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Dimethyl Sulfoxide in Cryopreservation: Mechanism, Application and its Role as the Gold Standard in Cryopreservation

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ABSTRACT

Review

Cryopreservation and cryomedia technologies play a central role in enabling the development, manufacture, and clinical deployment of advanced biotherapeutics by supporting the long-term storage, transport, and controlled use of biological materials. Since its introduction as a cryoprotectant in 1959, dimethyl sulfoxide (DMSO) has become the established gold standard cryoprotective agent across a wide range of biological cell types due to its unique physicochemical properties and mechanism of action. As a membrane-permeating cryoprotectant, DMSO protects cells during freeze–thaw cycles through coordinated intracellular and extracellular effects that regulate osmotic balance, stabilize cellular membranes, and inhibit damaging ice crystal formation. DMSO has demonstrated broad applicability across many mammalian cell systems, including hematopoietic, mesenchymal, and pluripotent stem cells, as well as immune cells such as T cells, NK cells, and CAR-T cells. In most applications, DMSO is used at concentrations between 5–10%, although lower concentrations may be effective for certain cell types when combined with complementary cryoprotectants. Over more than six decades of use, DMSO has established a long record of application in pharmaceutical and biotechnological settings, including cell banking and, more recently, the manufacture and delivery of cell and gene therapy products. This review provides an overview of the historical development of cryopreservation, the safety profile and mechanisms of action of DMSO, and key applications in which DMSO-containing cryomedia remain widely adopted for cell preservation.

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INTRODUCTION

History of cryopreservation in biotechnology and biopharmaceutical manufacturing and the role of DMSO in this evolution

Cryopreservation has been foundational to modern biotechnology and biopharmaceutical manufacturing, enabling the long-term storage, transport, and controlled use of biological materials. The field originated in the mid-twentieth century, when early experimental observations demonstrated that cells and tissues could survive exposure to subzero temperatures under carefully controlled conditions. The 1949 publication of the successful revival of spermatozoa following freezing in the presence of cryoprotective agents, established the principle that chemical additives could mitigate freezing-induced cellular injury and permit recovery of biological function after thawing [1]. This discovery laid the groundwork for the systematic development of cryobiology as a scientific discipline.

During the 1950s and 1960s, the mechanistic understanding of freezing injury expanded, with increasing recognition of intracellular ice formation and osmotic stress as key drivers of cell damage during cooling and thawing [2]. In this period, glycerol emerged as an early cryoprotectant, particularly in reproductive biology and hematologic applications; however, glycerol's limited permeability and cell-type specificity constrained its broader applicability across mammalian cell systems [3]. A major inflection point came with the identification of DMSO as a highly effective cryoprotective agent. Lovelock and Bishop showed in 1959 that DMSO could protect mammalian cells from freezing injury, marking one of the earliest demonstrations of a small, rapidly permeating cryoprotectant capable of intracellular protection [4].

DMSO's chemical properties, low molecular weight, amphipathic properties, and strong hydrogen-bonding interactions with water, enable rapid penetration across cell membranes and effective disruption of ice crystal formation. These attributes distinguished DMSO from earlier cryoprotectants and supported its rapid adoption across diverse mammalian cell types. By the 1960s and 1970s, DMSO-based cryopreservation protocols had become central to laboratory research, enabling routine banking of cell lines and primary cells, and facilitating reproducibility across experimental systems [5].

As biotechnology matured into an industrial enterprise in the late twentieth century, cryopreservation transitioned from a laboratory technique to a core manufacturing enabler. The rise of recombinant protein production and monoclonal antibody manufacturing required robust strategies for preserving production cell substrates, such as Chinese hamster ovary (CHO) and human embryonic kidney (HEK) cell lines. Regulatory frameworks and international guidance documents formalized the concept of Master Cell Banks (MCBs) and Working Cell Banks (WCBs), emphasizing the need for long-term genetic and phenotypic stability of cell substrates used in biologics manufacturing [6,7]. DMSO-containing cryomedia became a standard for mammalian cell banking due to their reproducibility, scalability, and well-characterized performance across freeze–thaw cycles.

In the twenty-first century, the advancement of cell and gene therapies (CGT) and regenerative medicine reframed cryopreservation as a critical unit operation directly linked to product quality and clinical performance. Cryopreserved cells increasingly represented the final drug product, rather than an intermediate or banking material. Consequently, contemporary cryopreservation strategies increasingly emphasize optimization of DMSO concentration and its use in combination with complementary cryoprotective agents to mitigate adverse effects, while maintaining robust post-thaw recovery and functional performance. This evolution has been accompanied by growing attention to formulation design, freezing and thawing protocols, and post-thaw handling as integral elements of process control. The reliance on DMSO-containing cryomedia is exemplified by the first wave of commercially approved chimeric antigen receptor T-cell (CAR-T) therapies. In 2017, Novartis' Kymriah® (tisagenlecleucel) and Kite Pharma's Yescarta® (axicabtagene ciloleucel) received regulatory approval in the United States for the treatment of hematologic malignancies. These products are cryopreserved using DMSO-containing formulations and administered by intravenous infusion, reflecting the successful translation of DMSO-based cryopreservation from development through commercial-scale manufacturing and clinical use. Commercial CAR-T therapies demonstrate that DMSO remains a clinically validated cryoprotectant for approved autologous cell therapies.

Today, DMSO remains a widely used and deeply validated cryoprotectant, particularly for cell banking and certain clinical

workflows, while simultaneously motivating innovation toward optimized and application-specific cryomedia. This evolution reflects the maturation of cryopreservation from a research practice to a controlled, regulated, and application-driven technology integral to modern biopharmaceutical manufacturing.

Importance of cryomedia in cell therapy, biologics production, and regenerative medicine

Cryomedia plays a central role in enabling the development, manufacture, and clinical deployment of advanced biotherapeutics. Rather than serving solely as a storage formulation, cryomedia directly influences post-thaw cell recovery, functional performance, stability during handling and transport, and consistency across manufacturing lots. As such, cryomedia selection and control are increasingly recognized as critical elements of product and process design in regulated biotechnology and biopharmaceutical applications.

