BioMed Visions Journal

Volume 2 | Issue 2 Article 10

7-15-2026

Cytotoxicity of Dimethyl Sulphoxide on Viability of Peripheral Hematopoietic Stem Cells

Rana Nidhamaldeen Al-Nuri
Medical Doctor, Head of Stem Cell Apheresis Unit, Nineveh Health Directorate, Al-Hadbaa Specialized Hospital for Hematology and Bone Marrow Transplantation, rananidam33@gmail.com

Francesco Ipsevich
Medical Doctor, Former Director of Stem Cell Collection Unit, San Camillo - Forlanini Hospital, Rome, Italy

Lazim Subhi Sheet
Head of Stem Cell Manipulation Unit, Nineveh Health Directorate, Al-Hadbaa Specialized Hospital for Hematology and Bone Marrow Transplantation

Follow this and additional works at: https://bmvj.alnoor.edu.iq/home

 Part of the Medical Sciences Commons

Recommended Citation

Al-Nuri, Rana Nidhamaldeen; Ipsevich, Francesco; and Sheet, Lazim Subhi (2026) "Cytotoxicity of Dimethyl Sulphoxide on Viability of Peripheral Hematopoietic Stem Cells," *BioMed Visions Journal*: Vol. 2: Iss. 2, Article 10.
DOI: https://doi.org/10.63100/3078-6738.1029

This Original Study is brought to you for free and open access by BioMed Visions Journal. It has been accepted for inclusion in BioMed Visions Journal by an authorized editor of BioMed Visions Journal.

BIOMED VISIONS JOURNAL 2026;2:138–144

Scan the QR to view
the full-text article on
the journal website

ORIGINAL STUDY

Cytotoxicity of Dimethyl Sulphoxide on Viability of Peripheral Hematopoietic Stem Cells

Rana Nidhamaldeen Al-Nuri [a,*], **Francesco Ipsevich** [b], **Lazim Subhi Sheet** [c]

[a] Medical Doctor, Head of Stem Cell Apheresis Unit, Nineveh Health Directorate, Al-Hadbaa Specialized Hospital for Hematology and Bone Marrow Transplantation
[b] Medical Doctor, Former Director of Stem Cell Collection Unit, San Camillo - Forlanini Hospital, Rome, Italy
[c] Head of Stem Cell Manipulation Unit, Nineveh Health Directorate, Al-Hadbaa Specialized Hospital for Hematology and Bone Marrow Transplantation

ABSTRACT

Background: Hematopoietic stem cells (HSC) are cells that can differentiate according to the tissue they are lying in. The transplantation of such cells is either autologous or allogeneic. Autologous storage is required until the patient finishes his intensive chemotherapy. Whilst allogenic storage is required until finding the suitable matched recipient for this cellular therapy. Storage is in deep frozen state (–80° C) with cryoprotectant compound. Dimethyl sulphoxide (DMSO) has been used as cryoprotectant due to its electrophilic property that makes H-bond between DMSO and H_2O, which is 2.6 times stronger than the H bond between two water molecules. As a result, minimal concentration of DMSO prevents formation of water crystals during freezing storage of cells.

Objectives: Assessing the cytotoxicity of DMSO on stored HSC, by determining the cells' viability after adding DMSO with concentration of 10%, after deep-freezing in specialized deep freeze at (–80° C), then in ordinary freeze at (–20° C), in room temperature, in ordinary refrigerator at (+4° C), and lastly in water bath incubation at (+37 ° C), each for 24 hours.

Methods: Using trypan blue dye, the vitality was assessed by counting the alive cells (uncolored), and dead cells (blue colored).

Results: Cells retain high membrane integrity when kept at (–80° C) and (–20° C), but when exposed to room temperatures (+22° C) and physiological temperature (+37° C), this will rapidly decrease cell viability.

Conclusions: Although prolonged incubation time further decreases viability, the essential driver of DMSO cytotoxicity is elevated temperature. The post-thaw cytotoxicity of 10% DMSO cryopreserved peripheral HSCs is greatly temperature dependent.

Keywords: Dimethyl sulfoxide, Cryopreservation, Cytotoxic effect, Hematopoietic stem cells, Viability

1. Introduction

Hematopoietic stem cells (HSC) are cells that can differentiate according to the tissue they are lying in. Stem cell transplantation is either autologous or allogenic transplantation (Baldomero et al., 2011; Bensinger, 2012; Hristova and Zhivkov, 2023).

