Transient Warming Events and Cell Viability of Placental/Umbilical Cord Blood ("PCB")

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BACKGROUND

Transient Warming Events ("TWEs") are brief exposures of a frozen blood component to ambient air during post-freeze processing, storage, shipping and transfer between cryogenic containers. The viability of cryopreserved hematopoietic stem and progenitor cells from PCB units may be reduced as a result of the TWEs that affect them after initial freezing. We question whether the loss of viability is sufficient to threaten the clinical effectiveness of PCB transplants. In these experiments we assessed temperature raise and multiple exposures.

EXPERIMENTS

A. Cell viability after multiple exposure of frozen (-196°C) to different temperatures.

- PCB leukocytes were controlled-rate-frozen in 10% DMSO to –196°C in 1 ml aliquots.
- Vials were transferred to alcohol baths maintaining -25°C, -40°C or -80°C, for 4 minutes and then returned to LN2.
- After 5 minutes in LN2, the exposed aliquots were returned to the same alcohol baths. Each aliquot underwent a total of five identical cycles from LN2 to either -25°C, -40°C or -80°C and back into LN2.
- Control aliquots were maintained at –196°C.
- All exposed and control aliquots were thawed and DMSO removed ([1]).
- Recoveries of total and viable leukocytes were assessed by acridine-orange and ethidium bromide staining.
- Recoveries of viable progenitor cells were ascertained by double staining of CD 34+ cells and formation of hematopoietic cell colonies, (CFU-C).
- The results are shown in Table 1.

Summary Conclusion: Definite reduction of viability with increased temperature and higher number of exposures was encountered in the three tests.

B. Timed exposure to room temperature and humidity.

- Placental blood collections, matched for ABO group, were combined into two separate pools (Pool 1 and Pool 2), each containing $>10^9$ total viable nucleated cells.
- Hydroxyethyl starch was added to 1% w/v (standard technique, ref 1).
- After 6 minutes centrifugation at 50 x G the leukocyte-rich plasma from each was collected. The supernatant from each pool was aliquoted into five processing bags and concentrated to a volume of 20 ml per bag by centrifugation (400 x G, 13 minutes).
- Five ml of 50% DMSO / 5% Dextran 40 was added and each mixture transferred to a freezing bag (Pall Medical Model 791-05) and overwrapped.
- The cryoprotected units were controlled-rate-frozen and stored (immersed in LN2) in a BioArchive® System (ThermoGenesis Corp.) for at least 48 hours.
- Units were retrieved from LN2 and kept in ambient air for either 2, 4, 6 or 8 minutes (21°C, 60% humidity) and then put back into LN2.
- Warming was monitored by thermocouples from a validated temperature logger. Exposed and control bags, were thawed, DMSO washed off and the number and viability of leukocytes and hematopoietic progenitor cells determined ([1,2]).
- The results are shown in Table 2.

C. Cumulative effect of multiple timed exposures to ambient air.

- Units from two additional pools (Pool 3 and Pool 4) were retrieved from LN2 and kept for either 1, 2 or 3 minutes, at room temperature (21°C) and then returned to LN2 for 2 minutes.
- The same units were again brought to air for the same time period and back to LN2 for 2 minutes for a total of five identical cycles.
- All exposed units and unexposed controls were kept in LN2 until tested.
- Temperature was monitored for each unit.
- All units were then thawed ([1]), DMSO washed off and viable leukocytes, CFU-C ([2]) and CD34+ cells counted. Cell recoveries were then calculated.
- The results are shown in Table 3.