In cell therapy, cryopreservation enables decoupling of manufacturing from clinical administration, supporting centralized production models, lot release testing prior to patient dosing, and distribution to geographically dispersed treatment sites. Cryomedia composition directly affects the feasibility of thaw-and-administer workflows, allowable post-thaw hold times, and the reproducibility of delivered viable cell dose across clinical settings. As cell and gene therapies progress from early-stage development to commercial-scale manufacturing, cryomedia has emerged as a key determinant of supply chain robustness and operational flexibility [8,9].

In biologics production, cryomedia underpins the establishment and maintenance of cell banking systems that ensure manufacturing continuity and product consistency throughout the lifecycle of a therapeutic product. Master and Working Cell Banks provide the foundation for controlled manufacturing starts, genetic stability, and reproducibility. Regulatory expectations for biologics emphasize traceability, characterization, and long-term stability of cell substrates, all of which depend on validated cryopreservation processes and well-controlled cryomedia formulations [6,7,10].

Regenerative medicine and tissue engineering further expand the importance of cryomedia by introducing the need to preserve complex cellular phenotypes and, in some cases, multicellular or three-dimensional constructs. In these applications, cryopreservation must maintain not only cell survival but also differentiation status and functional attributes that are essential for therapeutic efficacy. Reviews in this field consistently identify cryomedia as a key factor influencing translational success, particularly as therapies move from experimental platforms toward clinical and commercial implementation [11].

From a quality and regulatory perspective, cryomedia is embedded within Chemistry, Manufacturing, and Controls (CMC) expectations for investigational and licensed products. Compendial guidance recognizes cryopreservation as a controlled process that requires defined materials, standardized procedures, and appropriate validation to ensure product quality and consistency [12]. As a result, cryomedia selection is increasingly viewed as a strategic decision that impacts product performance, manufacturing feasibility, and regulatory compliance.

Together, these considerations position cryomedia as a critical enabling technology across biotechnology, biopharmaceutical manufacturing, and regenerative medicine. Today, sterile DMSO remains the cryoprotectant of choice with distinct advantages over other cryoprotectants including 'DMSO free' surfactant-based products.

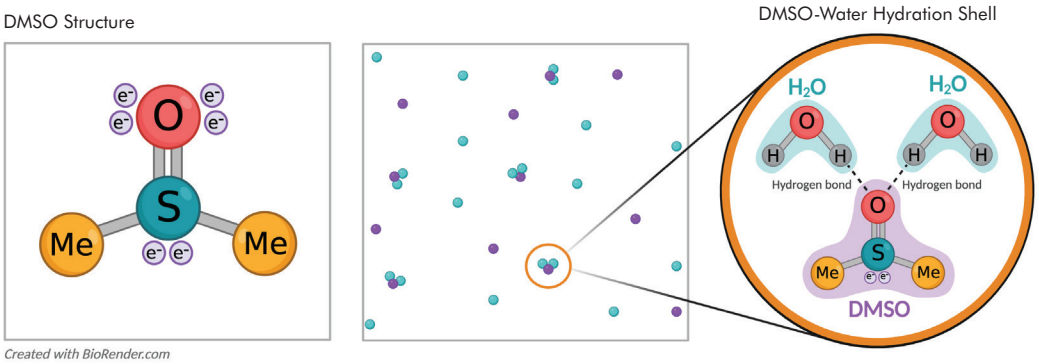
DMSO was first identified in the 19th century as a byproduct generated during the production of paper from wood pulp, however, a variety of more simple, less environmentally toxic methods have been developed for its synthesis [13]. These methods are also more cost-effective and increase product output, making DMSO highly accessible for a variety of scientific and industrial purposes [14]. For example, dimethyl sulfide oxidation followed by potassium hydroxide purification and distillation can produce high-purity DMSO that meets pharmacopeial standards [15]. This review will discuss DMSO as a cryoprotective agent, including its mechanisms of action and key applications. Comparisons of different cryoprotection agents will not be covered in this review paper but will be the focus for a future publication.

Mechanism of Action of DMSO in Cryopreservation

Molecular structure of DMSO

DMSO is an organic molecule with one hydrophilic sulfoxide group and two hydrophobic methyl groups, as illustrated in Figure 1. This mixed polarity promotes favorable interactions with water molecules in the freezing medium and lipid components of cell membranes, making it a highly effective cryoprotective agent (CPA). CPAs can be classified as permeating or non-permeating agents. Non-permeating CPAs cannot cross cell membranes. This restricts their cryoprotective mechanisms to the extracellular spaces [16]. In contrast, permeating CPAs such as DMSO, ethylene glycol (EG) and glycerol are small molecules capable of crossing cellular membranes, with cryoprotective effects that extend to both

Figure 1. DMSO-Water Interactions
DMSO is a polar aprotic molecule that forms hydrogen bonds with water molecules in solution.



intracellular and extracellular environments [17]. DMSO's small size and amphipathic nature facilitate rapid membrane permeation, enabling cryoprotective effects through multiple coordinated mechanisms. These unique properties contribute to the wide spread use of DMSO as an effective cryoprotective agent for various cell types [18,19]. Other factors include its cost-effectiveness, availability and minimal toxicity [20].

