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Hematopoietic Stem Cell Transplantation with Cryopreserved Grafts: Adverse Reactions after Transplantation and Cryoprotectant Removal Prior to Infusion

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Abstract

Transplantation of hematopoietic stem cells (HSC) has been successfully developed as a part of treatment protocols for a large number of clinical indications, and cryopreservation of both autologous and allogeneic sources of HSC grafts is increasingly being employed to facilitate logistical challenges in coordinating the collection, processing, preparation, quality control testing and release of the final HSC product with delivery to the patient. Direct infusion of cryopreserved cell products into patients has been associated with the development of adverse reactions, ranging from relatively mild symptoms to much more serious, life-threatening complications, including allergic/gastrointestinal/cardiovascular/neurological complications, renal/hepatic dysfunctions, etc. In many cases the cryoprotective agent (CPA) used — which is typically dimethyl sulfoxide (DMSO), is believed to be the main causal agent of these adverse reactions and thus many studies recommend depletion of DMSO before cell infusion. In this paper, we will briefly review the history of HSC cryopreservation, the side effects reported after transplantation, along with advances in strategies for reducing the adverse reactions, including methods and devices for removal of DMSO. Strategies to minimize adverse effects include medication before and after transplantation, optimizing the infusion procedure, reducing the DMSO concentration or using alternative CPAs for cryopreservation, and removing DMSO prior to infusion. For DMSO removal, besides the traditional and widely applied method of centrifugation, new approaches have been explored in the last decade, 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 some instruments (CytoMate™, Sepax S-100, Cobe 2991, microfluidic channels, dilution-filtration system, etc.) as well. However, challenges still remain: development of the optimal (fast, safe, simple, automated, controllable, effective, and low-cost) methods and devices for CPA removal with minimum cell loss and damage remains an unfilled need.

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Keywords

Hematopoietic stem cells (HSCs); cellular therapy; dimethyl sulfoxide (DMSO); side effects; removal of DMSO

INTRODUCTION

Since the pioneering, Nobel-prize winning work by Thomas et al. on transplantation of bone marrow in the 1950s (1), hematopoietic stem cell (HSC) transplantation as a treatment option has been evaluated and successfully applied to a wide variety of malignancies and bone marrow failure syndromes, including Hodgkin's and non-Hodgkin's lymphoma (2–10), other lymphoid/myeloid (2–6,8,11–13) or leukemia malignancies (5–8,14–18), myelodysplastic syndromes (7,15), certain solid tumors (3,5,6,12,13), sarcomas (3,19), amyloidosis (2,8,20), and Fanconi anemia (18). Stem cell transplantation has been performed using HSC from allogeneic, autologous and syngeneic donors. In addition to bone marrow, HSC collected from mobilized peripheral blood or umbilical cord blood are currently in wide-spread clinical use, with the potential for transplantation of HSC derived from embryonic stem cell or induced pluripotent stem cell sources- in the not-too-distant future (21,22). Each of these HSC-containing populations can have certain advantages/disadvantages relative to the other sources, such as more rapid availability, easier collection, reduced risk to donors, reduced incidence of graft versus host disease (GVHD) and lower requirement of human leukocyte antigen (HLA) compatibility between donors and recipients (16,18).

Importantly, for most types of transplants, cryopreservation of HSC is a necessary and essential component of the clinical protocol. Long-term storage provides a solution to various logistical aspects such the obligatory time interval needed between collection of the patient's HSC product, treatment with high-dose therapy, and subsequent infusion of the product in the case of autologous transplantation, or in the case of cord blood transplantation the mismatch between supply (when the baby is born) and demand (when the patient is ready to receive the unit). Cryopreservation also supports better HSC product characterization and quality control, improved donor screening for HLA or other markers that can impact successful outcomes, and optimal transportation from the point of collection to the site of infusion. Since the first studies of HSC freezing by Barnes and Loutit in 1955 (23), many experiments have been performed to optimize cryopreservation protocols to enhance overall recovery and functional capacity of HSC after freezing-thawing and transfusion. Numerous excellent reviews of stem cell cryopreservation have been published, ranging from basic scientific principles to clinical cell processing protocols (24–28). The most widely applied cryopreservation protocols for HSC have the following general features: after collection, cells are washed and resuspended in a basal salt solution supplemented with some protein, which also contains one or more cryoprotective agents (CPA). Dimethyl sulfoxide (DMSO) is the most commonly used CPA, typically at a final concentration of 5–10% (v/v). The cell suspension is frozen using a rate controlled freezer or mechanical passive cooling methods with an optimal cooling rate of −1 to −2.5°C/min

(27,28) to a low temperature such as −80°C (25,27,29,30), then transferred to a liquid nitrogen tank for long-term storage at temperatures < −150°C.

