FASTFACTS

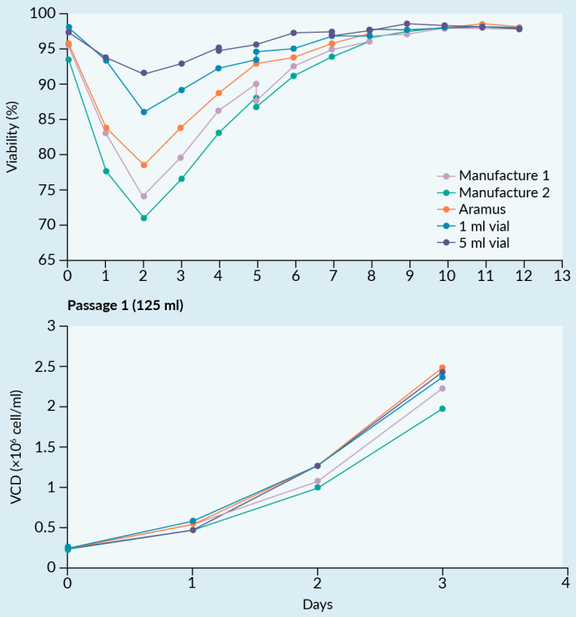
Benefits of upstream seed train intensification & high-density cell banking

Patrick Shu Miao

High-density cell banking (HDCB) involves freezing high concentrations of cells in large single-use bags. It increases productivity and reduces manufacturing costs by eliminating intermediate steps. HDCB also reduces the risk of contamination and the number of consumables needed, while enabling rapid cell recovery and high viability.

Aramus™ single-use fluoropolymer bags excel at HDCB, allowing cells to be frozen and stored at substantial volumes with a high cell concentration. After thawing, these large-volume, high-density cell cultures allow seeding direct into a bioreactor, thereby eliminating intermediate steps of the traditional seed train. The seed train process can be redesigned from vials and flasks to Aramus single-use fluoropolymer bags. The Aramus bag assembly is a closed system, which reduces the risk of contamination in the cell culture process, has lower surface energy, allowing recovery of every drop of valuable drug product, and has lower particulates per volume versus other bags. Additionally, Aramus ultra-pure low E&L profile has only one contributory element, resulting in reduced CAPA(s) due to unknown contaminants.

Figure 1. Cell viability over a 13-day CHO cell culture period (top) and cell density during the first passage of the same culture (bottom).



ARAMUS SINGLE-LAYER FLUOROPOLYMER ASSEMBLY FEATURES:

- Universal material chemical compatibility—resistant to solvents like DMSO, aggressive and highly concentrated chemicals
- Widest temperature operating range—fluoropolymer material maintains flexibility at -196°C
- Gamma-stable fluoropolymer film
- Built to be tough and 100% pressure tested, eliminating leaking welds and port fitments that can cause significant loss

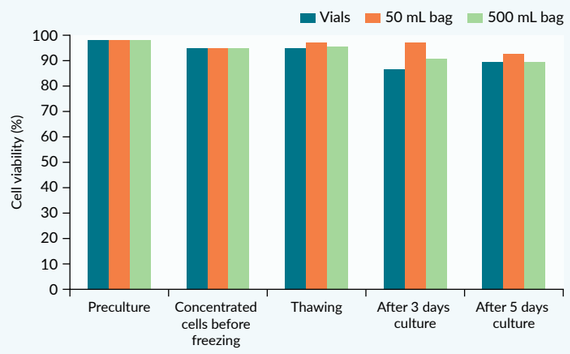
RECOVERY OF A HIGH-DENSITY CHINESE HAMSTER OVARIAN (CHO) CELL CULTURE

The recovery of a high-density CHO cell culture, post cryopreservation, was compared to alternative cryobags and 1 and 5 mL cryovial controls. The cells were frozen in liquid nitrogen for 2 weeks, then thawed and cultured for two passages up to 13 days. The results are shown in **Figure 1**. The Aramus bags demonstrated the quickest recovery among the different cryobags used and returned to a cell viability of >95%. In addition, the cells recovered from the Aramus bags showed a comparable viable cell density (VCD) to the vials and faster growth compared to alternative cryobags.

CELL VIABILITY AFTER THE FREEZE & THAW PROCESS

Viability of cells was measured before freezing and after thawing of the cells. Cultures were performed using glass vials or 50mL and 500mL cryobags. The results are described in **Figure 2**. Compared to glass vials, the fluoropolymer bags demonstrate comparable cell viability in the preculture as well as after thawing and culturing for 5 days.

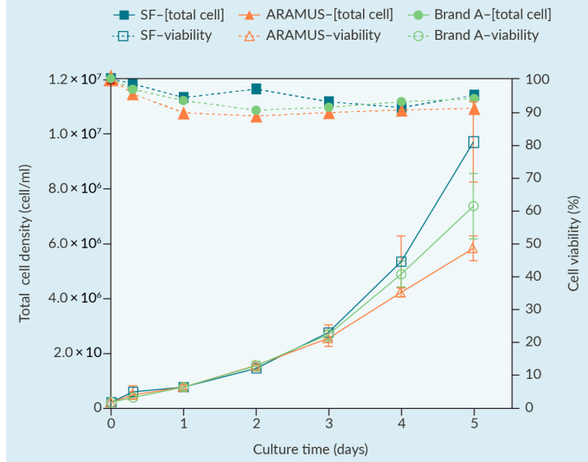
Figure 2. Cell viability during the various stages of the cell culture process (pre- and post-thawing).



CULTIVATION OF HEK293 CELLS

HEK293 cells were cultured for 5 days, and recovery was determined by measuring the total cell density and viability. Cell cultures were performed and compared between shake flasks (SF), Aramus- and brand A bags.

Figure 3. Cell density and cell viability in shake flask culture or cell culture performed using single-use bags.



The results are shown in **Figure 3** and demonstrate that the Aramus bags are suitable for cultivation of HEK293 cells.

CONCLUSION

Intensified HDCB using Aramus single-use cryobags can shorten cell culture times by weeks and reduce contamination risks by eliminating multiple manual cell transfer steps. It provides a wider window to start post-thaw seeding into the bioreactor, providing flexibility for scale-up production. Overall, the HCDB process leads to improved productivity, lower overall cost of goods, and better sustainability using fewer consumables.

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