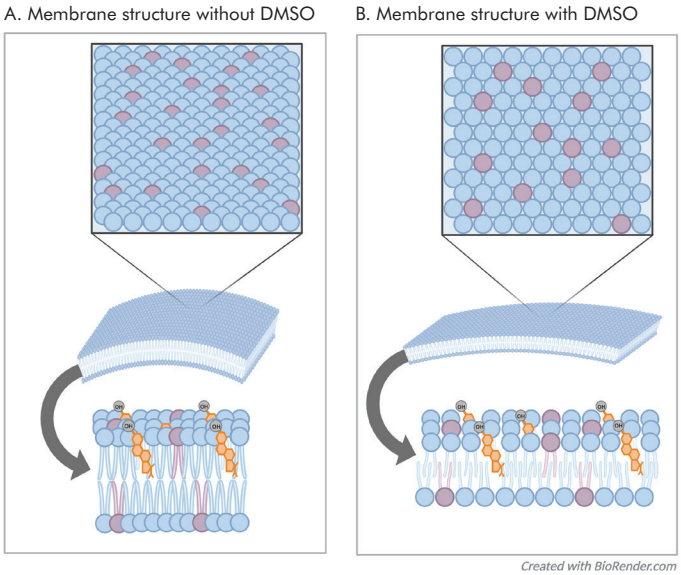
Safety of DMSO

Despite its effectiveness as a CPA, DMSO is an organic solvent with known cytotoxic effects. Direct DMSO-mediated cytotoxicity decreases at lower temperatures, and many of its associated toxic effects are therefore reduced during cryopreservation because exposure is brief and occurs mostly under sub-zero conditions [21]. However, DMSO cytotoxicity is not solely temperature dependent, but is influenced by a variety of factors including cell type, concentration, and variations in cryopreservation protocols such as freezing and thawing rates and temperatures, duration of exposure, and post-thaw dilution procedures [22,23,24]. Given its widespread use in biological research, careful assessment of DMSO-associated toxicity across all stages of cryopreservation along with the development of mitigation strategies is essential.

DMSO interacts with cell membranes to reduce lipid cohesion causing membrane thinning and increased surface area, as illustrated in Figure 2. Ultimately, these changes lead to enhanced membrane fluidity and permeability [25,26]. While these effects can be highly advantageous during cryopreservation, this also promotes interactions between DMSO and intracellular components, contributing to its associated toxicity at higher concentrations and prolonged exposure times. Intracellular interactions can lead to cellular damage including impaired intracellular signaling and enzyme activity, generation of reactive oxygen species (ROS), as well as the induction of apoptosis and abnormal differentiation of stem cells under cell culture conditions [22,23,27, 28].

Cellular exposure of EL-4 murine T lymphoma cells to DMSO under cell culture conditions has been associated with pro and anti-apoptotic effects, with pro-apoptotic effects mediated via mitochondrial apoptosis pathways as evidenced by translocation of cytochrome c and subsequent caspase activation [29]. While changes in mitochondrial pathways are linked to ROS production, as a radical scavenger, DMSO can also interact with free radicals and other ROS to reduce their ability to damage cells [28]. The high reactivity of DMSO molecules can also mediate hydrophobic interactions with intracellular proteins and the disruption of redox processes involved in ATP production leading to the destabilization of enzymes and

Figure 2. Alterations to Membrane Structure
A. Phospholipids are amphipathic, forming cohesive, thick bilayers through tight lipid-lipid packing which allows selective membrane permeability; B. DMSO interacts with phospholipids to reduce membrane cohesion, leading to membrane thinning and increased membrane surface area. These changes result in higher membrane fluidity and permeability.



altered cellular metabolism, respectively [23,28]. Importantly, the exposure conditions of these studies do not represent DMSO exposures during cryopreservation, which instead utilize short exposure times at reduced temperatures alongside post-thaw dilutions and rapid DMSO removal.

DMSO-free alternatives are being explored to reduce DMSO-related toxicity during cryopreservation. However, eliminating DMSO from cryomedia (CM) also removes the key cryoprotective advantages that underlie its gold standard status in cryopreservation. Wide variability in DMSO-associated cytotoxicity has been reported, likely reflecting cell-type-specific differences, including variation in membrane composition [26]. For example, human umbilical vein endothelial cells (HUVECs) are significantly more sensitive to concentrations of DMSO as low as 5%, while mesenchymal stem cells (MSCs) were not significantly affected. Mizuno et al. [27] further showed that, relative to MSCs, HUVECs exhibit higher baseline membrane fluidity and lower antioxidant capacity, which may underlie their greater sensitivity. In HUVECs, reducing membrane fluidity with a stearoyl-coA desaturase (SCD1) inhibitor and supplementing DMSO-containing CM with glutathione (GSH) significantly improved freeze–thaw survival by increasing DMSO-tolerance. Inhibition of the SCD1 enzyme reduced the desaturation of fatty acids in the cell membrane to minimize its fluidity, while the antioxidant, GSH, likely played a key role in reducing ROS-related damage [27]. These findings suggest that cell-specific modulation of membrane fluidity and antioxidant capacity may help reduce DMSO-associated toxicity during the cryopreservation of diverse cell types.

Other strategies to reduce DMSO-related toxicity include lowering the concentration used during cryopreservation and/or post-thaw removal of DMSO. DMSO is considered relatively safe at low, controlled concentrations, therefore use at its lowest effective concentration is typically advised. In many cell types, high concentrations of DMSO (>10%) may not be required to elicit effective cryoprotection, especially if other CPAs are used simultaneously within the CM. For example, DMSO concentrations as low as 3.5% provide sufficient cryoprotection of hematopoietic stem cells (HSCs) [18,30]. Peripheral blood progenitor cells (PBSCs) were also effectively preserved at 3.5% DMSO, when combined with 2.5% hydroxyethyl starch (HES), and T-cell preservation with 2% DMSO alongside pentaisomaltose, reported superior protection compared to 10% DMSO alone [31,32].