In autologous setting, after collection of the autologous stem cells, the patient will receive chemotherapy where the malignant and healthy mature leukocytes are destroyed, the patient will be then infused with his own stem cells. During this interval, the cells are kept in a deep freeze state at a temperature of (–80° C) for few weeks or months. Whereas, in allogenic one, after chemotherapy treatment, the patient receives HSC from a healthy human leukocyte antigen (HLA) matched donor. The process of donor selection, in allogeneic setting, is difficult and time consuming, because the requirement of maximal matching of the HLAs between the stem cells of the donor with that of

Received 5 February 2026; revised 22 April 2026; accepted 9 May 2026.
Available online 15 July 2026

* Corresponding author.
E-mail address: rananidam33@gmail.com (R. N. Al-Nuri).

https://doi.org/10.63100/3078-6738.1029
3078-6738/© 2026 Al-Noor University College. This is an open-access article under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

the recipient. Therefore, the donors' stem cells could be stored for months or years until an appropriate recipient is in need for these stem cells.

At room temperature, the collected stem cells have an intensive metabolism, which must be minimized to save the cell viability. So, the cells have to be cooled down, if it was intended to be stored for a short time, or to be deeply frozen with cryoprotective solution in a freezer at a temperature of –80° C, if it was intended for prolonged storage (Hristova and Zhivkov, 2023). Cryoprotective solutions is a chemical compound that act by preventing the formation of ice crystals. (Agarwal and Singh, 2023).

Dimethyl sulphoxide's (DMSO) cryoprotective characteristic is attributed to two strong electrophilic atoms (s and o), which have negative partial charge that leads to subsequent increase in potential of proton acceptance, so even one molecule of DMSO will form hydrogen bonds with two water molecules. The strength of one H-bond between DMSO and H_2O is 2.6 times stronger than the strength of a hydrogen bond between two water molecules (Oh and Baiz, 2018). As a result, DMSO with such an electrophilic property, even at very low concentration will prevent the development of water crystals during the process of freezing (Mohanna et al., 2023).

A crucial requirement for cryoprotectant is that they should be nontoxic to the cells, but there are many data showing that DMSO is cytotoxic at temperature above (+4° C) (Kang et al., 2002; Best, 2015).

Although the wide use of DMSO as intracellular cryoprotectant for variety of animal cells, in addition to stem cells, the influence of temperature on DMSO cytotoxicity after longer incubation is not fully explored (Tonev et al., 2020).

The purpose of this current research is to study the effect of incubation temperature and time of exposure on the cytotoxicity of DMSO on the human HSC cryopreserved with 10% DMSO for up to 24 hours. To determine the cytotoxic effect of DMSO at different temperatures: (–80° C) in deep freezing condition, (–20° C), in an ordinary freezer, (+4° C) in an ordinary refrigerator, (+22° C) in room temperature, and (+37° C) in physiological temperature, during incubation for 10, 30, and 60 minutes respectively. All these conditions were tested to simulate and manage any emergency conditions threatening the frozen stem cells such as shutdown or shortage of electricity or power instability, or even any technical issues or failure inside the deep freeze.

2. Material and methods

Experimental study was done to evaluate the post-thaw cytotoxicity of 10% DMSO on HSCs. Ten cryovials containing frozen peripheral stem cells belonging to a single donor. These vials represent technical replicates sourced from a single donor's aphaeretic collection, which had been aliquoted and cryopreserved under standard hospital protocols, for one month in specialized deep freeze at (–80° C), then transferring to different temperature, for different incubation time. Examination is then done via counting the viable stem cells, using trypan blue dye (0.4% solution) to test the percentages of viable stem cells.

This trypan blue viability test depends on the staining of dead cells as blue, as the dye is able only to penetrate the dead cells through damaged membranes.

Ethical Considerations. This study adhered to the ethical principles outlined in the World Medical Association's Declaration of Helsinki for medical research involving human subjects. This study was also approved by the Ethical Committee of Research at the Nineveh Directorate of Health on May 8th, 2025 (number: 19772). Patient's written consent form for publication was also obtained.

Data from experiment were analyzed using a two-way ANOVA statistical test (temperature x time).

Commercial DMSO (OriGen Biomedica- CryoPur™, with 99.9% purity) was used as cryoprotective solution in a concentration of 10% here in this study.

