The Need for Standardized Cryoprocessing: Process Impacts on Preservation Outcomes

Lukas Underwood, PhD; Jacqueline Mikhaylov, BS; Quinn Osgood, BS; Nilay Chakraborty, PhD
ATCC, Manassas, VA 20110 | Email: lunderwood@atcc.org

Background

- Characteristics of ice formation during cryopreservation can impact preservation outcomes in eukaryotic cells.
- Ice formation can be modulated by various chemical and physical approaches such as anti-freeze proteins, ice nucleation, and controlled rate freezing (CRF).
- It is a common practice to use passive freezing containers for small-scale cryopreservation. However, such freezing conditions have limited scalability and offer little control over the freezing process.
- Certain non-adherent human cell lines have poor post-cryopreservation outcomes characterized by significant loss of viability 24 hours post-thaw.
- The current work highlights the need for standardization of biomaterial cryopreservation in production environments using CRFs.

Materials and Methods

- In this study, the preservation outcome of THP-1 human monocytes isolated from a monocytic leukemia patient (ATCC® TIB-202™) was compared for two CRF profiles and a passive freezing container (PFC) (Figure 1).
- Cell recovery was measured using standard viability and growth. A genetically modified version of THP-1 (ATCC® TIB-202-NFkB-LUC2™) was used to assess post-thaw functionality (Figure 2-4).
- The thermal characteristics for each CRF profile were analyzed using differential scanning calorimetry (DSC) (Figure 5).

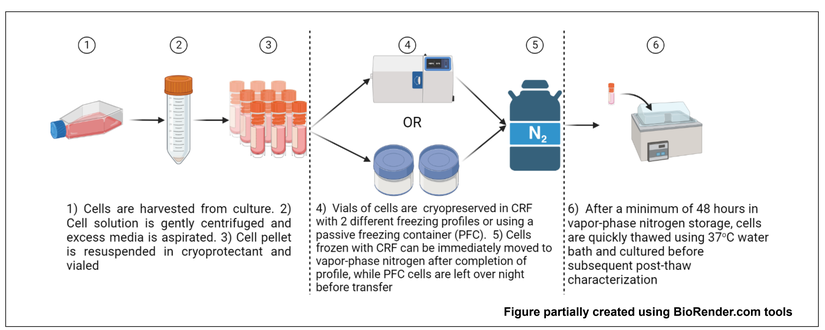

Figure 1: Technical approach for cryopreservation processing

Results

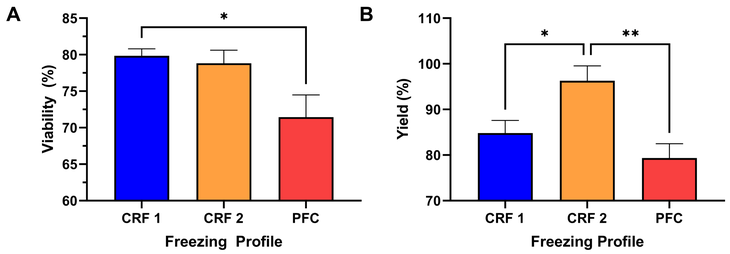

Figure 2: Evaluation of post-thaw yield and viability. (A) Post-thaw viability is the ratio of live/dead cell. Cells that were cryopreserved using the passive freezing container have lower post-thaw viability indicating less favorable freezing conditions. (B) Yield is the percentage of live cells recovered following post-thaw processing compared to the pre-cryopreservation measurement. CRF Profile 2 gives a significantly higher yield compared to the other methods. n=6, ±SEM, * p=0.05, ** p=0.01.

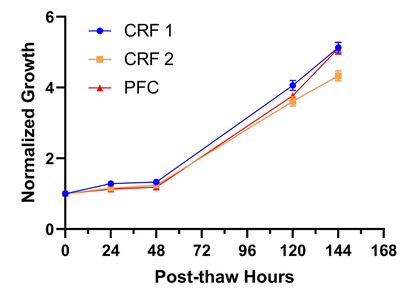

Figure 3: Comparison of post-thaw growth characteristics. Though significant differences were observed for the initial growth and yield, there is very little variation in long-term growth. This result indicates that the freezing process does not significantly damage cellular structures involved in growth. n=3, ±SEM.

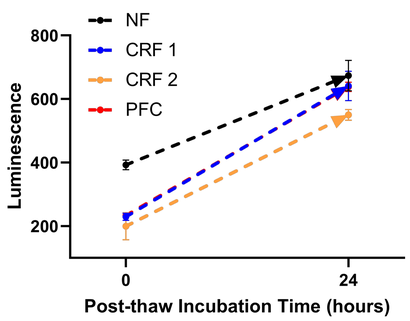

Figure 4: Functional recovery of cellular luminescence after 24 hours. Immediately post-thaw, all conditions have significantly lower luminescence compared to the non-frozen (NF) control. All conditions, including the NF control, have large increases in luminescence after 24 hours of incubation. This indicates that a 24-hour recovery period is needed for proper functional recovery. We theorize that even standard culture processing can reduce luminescence based on 24-hour recovery of NF cells. n=3, ±SEM.

Results Continued

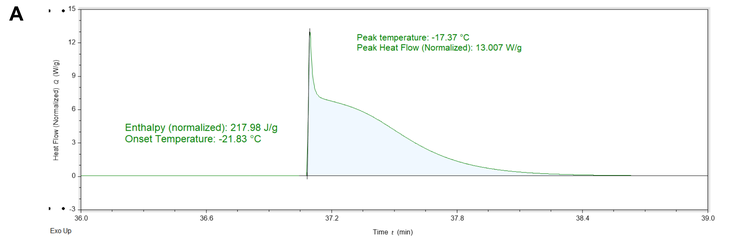

Figure 5: DSC evaluation of thermal characteristics. (A) A representative thermogram including key analytic parameters such as enthalpy, temperature, and maximal heat flow of the ice nucleation event. An inverse linear correlation between the (B) maximal heat flow and the (C) temperature of ice nucleation. These thermodynamic differences between profiles could partially explain the enhanced post-thaw viability for the cells cryopreserved using the CRF profiles. n=3, ±SEM, ** p=0.01, *** p=0.001.

Conclusions

- Employing a controlled-rate freezer for cryopreservation enables control and standardization for large-scale cellular preservation.
- Initial viability was significantly improved using the two CRF profiles as compared to the PFC.
- There were very minimal differences between post-thaw growth and functionality of the cells between freezing profiles. However, CRFs grant scientists control to optimize and standardize these parameters.