Use of combination CM can improve cell viability and functioning, with mixtures often containing both permeating and non-permeating CPAs. In these circumstances, the enhanced protection may be a direct result of lowering DMSO concentration, which reduces potential for toxicity. However, use of combination CM may also provide additional protection throughout the cryopreservation process, depending on the alternative CPAs used and the diversity in their mechanisms of action. For example, DMSO can be highly effective when paired with non-permeating cryoprotectants such as HES, pentastarch (PS), or dextran [18,33]. Non-penetrating CPAs such as HES, provide protection by osmotically dehydrating the cell to further minimize intracellular ice formation [34]. Moreover, a synergistic effect has been reported when DMSO is combined with polyampholytes, enabling high post thaw recovery of up to 90% for stem cells, red blood cells, and human lung cells [19]. Polyampholytes are large polymers with positive and negative side chains that provide ice recrystallization inhibition (IRI) effects and interact with cellular membranes to increase their flexibility. Polyampholytes are not replacements for DMSO as their cryoprotective effects are severely limited without it, likely due to their large size and resulting lack of membrane permeability, which restricts their protective abilities to the extracellular environment [19]. Xue et al. [35] found that a combination CM containing 10% DMSO, 1% methylcellulose, 10% ethylene glycol, and 10 μM Y27632 provided superior cryoprotective effects in cortical organoids derived from human embryonic stem cells (hESCs) compared to other tested CPA formulations. This medium, termed MEDY, was associated with the upregulation of genes involved in neural development, neuron projection, and synaptic function, and the downregulation of genes involved in endoplasmic reticulum-mediated apoptotic pathways [35]. Additionally, methylcellulose further supported cell survival by forming a protective film and a hot gel during thawing. Although DMSO (10%) alone was associated with the upregulation of genes involved in promoting regulated cell death after thawing, it remained an essential component of the optimal cryopreservation formulation, suggesting that it may be most beneficial when used alongside complementary CPAs that provide additional protective mechanisms [35].

Cryopreservation using vitrification requires high CPA concentrations, which may appear concerning, however, this is paired with rapid freezing rates and small overall volumes. These conditions induce a glassy or vitreous state, which avoids the formation of damaging ice crystals and minimizes exposure to DMSO [17,36,37]. Conventional vitrification involves the rapid freezing of samples by placing them directly in liquid nitrogen which risks sample contamination.

Alternatively, surface-based vitrification techniques are indirect, via contact of the cryogen with a cooled surface, improving biosafety and handling. Rapid cooling rates limit cellular exposure to high concentrations of DMSO at temperatures above 0°C and shorten the time available for ice nucleation to ensure that the only ice crystals that form, are small and "non-harmful." [17].

CPA toxicity is concentration-dependent, and can be further mitigated during vitrification procedures by using combination CM. Because CPAs have differences in toxic effects and mechanisms, increasing the number and variety of CPAs within a solution reduces the total toxicity [17]. For example, vitrification is the current method of choice for human clinical oocyte cryopreservation. Furthermore, studies have shown successful cryopreserving of mouse oocytes using a combination CM consisting of 15% DMSO, 15% EG, 0.5 M sucrose and 12% serum synthetic substitute [38]. Although DMSO is often regarded as harmful, it may also provide distinct benefits when incorporated into CM. For example, enhanced oocyte survival may be partly attributable to DMSO-induced differential gene expression. A study by Wiltshire et al. [39] found that vitrification with DMSO led to an upregulation in genes associated with improved oocyte developmental competence and downregulation in genes known to impair it. Following thawing, vitrification protocols generally recommend prompt removal of the cryomedia.

Post-thaw DMSO removal strategies are routinely implemented following both vitrification and slow-cooling cryopreservation protocols to minimize continued cellular exposure and mitigate DMSO-associated toxicity. This is especially important when cryopreserved cells are intended for clinical application. Direct infusion of cells preserved using DMSO-containing solutions, such as HSCs, has been associated with adverse reactions ranging from mild symptoms such as nausea and vomiting to life threatening events including cardiac arrest and epileptic seizures [18,21]. Although DMSO has been associated with infusion-related adverse reactions, these reactions may not be attributable to DMSO alone. Shu et al. [21] provide a comprehensive review of these contributing factors. Thus, minimizing DMSO concentration and ensuring rapid post-thaw removal are commonly recommended to improve safety, especially when concentrations above 10% are used to preserve cells intended for patient administration. While removal of DMSO prior to infusion has been shown to reduce patient adverse effects, this process can be time consuming, expensive, and may lead to contamination, cell clumping, and cell loss, depending on the cell type and method used [21, 40]. Post-thaw removal techniques include centrifugation, filtration, diffusion-based techniques, and dialysis, among others. Removal of DMSO using gradual stepwise dilution is often implemented. This process can be performed manually or mechanically using a device, such as a diffusion-based microfluidic device. Use of automated devices can allow up to 85% removal of DMSO with high cell recovery rates, depending on the cell type [41]. However, limitations to these techniques include the small sample volume and the high cell density required, as well as availability of cell-processing equipment.

Following DMSO removal, cell recovery appears to be cell-type specific, with no adverse effects observed in stem cells, compared to reports of poor recovery in peripheral blood mononuclear cells [30, 40]. Overall, although DMSO induces conditional toxicity in various cell types, when used under optimized protocols and appropriate formulations, the cryoprotective advantages of DMSO outweigh its potential cytotoxicity, supporting its continued widespread use in both research and clinical applications.

Mechanism of Action

Osmotic buffering and reduced membrane stress

As temperatures decrease, water in the aqueous cryomedia will form pure, organized, crystal lattice structures that exclude solutes in the medium [42]. As more ice crystallizes, solutes in the residual aqueous medium become increasingly concentrated. This highly concentrated extracellular environment creates an osmotic gradient that drives water out of the cell. When appropriately regulated, this process is cryoprotective because it reduces the volume of water inside the cell during freezing, thereby minimizing intracellular ice formation. However, excessive, uncontrolled cellular dehydration is highly damaging and irreversible [19]. This gradient will strengthen during freezing, because as temperatures decrease, ice crystals continue to form, ultimately leading to increased osmotic pressure across the membrane and a higher risk of membrane rupture and cellular collapse [24, 43]. These mechanisms of osmotic injury are further exacerbated by high freezing rates and low cell membrane permeability [24].