Just prior to transplantation, most cryopreserved cell products are thawed quickly in a 37°C water bath and infused immediately into the patient. Infusion of thawed products has been associated with several types of adverse reactions (AR), ranging from mild events like nausea/vomiting, hypotension or hypertension, abdominal cramps, diarrhea, flushing and chills to more severe life-threatening events like cardiac arrhythmia, encephalopathy, acute renal failure and respiratory depression (4,5,8,20,31–49). In some cases, these adverse reactions have been directly attributed to DMSO (20,33,36,44), while others have suggested additional factors such as red cell lysate (46–48), or infusion of high numbers of damaged granulocytes that do not survive cryopreservation (4,8,37,45) are the main causal trigger of these adverse reactions. To minimize such adverse infusion reactions, many institutions have chosen to limit the total amount of DMSO that can be infused at any one time, while others have evaluated washing protocols to first remove the DMSO and other damaged cell products prior to infusion (2,5,7,16,32,39,50–60). This review article will focus on summarizing the reports of AR seen after stem cell transplantation with cryopreserved products, the role of DMSO in these adverse events, and new options for removal of DMSO before transfusion in an attempt to reduce these adverse reactions.

ADVERSE REACTIONS AFTER INFUSION OF CRYOPRESERVED HSC

Listed in Table 1 are a summary of the categories of adverse events reported after infusion of cryopreserved HSC, restricted mostly to publications from the last 10 years.

MANY FACTORS MAY CONTRIBUTE TO ADVERSE REACTIONS

The biological mechanisms that cause adverse reactions after cryopreserved HSC infusion are complex and not yet completely understood. Likely factors include:

1. DMSO itself, by virtue of direct physiological impact (13,15,62)
2. Post-thaw cell aggregation and dead cell debris (19)
3. Lysis of red blood cells, with release of hemoglobin, electrolytes and membrane fragments (13)
4. Total nucleated cell content and volume of cell suspension (4,5,8)
5. Low temperature of infused products (13)
6. Electrolyte imbalance (13,20)
7. Premedication given before transfusion, e.g., anti-emetics, corticosteroids, diuretics, and anti-histamines, which are used to neutralize DMSO-induced histamine release but may cause bradycardia at the same time (8,13)

In addition, patient-specific factors such as age, weight, gender, specific disease can also contribute to development of adverse infusion reactions (e.g., older and male patients have a lower incidence of adverse events compared to younger and female patients, and more adverse reactions occur in patients with Hodgkin’s lymphoma compared to non-Hodgkin’s

lymphoma or multiple myeloma (8)) or the type of prior treatments given and chemotherapeutic agents received (15), as can the infusion procedure itself (speed of injection, pausing for short periods, and the time gap between thawing of frozen cells and infusion can influence the risk for development of adverse reactions) (8,55).

PHYSIOLOGICAL ROLE OF DMSO IN ADVERSE REACTIONS

The first trial of DMSO usage for prevention of freezing damage to living cells was reported by Lovelock and Bishop in 1959 (78). Since then DMSO has become the most widely used cryoprotective agent for freezing of both cells and tissues. As part of its protective mechanism of action, DMSO can readily permeate across cell membranes to both inhibit intracellular ice formation, and to prevent cell injury triggered by severe dehydration as extracellular ice causes withdrawal of water from the intracellular milieu. The chemical structure of DMSO [$(CH3)_2SO$] results in an amphipathic molecule with one highly polar and two nonpolar domains, making it soluble in both aqueous and organic media, and thus useful for diverse laboratory and clinical purposes. DMSO is a very efficient solvent for water-insoluble compounds, a hydrogen-bond disrupter, a cell-differentiating agent, a hydroxyl radical scavenger, an intracellular low-density lipoprotein-derived cholesterol mobilizing agent, and so on. It first became commercially available as a solvent in the 1950s and following several clinical use studies in the 1960–70s, it was approved by the United States Food and Drug Administration for the treatment of interstitial cystitis in 1978. Subsequently DMSO has been evaluated for brain edema, amyloidosis, schizophrenia, urinary musculoskeletal and gastrointestinal disorders, pulmonary adenocarcinoma, rheumatologic and dermatologic diseases, chronic prostatitis, Alzheimer's disease, and as a topical analgesic (18,79–81).

