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A comparative review of MSC thaw techniques, source-specific performance, and clinical implications

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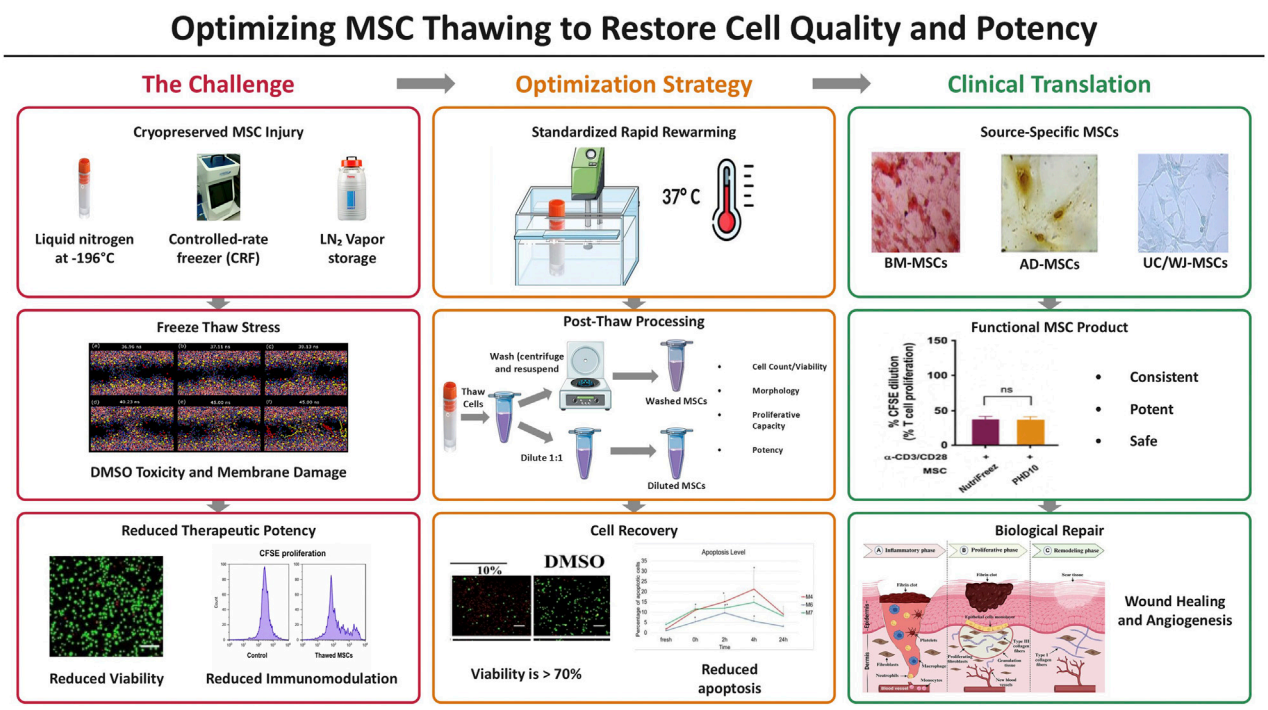
Introduction: Mesenchymal stem cells (MSCs) are the most widely investigated cellular therapeutic in regenerative medicine. There is an increased interest in cryopreserved over-the-counter products in clinical practice. The thawing step, although often treated as trivial, has emerged as a critical determinant of post-thaw viability, immunomodulatory potency, and ultimately therapeutic efficacy.
Methodology: This review compares major clinical thawing techniques. These include 37 °C water bath, automated dry-warming devices, slow rewarming, body-temperature thawing, and vitrification rewarming and supplements. This review compares detailed numerical data from the primary studies using viability percentages, fold-changes, and clinical efficacy estimates. A substantially expanded source-comparison section synthesizes head-to-head data on bone marrow adipose-derived umbilical cord/Wharton's jelly (UC/WJ), placental, and other mesenchymal stem cell sources.
Results: According to the previously conducted studies, UCMSCs demonstrated promising post-thaw viability (~81% with standard 10% dimethyl sulfoxide) and favorable proliferative recovery in several studies. However, direct and standardized comparisons across MSC sources remain limited. ADMSC survival during freeze–thaw (notable loss of α4-integrin and CFU-F counts) is comparatively less than other sources. However, they exhibit the strongest immunosuppressive secretome. BMMSCs have been used extensively in clinical research and produce the most consistent angiogenic effect. However, they are cleared from the lungs faster than UCMSCs or ADMSCs.
Conclusion: We provide a practical, evidence-based protocol and identify potential interventions for clinical sites adopting cryopreserved MSC programs.