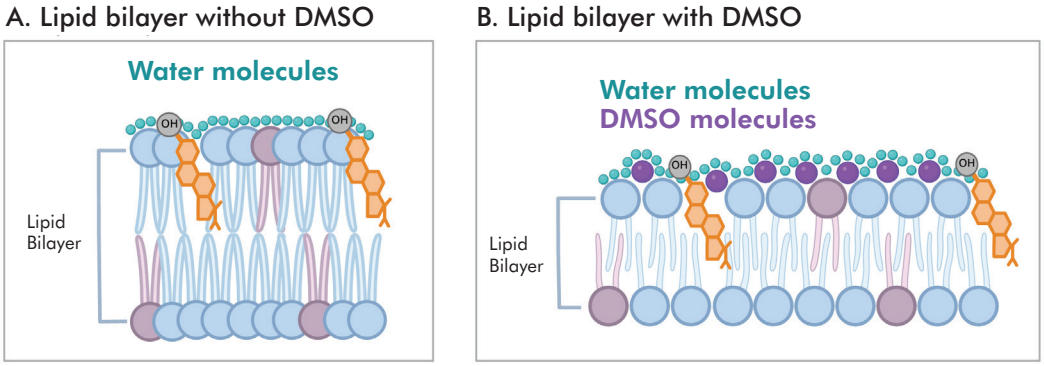
As a permeable CPA, DMSO passively diffuses across the plasma membrane and into the cell down its concentration gradient [44]. Movement of DMSO through the lipid bilayer reduces osmolarity differences across the membrane, preventing the formation of extreme osmotic gradients that uncontrollably drive water from the cell [36]. DMSO also directly influences the structure and function of the lipid

bilayer in a concentration-dependent manner [25,26, 45, 46]. These interactions are advantageous as they reduce membrane rigidity and structural stress.

Notably, these effects rely on bilayer structure and composition, which varies between cells. A study by Kundu et al. [26] found that variance in lipid headgroups led to cell-specific differences in DMSO-induced membrane fluidity. Specifically, the fluidity of 1,2-dipalmitoyl-rac-glycero-3-phosphatidylcholine (DPPC) bilayers was shown to significantly increase following exposure to DMSO. This is relevant because DPPC bilayers are made of phosphatidylcholine (PC), the major phospholipid present in most animal cell membranes. Furthermore, this study acknowledged the simplicity of DPPC models, noting that true animal cell membranes are far more heterogeneous [26]. This greater heterogeneity likely increases cell membrane resistance to the effects of DMSO compared to more homogenous membrane models.

Cells cryopreserved in DMSO-containing solutions, exhibit increased permeability and fluidity resulting from DMSO interactions with the phospholipid heads of lipid membranes and the other water molecules in solution (see Figure 1). Water molecules typically hydrate lipid membranes, forming hydrogen bonds with hydrophilic lipid head groups, as illus-trated in Figure 3A. These interactions are associated with the stability and selective permeability of cellular membranes. When added to cryomedia, DMSO interacts strongly with water molecules, pulling them away from the surface of the membrane. This semi-dehydrates the membrane and facili-tates DMSO-lipid interactions, as illustrated in Figure 3B. These interactions cause lipid disorganization and reduced packing arrangement, increasing the area per lipid and reduc-ing the space between lipid bilayers [45]. Concentrations of approximately 10% DMSO have shown to induce membrane thinning and improve the fluidity of cell membranes, with increasing concentrations resulting in the formation of water pores and ultimately, membrane disintegration [45,46]. Gurtovenko et al [45] used atomic-scale Molecular Dynamics (MD) simulations to visualize these distinct stages in DPPC bilayers. At approximately 10%, DMSO induces water pore formation in the lipid bilayer. This can facilitate the movement of water between the intracellular and extracellular spaces to further minimize membrane stress induced by high extracel-lular osmolarity that occurs during freezing [18,26]. Overall, DMSO regulates cellular dehydration by promoting con-trolled removal of intracellular water during freezing through osmotic buffering and altered membrane fluidity and permeability, thereby minimizing osmotic stress on the membrane and preserving its structural integrity. While controlled cellular dehydration indirectly lowers the risk of intracellular ice formation, DMSO also has direct effects on ice nucleation, growth, and recrystallization through its interactions with water molecules, see Figure 4.

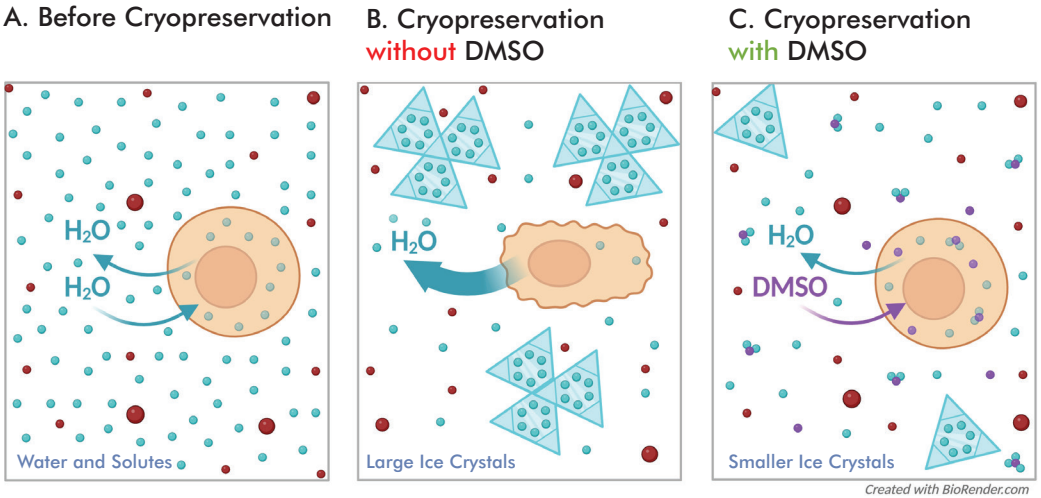
Figure 3. DMSO-Lipid Bilayer Interactions
A. Water molecules hydrate the membrane, forming hydrogen bonds with hydrophilic lipid head groups. These interactions are associated with the tightly packed membrane structure; B. DMSO semi-dehydrates the membrane, replacing some of the water molecules. DMSO is polar, allowing it to interact closely with lipid head groups. These DMSO-lipid interactions cause increased area per lipid and decreased membrane thickness.



