The Impact of Thaw Rate on the Recovery and Proliferation of MSCs and T cells

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Background

Understanding the impact of thaw rate on cryopreserved material can be beneficial in achieving the best quality cells in terms of viable cell recovery and functional performance. While a common perception is that faster thaw rates are beneficial to cell quality, there is literature both supporting[1, 2, 3] and against[4, 5] this establishment. Furthermore, the cryopreservation medium[1] and cell type[6] are also contributory factors in determining cell quality post thaw. The aim of this study was to therefore elucidate the impact of thaw rate and cryopreservation medium on T cells and Mesenchymal Stem Cells (MSCs) in order to inform the development of vial thawing devices.

Methods

Two cell banks were produced to conduct this study. MSCs were cultured in ten layer cell factories and T cells in a 2L wave bioreactor and both cryopreserved (using controlled rate freezers at -1°C/min) in vials (2mL Nunc cryovials (CV) and 6mL Aseptic Technology (AT) crystal vials), with and without 10% DMSO.

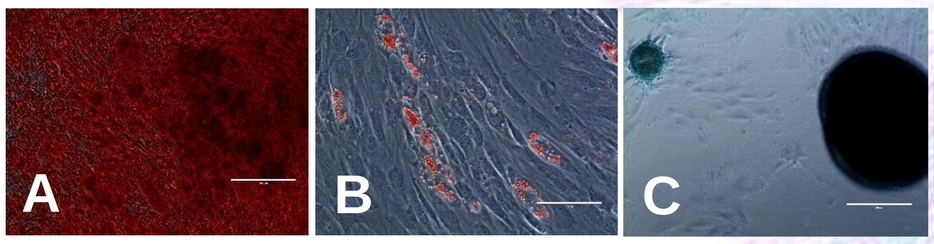

Image 1: Banked MSCs confirmed to be multipotent by differentiation into osteoblasts (A) scale: 400um, adipocytes (B) scale: 100um and chondroblasts (C) scale: 400um.

The cryopreserved MSCs and T cells (>86% CD3 positive cells) were thawed to a small ice crystal at a range of thaw rates. These rates were achieved by immersing and agitating vials at set temperatures in a water bath. Once thawed, cells were processed to determine the recovery of viable cells (%) (**Eq.1**) and proliferative performance (xCELLigence adherence assay measuring cell index (electrical impedance caused by cell attachment) for MSCs and CFSE cell proliferation assay for T cells).

Thaw temperature and time			
2mL Cryovial 0.5mL fill		6mL AT vial 3mL fill	
95 °C	56s	95 °C	2m 25s
65 °C	1m 16s	65 °C	3m 42s
37 °C	1m 39s	37 °C	4m 52s
10 °C	3m 10s	10 °C	12m 20s
Air Note: water bath at 25 °C	9m 45s	Air Note: water bath at 25 °C	33m 40s

Recovery Eq.1:

$$\%\ viable\ cell\ recovery\ (VCR) = \frac{Vout \times Tout}{Vin \times Tin}$$

V = % cell viability
T = Total cell number
Out = Value on thaw
In = Value on harvest

Results

Thaw Rate Impact on VCR:

The VCR was found to be approximately 70% or above for the majority of conditions indicating that thawing at temperatures below 37°C do not compromise cell viability. In addition, poor VCRs (<50%) were achieved when using the DMSO free cryopreservation medium for T cells.

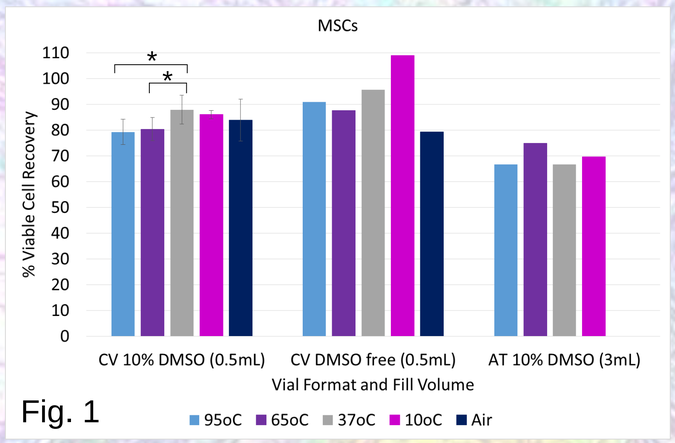

Fig. 1

Figure 1: Thaw rate impact on VCR of MSCs. CV 10% DMSO (0.5mL) n = 5 thaws, CV DMSO free (0.5mL) n = 1 thaw, AT 10% DMSO (3mL) n = 2 thaws.