KEYWORDS
cell viability, cryopreservation, DMSO, mesenchymal stem cells, thawing

1 Introduction

Mesenchymal stem cells/mesenchymal stromal cells are plastic-adherent, fibroblast-like cells (Renesme et al., 2025). They are widely investigated for cellular therapy in regenerative medicine, with multiple registered clinical trials spanning graft-versus-host disease, osteoarthritis, ischemic cardiomyopathy, chronic wounds, and orthopedic injury. Almost all clinical MSC programs deliver a cryopreserved product (Wragg et al., 2020). Cryopreservation enables release testing, scalable manufacturing, and just-in-time



GRAPHICAL ABSTRACT

delivery. However, it also imposes a thaw stress that is now widely recognized to attenuate the therapeutic phenotype of the infused cells (Cottle et al., 2022; Safwan et al., 2025; Gao et al., 2021). The thaw step is on a short, unforgiving clock. After thawing, the cytotoxic effects of dimethyl sulfoxide (DMSO) become progressively more pronounced (de Ménorval et al., 2012). As the cell suspension warms above freezing, minimizing the duration of post-thaw exposure to DMSO before dilution or removal is recommended (Niebergall-Roth and Kluth, 2025). When warming is too slow, intracellular ice recrystallization damages cells mechanically (Traversari et al., 2022). Once the cells reach the patient, even small reductions in immunomodulatory secretome or homing molecule expression translate into measurable losses of efficacy. Whaley et al. (2021) conducted a similar review on cryopreservation. However, the focus of their study revolved around the different parameters and principles of cryopreservation and not the different thawing techniques and their impact. Wang et al. (2026) also discussed how cryopreservation impacts biologics at a cellular level with an emphasis on the GMP aspect of their production. This review synthesizes the current evidence on clinical MSC thawing in two complementary ways. First, the review compares the principal thawing techniques and supporting post-thaw handling steps, with specific numerical data extracted from the primary literature. Secondly, the source comparison was expanded. Using previously published studies, different tissue sources, bone marrow (BM), adipose tissue (AD) or umbilical cord/Wharton's jelly (UC/WJ) were considered to see the differences noticed with their use on post-thaw viability and potency. The clinical question is no longer whether to cryopreserve, but rather how to recover the most functional cells at the bedside.

2 Cryobiological background: why the thaw matters

Cryopreservation of MSCs is usually performed by slow controlled-rate freezing (~−1 °C/min) in the presence of dimethyl sulfoxide (DMSO; typically, 5%–10% v/v), often supplemented with human serum albumin (HSA) or human platelet lysate (Martín-López et al., 2023). This protocol minimizes the competing injuries that include solution effects from progressive concentration of extracellular solutes during slow freezing, and intracellular ice formation during fast freezing (Cottle et al., 2022; Wragg et al., 2020). While the cooling phase has historically received the bulk of research attention, the warming phase is getting similar recognition. During warming, any small intracellular ice crystals that formed during cooling tend to coalesce into larger, more damaging crystals through recrystallization (devitrification). Recrystallization proceeds most aggressively between roughly −80 °C and the melting point (Fahy and Wowk, 2021; Gangwar et al., 2022). Slow rewarming gives ice time to grow. Rapid rewarming through this hazardous zone prevents crystal expansion. This is the central biophysical reason every modern MSC thawing protocol emphasizes rapid warming. Osmotic stress is the second principal injury pathway. During warming, water influx can outpace DMSO efflux, resulting in transient osmotic swelling. Prolonged warming exposes biologics to greater osmotic imbalance. Abrupt post-thaw dilutions may exceed the cell's osmotic volume tolerance and trigger lysis. Stepwise dilution post-thaw is therefore an essential component of an optimized thawing protocol (Safwan et al., 2025; Hunt, 2019). DMSO toxicity is the third constraint. DMSO exhibits relatively low cytotoxicity at cryogenic temperatures but becomes increasingly cytotoxic as temperature rises. Prolonged exposure of thawed cells to

DMSO at room temperature progressively impairs cell viability and function. Many clinical and laboratory protocols recommend limiting the interval between thawing and DMSO dilution or removal to approximately 30–60 min, although acceptable handling times vary depending on the cell product and protocol (Safwan et al., 2025; Regan et al., 2010; Rowley et al., 1999).

3 Mechanisms of post-thaw injury in MSCs

Although post-thaw viability commonly exceeds 80%, freshly thawed MSCs frequently exhibit functional deficits compared with continuously cultured MSCs despite appearing viable by standard assays (Antebi et al., 2019; Moll et al., 2016; Navakauskienė et al., 2023). Freshly thawed bone marrow MSCs exhibit transient reductions in CD44 and CD105 surface expression that resolve over 24 h of culture (Antebi et al., 2019). Freshly thawed adipose-derived MSCs show a sharp drop in α4-integrin (CD49d), an adhesion molecule critical for endothelial homing (Irioda et al., 2016). Thawed MSCs show elevated active caspase-3, annexin-V positivity, and intracellular reactive oxygen species. In thawed MSCs, pro-apoptotic BAX and p53 are upregulated, and the anti-apoptotic p21 is downregulated relative to fresh cells (Demirci et al., 2014; Ikeda et al., 2018). Many of these stress-associated markers partially or completely normalize following several hours to 1 day of recovery culture (Antebi et al., 2019). A previously conducted study found plating efficiency and colony-forming unit–fibroblast (CFU-F) counts of immediately thawed MSCs to be reduced compared with fresh cells from the same lot. This likely reflects both reduced integrin-mediated adhesion and freezing-induced cytoskeletal disruption (Antebi et al., 2019; Irioda et al., 2016). Among the functional alterations reported after thawing, impaired immunomodulatory activity has received clinical attention. Freshly thawed MSCs are markedly less effective at suppressing T-cell proliferation, display reduced responsiveness to IFN-γ stimulation, resulting in diminished induction of IDO and PGE2. Antebi and colleagues showed that 24 h of post-thaw culture fully restored T-cell suppression and IFN-γ-induced potency, while immediately thawed cells were the weakest immunomodulators in their assay panel (Antebi et al., 2019). Cryopreserved MSCs are more susceptible to the instant blood-mediated inflammatory reaction (IBMIR), characterized by complement activation, coagulation, and platelet activation immediately after intravascular infusion. They express elevated tissue factor and bind complement more avidly than fresh MSCs, predisposing them to clearance and even microvascular thrombotic events when infused intravenously (Moll et al., 2016). These findings suggest that post-thaw cellular alterations influence not only MSC survival but also the safety and efficacy of intravenous administration. Niekamp et al. (2026) validated a potency assay matrix confirming that bone marrow-derived MSCs consistently suppress T-cell proliferation, promote regulatory T-cell differentiation, and induce anti-inflammatory M2 macrophage polarization, with immunomodulatory function further enhanced by interferon-γ priming prior to cryopreservation.