Disruption of ice crystal growth and recrystallization

Ice crystallization poses a significant risk t o cell viability during initial freezing and post-preservation thawing. The formation of large intracellular and extracellular ice crystals can puncture cell membranes, causing fatal mechanical injury [22]. Additionally, recrystallization; the fusion of small ice crystals into larger, more damaging structures, can occur during the cell thawing process [22,24]. The molecular structure of DMSO allows it to form strong interactions with water molecules, which can explain its ability to reduce initial ice crystal formation and post-preservation recrystallization [47]. DMSO molecules contain a sulfinyl group (S=O), capable of forming two hydrogen bonds with surrounding water molecules to create a hydration shell, as illustrated in Figure 1 [48].

Figure 4. Osmotic Injury During Cellular Freezing
A. The cell is in dynamic equilibrium; B. During freezing, extracellular ice crystal formation creates an osmotic gradient that drives rapid cellular dehydration and membrane destabilization; C. DMSO penetrates the cell membrane, displaces water molecules and interacts with water molecules in solution to reduce frequency and size of extracellular ice formation, leading to reduced osmotic pressure and controlled cellular dehydration.



As DMSO molecules become hydrated, the viscosity of the solution increases, which impacts the kinetics of nucleation [23]. Nucleation is required for the formation of ice crystals; however, water molecules must be able to move freely to either arrange themselves into a critical nucleation radius or to form extensive hydrogen-bond networks around a catalyst [23]. With increasing viscosity and less free water molecules available, DMSO can effectively delay the onset of nucleation, resulting in slower ice crystal formation and growth [18,23,48]. Water that strongly associates with solutes is considered "bound," as these interactions render them osmotically inactive and less available for hydrogen-bond network formation [23]. Therefore, ice crystals that do form remain smaller and less mechanically damaging [49]. Although the warming rate is especially important in preventing recrystallization during the thawing process, DMSO exhibits effective IRI activity in a concentration-dependent manner, with concentrations as low as 2.5% demonstrating measurable inhibitory effects [24,36].

An additional source of mechanical injury during cryopreservation is eutectic crystallization of sodium chloride (NaCl) within the media. As temperatures decrease and water crystallizes, solutes that are excluded from these structures become suspended in small, highly concentrated volumes of aqueous medium [42]. At low enough temperatures, these highly concentrated solutions containing NaCl either crystallize or vitrify [42]. Eutectic crystallization of NaCl forms dihydrate structures with water that have shown to associate closely with the plasma membrane, causing direct cell injury [50]. The mechanism behind DMSO-mediated inhibition of eutectic crystallization is uncertain. It has been hypothesized that DMSO acts as a dilutant, that either reduces overall solute concentration in the unfrozen medium or alters solution thermodynamics in a way that favors dissolved NaCl over the dihydrate crystalline salt formation [50].

At high concentrations and rapid cooling rates, DMSO can promote vitrification, resulting in the formation of an entirely amorphous, ice-free state [19]. Although vitrification requires high CPA concentrations, recent studies suggest that at concentrations higher than 2%, DMSO may still promote the formation of an amorphous freeze-concentrated phase [50-52]. The presence of this phase could provide a protective physical barrier by existing between cell membranes and extracellular ice crystals [51,52]. Evidence suggests that for slow cooling protocols using DMSO, this amorphous phase may still provide concentration-dependent levels of protection [51]. Overall, while higher DMSO concentrations can provide stronger protection against ice crystal formation, lower concentrations still offer measurable cryoprotective effects while reducing potential for toxicity.

Key Applications Where DMSO-Containing Media Is Most Suitable

Hematopoietic stem cell transplantation (HSCT)

For successful autologous and allogeneic hematopoietic stem cell transplantation (HSCT), hematopoietic stem cells (HSCs) often require cryopreservation because they are commonly harvested pre-emptively or transported between clinical sites [21,25]. DMSO is widely validated for HSC cryopreservation at a concentration of 10% [18]. DMSO influences cell membrane structure and fluidity, therefore it is proposed that DMSO-interactions with membranes of hematopoietic stem and progenitor cells (HSPCs) lead to inhibited chemokine

receptor (CXCR4) internalization, facilitating increased receptor activation, enhanced cell motility and improved engraftment [25]. Furthermore, prior work shows that a 10-minute pre-incubation with 10% DMSO increases the chemotactic, homing activity of HSPCs, and overall survival of HSPC transplants [25].

Importantly, direct infusion of DMSO at high concentrations in clinical settings can induce mild to severe adverse patient side effects at a dose-related dependency and for treatments requiring multiple doses, these effects can be cumulative [18,21,53]. Compared to 10%, preservation of HSCs in 5% DMSO significantly reduces infusion-related toxicity while maintaining hematopoietic recovery and clonogenic potential [53,54]. To compensate for the reduced cryoprotective efficacy observed at DMSO concentrations below 10%, adding supplementary agents to 5% DMSO CM can improve post-thaw viability, extend storage stability, and simplify processing. For example, after 17.8 years of cryopreservation, HSC grafts stored in 5% DMSO supplemented with 5% HSA retained cell viability and engraftment potential equivalent to those preserved in 10% DMSO, supporting the feasibility of reduced-DMSO formulations for long-term storage [55]. Other components such as pentastarch, hydroxyethyl starch (HES), dextran and/or trehalose, have also proven to be effective in allowing successful reduction of DMSO concentrations to as low as 3.5% [18,30,31,33]. Various studies using supplemented 3.5-5% DMSO have shown success using both controlled and uncontrolled freezing methods as well as both long-term storage in -80°C mechanical freezers and liquid nitrogen [21,31,53]. The use of DMSO for the cryopreservation of HSCs is highly valuable as it has been shown to provide protection against cell loss and maintenance of cell function leading to high engraftment success rates while allowing for flexibility in procedural options. Overall, growing evidence suggests that supplemented 5% DMSO-containing cryopreservation media may be preferable to 10% DMSO in HSCT, with potential relevance for other clinical stem cell therapies.