Studies have shown that prolonged exposure to DMSO can directly impact cellular function and growth by affecting metabolism, enzymatic activity, cell cycle and apoptosis (82,83). DMSO is also thought to interfere with intracellular calcium concentration (80). DMSO can affect (induce or inhibit) cell apoptosis and differentiation (81–90). This effect depends on type of cell, the stage of cell development and differentiation, the specific DMSO concentration and the duration of exposure (83,89). Lin et al. found that DMSO at concentrations higher than 1–2% could induce apoptosis in lymphoma cells (90). Ji and Hegner found that DMSO can promote uncontrolled differentiation of stem cells (89,91). Zyuz'kov et al. reported that DMSO can inhibit proliferation, stimulate maturation or change biological properties of the transplanted bone marrow stem cells even when the DMSO concentration was low (0.02–0.25%)(88). Pal et al. studied exposure of embryoid bodies to DMSO and found effects on phenotypic characteristics, alternations in gene expression, differentiation patterns, and functionality of derived hepatic cells (83). All these findings imply that DMSO exposure could affect the function of HSC and influence short and long-term engraftment ability, but it seems likely the short-term exposure and cold temperatures minimize any detrimental impact. It is also important to acknowledge that a typical 10% DMSO concentration is very hyperosmotic (2500–3000 mOsm), and thus rapid infusion of cryopreserved cells (with 10% DMSO inside the cells) into a normal isosmotic blood system can cause extreme cell volume expansion and potential osmotic injury to cells,

leading directly to cell death (92,93). Thus, loss of cell viability can occur right after transfusion of HSC-DMSO suspension, again potentially affecting engraftment.

A significant uncomfortable response of injected DMSO is a garlic-like odor and taste, caused by its metabolite—dimethyl sulfide (DMS). About 45% of infused DMSO can be excreted through the urine, but a proportion of the injected DMSO is reduced to DMS in the body and subsequently secreted through the skin, breath, feces, and urine for up to 2 days after infusion, causing the “noxious” malodor. DMSO can also induce histamine release and can affect the central limbic-hypothalamic pathways, leading to nausea, vomiting, diarrhea, headache, flushing, fever, chills, dyspnea, anaphylaxis, vasodilatation and hypotension, pulmonary or abdominal complaints and complex reactions of cognition and emotion, etc. (3,4,10,13,18,31,42,44,62,77,94,95). As such, premedication with antihistamines is typically prescribed to minimize/neutralize DMSO-induced histamine release, especially in cases where it may cause some other more serious complications, such as bradycardia (13).

DMSO, in a dose-dependent manner, has been associated with neurotoxic adverse reactions (4,10,12,43,96–99). Hanslick et al. found that DMSO produced widespread apoptosis in the developing central nervous system (96). Cavaletti et al. reported that DMSO administration could induce a reduction in nerve conduction velocity and structural changes in the sciatic nerves of rats (98). Animal studies also showed that DMSO affected the sleep structure in rats by increasing light slow wave sleep and reducing deep slow wave sleep (80). Similarly, DMSO can cause renal, hepatic dysfunctions and cardiovascular complications after transplantation (20,63,79). Ruiz-Delgado et al. found that cryopreserving hematopoietic stem cells with 5% rather than 10% DMSO could result in less toxic reactions of cardiac dysfunction and acute renal failure (18). Donmez et al. found that DMSO content was significantly higher in patients with side effects than those without side effects, and higher in patients with cardiac side effects compared to non-cardiac side effects (8). Infusion of DMSO can cause acute vasospasm in swine, suggestive of angiotoxicity (100). Pal et al. suggested potential DMSO-induced hepatotoxicity by severely affecting the endodermal and hepatic lineage in a concentration-dependent manner (83).