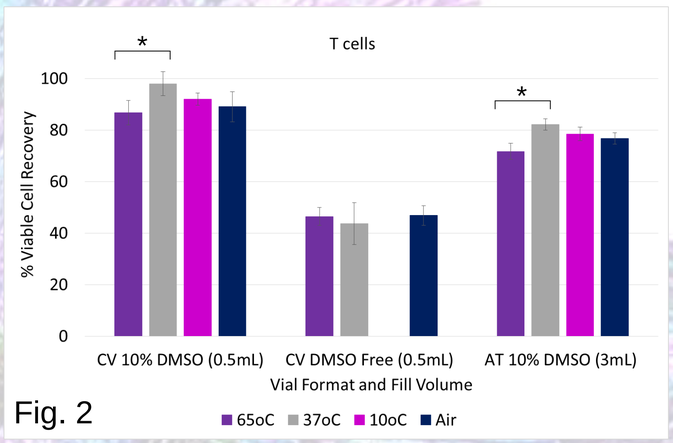

Fig. 2

Figure 2: Thaw rate impact on VCR of T cells. All conditions n = 5 thaws.

Thaw Rate Impact on Proliferation & Function:

MSCs: Thawed cells were seeded on xCELLigence adherence plates and the growth monitored for a period of 200 hours. The rate of growth (cell index/hr) during the exponential growth phase was calculated at each condition and compared to freshly harvested material (**Fig. 3**).

T cells: Thawed cells were stained with CFSE and stimulated with CD3/CD28 dynabeads for four days. The level of proliferation was calculated from the decrease in CFSE fluorescence of stained cells via flow cytometry analysis (**Fig.4 and Fig. 5**).

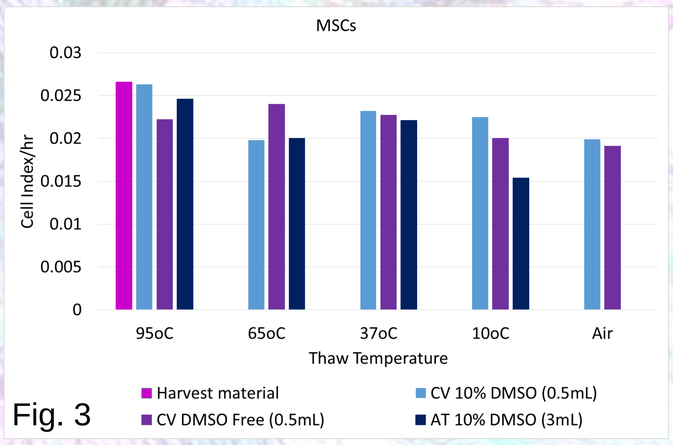

Fig. 3

Figure 3: Growth rate achieved at each condition compared to freshly harvested MSCs.

Similar levels of T cell proliferation were achieved from cells thawed at 65°C, 10°C and air compared to 37°C. The condition of the cells was determined using a ELISA to assess the level of IL4 and TNF alpha production at each condition (**Fig.6 – Fig. 9**). High levels (IL4 > 20pg/mL, TNF alpha > 1000pg/mL) of both cytokines were detected at each thaw rate. Cells formulated in the DMSO free cryopreservation medium were found to have poor proliferative potential and functional activity compared to those in 10% DMSO.