Post-thaw injury in MSCs results from a combination of physical and biochemical stresses occurring during freezing and rewarming. During freezing, extracellular ice formation increases solute concentration in the remaining unfrozen fraction, leading to cellular dehydration and osmotic stress, whereas intracellular ice formation can cause direct structural damage to cellular membranes and organelles. Rewarming can introduce additional stress through changes in temperature, osmotic conditions, and ice recrystallization. These cryopreservation-associated stresses can cause mitochondrial dysfunction, reactive oxygen species (ROS) generation, DNA damage, and activation of apoptotic pathways, resulting in delayed cell death and impaired cellular function after thawing (Cottle et al., 2022). In MSCs specifically, cryopreservation has been shown to induce delayed apoptosis associated with DNA double-strand breaks arising in labile replicating DNA during the freeze-thaw process, particularly in cells frozen during active S-phase replication (Johnstone et al., 2023). Consequently, post-thaw injury manifests not only as an immediate reduction in viability but also as impairments in metabolic activity and adhesion potential that persist beyond the first 24 h post-thaw (Bahsoun et al., 2020).

4 Pre-thaw considerations

4.1 Cell cycle

Johnstone et al. (2023) found that mechanistically, cell-cycle status during cryopreservation is a significant factor in post-thaw performance. Delayed apoptosis was observed to be significantly higher, and T-cell suppression activity was lower in BMMSCs after thawing when they were frozen during S-phase. By contrast, BMMSCs synchronized in G0/G1 prior to freezing maintained the capacity to survive, form colonies, and exhibit immunomodulatory activity at a similar level to the pre-freeze controls.

4.2 Reconstitution media

Human serum albumin (HSA) and human platelet lysate (HPL) serve distinct, non-interchangeable roles in MSC processing. During thawing, HSA functions as a protective protein supplement. Aabling et al. (2023) demonstrated that isotonic saline supplemented with 2% HSA preserved 90%–95% cell survival, whereas protein-free formulations caused instantaneous losses of nearly half the cell population, with HSA-containing saline maintaining >90% survival for at least 4 h post-thaw, but cells suspended in PBS or normal culture media showed >40% loss within 1 h. Additionally, low-density preparations ($<1 \times 10^3$ cells/mL) showed quick losses of 40%–50% ($p < 0.001$). In contrast, HPL's established role is as a growth-factor-rich supplement for MSC expansion culture, supporting adhesion, survival, and proliferation of actively growing cells (Guiotto et al., 2020). Notably, substituting HSA with HPL specifically within cryopreservation/freezing media has been shown not to enhance post-thaw viability, recovery, or metabolic activity, nor to rescue thaw-induced susceptibility to T-cell–mediated lysis, indicating that HPL's benefits are specific to the expansion phase and do not extend to improving the immediate post-thaw recovery process itself (Chinnadurai et al., 2016).

Petrenko et al. (2019) who demonstrated that a trehalose-based hypothermic storage solution supported significantly higher MSC recovery and attachment capacity than Plasma-Lyte 148 or Ringer's solution alone during extended cold storage. Similarly Mirabel *et al* (Mirabel et al., 2018) demonstrated that formulating MSCs in Plasma-Lyte 148 supplemented with 2% recombinant albumin (AlbIX), both during cryopreservation and in the final post-thaw suspension, extended clinically acceptable stability from 24 h to 72 h, with mechanistic analysis showing that the albumin additive delayed progression of cells into late-stage apoptosis.