Many studies have suggested the adverse effects related to DMSO are dose-dependent and can even be cumulative when multi-dose cell therapies are implemented (4,18,19,42,44,59,63,77,83,95,98,101). Studies of HSC transplants in children have shown that side effects in this pediatric population were more severe (77,102,103), perhaps because of their lighter bodyweight. On that basis, Junior et al. recommended the maximal dose of DMSO to be infused should be adjusted to bodyweight (1g DMSO/kg) (10). It should again be pointed out the adverse reactions described above are likely multifactorial in origin, often it is difficult to directly confirm whether the pathogenesis of the complications was due to only to infusion of the DMSO or whether other characteristics of the HSC graft and patient specific factors played a role as well. In that vein, there is still some debate in the field on the benefits of removing DMSO before transfusion. Cordoba et al. found that, despite DMSO depletion and adequate histamine blockage, side effects continued to appear, suggested other factors such as number of granulocytes in the thawed product were more important than DMSO content, and perhaps removal of DMSO was not needed (4). However, most investigators believe removing DMSO before infusion is beneficial (2,3,5,8–

11,13,15,18,20,36,52,56,59,62,64,69,73,74,104). In addition, most DMSO depletion strategies will also concomitantly remove cell debris and reduce neutrophil, platelet and other blood cell-derived soluble mediators, which may further contribute to decreasing the adverse event incidence and severity (2). Indeed, many studies have suggested that DMSO depletion can reduce adverse reactions, with minimum effects or even improvements on engraftment after HSC transplantation (2,5,7,13,55,105,106). Given the lack of consensus, no specific requirements regarding removal of DMSO from HSC grafts prior to infusion have been issued by the regulatory agencies or accreditation associations, instead leaving the decision to the discretion of physicians and clinical institutions to set their own policies and guidelines.

REDUCING THE INFUSIONAL SIDE EFFECTS OF CRYOPRESERVED HSC GRAFTS

Many approaches have been applied to reduce the adverse effects of cryopreserved hematopoietic stem cell transplantation, such as: (1) systematic premedication before infusion (62), (2) hydration and allopurinol administration after infusion (62); (3) slowing down the infusion speed and prolonging the infusion time (2,62), (4) dividing the infusion into multiple aliquots given several hours or days apart (10,62); (5) further concentrating HSC grafts to reduce the cryopreservation volumes and corresponding DMSO content (2); (6) reducing % DMSO concentration for cryopreservation to lower than 10%, or use alternative CPA to mix with or replace DMSO (2,108–110); and (7) removing DMSO before infusion (2,5,7,13,55,105,106). Since the side effects are idiosyncratic thus unpredictable so far to our knowledge, all these approaches are suggested to be combined to reduce the reaction incidence as low as possible. Several studies examining the use of DMSO with lower concentrations or alternative CPA are listed in Table 2. Simply reducing % DMSO concentration may decrease the toxicity and improve the kinetics of engraftment (108,109); however, it is also likely to reduce the recovery rate of the HSC after cryopreservation and thawing as well. Therefore, other cryoprotective agents, such as hydroxyethyl starch or trehalose, are recommended to be combined with any proposed reduction in % DMSO.

REMOVAL OF DMSO

A summary of methods and devices used for removal of DMSO from cryopreserved products is presented in Table 3. Conventional manual methods of removing DMSO from cell suspensions based on centrifugation have changed little since the 1970s. The most widely used procedure was proposed in 1995(57). This process can result in cell clumping and HSC loss, cell activation, and carries a risk of product contamination. This procedure is also time-consuming and labor intensive. Several devices, commercially developed for other purposes, have been evaluated for CPA removal, such as the CytoMate™, Sepax S-100 and Cobe-2991 instruments. Using user-definable programs DMSO can be efficiently reduced by these automated systems, resulting in reduced labor and risk of contamination due to the closed fluid path. However, these devices are expensive, and since they are all still based on centrifugation as their primary mode of operation can again cause cell clumping, osmotic injury, and loss of cells.