Mesenchymal stem/stromal cells (MSCs) banking and therapies

Mesenchymal stem cells (MSCs) are a promising cell therapy for a variety of diseases due to their multilineage differentiation capacity and their immunosuppressive and anti-inflammatory properties [56]. Furthermore, MSCs can be easily expanded *in vitro*, making them useful for cell-banking strategies to ensure clinicians and researchers have immediate access to functional, high-quality cells [57]. Cell storage and banking require effective preservation of MSCs with maintained cell morphology, function, and overall viability. However, MSCs have proven difficult to cryopreserve due to their freeze-thaw sensitivity [58]. Effective cryopreservation of MSCs is typically achieved with 5–10% DMSO, although the optimal concentration may vary depending on intended use and MSC tissue origin [59]. Adipose-derived mesenchymal stem cells (ASCs) are an ideal source of MSCs due to their accessibility, rapid proliferation, and high cellular activity [60]. Thirumala et al. [61] found that ASCs preserved in various DMSO concentrations ranging between 2-10% were all successful with no significant differences in cell viability and apoptosis. For ASCs, concentrations of DMSO greater than 2% are required to maintain cellular activity, with lower concentrations leading to significant post-thaw cell loss. Although 10% DMSO preserves post-thaw differentiation capacity, it may temporarily reduce MSC immunosuppressive activity, which returns to baseline after three days of cell culture [58,62]. Human bone mesenchymal stem cells (hBMSCs) cryopreserved in 10% DMSO-containing media demonstrate increased production of ROS, which may play a key role in the increased post-thaw cell apoptosis, DNA damage and cell cycle arrest seen in cryopreserved hBMSCs compared to their fresh counterparts [56]. Notably, the addition of glucose or high-polymer anhydrous dextrose to DMSO can significantly improve post-thaw outcomes, with studies showing improved MSC viability and proliferation [58].

When using MSCs for cell therapy, the use of non-human additives and the reduction or removal of DMSO prior to clinical use is strongly advised to reduce the potential for *in vivo* sample contamination and patient adverse effects [56,59]. 5% DMSO remains a suitable option for MSCs cryopreserved for clinical use, with synovial MSCs cryopreserved in 95% FBS with 5% DMSO showing sustained cell colony formation and chondrogenic abilities comparable to fresh MSCs [63]. Overall, DMSO is highly effective in MSC banking and therapeutic formulations due to its ability to preserve cell viability and function during cryopreservation. Optimal DMSO concentrations for MSC cryomedia generally range from 5–10%, with 5% DMSO often preferred, although concentrations may vary depending on downstream applications and the presence of other cryoprotectants.

Immune Cell-Based Therapies

Immune cell-based therapies offer a promising approach for treating a range of diseases because immune cells can target

and destroy virally infected, foreign, and/or cancerous cells [64]. The most common starting material for immunotherapies is human peripheral blood mononuclear cells (PBMCs), however, immune cells can also be sourced from induced pluripotent stem cells (iPSCs) and cord blood. PMBCs are the most direct source, consisting of a mixed population of mature immune cells, including monocytes and lymphocytes [65]. Although 10% DMSO is currently the most established approach for the cryopreservation of various cells, this concentration can be toxic to certain subpopulations of PBMCs, which can be masked by viability assessments that determine overall recovery of the heterogenous cell population [18,65]. For effective cryopreservation of subpopulations, isolation can be performed prior to cryopreservation of PMBCs, thus allowing for subset-specific optimization protocols.

T cell subsets respond differently to DMSO cryopreservation, a phenomenon likely influenced by cell-to-cell differences including metabolic profile and membrane permeability [32,66]. While 10% DMSO is the standard cryopreservation method for T effector cells and gamma delta T cells, reducing the concentration to 5% has shown positive effects on regulatory T-cell recovery rate and cell viability [64, 66]. Furthermore, Haastrup et al. [32] found that adding 10% pentaisomaltose to cryomedia enabled DMSO concentrations to be reduced to 1-2% without compromising T-cell viability, proliferation, or migration compared with the clinically accepted 10% DMSO formulation.

5-10% DMSO is also the preferred freezing medium for CAR-T cells [66,67]. CAR-T cells are a subset of T cells that are genetically engineered to target cancer cells expressing a specific antigen [68]. These cells must endure two freeze-thaw procedures. First, the cellular starting material is subject to the first freeze-thaw cycle, usually at 10% DMSO. However, Jandova et al. [20] reported that the inclusion of a secondary cryoprotectant such as HES (6%), dextran and/or human serum albumin (HSA) (4%), permits effective reduction of DMSO in the apheresis to 5-7.5% without risking cell viability and recovery rate of PMBCs [20]. Next, these cells undergo selection and transduction to create the modified CAR-T cell product which is then subjected to its second freeze-thaw cycle for preservation until therapeutic use [32]. Final CAR-T products may be cryopreserved in formulations containing 5% to 10% DMSO typically using controlled-rate freezing at approximately 1°C/min before long term storage in liquid nitrogen [20,64]. Notably, approved CAR-T therapies commonly employ 5% DMSO-based cryoprotectant formulations supplemented with varying concentrations of HSA [64]. Some studies have found that freeze-thawed CAR-T cells are comparable to fresh cells, while others have noted significant alterations in cell function including reduced release of cytokines, mitochondrial dysfunction, apoptosis signaling, gene over-expression and cell cycle damage [64]. One study reporting similar *in vitro* functional impairment post-thaw found, however, that *in vivo* clinical outcomes observed in patients were comparable between fresh and cryopreserved CAR-T products, indicating that these impairments may not be clinically detrimental [69].