After stimulation of healthy donor or patient to release stem cells from the bone marrow to the peripheral blood, then hematopoietic stem cells were isolated from the peripheral blood via leukapheresis using a cell separator.

Two cell separators are employed; Com.Tec Cell Separator, software 04.03.09, and Amicus Cell Separator, software 4.4, both from Fresenius Kabi.

The cell suspension was then concentrated by removing the plasma. Then a cryopreserved solution was added to the cellular suspension. This cryoprotectant solution consists of DMSO, and extracellular cryoprotectant: normal saline, and plasma, in a way that the final concentration of DMSO was 10%, while normal saline was 20% and plasma was 10%.

Then stored in specialized deep-freezing device at (–80° C) for one month. After that the viability testing started.

The ten cryovials were divided into pairs to undergo different temperature treatments.

The first two vials were thawed immediately. The remaining four pairs were stored for 24 hours under varying conditions: in an ordinary freezer (–20° C), an ordinary refrigerator (+4° C), at room temperature (+22° C), and in an incubator (+37° C). After the 24-hour period, the vials that were kept at –20° C, they were thawed.

Finally, trypan blue dye was added to all samples, and the viable cells were counted at 10, 30, and 60 minutes respectively.

Trypan blue test is based on the difference in the permeability of membranes of living and dead cells for dyes. Under optical microscope, the vitality was assessed by counting the alive cells (uncolored), and dead cells (blue colored).

In our bone marrow transplantation center, cryopreservation at deep freeze (–80° C) is adopted for a maximum of six months storage. During which the patient undergoes intensive conditioning with high dose chemotherapy for eradication of malignant cells and creating space for the newly infused cells.

Statistical analysis: Two-way ANOVA was used to evaluate the main effects of temperature and incubation time, and their interaction, on stem cell viability.

3. Results

Chart 1 illustrates the progressive and temperature dependent decline in stem cell viability following post thaw exposure to 10% DMSO at 10, 30 and 60 minutes intervals.

Viability across all groups was examined utilizing the trypan blue exclusion test. The data are presented as the mean percentage of viable cells ± standard deviation (Kang et al., 2002), standard error of the mean (SEM), and 95% confidence intervals for technical replicates (n = 2), as in Table 1.

A two-way ANOVA statistical test was applied to examine the effects of storage temperature, incubation time and their interaction on the viability of peripheral hematopoietic stem cells suspended in 10% DMSO.

The analysis revealed a highly significant main effect of temperature on cell viability ($p = 1.36 * 10^{-13}$). As temperature increased from (–80° C) to (+37° C), the mean cellular viability decreased.

Furthermore, there was a highly significant main effect of incubation time on viability ($p = 1.43 * 10^{-5}$), with cell viability decreasing across all temperature groups as the exposure time extended from 10 minutes to 60 minutes.

The interaction effect between temperature and time was statistically significant ($p = 0.414$). This indicates that cytotoxic effect of prolonged DMSO exposure is independent of the temperature conditions. Extended incubation time consistently reduces