Several new methods/technology for DMSO removal without using centrifugation have recently been developed. Fleming et al. investigated an elegant and effective microfluidic method for small samples based on diffusion (116,117). It is expected that this method could be scaled up to prepare HSC units for transplantation. Ding et al. proposed an effective dialysis method for DMSO removal using hollow fiber modules with semi-permeable membranes (118,119). Zhou et al. have recently developed a novel dilution-filtration method and system (93), which can be used to precisely control the removal process to effectively reduce CPA concentration and prevent cell osmotic injury. Research data suggests this method promises to be a fast, safe, easy to operate, automated, and cost-effective approach with low cell loss and low contamination risk.

To go along with these approaches, DMSO-washing solutions are needed (some examples are listed in Table 4). Generally, washing solutions consist of saline or cell culture medium together with non-permeable macromolecules (dextran, albumin and/or ACD), which are non-toxic, infusible and provide a mild hyperosmotic environment to help extract the DMSO from cells. This is also why slow addition of such solutions (e.g. dripping) is preferable, it allows the cells to slowly equilibrate to the changing osmotic environment, and minimize the rapid uptake of water that can damage the cell membranes.

Briefly speaking, much progress on effective devices and methods for removal of DMSO from cryopreserved HSC grafts has been achieved in the last decade, but challenges still remain: further studies are highly needed to develop the optimal (fast, safe, simple, automated, controllable, effective, and low-cost) methods and devices for CPA removal with minimum cell loss and damage.

QUANTIFICATION OF RESIDUAL DMSO CONCENTRATION IN WASHED CELL SUSPENSION

To help advance this field the development of a reliable methodology to accurately quantitate the residual amount of DMSO left after such removal interventions is needed. As indicated above, addition of DMSO to a solution will result in increased osmotic pressure, thus osmolality measurements with osmometer can be used to estimate residual DMSO concentration in a washed cell suspension. However, this technique measures total osmolality, including effects of not only residual DMSO, but also other electrolytes, macromolecules, and cells themselves. Capillary zone electrophoresis (13,50,120) and chromatography, such as high-performance liquid chromatography (HPLC)(55,56) or gas chromatography (54), were proposed to directly measure residual DMSO concentration and sometimes applied in clinical practice. But these methods have significant disadvantages including using special expensive chemical agents and devices, complex procedures, and taking long time to complete. Recently, Chen et al. found that CPA concentration and electrical conductivity of cryopreservation solutions have a deterministic correlation, thus they proposed a novel method of electrical conductivity measurements to predict CPA concentration in cryopreservation medium (121). This method is very simple, minimum invasive, and cost-effective.

ALTERNATIVE CRYOPROTECTIVE AGENTS FOR HSC CRYOPRESERVATION

Although DMSO has been widely accepted and utilized for HSC cryopreservation and transplantation, in some situations it may be desirable to employ other alternative CPAs, combining with or even replacing DMSO. The criteria of selecting optimal CPA include: (1) providing protective function to cells during cryopreservation; (2) no need to be removed prior to infusion, which means the CPAs should be non-toxic and can be metabolized or digested by the body with minimum effects; and (3) cost and availability. Some agents, such as ethylene glycol, hydroxycellulose, disaccharides sucrose, maltose, trehalose and some macromolecules (dextran, hydroxyethyl starch, etc.) could be potentially used as alternative CPAs. In the last two decades, trehalose has drawn lots of interests in this field due to its unique properties. It has very high glass transition temperature, and is extremely effective in forming a fragile glass state to protect cells during freezing/thawing and drying, maintaining the thermodynamic stability of cell membranes, and inhibiting lipid-phase transition and separation during freezing and drying (122–124). However, for HSC, DMSO is still the most widely used CPA. In the future, searching for alternative CPAs could be another strategy to reduce the adverse reactions after HSC transplantation with DMSO.

