/L (Day 5). Lactate concentration increased to ~ 1.5 g/L (Day 3) and remained constant until end of culture. The results show that the perfusion rate was sufficiently high to maintain nutrient and metabolite concentrations at levels that allow keeping cells in exponential growth phase throughout the culture.

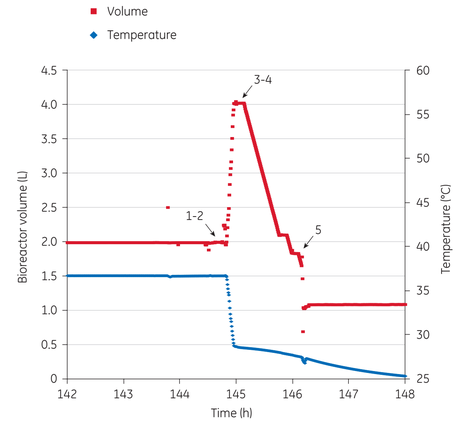

Fig 5. Cell bank generation. Perfusion and temperature control was stopped at ~ 50 × 10^6 cells/mL, after which chilled (4°C to 8°C), fresh medium supplemented with 15% DMSO was added at a 1:1 ratio (1,2). Agitation was lowered from 20 to 15 rpm and culture volume was reduced back to the original volume (2 L) using the cell retantion filter (3,4). The cell suspension was drained, aliquoted into 4.5 mL cryovials, and frozen (5).

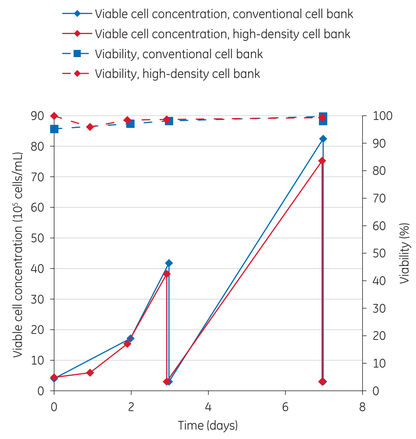

Fig 6. Cells from the high-density cell bank were revived with a viability of > 95% and growth after revival was similar to a conventional cell bank.

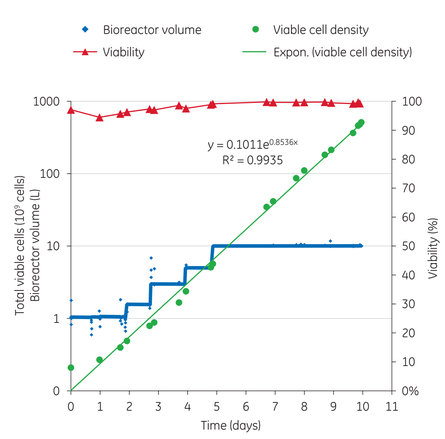

Fig 7. One-step seed culture process. Cells were maintained in rapid exponential growth throughout the culture. Average specific growth rate was 0.853 day^{-1} (doubling time ~ 19.5 h). Cell-specific perfusion rate was 140 pL/cell/d, starting from Day 5, and culture duration was 10 days. At harvest: 51.2 × 10^6 viable cells/mL, cell viability > 95%.

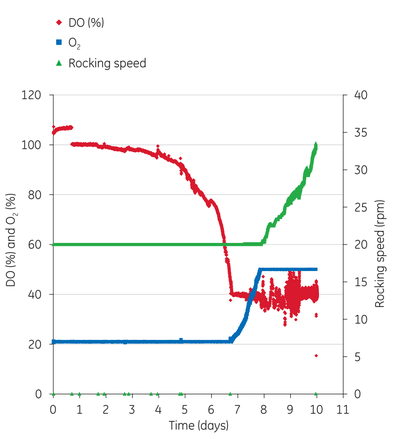

Fig 8. The bioreactor system allowed real-time monitoring and control of culture parameters. For example, dissolved oxygen was shown to be well-controlled in the culture, even at high cell density and large working volume.

Conclusion

The described culture process allowed:

- A simple method for generation of high-density cell banks in a closed system, without the need for a separate centrifugation step.
- Direct inoculation of a disposable bioreactor culture from one vial of the cryopreserved high-density cell bank, without prior cell expansion in shake flasks.
- Efficient one-step seed culture process, generating high cell densities at a culture volume that obviate the need for intermediate seed bioreactors.

Perfusion cell culturing in combination with the high density cell bank enabled expansion of cell numbers sufficient for seeding of a 2000 L bioreactor in 10 days from revival of the cell bank.

29156980 AB 06/2015