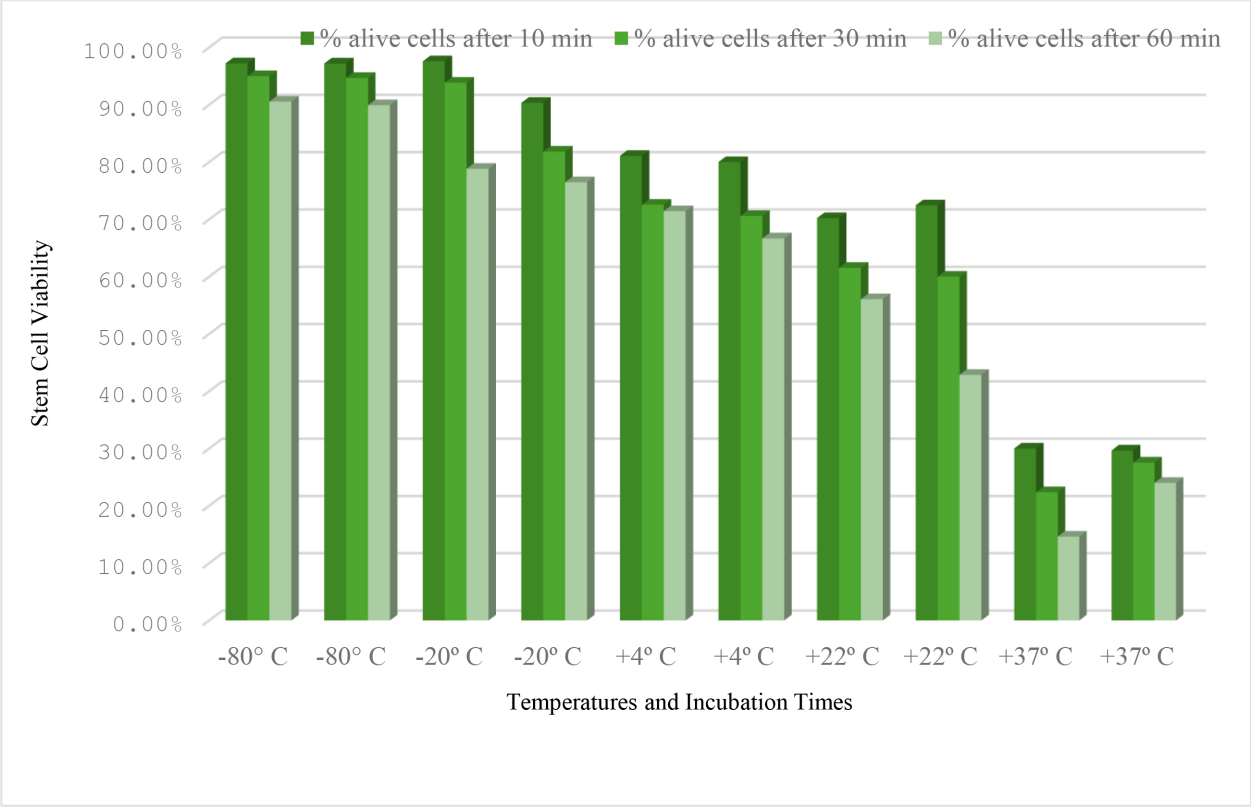

Chart 1. Percentage of viable HSCs across varying temperatures and incubation times.

Table 1. Mean viability and statistical variance of HSCs across different temperatures and incubation conditions.

Temperature	Incubation Time	N	Mean Viability (%)	SD	SEM	95% CI Lower	95% CI Upper
+37° C	10 min	2	29.835	0.247487	0.175	27.61141	32.05859
+37° C	30 min	2	24.96	3.662813	2.59	–7.94907	57.86907
+37° C	60 min	2	19.315	6.625591	4.685	–40.2136	78.84357
+22° C	10 min	2	71.335	1.576848	1.115	57.16758	85.50242
+22° C	30 min	2	60.77	1.088944	0.77	50.98622	70.55378
+22° C	60 min	2	49.46	9.33381	6.6	–34.401	133.321
+4° C	10 min	2	80.54	0.763675	0.54	73.67865	87.40135
+4° C	30 min	2	71.57	1.385929	0.98	59.11792	84.02208
+4° C	60 min	2	69.05	3.365828	2.38	38.80923	99.29077
–20° C	10 min	2	93.96	5.119453	3.62	47.96354	139.9565
–20° C	30 min	2	87.835	8.506495	6.015	11.40718	164.2628
–20° C	60 min	2	77.645	1.661701	1.175	62.71521	92.57479
–80° C	10 min	2	97.21	0.014142	0.01	97.08294	97.33706
–80° C	30 min	2	94.84	0.226274	0.16	92.80701	96.87299
–80° C	60 min	2	90.235	0.445477	0.315	86.23255	94.23745

viability, regardless of the temperature at which the cells are kept.

The Figs. 1 to 5 below show the microscopical images of HSC suspension incubated at 10% DMSO at different temperatures (–80° C, –20° C, +4° C, +22° C and +37° C) respectively, and were stored for 24 hours, then were defrosted and stained with trypan blue dye, then finally were examined under microscope after 10, 30, and then after 60 minutes.

4. Discussion

Although the wide use of DMSO as a cryoprotectant for human cells, there are few studies focusing overtly