4.3 Administration timing

Another significant element influencing product quality is the time between thawing and administration (Tan et al., 2024). In the study by Nguyen et al. (2024) cryopreserved ADSCs and UCMSCs were transferred from liquid nitrogen to an −86 °C freezer overnight and subsequently thawed at room temperature. Complete thawing occurred within 5–10 min, after which the cell suspension was mixed with After freeze solution at a 1:1 ratio. For the infusion experiment, the thawed cell suspension was administered into Lactated Ringer's solution, and cell viability was assessed at defined time points. Cell Carrier was subsequently evaluated as an alternative infusion solution and was described as phosphate-buffered saline (PBS) supplemented with 5% human serum albumin. Lactated Ringer's solution is an electrolyte-containing crystalloid solution, whereas Cell Carrier is a PBS-based solution supplemented with human serum albumin. Nguyen et al. (2024) reported a progressive decline in MSC viability during infusion in Lactated Ringer's solution, whereas viability remained higher with Cell Carrier. According to Nguyen et al. (2024), ADSC and UCMSC viability decreased at an average rate of approximately 6.86 and 5.45 percentage points per hour ($p < 0.05$) post-thaw after transfusion. However, when the cells were supplemented with a solution of HSA and PBS in a separate investigation, they observed improved cell viability (~0.446 and 0.603 percentage points per hour for ADSC and UCMSC), respectively. These results emphasize the need to minimize delays prior to administration and optimize carrier solutions. Another study showed that the membrane electrophysiological properties of the MSC do not revert to normal levels until about 72 h into culture following thaw. The authors attributed the residual DMSO exposure to delayed recovery and concluded that post-thaw processing is important regardless of the warming device used (Johnson et al., 2025). Standardized post-thaw handling procedures and guidelines with a general consensus for various MSC products are still scarce, despite these developments. Cell recovery and biological activity can be affected by differences in reconstitution media, storage times, and infusion techniques, underscoring the need for further research to inform standardized clinical recommendations.

5 Post-thaw considerations

Post-thaw handling is an important but often under-recognized factor that can affect the viability, function, and therapeutic efficacy of cell products preserved by cryopreservation (Cottle et al., 2022). Cryopreservation can be used to store cells for extended periods; however, thawing can cause significant physiological and chemical stress on cells (Wang et al., 2023). Biological products are often diluted or reformulated post-thaw to reduce exposure to DMSO. This may cause biological damage (e.g., changes in osmolarity, oxidative stress, membrane disruption, mitochondrial dysfunction, and activation of apoptotic pathways) depending on temperature and time of exposure (Wang et al., 2026; Uhrig et al., 2022). There are several factors that need to be considered for cryopreservation and post-thaw handling of biologics. These include the medium used for reconstitution and cell concentration during product preparation, the administration time of the product, and removal of DMSO, washing, filtration, and acclimation post-thaw (Cottle et al., 2022).

5.1 DMSO removal

The most commonly used cryoprotective agent is DMSO/Me_2SO, which can penetrate cellular membranes to inhibit intracellular ice formation (Silva et al., 2025). When thawed, however, DMSO becomes toxic at physiological temperatures, causing membrane instability, oxidative stress, mitochondrial dysfunction, and apoptosis (Wang et al., 2023). In clinical practice, removing DMSO after thawing is an important goal in the cell processing system, as residual DMSO has been linked to infusion-related adverse events, including hypotension, nausea, vomiting, and allergic reactions (Cottle et al., 2022; Ekpo et al., 2022). The conventional method of removal is through stepwise dilution with isotonic solutions or HSA-containing solutions (1:1, 1:3, 1:7), followed by centrifugation (400×g, 10 min, 4 °C) and resuspension. Osmotic stress-induced membrane damage is a primary concern during gradual dilution, but mechanical stress and cell damage during centrifugation can also occur, especially in fragile populations like MSCs (Chow et al., 2017). In addition, there is a need to reduce the delay between thaw and administration to account for time-dependent toxicity. Tan et al. (2024) showed that MSCs had stable viability for 6 h after freezing at 9×10^6/mL. When the cells were frozen at 6×10^6/mL, viability decreased from 92% ± 4.3%–84% ± 2.7%. The effects of delayed DMSO removal may be minimized by premedication (steroids) and having venous access in place before the thaw (Fernández-Santos et al., 2022). Based on the literature, there is no major safety signal, as typical DMSO doses used with MSCs are 2.5–3.0 times lower than hematopoietic thresholds, suggesting that complete removal of residual DMSO may not always be necessary (Fernández-Santos et al., 2022). The manufacture of next-generation low-DMSO and DMSO-free cryomedia further reduces the post-thaw DMSO removal requirements. A multicenter trial of a sucrose glycerol isoleucine (SGI) solution observed slightly lower viability in BMMSCs or ATMSCs, but better viable recovery and a similar phenotype and gene expression compared with 5%–10% DMSO media (Mamo et al., 2024). Similarly, XT-Thrive (DMSO-free) reagent was tested with BMMSCs. The researchers noticed post-thaw viability to be >85% at 6 h with this reagent and observed that it supported improved expansion and osteogenic differentiation compared with CS10 cryomedia (post-thaw viability of <75%) (Lam et al., 2026).

5.2 Washing

Following DMSO removal, thawed MSCs are subjected to an additional washing step. Unlike the DMSO-removal step, this wash is performed to clear debris, dead cells, and other contaminants rather than to reduce cryoprotectant exposure. This involves both dilution and centrifugation. However, it has its drawbacks. In a previously conducted septic mouse model, cells treated with MSC-based therapy were washed to remove DMSO, resulting in a 45% reduction in total cells. However, when cells were simply diluted to 5% DMSO, both cell viability and therapeutic activity were preserved (Wang et al., 2025). In a separate cryomedia comparison study, MSCs were washed and re-suspended in cold PBS containing 3% fetal bovine serum (FBS, Life Technologies) and were stained following the protocol for antibodies. Washed products had high viabilities but "significant decreases" in recovered cell numbers, and clinical trials using these indicated broad final viabilities (30%–80%) (Tan et al., 2024). Post-thaw washing involves significant labor and equipment and carries the risk of cell damage, loss, and variability. Hence, the majority of trials now include DMSO during the thawing stage and wash the cells at the bedside (Niebergall-Roth and Kluth, 2025). Likewise, a review by Wang and Li (2024) highlighted the importance of improving the removal of CPAs, as centrifugation after thaw results in "considerable" cell loss. The available evidence suggests that washing is not always beneficial to product quality, but in certain cases, it can have a detrimental effect on the final therapeutic dose. More recently, Wang et al. (2025) compared post-thaw washing with dilution of a 10% DMSO-containing MSC product. Diluted MSCs showed significantly higher cell recovery, while viability remained comparable between groups for up to 24 h and functional potency was maintained. These findings suggest that post-thaw dilution may provide higher cell recovery than washing, although the optimal approach should be validated for each MSC product.