CONCLUSIONS

Adverse reactions after infusion of cryopreserved-HSC transplantation grafts are generally believed to be directly or indirectly related to the concomitant infusion of the CPA, DMSO. Fortunately, by premedication, limiting exposure, and other techniques, most patients' adverse reactions are not severe. Several studies have investigated removal of DMSO from cryopreserved HSC suspension before infusion, these have suggested one can reduce but probably not completely eliminate these side effects. Currently-used DMSO removal techniques are mostly centrifugation-based; these can generate mechanical and osmotic stress to HSCs, causing osmotic injury, aggregation and cell loss. Along with concerns about potential contamination of grafts by additional post-thaw manipulations, this means that at present most cryopreserved HSCs are infused into patients without any attempt to remove DMSO. Some progress has been made in alternative DMSO removal methods and technology that do not rely on centrifugation; however the development of more optimal (fast, safe, simple, automated, controllable, effective, and low-cost) methods and devices for DMSO removal with minimum cell loss and damage remains an unfilled need. Any efforts and significant progress to meet this urgent and increased need will be greatly beneficial for HSC transplantation, in particular, and for the growing field of cellular therapy, in general.

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Table 1

Adverse Reactions (AR) after Cryopreserved HSC Transplantation

Adverse Reaction category	Symptoms	Reference	Adverse Reaction (AR) Incidence
Allergy	Flushing, rash, pruritus, erythema	(5,10,18,39,59,61–64)	• Cordoba(4): 67.36% developed AR. Specifically, – 43.75% allergic reactions – 25% gastrointestinal symptoms – 20.83% respiratory symptoms – 11.81% cardiovascular – 3.47% neurological symptoms • Donmez(8): 25.25% developed AR. Specifically, – 7.73% cardiac AR – 1.54% sinus bradycardia – 15.97% non-cardiac AR • Kersting(14): – Nephropathy incidence 0~29% • Konuma(15): – 58% systolic hypertension, 64% diastolic hypertension – 32% bradycardia • Alessandrino(31): – 8% non-cardiac complications – 57.33% cardiac AR, where 36% – hypertension • Zambelli(44): 50% developed AR, wherein – 22% hypotension – 4.54% hypertension
	Edema, anasarca	(8,18)	
	Bronchospasm	(61)	
Gastro-intestinal	Headache	(5,7–9,15,18,69)	
	Chest tightness, dyspnea	(5,9,15,62,63)	
	Abdominal cramping/pain, gastrointestinal distress, diarrhea	(5,8,14,16,18,59,61–63,69)	
	Nausea, emesis, vomiting	(5,8,12,15,16,18,39,43,63)	
Renal	Hemoglobinuria, proteinuria, mild azotemia	(59,69,70)	
	Hemolytic-uremic syndrome (HUS)	(70)	
	Urine incontinence	(11,71)	
	Thrombocytopenia, hemolytic anemia	(14,70)	
	Renal insufficiency, nephropathy, acute renal failure	(14,64,65,70,72,73)	
Cardio-vascular	Hypotension, hypertension	(5,8,12,14–16,18,31,40,61–63,67)	
	Arrhythmias/bradycardia, tachycardia	(6,8,12,15,17,18,20,43,61–63,67,69,73)	
	Hypothermia, hyperthermia	(5,8,16,17,70,73)	
	Rigor, tremor	(8,39,70)	
	Ischemia, hypoxia	(39,40,43)	
	Syncope, coma, somnolence, shock, loss of consciousness, trismus	(10,11,19,40,43,74–76)	
	Respiratory arrest, shortness of breath, cardiac arrest, coronary artery spasm	(10,12,16,18,19,33,40,43,59,61,64, 77)	
	Seizure	(5,10,12,14,19,73, 74)	
	Heart block	(18,63,67)	
Neurological	Mydriasis, miosis, Dysarthria, bilateral thalamic infarction, ophthalmic deviation, blurred vision	(9–12,19,71)	
	Dysgeusia	(59)	
	Reversible leukoencephalopathy (RPL), severe encephalopathy,	(39,40,71,73,74)	
	Central nervous system affected	(10,64)	
	Transient global amnesia	(9,19,36,43,71)	
	Cerebral infarction	(36,43)	
	Cognition problem	(9,39,43,71)	
	Numbness, muscle weakness	(8,9,40,43,71)	
	Mental acuity, anxiety	(39,71)	
Hepatic	Progressive jaundice	(64,70)	

