

Effects of long-term cryopreservation on hematopoietic progenitor cells in umbilical cord blood

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Summary:

There is considerable interest in developing banks of frozen umbilical cord blood cells for transplants but it is uncertain how long frozen cells survive. Our objective was to determine the recovery of frozen umbilical cord blood cells. We quantitated recovery of hematopoietic progenitor cells (CFU-GM, BFU-E, and CFU-GEMM) from frozen umbilical cord blood cells stored for up to 12 years. Decay rates of CFU-GM, BFU-E and CFU-GEMM (d, expressed as percent of viable cells recovered (95% confidence interval) were 0.9930 (0.9889–0.9970), 0.9840 (0.9769–0.9911) and 0.9817 (0.9707–0.9927). Time-dependent recoveries, calculated by the formula d^k , (k = frozen storage interval in years) were >90% at 10 years. We conclude that frozen cord blood cells can be stored safely for prolonged intervals without substantial loss in hematopoietic progenitor cells.

Keywords: umbilical cord blood; long-term cryopreservation

Umbilical cord blood cells are increasingly used for transplant, especially when no HLA-identical sibling donor is available. Consequently, there is growing interest in developing frozen HLA-typed cord blood banks.

Umbilical cord blood cells in these banks may be stored for several years before use. However, there are few data on long-term survival of frozen cord blood cells; most reports focus on relatively short storage intervals.¹ We studied viability of hematopoietic progenitor cells in 12 umbilical cord blood samples frozen for up to 12 years.

Materials and methods

Between July 1985 and September 1985, cord blood samples were obtained from the placenta of 12 healthy volunteer mothers at delivery (38 to 41 weeks of gestation). Informed consent was obtained from all. Immediately after delivery of the baby, while the placenta was still *in utero*,

the umbilical cord was double-clamped 6 to 8 cm from the baby and cord blood collected. Before collection, the venipuncture site was cleaned with alcohol and betadine. An 18 gauge needle was inserted into the umbilical vein and cord blood was aspirated into a 50 ml syringe containing 5 ml of ACD. The volume of cord blood collected ranged from 28 ml to 45 ml. The umbilical cord blood was processed using standard techniques.² Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation was used to isolate the mononuclear cell fraction, which was resuspended at concentration of 2×10^7 /ml. Mean (s.d.) mononuclear cell recovery was $48 \pm 11\%$. Umbilical cord blood mononuclear cells were cryopreserved using a programmable freezer (model 801 CryoMed, Mt Clemens, MI, USA), using standard cryopreservation programs, in RPMI-1640 media, 10% DMSO and 20% fetal calf serum³ and stored in the liquid phase of liquid nitrogen.

Cells were thawed after 1 and 6 months and 1, 2 and 12 years and a small aliquot removed to assess viable cell recovery (trypan-blue exclusion test) and to assay hematopoietic progenitor cells (CFU-GM, BFU-E and CFU-GEMM) by standard techniques.³ Recovery was calculated by comparing numbers of cells to 1 month values.

Statistics

A linear model was applied to the logarithm of cell viability results. Logarithmic transformation was used to stabilize variance in observed recoveries. For a given progenitor cell assay, the model is:

$$\text{Log (viability)} = a + b \text{ interval cryopreserved}$$

when the estimate of slope (b) = 0, there is no trend for cell loss over time. Results were expressed as a decay factor (d) with 95% confidence intervals.

Decay over time (k) was calculated as d^k .

Results

Recoveries of CFU-GM, BFU-E and CFU-GEMM are shown in Table 1 and slope of cell loss (b) in Table 2. There was a modest but statistically significant time-dependent decay of the three cell types. In Table 3, we calculated an annual decay factor (d) for each cell type. These data confirm the very small loss of cell viability, even over pro-

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Table 1 Hematopoietic progenitor cell recoveries after freezing

	6 months	2 years	12 years
CFU-GM	98 ± 3% ^a	93 ± 10%	91 ± 7%
BFU-E	95 ± 15%	89 ± 9%	81 ± 10%
CFU-GEMM	93 ± 15%	82 ± 13%	77 ± 8%

^aMean ± s.d.; control is 1 month value.

Table 2 Slope (b) of hematopoietic progenitor cell loss

	(b)	95%CI	P for no trend
CFU-GM	-0.0070 ^b	0.0020	0.0011
BFU-E	-0.0162	0.0036	0.0017
CFU-GEMM	-0.0185	0.0056	0.0001

^bUnits are log (% cells/year).

Table 3 Annual decay (d) of hematopoietic progenitor cell

	d	95% CI
CFU-GM	0.9930	0.9889-0.9970
BFU-E	0.9840	0.9769-0.9911
CFU-GEMM	0.9817	0.9707-0.9927

longed storage. For example, loss of CFU-GM over 10 years is 0.9930¹⁰ or <10% CFU-GM.

Discussion

Successful engraftment following umbilical cord blood transplant, critically depends on harvesting an adequate

number of hematopoietic progenitor cells from cord blood and the subsequent successful cryopreservation of these cells without substantial losses in viability. The long-term effects of cryopreservation on hematopoietic progenitor cell viability in human bone marrow have been studied,⁴ but there are few similar reports on cord blood cryopreservation, with most studies reporting only short storage intervals.

Our data indicate that umbilical cord blood cells can be stored for prolonged intervals without substantial decline in recovery of hematopoietic progenitor cells.

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