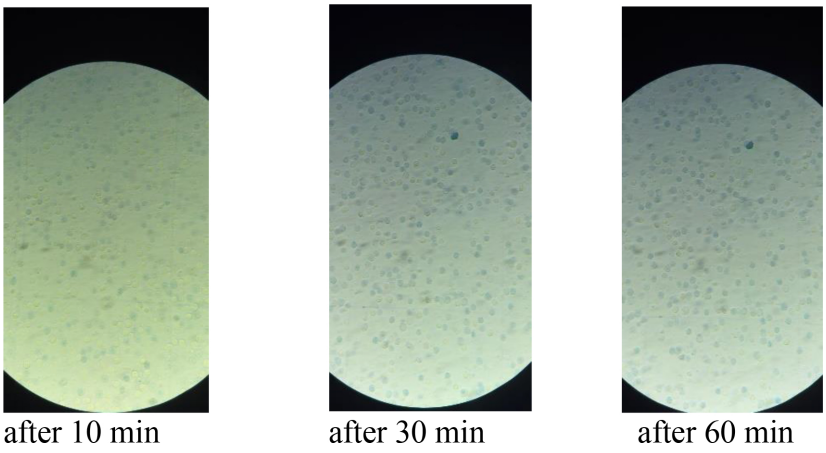

Fig. 1. Microscopic images of HSCs that were stored in deep freeze at (–80° C).

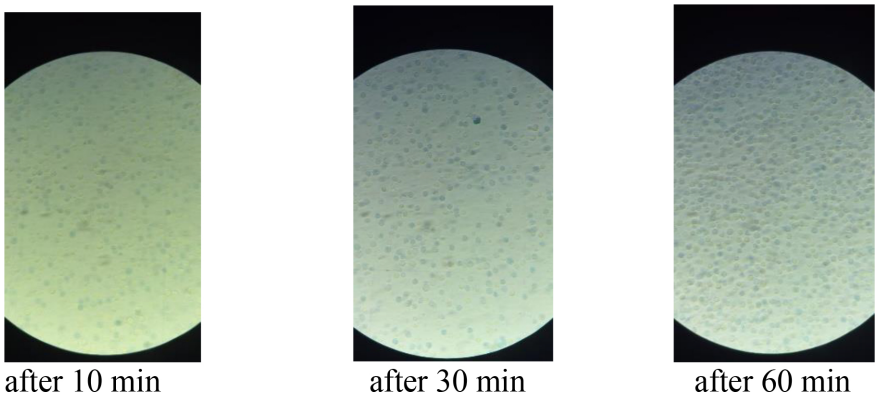

Fig. 2. Microscopic images of HSCs that were stored (–20° C).

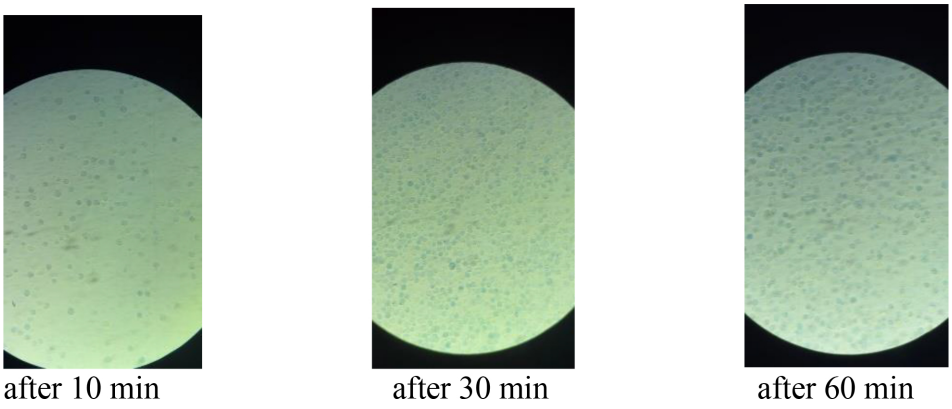

Fig. 3. Microscopic images of HSCs that were stored at (+4° C).

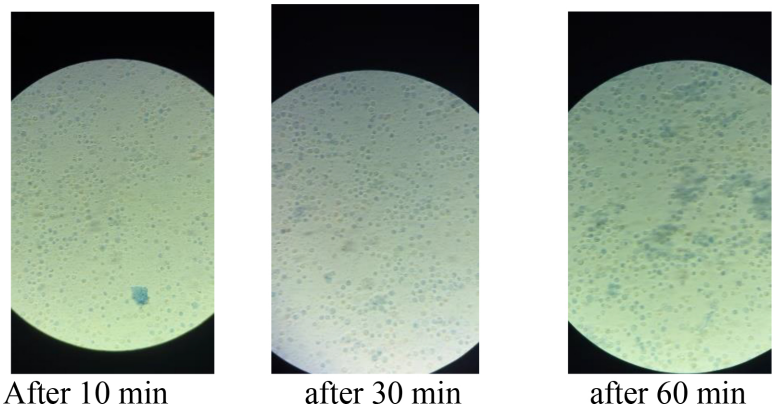

Fig. 4. Microscopic images of HSCs that were stored at (+22° C).

