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Review Article

Cryopreservation Protocols and Post-Thaw Viability of Haematopoietic Stem Cell Grafts: A Review

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Abstract

Background: Autologous haematopoietic grafts are routinely frozen under a protocol that is inherited rather than optimised.

Objective: To review the evidence on cryoprotectant concentration, freezing method and storage, and on what post-thaw assays predict.

Methods: Narrative review of clinical and laboratory studies of progenitor cell cryopreservation.

Findings: A randomised comparison of 10%, 7.5% and 5% dimethyl sulfoxide found no difference in engraftment and fewer adverse reactions at 5%. Post-thaw *recovery* does not predict engraftment; the absolute reinfused viable CD34+ dose does. Viability dyes, colony assays and aldehyde dehydrogenase activity disagree.

Conclusions: Reducing DMSO is well supported. Post-thaw viability is a release check on the dose delivered, not a proxy for graft function.

Keywords: haematopoietic stem cells; cryopreservation; dimethyl sulfoxide; post-thaw viability; engraftment

Introduction

Cryopreservation sits between collection and infusion in every autologous transplant and in unrelated cord blood transplantation, yet the protocol most centres use — 10% dimethyl sulfoxide, controlled-rate freezing, vapour-phase liquid nitrogen — was fixed decades ago. The graft is the product; freezing is the step most likely to damage it.

Two questions follow. How should the graft be frozen: which cryoprotectant at what concentration, cooled how fast, stored how? And what do the post-thaw measurements tell the transplanting clinician? The two are connected: the assays used to justify protocol choices are poorly correlated with the outcome that matters.

Materials and Methods

Studies were retrieved from PubMed, Europe PMC and Crossref for the terms *cryopreservation*, *haematopoietic progenitor cell*, *dimethyl sulfoxide*, *post-thaw viability*, *CD34* and *engraftment*, without date restriction. Priority went to randomised or directly comparative clinical studies and to laboratory work reporting more than one post-thaw assay on the same material. Bibliographic records were confirmed against Crossref or the publisher, and quantitative values taken from the source, not secondary citation.

Cryoprotectant concentration

Dimethyl sulfoxide at 10% is conventional rather than optimal. It is toxic to cells at room temperature, and the infused dose is responsible for the acute reactions during reinfusion.

The decisive study is a prospective randomised comparison of 10%, 7.5% and 5% DMSO in autologous peripheral blood transplantation: of 150 patients randomised and 143 transplanted, median leukocyte recovery was 10 days (p = 0.36), neutrophil recovery 10 days (p = 0.2) and platelet recovery 15 days (p = 0.61), with no difference between arms — while adverse reactions were least frequent in the 5% arm (p = 0.02) [1]. A systematic review and meta-analysis of controlled studies reached the same conclusion [2]. Earlier work showed that 5% DMSO without rate-controlled freezing preserved progenitors adequately [3].

No alternative to DMSO has displaced it. Trehalose gave colony counts comparable to DMSO after five weeks of long-term culture — 172 ± 28 against 170 ± 52 colony-forming units — but with variance wide enough to limit the inference [4]. Screening four commercial DMSO-free solutions against a DMSO control for cord blood grafts found one that matched or exceeded it on post-thaw viability, viable CD45+ and CD34+ recovery and progenitor expansion [5]. Cell concentration at freezing also affects recovery [6].

Freezing method and storage

Controlled-rate freezing at approximately 1 °C per minute is standard [7], but the evidence that it is necessary is weaker than its universality implies. A direct comparison against uncontrolled freezing at –80 °C found comparable long-term haematologic reconstitution [8], and a recent analysis reported passive freezing equivalent to controlled-rate freezing for engraftment [9]. Given the equipment and staffing it requires, this matters for smaller centres.