5.3 Filtration

Following washing, the resuspended MSC preparation is passed through a filtration step prior to final formulation. Freeze–thaw cycling, together with the mechanical stress of resuspension, can generate cell aggregates and residual debris that are not fully cleared by washing alone. In an intrathecal delivery model, MSC aggregation mediated by integrin α4/VCAM-1 interactions was shown to obstruct cerebrospinal fluid flow and impair motor function following transplantation, underscoring the clinical relevance of aggregate removal prior to administration (Ran et al., 2022). As the finding is preclinical and specific to intrathecal delivery, the direct extrapolation to MSC administration via other routes should be made cautiously. In practice, mesh sizes in the 40–70 μm range are commonly applied to single cell suspensions to remove aggregates and debris while minimizing loss of viable single cells. Cunha et al. (2015) made similar observations using human MSCs, where a pore size greater than 70 μm was able to filter cell carriers without compromising cell viability. Cottle et al. (2022) reviewed several cell types and automated washing devices, filtration spinning membranes, hollow fiber membranes, and microfluidic channels. These can remove DMSO without centrifugation, thus minimizing shear and osmotic stress (Cunha et al., 2015). It is important to note that these filtration methods have been proposed for cell therapy products. However, MSC-specific validation remains limited. Niebergall Roth *et al* (Niebergall-Roth and Kluth, 2025) reviewed DMSO safety and noted that developers are trying to minimize DMSO exposure by using filtration to reduce cell damage and variability, especially after repeated centrifugation. Wang et al. (2026) used a standardized, closed dry-thaw system combined with carefully monitored post-thaw to limit DMSO toxicity and handling variability. Overall, filtration/automated systems are promising methods for minimizing and/or standardizing DMSO elimination, but more comprehensive performance testing is required for each specific MSC.

5.4 Acclimation

The period between thawing and cells being infused or assayed is called acclimation. Mechanistic studies have shown that cryopreserved MSCs produce higher levels of ROS, DNA damage, mitochondrial dysfunction, and activation of apoptotic pathways following thaw. Ding et al. (2024) reported that 10%–15% of cells underwent apoptosis immediately after thawing. Oxidative stress caused DNA damage after 48 h, and transient G0/G1 cell-cycle arrest. The researchers observed diminished migration and differentiation potential as well as increased DNA damage after thawing cryopreserved bone MSCs compared with fresh MSCs. However, they also observed that after thawing, cryopreserved MSCs remained more viable than fresh ones for a longer period of time. Similarly, electrophysiological analysis by Johnson et al. (2025) revealed a significant difference in "health" metrics between the state immediately following thaw and that after approximately 3 days. Time-course evaluations reveal that viability, metabolic activity, and cellular adhesion decrease in the first 4 h after thawing. Cellular function may be restored after 18–72 h, although viability and apoptotic markers may improve within 24 h. For this reason, some investigators have suggested adding a recovery period prior to clinical or experimental application. Some reviews indicate that 24 h may not be sufficient to achieve full functional recovery, and that culture recovery periods of 1–2 days may enhance the potency and therapeutic efficacy of MSCs (Johnstone et al., 2023; Linkova et al., 2022). Several strategies have been tested to speed acclimation. Pre-freezing cell-cycle synchronization in the G0/G1 phase reduces delayed apoptosis and preserves pre-freezing viability, colony growth, and T-cell suppression. This reduces the post-thaw recovery time (Linkova et al., 2022). Cytokine preconditioning (IFN-γ/TNF-α) converts MSCs into a glycolytic state that has been preserved upon freezing and allows rapid recovery of immunomodulatory functions, including IDO-1 and pro-inflammatory cytokines (IL-6, IL-8) upon thawing after 6–24 h. Simultaneously, other indications exhibit only slight acclimation, such as in bone regeneration, where viable cells became similar within 2 h and differed in gene expression, inflammatory responses, vasculogenesis, and bone formation (Marie et al., 2026). In general, post-thaw handling has a significant effect on the quality, safety, and therapeutic efficacy of MSCs. Removal of DMSO and washing can minimize exposure to the cryoprotectant, but excessive handling may reduce cell recovery and potency. New filtration technologies and DMSO-free cryopreservation methods are being developed that

TABLE 1 Post-thaw viability, vehicle and recovery over time.