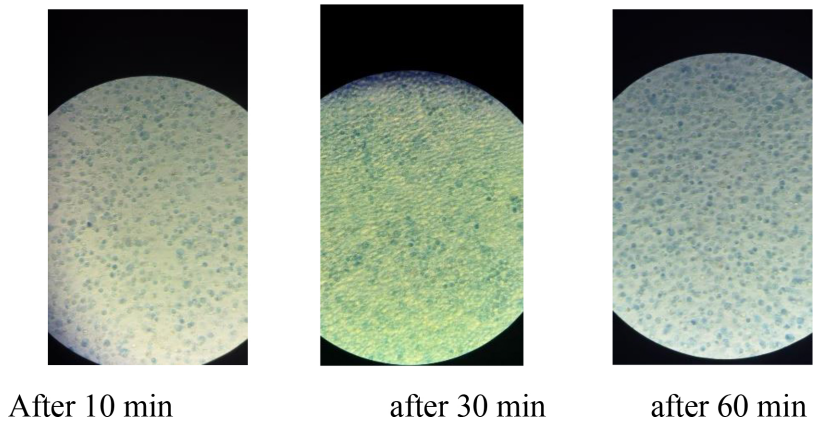

Fig. 5. Microscopic images of HSCs that were stored at (+37° C).

on its post-thaw cytotoxicity under varying temperature conditions.

According to previous literatures, DMSO leads to significant modification in the function and structure of the cell membrane, nucleic acids and proteins (Best, 2015).

This intrinsic cytotoxicity together with logistical challenges, as the need for specialized equipment and strict temperature-controlled supply chain, makes it difficult to maintain ideal post-thaw conditions in resource-limited clinical settings (Hunt, 2019).

In the current study, cell viability was assessed only by using the trypan blue exclusion test, to ensure strict methodological standardization. This method is highly dependable, economical, and rapid in application (Strober, 2015). The test mainly depends on

membrane permeability. Living cells have an intact lipid bilayer that serves as an impermeable barrier to the large, negatively charged trypan blue dye. As a result, living cells remain uncolored, while dead or damaged cells are penetrated by the dye and are stained blue (Hristova and Zhivkov, 2023; Tonev et al., 2020).

The primary objective of this study was to assess the cytotoxicity of 10% DMSO on cryopreserved HSCs across different temperatures (–80° C, –20° C, +4° C, +22° C and +37° C) and at different incubation times (10, 30 and 60 minutes). These specific variables were selected to simulate real world technical emergencies, such as power outage or freezer failures, which are practical concerns in our hospital's specific infrastructure.

Our results reveal that DMSO cytotoxicity is highly temperature dependent. When stem cells were stored at (–20° C) for up to 60 minutes, there was a non-significant decrease in percentage of vital cells. However, keeping the cells at room temperature (+22° C) reduced stem cell viability to an average of (60.77%) by 30 minutes, and incubation at (+37° C) quickly decreased viability to about (24.96%) in the same timeframe.

The two-way ANOVA proved a highly significant primary effect of temperature on the cell death ($p = 1.36 * 10^{-13}$).

Interestingly, the statistical interaction between temperature and time was non-significant ($p = 0.414$), indicating that while higher temperature extremely reduces cell viability, the main damage is really temperature dependent rather than compounding significantly over the 10-to-60-minute windows tested.

These findings align with Tonev et al. (Tonev et al., 2020), who found that upon incubation at lower temperatures, the number of dead cells remains relatively stable over time, whereas at higher temperatures, the number of dead cells increases significantly.

Furthermore, our results are consistent with Hristova and Zhivkov (Hristova and Zhivkov, 2023), who showed that the percentage of viable stem cells significantly decreases as incubation temperature increases. They also concluded that the deep-freezing process itself induces additional delayed damaging effect on the cells, rendering them much more vulnerable to DMSO during thawing.

This contrast with findings by Elmoazzen et al. (Elmoazzen et al., 2007), who observed that in human chondrocytes, the incubation temperature did not significantly affect the vitality of cells suspended in 8% DMSO for 120 minutes. However, comparing chondrocytes to HSCs gives sever physiological limitations, because HSCs are single-cell suspension with rapid DMSO equilibrium because the cells are not surrounded by a dense matrix, whereas chondrocytes in intact articular cartilage are protected by a dense extracellular matrix that severely delays DMSO infusion.