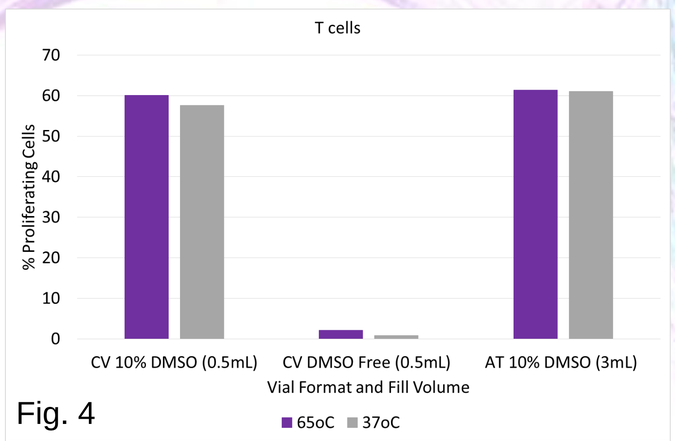

Fig. 4

Figure 4: % of proliferating cells at 65°C compared to 37°C.

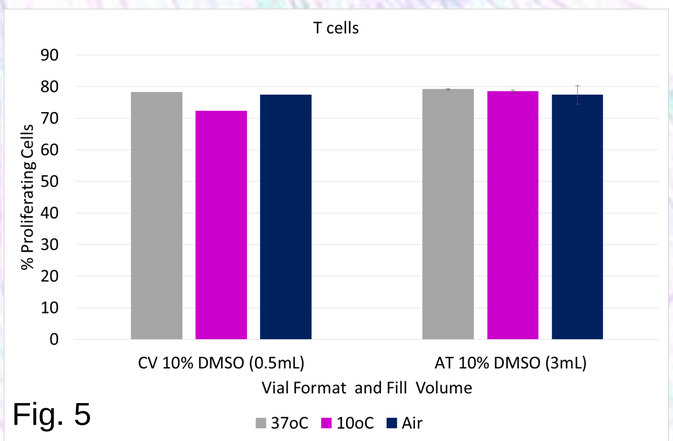

Fig. 5

Figure 5: % of proliferating cells at 10°C and Air compared to 37°C. AT 10% DMSO (3mL) n = 2 thaws.

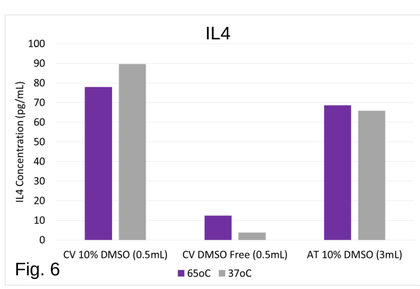

Fig. 6

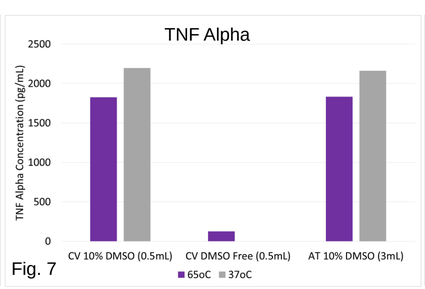

Fig. 7

Figure 6 & 7: IL4 and TNF alpha expression at 65°C compared to 37°C.

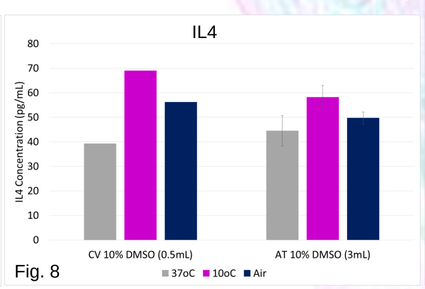

Fig. 8

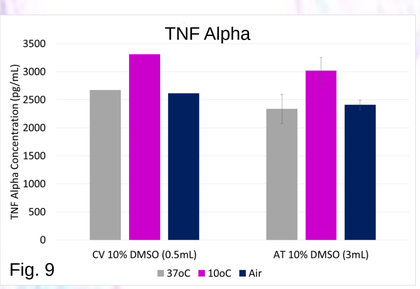

Fig. 9

Figure 8 & 9: IL4 and TNF alpha expression at 10°C and Air compared to 37°C. AT 10% DMSO (3mL) n = 2 thaws.

Conclusions & Future Work

When thawing at the rates tested which corresponded to temperatures slower (in 10°C water and in air) than a standard 37°C water bath, no significant difference in VCR or proliferative performance was observed using T cells or MSCs. In addition, DMSO free media was found to be a poor freezing formulation for T cells, but was effective with MSCs. This data is being used to inform the development/design of vial thawing devices ensuring robust and controlled thawing of cell therapies in a range of vial formats.

References

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