Reference	Cell source	DMSO (% and type)	Holding vehicle (Post-thaw)	Post-thaw Time (h)	Viability trend	Recovery
(Tan et al., 2024)	MSC	10% DMSO (NutriFreez, PHD10, CS10), 5% DMSO (CS5)	PlasmaLyte-A + 5% Human Albumin	0–6	10% DMSO: stable >80% CS5: decreasing; dilution improved viability	Slightly ↓ with higher dilution
(Aabling et al., 2023)	MSC	DMSO-based	Saline; PBS ± 2% HSA; culture medium	0–4	Saline: >90% by 4 h; PBS/medium: <80% by 1 h; HSA improved stability	Strongly vehicle-dependent
(Bahsoun et al., 2020)	BM-MSC	10% DMSO	Standard MSC culture medium	0–24	Early viability ↓ (0–4 h), then partial recovery (24 h)	Partial recovery; metabolic activity and adhesion remained impaired
(Mamo et al., 2024)	MSC	5%–10% DMSO	SGI in Plasmalyte A (DMSO-free)	0–3	Viability drop: DMSO (11.4%) > SGI (4.5%)	Recovery: DMSO-free (92.9%) > DMSO (87.3%)
(Lam et al., 2026)	BM-MSC	10% DMSO (CS10), XT-Thrive	Culture medium	0–6	6 h viability XT-Thrive (≥85%) > DMSO (60%–70%)	XT-Thrive superior at 6 h

Abbreviations: BM-MSC: bone marrow-derived mesenchymal stem cell; DMSO: dimethyl sulfoxide; NutriFreez: NutriFreez® cryopreservation medium; PHD10: PlasmaLyte-A + 5% human serum albumin +10% DMSO; CS: CryoStor®; PBS: phosphate-buffered saline; HSA: human serum albumin; SGI: sucrose, glycerol, and isoleucine medium; XT-Thrive: XT-Thrive® cell cryopreservation medium (DMSO-free); h: hours.

TABLE 2 Viability/recovery and functional potency assessment methods reported in primary MSC studies.

MSC source	Reference	Viability test used	Potency test used
BM-MSC	(Antebi et al., 2019)	Annexin V-FITC/PI flow cytometry (apoptosis); Live/Dead Cell Viability Kit (fluorescent staining)	T-cell proliferation suppression assay; IFN-γ stimulation assay; IDO/PGE2 induction assay
BM-MSC	(Bahsoun et al., 2020)	Flow cytometry (apoptosis and immunophenotyping); CFU-F colony-counting assay	Not assessed
AT-MSC	(Irioda et al., 2016)	Annexin V and 7-AAD staining by flow cytometry; colony-forming unit (CFU) assay	α4-integrin (CD49 d) immunophenotyping by flow cytometry
UC/WJ-MSC	(Shivakumar et al., 2016)	Hoechst 33,342 and propidium iodide (PI) staining	Trilineage differentiation assay (osteogenic, adipogenic, and chondrogenic); hepatocyte differentiation also assessed; exact staining method not reported
UC/WJ-MSC	(Izaguirre-Pérez et al., 2025)	Flow cytometry (exact dye not reported); karyotyping (chromosomal stability)	Trilineage differentiation assay (exact staining method not reported)
UC-MSC	(Rivera-Crespo et al., 2025)	MTT assay (metabolic activity, Abcam Cell Proliferation Kit); viability dye/method not reported	LPS-induced M1 macrophage polarization inhibition assay with IFN-γ priming (20 ng/mL, 48 h), measured by GFP fluorescence intensity (460/505 nm)

Abbreviations: CFU-F, colony-forming unit-fibroblast; PI, propidium iodide; 7-AAD, 7-aminoactinomycin D; IFN-γ, interferon-gamma; IDO, indoleamine 2,3-dioxygenase; PGE2, prostaglandin E2; LPS, lipopolysaccharide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

could reduce processing-related stress and variation. Despite growing evidence supporting optimized post-thaw processing, standardized protocols for DMSO removal, washing, filtration, and recovery culture remain limited. Further studies are needed to define evidence-based procedures that maximize MSC viability, potency, and clinical consistency. Similarly, Tiago *et al* (Tiago et al., 2026) evaluated selected MSC functional properties immediately after thawing and following a 48-h recovery period and found no significant differences in the functional assays examined. These findings suggest that a prolonged recovery period may not be necessary for all MSC products. However, the requirement for post-thaw recovery should be determined using product-specific potency and quality criteria. Also, these post-thaw handling approaches should not be considered universally applicable, as their effects may vary according to MSC source, cryopreservation formulation, post-thaw processing, assessment methods, and product characteristics; therefore, product-specific validation is required before clinical application.

Evidence of post-thaw holding conditions, viability, and cellular recovery is summarized in Table 1.

6 Cryopreserved MSCs from different tissue origins

MSCs may be isolated from several sources, such as bone marrow (BM), adipose tissue (AT), umbilical cord (UC), and Wharton's jelly (WJ). They differ with respect to donor age, proliferation potential, secretion characteristics, and sensitivity to the stress of the cryopreservation procedure. Viability and functional potency after thawing are important quality attributes, as most clinical MSC products are produced as counter-top cryopreserved therapies. Current evidence suggests that post-thaw performance is influenced more by cryopreservation formulation, thawing conditions, and post-thaw handling than by tissue source alone. Although, UC-MSCs often demonstrate robust proliferative capacity, and BM-MSCs have been characterized clinically, no consensus exists that one MSC source consistently exhibits superior post-thaw viability or potency under standardized conditions (Semenova et al., 2021). Assessment of post-thaw MSC quality varies across the primary studies discussed in this section, with viability, recovery, and functional/potency measured using different methods. These assessment methods are summarized in Table 2.