Furthermore, Elmoazzen et al. showed that DMSO toxicity at (+4° C) for 24 hours was significantly higher on deep frozen cells compared to non-frozen controls. This really supports our basic idea; the physiological and osmotic stress of deep freeze storage compromises membrane integrity, making HSCs significantly more sensitive to the cytotoxic effects of DMSO during post thaw holding.

5. Limitations of the study

This study tried to present a valuable practical understanding for resource-limited settings, but there are several limitations.

First, the sample size was limited to 10 cryovial technical replicates derived from single donor's stem cells, due to serious resources constraints and limited sample accessibility at our center during our study period. We acknowledge that the absence of biological replicates limits the broad generalization of our statistical findings across a diverse patient population.

Second, as our study used pre-existing clinical inventory, all samples already contained the standard 10% DMSO concentration. Consequently, we could not examine a DMSO- free control group or lower DMSO concentrations to completely isolate chemical toxicity from freeze-thaw mechanical injury.

Finally, viability was examined only by trypan blue exclusion, which measures membrane integrity, but does not confirm functional biological capacity as $CD34^+$ engraftment.

Future studies utilizing functional assays and larger sample size with controlled groups are recommended.

6. Conclusions

The post-thaw cytotoxicity of 10% DMSO cryopreserved peripheral HSCs is greatly temperature dependent. Cells retain high membrane integrity when kept at (–80° C and –20° C), but when exposed to room temperatures (+22° C) and physiological temperature (+37° C), this will rapidly decrease cell viability. Although prolonged incubation time further decreases viability, the essential driver of DMSO cytotoxicity is elevated temperature.

These findings highlight the vital role of providing strict temperature control after thawing cryopreserved HSCs. Especially in resource limited settings

were equipment shortage, logistical delays or power instability may happen.

It is necessary to reduce the duration that the cells spend at elevated temperatures. To increase viability for cellular therapy, thawed stem cells suspended in DMSO must be reinfused as early as possible.

Acknowledgment

I hereby acknowledge and thank the University of Mosul/College of Science for their support.

Source of funding

Nil.

Conflict of interest

There are no conflicts of interest to declare.

References

Baldomero, H., *et al.*, (2011) The EBMT activity survey 2009: trends over the past 5 years. *Bone marrow transplantation,* 46(4), 485–501.

Bensinger, W. I., (2012) Allogeneic transplantation: peripheral blood vs. bone marrow. *Current opinion in oncology*, 24(2), 191–196.

Hristova, S. H. and Zhivkov, A. M. (2023) Cytotoxicity of Dimethyl Sulfoxide on Human Hematopoietic Stem Cells at Different Temperatures. *Journal of Applied Hematology*, 14(4), 263–267.

Agarwal, V. and Singh, M. (2023) Cryopreservation of cell lines, in Animal Cell Culture: Principles and Practice. *Springer*. 147–161.

Oh, K.-I. and Baiz, C. R. (2018) Crowding stabilizes DMSO–water hydrogen-bonding interactions. *The Journal of Physical Chemistry B,* 122(22), 5984–5990.

Mohanna, A., *et al.* (2023) Validation of long-term handling and storage conditions for hematopoietic stem cell products for autologous transplants. *Journal of Medicine and Life,* 16(4), 515.

Kang, E. M., *et al.* (2002) Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. Blood, *The Journal of the American Society of Hematology*, 99(3), 850–855.

Best, B. P. (2015) Cryoprotectant toxicity: facts, issues, and questions. *Rejuvenation research*, 18(5), 422–436.

Tonev, I., *et al.* (2020) Cytotoxic effect of dimethyl sulfoxide (DMSO) on hematopoietic stem cells: Influence of the temperature and the incubation time. *Bulgarian Chemical Communications*, 52, 40–43.

Hunt, C. J. (2019) Technical considerations in the freezing, low-temperature storage and thawing of stem cells for cellular therapies. *Transfusion Medicine and Hemotherapy*, 46(3), 134–150.

Strober, W. (2015) Trypan blue exclusion test of cell viability. Current protocols in immunology, 111(1), A3.B.1–A3.B.3.

Elmoazzen, H. Y. *et al.* (2007) Dimethyl sulfoxide toxicity kinetics in intact articular cartilage. *Cell and tissue banking*, 8, 125–133.