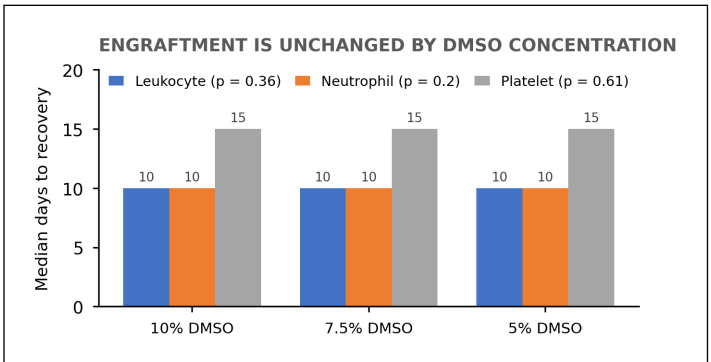

Figure 1: Median days to leukocyte, neutrophil and platelet recovery in a randomised comparison of three dimethyl sulfoxide concentrations, 143 autologous transplants. No endpoint differed between arms. Data from Mitrus et al. [1].

Storage duration appears forgiving. Functional progenitor and stem cells were recovered at high efficiency from cord blood cryopreserved for 15 years [10], and grafts stored for prolonged periods retained engraftment potential in salvage autologous transplantation [11]. Storage temperature is the more meaningful variable: –80 °C is generally considered acceptable only for shorter intervals, with vapour-phase liquid nitrogen beyond that [12,7]. That convention may be conservative. In 72 products held at –80 °C for a median of 868 days, median post-thaw viability was 94.8%, falling by about 1% per 100 days, and neither storage duration nor donor age was associated with engraftment [13] — a single-arm series, not a head-to-head comparison, so it weakens the case for the convention without settling it.

Post-thaw assays and what they measure

Four assays are in routine or near-routine use, and they disagree. Flow cytometric CD34+ enumeration follows the ISHAGE guidelines [14], refined into single-platform absolute counting [15]. Colony-forming unit assays measure proliferative capacity over one to two weeks. Aldehyde dehydrogenase activity has been validated as a rapid cord blood potency assay [16].

The disagreement has identifiable causes. DMSO itself interferes with post-thaw viability assessment by dye exclusion in both cord blood and mobilised peripheral blood [17]. The ALDH assay excludes early apoptotic cells [18], which viability dyes score as live — the two measure different populations by design, not by error. Recovery also differs between leukocyte subpopulations within the same product [19], so a single whole-product figure conceals variation.

Does post-thaw viability predict engraftment?

The answer is more specific than the literature's usual framing.

Table 1: Post-thaw assays used on haematopoietic grafts, what each measures, and why they disagree.

Assay	Measures	Principal limitation	Ref.
Viability dye (7-AAD)	Membrane integrity	DMSO in the sample distorts dye exclusion	[15]
CD34+ flow (ISHAGE)	Progenitor number	Counts cells, not their function	[12,14]
Single-platform count	Absolute CD34+/µL	Requires bead standard and strict gating	[14]
CFU-GM colony assay	Proliferative capacity	One to two weeks; too slow for release	[19]
ALDH activity	Functional potency	Excludes early apoptotic cells that dyes score live	[16,17]
Whole-product viability	Bulk survival	Recovery differs widely between subpopulations	[18]

Yang and colleagues examined 78 collections from 52 patients: median post-thaw recovery of viable CD34+ cells was 66.4% (range 36.1–93.6%) and of CFU-GM 63.0% (28.6–85.7%). Neither *recovery percentage* correlated with neutrophil engraftment ($p = 0.136$ and $p = 0.417$) or platelet engraftment ($p = 0.88$ and $p = 0.126$). The *absolute reinfused viable CD34+ dose per kilogram* did: $p = 0.0001$ pre-cryopreservation and $p = 0.001$ post-cryopreservation for neutrophils, and $p = 0.023$ and $p = 0.010$ for platelets [20]. The distinction between proportion recovered and absolute number delivered is the whole of it.