6.1 Bone marrow-derived MSCs (BM-MSCs)

Bone marrow-derived MSCs are the most extensively characterized MSC source in clinical research. A systematic review by Bahsoun *et al* (Bahsoun et al., 2019) concluded that standard cryopreservation protocols generally preserve BM-MSC morphology and surface marker expression, and that cells stored for over a decade retained multipotency, suggesting that cryogenic quiescence itself does not degrade stemness over time. However, quantitative post-thaw kinetic studies have shown that these preserved endpoints do not tell the whole story. Antebi *et al* (Antebi et al., 2019) demonstrated that human BMMSCs exhibit reduced viability, increased apoptosis, and impaired metabolic and adhesion activity within the first 4 h post-thaw. They observed only partial recovery by 24 h, a window directly relevant to thawing protocols intended for near-bedside administration.

Efforts to refine BMMSC cryopreservation formulations have focused on reducing DMSO exposure. Liu *et al* (Liu et al., 2011) showed that reducing DMSO to 7.5% (v/v) in combination with PEG or BSA produced post-thaw viability comparable to conventional 10% DMSO, indicating that partial DMSO substitution may be feasible without compromising recovery. Separately, cryopreservation at higher cell densities (up to 10 million cells/mL) has been shown not to compromise post-thaw viability, apoptosis, metabolic activity, or osteogenic differentiation, supporting the feasibility of concentrated BM-MSC banking for logistical and cost efficiency (Bahsoun et al., 2020). Recent findings have shown that the post-thaw performance is affected by the cryopreservation strategy used. Lam *et al* (Lam et al., 2026) compared a DMSO-free XT-Thrive formulation with CryoStor CS10 for BM-MSC cryopreservation and reported improved post-thaw viability, proliferative capacity, and growth-factor secretion with the DMSO-free formulation. These findings indicate that cryopreservation conditions may influence post-thaw viability and regenerative potency.

6.2 Adipose tissue derived MSCs (AT-MSCs)

Adipose tissue-derived MSCs are increasingly investigated as a cell source due to their relative abundance and high expansion potential compared with other MSC origins (Hamada Abd El-Hafeez Thabet et al., 2025). Abraham and Goel (Abraham and Goel, 2025) evaluated conventional DMSO and FBS cryopreservation protocols in goat and buffalo ADSCs and reported high post-thaw recovery and metabolic activity along with increased oxidative stress and apoptosis relative to fresh controls. Supplementing the medium with exocellular agents trehalose, BSA, and PEG mitigated oxidative damage and improved post-thaw preservation. The authors proposed that the lipid-rich composition of adipocyte-lineage cells contributes to a source-specific cryobiological response, distinct from that of non-adipose MSCs (Abraham and Goel, 2025). These findings are consistent with a broader body of work on human ADSCs. In a systematic review of fourteen studies, Crowley *et al* (Crowley et al., 2021) found that trehalose combined with DMSO consistently improved post-thaw outcomes for human adipose tissue and AT-MSCs relative to DMSO alone, although non-permeating trehalose alone was inferior to DMSO unless intracellular delivery methods (e.g., electroporation) were used to load the sugar into the cells. More recent efforts have pursued DMSO-reduction or DMSO-free strategies specifically for adipose-lineage cells, including microfluidic-based approaches and metformin-supplemented cryopreservation media, reflecting ongoing efforts to reduce DMSO-associated cytotoxicity while preserving lipid membrane integrity unique to this tissue source. Available data suggest that AT-MSCs can maintain their characteristic immunophenotypic markers and proliferative capacity following optimized cryopreservation, though direct head-to-head comparisons of post-thaw potency between AT-MSCs, BM-MSCs, and UC-MSCs remain limited.

6.3 Umbilical cord-derived MSCs (UC-MSCs)

UC-MSCs, and Wharton's jelly (WJ)-derived MSCs specifically, have attracted interest owing to their non-invasive harvest and comparatively high proliferative activity relative to BM-MSCs. Studies cryopreserving intact WJ tissue (rather than isolated cells) have shown that programmed freezing protocols combined with a multi-component cryoprotectant "cocktail" solution yield higher post-thaw cell survival than conventional slow-freezing with DMSO alone, with post-thaw WJ-MSCs retaining surface marker expression, pluripotency-associated factor expression, and trilineage/hepatocyte differentiation capacity comparable to fresh tissue (Shivakumar et al., 2016). Serum-free and reduced-DMSO formulations (e.g., DMSO with sucrose, or glycerol-based media) have similarly supported robust post-thaw expansion and maintenance of MSC-characteristic markers, indicating that UC-MSCs may tolerate a wider range of cryoprotectant substitutions than lipid-rich AT-MSCs. Clinically oriented studies extend these observations to functional potency. Notably, Barcia *et al* (Bárcia et al., 2017) reported that umbilical cord tissue-derived MSCs maintained both immunomodulatory and angiogenic potency after cryopreservation and thawing, in contrast to earlier reports of impaired immunosuppressive function in cryopreserved BM-

TABLE 3 Comparative post-thaw viability and potency characteristics of MSCs derived from different tissue sources.