Table 1: Loss of Cell Viability from TWEs

Temperature		-80 °C	-80 °C	- 40 °C	- 40 °C	- 25 °C	- 25 °C
No of TWEs		x 1	x 5	x 1	x 5	x 1	x 5
Leukocytes (% Loss)	Median Range	0 0 - 4 (n=3)	6 3 - 10 (n=3)	10 9 - 11 (n=2)	13.5 13 - 14 (n=2)	8 - (n=1)	41 - (n=1)
CD34+ cells (% Loss)	Median Range	2 0 - 4 (n=2)	6.5 2 - 11 (n=2)	10 9 - 11 (n=2)	21 18 - 24 (n=2)	Nd*	nd
CFU-C (% Loss)	Median Range	9 3 - 14 (n=2)	22 18 - 25 (n=2)	28 18 - 38 (n=2)	52 48 - 55 (n=2)	nd	nd

Table 2: Timed Exposure to Room Temperature

		CFU Control	Control	Exposed Time			
		Post-Thaw	%	2 min.	4 min.	6 min.	8 min.
% TNC Viability	Pool 1		76	75 (1)	75 (1)	69 (7)	63 (13)
(% Loss)	Pool 2		87	81 (6)	79 (8)	71 (16)	67 (20)
% CFU	Pool 1	450,000	100	100	94	78	67
Recovery	Pool 2	650,000	100	72	69	65	46

Table 3: Cumulative Effect of Multiple Timed Exposures

		CFU Control	Control	Exposed Time		
		Post-Thaw	%	1 min. x 5	2 min. x 5	3 min. x 5
% TNC Viability	Pool 3		72	63 (9)	62 (10)	58 (14)
(% Loss)	Pool 4		74	72 (2)	70 (4)	65 (9)
CFU Recovery	Pool 3	533,333	100	100	94	87
(%)	Pool 4	300,000	100	100	100	89
% CD34+ Viability	Pool 3		87	82 (5)	81 (6)	82 (5)
(% Loss)	Pool 4		65	64 (1)	76 (0)	61 (4)

CONCLUSIONS

1. Viability loss was demonstrated in several of the TWE modes.
2. Viability losses upon repeated exposure were additive.
3. These losses, UNDER CONDITIONS THAT MAY OCCUR IN ROUTINE PRACTICE, suggest that units that may be used for transplantation should be monitored for TWE and all incidents recorded.
4. Documentation of TWE should include minimally the time and the temperature of each exposure.

REFERENCE:

1. Rubinstein et al. *Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution.* PNAS 1995;10119-22.
2. Migliaccio et al. *Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity.* Blood 2000; 96: 2717-2722.

Figure 1: Cryo Bag 25 ml (Model 791-05)

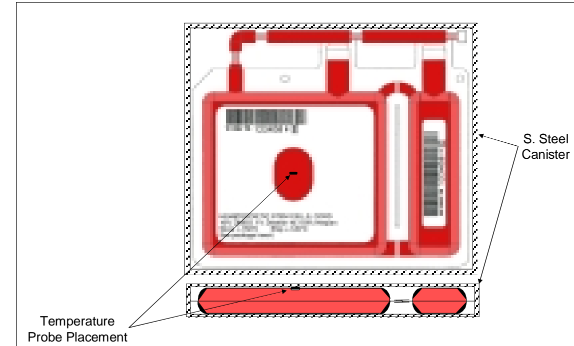

Figure 2: One TWE, 8-Minute Exposure to Ambient Air Pall Medical (Model 791-05) Cryo Bag 25 ml with Overwrap Bag in S. Steel Canister

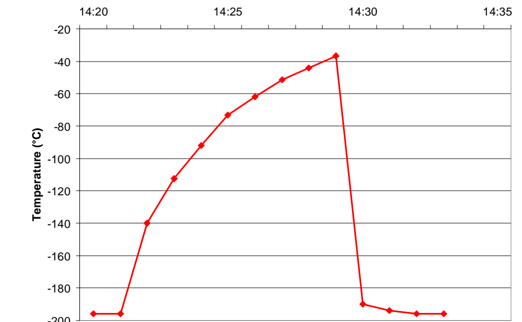

Figure 3: Five TWEs, Exposures to Ambient Air Pall Medical (Model 791-05) Cryo Bag 25 ml with Overwrap Bag in S. Steel Canister