A second study agrees: the viable transplanted CD34+ dose measured post-thaw does not predict engraftment kinetics better than the total dose measured pre-freeze, in patients receiving more than 2×10 CD34+ cells/kg [21]. Not all reports agree — post-thaw viable CD34+ count has been described as a valuable predictor of engraftment [22] — but the threshold effect offers a reconciliation: above an adequate dose, further variation in viability is not limiting, and classic dose-finding work established where that threshold lies [23].

DMSO toxicity and cord blood

Infusion reactions attributable to DMSO are not rare. An EBMT survey of 97 responding autologous centres put the incidence of DMSO toxicity at approximately one in 70

transplants, predominantly cardiovascular and respiratory, with a trend toward fewer complications at lower concentration or with washing [24]. DMSO depletion before autografting has been examined for its effect on recovery, side effects and toxicity [25]; washing removes the agent but costs cells, so practice remains divided.

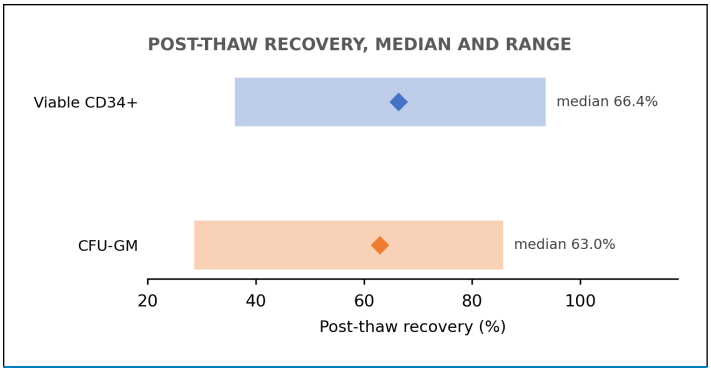

Figure 2: Post-thaw recovery of viable CD34+ cells and CFU-GM across 78 collections from 52 patients, shown as median and full range. Neither recovery percentage correlated with engraftment. Data from Yang et al. [20].

Cord blood is a distinct problem. The volume-reduction and cryopreservation methods developed for unrelated banking [26] handle a graft that is small, fixed in size and cannot be topped up, so proportional losses matter far more than for a mobilised apheresis product — which is why a validated potency assay was pursued for cord blood first [16].

Discussion

Three conclusions are supportable. First, 5% DMSO is as effective as 10% for engraftment and causes fewer adverse reactions, on randomised evidence supported by meta-analysis. There is little justification for 10% as a default.

Second, controlled-rate freezing may not be necessary. Two independent comparisons found uncontrolled or passive freezing equivalent for engraftment, which matters for centres where the equipment is a barrier.

Third, post-thaw viability is widely misread. Recovery percentage does not predict engraftment; the absolute viable CD34+ dose reinfused does. A product releasing at 60% recovery but delivering an adequate dose per kilogram is not a compromised graft, and reporting recovery without the absolute dose is uninformative.

A fourth point is procedural. Because recovery and absolute dose are reported inconsistently between centres, and the assays are not interchangeable, comparisons across published series are weaker than they look. Accreditation frameworks address process rather than assay equivalence [27], so two compliant laboratories can produce viability figures that are not comparable. A centre changing cryoprotectant concentration or freezing method should validate locally against its own engraftment data and record which assay generated each figure.

This is a narrative review without systematic search or risk-of-bias assessment. Several key comparative studies are single-centre and non-randomised, assay methods differ, and for a number of the cited papers only the bibliographic record and abstract could be verified, so quantitative claims are restricted to those confirmed at source.

Conclusion

The conventional cryopreservation protocol is defensible but not optimal, and two of its components — 10% DMSO and controlled-rate freezing — are supported more by inheritance than by evidence. Post-thaw assays measure different things, and disagree for reasons that are understood. The practical recommendation: reduce DMSO where local validation permits, report the absolute reinfused viable CD34+ dose alongside any recovery percentage, and treat post-thaw viability as a release specification rather than a prediction of graft function.

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Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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