

Removal of potentially cytotoxic DMSO from cell therapy cryopreservation formulations

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Abbreviations: DMSO, dimethylsulfoxide; HSCs, hematopoietic stem cells

Opinion

Hematopoietic stem cells (HSCs) have been stored for >20years using current slow rate cooling freezing methods that utilize the cryoprotectant dimethylsulfoxide (DMSO) with long-term preservation below -135°C. In spite of the fact that there is excellent retention of HSC survival¹ during such frozen storage, current opinion in the field of cryopreservation is that DMSO should be removed before cells are infused in to patients. Removal of DMSO requires washing of the cells resulting in cell losses. For DMSO removal, besides the traditional and widely applied method of centrifugation, new approaches have been explored,² such as filtration by spinning membrane, stepwise dilution-centrifugation using rotating syringe, diffusion-based DMSO extraction in microfluidic channels, dialysis and dilution-filtration through hollow-fiber dialyzers and instruments such as CytoMate, Sepax S-100, Cobe 2991, microfluidic channels, and dilution-filtration systems, etc.). Development of optimal (fast, safe, simple, automated, controllable, effective, low cost) methods and devices for cryoprotectant removal with minimum cell loss and damage has not been successful. The net result is that many transplant groups still infuse their HSCs in DMSO directly into the patient. While DMSO is considered safe and non-toxic to stem cells,³ there have been many reports from transplant centers of serious side effects in patients as a result of DMSO toxicity. Zambelli⁴ reported nausea, vomiting and abdominal cramps occur in about half of all cell transplant cases. Serious side effects have also been reported in patients' cardiovascular, respiratory, and renal systems including some fatalities. For example, a search of the literature in the year 2000 reported neurological events associated with infusion of cryopreserved bone marrow and/or peripheral blood in three of 179 patients,⁵ fatal cardiac arrhythmia⁶ and a reversible encephalopathy.⁷ Clinical concerns about DMSO toxicity have continued (Caselli, 2009; Junior, 2008; Mueller, 2007; Otrick, 2008; Schlegel, 2009). The mechanism of DMSO cytotoxicity has not been determined, however, its ability to modify membrane fluidity, induce cell differentiation and cytoplasmic microtubule changes, and form metal complexes has been well documented. DMSO also decreases expression of collagen mRNAs in a dose-dependent manner.⁸ Comprehensive reviews of the published literature identified several hundred adverse reactions (e.g. nausea, chills, cardiac arrhythmias, neurological symptoms and respiratory arrest) associated with the transplantation of stem cells cryopreserved with dimethyl sulfoxide.^{2,8,9} It is for these reasons that DMSO should not be used.

This leaves us with the question why is it still being used if there are side effects and even fatalities attributed to DMSO toxicity? The answer is quite simple, without a cryoprotectant the cells can't survive the freezing process. If the cells don't survive cryopreservation,

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there is no therapy and the clinical community is locked into DMSO because that is what they have used for more than 20years. There are other cryoprotectants available which do not have the associated toxicity of DMSO that could be infused directly into patients without concern. Trehalose is a non-reducing disaccharide of glucose that possesses an exceptional ability to stabilize and preserve cells and cellular structures during freezing. The major obstacle for utilization of trehalose as a cryoprotectant is getting it across mammalian cell membranes. Mammalian cells do not metabolize trehalose so there are no active cellular mechanisms for transport across the cell membrane. The cell membrane barrier to trehalose can be overcome using an ATP4-activated receptor called P2X7 to open transmembrane pores to deliver trehalose into eukaryotic cells (US Patent)¹⁰ and HSC cytoplasm.¹¹

We have independently verified parts of Buchanan's work on HSC survival post-freezing¹² using the TF-1 human hematopoietic stem cell line.¹³ We also found that a rapid 100°C/min cooling rate in combination with trehalose resulted in the same TF-1 cell survival as DMSO at 1°C/min. Reports of effective trehalose cryopreservation strategies for various cell types are accumulating in the literature.

Trehalose preservation would include the following advantages over current DMSO freezing methods:

1. Trehalose preserved HSCs will be directly infused in patients without any intermediate washing steps following thawing;
2. No recipient reaction due to DMSO;
3. No HSC cytotoxicity due to DMSO if sample is exposed to physiological temperatures before further processing during cryopreservation or delivery to patients;
4. Long-term preservation capability at -80°C as well as below -135°C;
5. Reduced risk of microbial contamination at -80°C compared with use of liquid nitrogen;
6. Reduced risk of packaging material deterioration, -80°C compared with nitrogen exposure;
7. Lower probability of product loss due to temperature deviations during storage, transport or handling;

8. Reduced costs, no liquid nitrogen required for -80°C storage;
9. Reduced costs, dry ice instead of expensive dry shippers for distribution of product;
10. In conclusion, removal of DMSO from cryopreserved cell therapy procedures such as HSC preservation is an unmet need. Replacement with a non-toxic cryoprotectant that is not metabolized by humans such as trehalose would remove the need for complicated post-thaw handling procedures and minimize costs.

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Conflict of interest

The author declares no conflict of interest.

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