Adverse Reaction category	Symptoms	Reference	Adverse Reaction (AR) Incidence
			– 4.5% bradycardia • Hazar(65): for children – 34–50% renal insufficiency in the early phases – 41%, 31% and 11% renal inefficiency at 1, 3 and 7 years after infusion, respectively • Graves(66): – 0.4% severe AR and 50% non- cardiac AR • Keung(67): – 65% sinus bradycardia – 29.41% heart block – 41% hypertension – 82% arrhythmias • Lopez-Jimenez(68): – 41% non-cardiac AR – no bradycardia and arrhythmias

Table 2

Trials using lower DMSO concentrations in cryopreservation

DMSO concentration	References	Cryopreservation medium and comments
10%	(2,5,13,16,18, 19,24,36,39,40,43,50,55–57,62,64,68,69,73,74)	• Generally, 10% DMSO + plasma (concentration: 2–22.5%) in saline, culture media or electrolyte injection solutions (e.g., Normosol-R).
9%	(12)	
7.5%	(8,64,104)	• Donmez (8): 3% HES was added.
5%	(3,7,64,67,108,110–113)	• 5% DMSO instead of 10% DMSO could – decrease DMSO toxicity (109) – reduce the release of intracellular components from dead cells (109) – improve the kinetics of engraftment (108) • Hayakawa (110): 5% DMSO+6% pentastarch+25% human albumin, heparin and DNAse, which was equivalent to/better than the 10% DMSO medium. • Keung and Rowley (67,108): 6% HES + 5% DMSO
<5%	(64,109,114)	• Cell recovery rate may be reduced with reduced DMSO concentration • Other CPAs (e.g., HES, trehalose) are recommended to be combined.

* HES: hydroxyethyl starch

Table 3

Methods and Devices for DMSO Removal

Methods or devices	Mechanism	Comments, pros and cons	References
Centrifugation	Centrifugation	• Most widely applied procedure for CPA removal so far. • Pros: conventional devices available widely • Cons: high time and labor consumption, cell loss, high risk of contamination, etc.	(2,7,13,16,39,50,56–58,74)
CytoMate™	Filtration by spinning membrane	• Pros: automated, effective and allowing a step-by-step user definable programming, low risk of contamination • Cons: high cost and cell loss due to clumping	(13,54,107)
Sepax S-100	Consisting steps of dilution and centrifugation using a rotating syringe	• Pros: fast, automated processing, low risk of contamination • Cons: high cost and cell loss due to clumping	(56,58)
Cobe 2991	Centrifugation	• Pros: fast, automated processing, low risk of contamination • Cons: high cost and cell loss due to clumping	(5,50,115)
Microfluidic method	Diffusion-based extraction in microfluidic channels	• Pros: automated processing, elegant, effective for CPA removal for samples with small volumes • Cons: hard to be scaled up for samples with large volume	(116,117)
Dialysis through hollow-fiber dialyzer	Dialysis across semi-permeable hollow fiber membranes	• Pros: automated processing, effective CPA removal, low risk of contamination • Cons: optimization needed for samples with small volume	(118,119)
Dilution-filtration through hollow-fiber dialyzer	Controlled dilution and controlled filtration through semi-permeable hollow fiber membranes	• Pros: fast, automated processing, low risk of contamination, low-cost, controllable, effective CPA removal • Cons: optimization needed for samples with small volume	(93)

Table 4

DMSO-washing solutions

DMSO-washing solution	References
10% dextran-40 and 5% HSA* in saline	(39)(51)
PBS supplemented with 5% dextran-40, 5% ACD**-A and 1% HSA.	(13)
10% ACD in saline	(2)(50)
7.5% dextran-40 and 5% human albumin in saline	(56)
One-third ACD-A anticoagulant+two thirds albumin 4%	(74)
Saline solution with 10% acid citrate dextrose anticoagulant	(5)
2.5% w/v HSA and 5% w/v dextran-40 in isotonic saline	(57)
15% ACD-A in RPMI-1640 medium	(7)
5% dextran, 2.5% human albumin, 10% acid citrate dextrose (ACD-A)	(58)

* HSA: human serum albumin,

** ACD: acid citrate dextrose.