Natural killer (NK) cells are particularly sensitive to cryopreservation. Some studies investigating the effect of cryopreservation using DMSO report reduced NK cell cytotoxic abilities, migratory capacity and viability, while others find functionality comparable to fresh counterparts [70]. Cryopreservation of NK cells derived from PBSCs using 4-5% DMSO demonstrated the best post-thaw recovery, while 10% DMSO led to reduced cytotoxic and secretory post thaw functional abilities [64,67]. A recent study by Berjis et al. (2024) observed that 5% and 10% DMSO led to significant NK cell death 12 hours post thaw. Upon investigation, they found that post thaw NK cell death was not a direct result of cryoprotectant mediated lysis or physical injury, but rather internally programmed GZMB-mediated apoptosis. Pre-treatment with a combination of interleukins; IL-15 and IL-18, was found to significantly improve viability to ~90-100% and functionality of thawed NK cells [71]. Despite potential biological trade-offs, cryopreservation is widely used across NK cell therapy platforms and has effectively become the enabling strategy for scalable, "off-the-shelf" approaches. Leading programs such as those from Fate Therapeutics and the MD Anderson Cancer Center rely on cryopreserved NK products derived from iPSCs or cord blood to support batch manufacturing, inventory, and multi-patient dosing, while Nkarta Therapeutics has specifically optimized its processes to preserve post-thaw potency of peripheral blood-derived CAR-NK cells. Similarly, cell line-based platforms developed by ImmunityBio inherently use frozen cell banks as part of a biologics-like production model. Overall, DMSO remains the leading cryoprotective agent for NK cells, supporting satisfactory post-thaw viability and offering practical advantages for manufacturing flexibility, global distribution, and repeat dosing.

Mammalian cell line preservation in biomanufacturing

Cryopreservation allows large-scale cell preservation within the biopharmaceutical industry. However, methods cannot be generalized across all mammalian cell lines, therefore cell-type-specific cryopreservation approaches are necessary [72]. Mammalian cell banks are routinely curated by cryopreserving cells in 5-10% DMSO [72,73]. Biopharmaceutical production commonly employs mammalian cell lines, Chinese hamster ovary (CHO) and human embryonic kidney (HEK), to produce therapeutic proteins and viral vectors [74,75]. Research demonstrates freeze-thaw resilience among recombinant CHO cell lines following cryopreservation, as evidenced by comparable cell performance, product quality, and flow cytometric profiles between cryopreserved and non-cryopreserved cells. Specifically, recombinant CHO cell lines cryopreserved using 7.5% DMSO followed by long-term storage in liquid nitrogen exhibited sustained cell-specific productivity post thaw [73]. Research and early clinical development using HEK cells also rely on cryopreservation techniques. HEK cells can be efficiently cryopreserved at a controlled freezing rate in 5% DMSO followed by long-term storage in liquid nitrogen [76]. Research using frozen high-density HEK-293 cells to produce adeno-associated virus (AAV) vectors has demonstrated that this cryopreservation process effectively maintains cell productivity, with experimental concentration of post-thaw cells comparable to conventionally prepared fresh HEK cells [76]. Cryopreservation using DMSO allows large-scale cell line manufacturing of reliable, high performing host cells, which promotes the standardized, timely production of AAV vectors for gene therapies. Overall, DMSO remains a key player in the cryopreservation of mammalian cell lines, specifically CHO and HEK, which are essential for the development of biotherapeutics.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)

The self-renewal capacity and pluripotency of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) make them valuable for a wide range of biomedical applications, including regenerative medicine, cell therapy, and drug screening [77]. As pluripotent cells, they can give rise to multiple types of differentiated mesoderm, ectoderm and endoderm cells, however, freeze-thaw cycles during cryopreservation can cause damage that may compromise their differentiation potential, among other cellular functions [37]. Conventional cryopreservation methods for ESCs and iPSCs typically rely on 5% to 10% DMSO, a controlled rate freezing (1°C/min) and rapid post-thaw DMSO removal techniques. In addition, reducing the DMSO concentration needed for effective cryopreservation may improve the safety of stem cell therapies. For example, a recent study by Kim et al. [74] found that the addition of a recombinant elastin-like peptide (REP) with an RGD motif (RGD-REP) to 10% DMSO improved ESC viability and pluripotency following cryopreservation. Specifically, RGD-REP enhanced cell survival by activating the FAK-AKT signaling cascade following integrin binding, thereby inhibiting FoxO3a activity and reducing apoptosis-related signaling. iPSCs are particularly advantageous because they are highly accessible and their use is not associated with the ethical concerns often linked to ESCs [78]. Mommaerts et al. [79] demonstrated that DMSO concentrations of approximately 5% remain effective for the cryopreservation of iPSCs when supplemented with IRIs. The addition of 15 mM of IRIs to 5% DMSO cryomedia, led to improved post-thaw recovery and viability, sustained pluripotency, and minimal transcriptomic change [79]. Strategies that preserve the cryoprotective efficacy of DMSO while reducing its required concentration may improve the safety and translational applicability of these therapies.

CONCLUSIONS

DMSO has played a foundational role in the development and evolution of cryopreservation technologies across biotechnology and biopharmaceutical manufacturing. Since its introduction as a permeating cryoprotectant in the late 1950s, DMSO has enabled reliable preservation of a wide range of mammalian cell types by mitigating freezing-induced injury through coordinated intracellular and extracellular mechanisms. Its small molecular size, amphipathic structure, and strong interactions with water molecules underpin its ability to regulate osmotic flux, reduce membrane stress, and disrupt ice nucleation, growth, and recrystallization. These properties have supported decades of reproducible use in research, cell banking, and large-scale biomanufacturing, establishing DMSO as the benchmark against which alternative cryoprotective strategies continue to be evaluated.

Importantly, DMSO's long history of clinical and commercial application has generated a substantial body of practical experience supporting its safe and effective use when applied within optimized concentration ranges and controlled freeze–thaw conditions, to well-tolerating patient populations. The successful translation of DMSO-containing cryomedia into approved cell therapies, including commercial CAR-T products, underscores its robustness

as a cryoprotectant in regulated, patient-facing applications. While ongoing innovation continues to refine cryopreservation approaches through composition optimization and combination with complementary cryoprotective agents, DMSO remains the currently validated gold standard for cryopreservation across cell therapy, regenerative medicine, and biopharmaceutical manufacturing. A subsequent Part 2 of this review series will address the technical, quality, regulatory, and supply-chain considerations associated with the use of DMSO in bioprocessing and clinical manufacturing applications, providing a practical framework and best practices for its implementation across development and commercial lifecycles.

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