Parameter	BM-MSCs	AT-MSCs	UC-MSCs
Donor invasiveness	High	Low	None (non-invasive collection)
Cell yield per harvest	Low–moderate	High	Low (initial isolation); high expansion potential after successful isolation
Donor age effect	Significant decline in MSC number and differentiation with donor age	Donor age negatively affects expansion and differentiation potential	Minimal (neonatal source)
Post-thaw viability	60%–70% viability at 6 h with CryoStor® CS10; >85% with XT-Thrive (Lam et al., 2026)	High post-thaw viability maintained with optimized cryopreservation media	Lower recovery than DMSO but functional properties retained with 0.5 M trehalose +10% E.G., (Izaguirre-Pérez et al., 2025)
Cryoprotectant response	XT-Thrive improved post-thaw viability and expansion compared with CryoStor® CS10	Trehalose + glycerol preserved post-thaw viability, proliferation and multilineage differentiation comparable to DMSO.	Trehalose + E.G., preserved viability, differentiation and genomic stability
Immunomodulatory potency	T-cell suppression reduced in S-phase frozen cells but preserved after G0/G1 synchronization	Post-thaw immunomodulatory activity retained under optimized cryopreservation conditions	PBMC suppressive activity preserved after cryopreservation
Paracrine activity	HGF secretion increased 2.1-fold and FGF2 1.8-fold with XT-Thrive (Lam et al., 2026)	Secretory function retained, with transient changes in growth-factor secretion immediately after thawing	Immunomodulatory and angiogenic potencies maintained after cryopreservation and thawing
Differentiation potential	Trilineage differentiation maintained; osteogenesis enhanced with XT-Thrive	Differentiation potential maintained after cryopreservation	Trilineage differentiation maintained after cryopreservation with trehalose + EG.
Clonogenic/Proliferative potential	CFU-F efficiency ~23% with XT-Thrive vs. ~15% with CryoStor CS10 (Lam et al., 2026)	Colony-forming ability decreased after cryopreservation (28.1% ± 7.1% vs. 21.5% ± 6.6%, $p < 0.01$) (Irioda et al., 2016)	Proliferative capacity maintained after long-term cryopreservation

Abbreviations: BM-MSC, bone marrow-derived mesenchymal stromal cell; AT-MSC, adipose tissue-derived mesenchymal stromal cell; UC-MSC, umbilical cord-derived mesenchymal stromal cell; CFU-F, colony-forming unit-fibroblast; DMSO, dimethyl sulfoxide; E.G., ethylene glycol; FGF2, fibroblast growth factor-2; HGF, hepatocyte growth factor.

MSCs, suggesting that susceptibility to cryopreservation-induced potency loss may itself be tissue-source-dependent and, in part, manufacturing-process-dependent rather than an intrinsic property of all MSCs. Izaguirre-Pérez *et al* (Izaguirre-Pérez et al., 2025) examined trehalose-based cryopreservation approaches and found that post-thaw recovery was lower than that with conventional DMSO formulations. UC-MSCs had no loss of chromosomal stability, morphology, or trilineage differentiation capacity. In particular, 0.5 M trehalose in 10% ethylene glycol successfully cryopreserved cells that showed osteogenic, adipogenic, and chondrogenic differentiation ability similar to the DMSO-preserved cells. Morphology and viability were not significantly altered by a second freeze–thaw cycle, and no changes in the expression of stress-related genes such as TP53 were observed. Further evidence from Rivera-Crespo *et al* (Rivera-Crespo et al., 2025) showed that MSCs cryopreserved on microcarriers using the Cryo-MASCS platform had post-thaw viability similar to that of standard DMSO suspension cultures. The ability of MSCs to inhibit M1 macrophage polarization in response to LPS was maintained following thawing of IFN-γ-primed cells, suggesting that the immunomodulatory function was retained. These results indicate the suitability of UC-MSCs complemented with microcarriers for scalable manufacturing approaches off the shelf. A comparative summary of the post-thaw viability, potency, and cryopreservation-related characteristics of BM-MSCs, AT-MSCs, and UC-MSCs is presented in Table 3.

7 MSC thawing techniques for clinical use

7.1 Rapid water-bath thawing (37 °C)

For the recovery of cryopreserved MSCs, rapid water-bath thawing at 37 °C is a commonly used technique in both research laboratories and clinical manufacturing environments. In this process, cryovials or cryobags are immersed in a 37 °C water bath and swirled until only a small ice fragment remains before immediate removal. Rapid warming (typically >100 °C/min during 37 °C water-bath thawing) minimizes the time cells remain within the critical temperature range where ice recrystallization, devitrification, and osmotic stress occur, thereby reducing membrane damage and improving post-thaw cell recovery (Wang and Li, 2024). Mamo *et al* (Mamo et al., 2024) tested MSC cryopreservation/recovery procedures across seven manufacturing centers and found supporting evidence for this approach, using a standardized 37 °C water-bath thawing procedure. An in-house formulation with DMSO achieved a mean pre-freeze viability of 94.3%, which fell by only 4.5% upon thawing ($p = 0.049$). These findings demonstrate that standardized rapid water-bath thawing, when combined with optimized cryopreservation protocols, can support high post-thaw viability across multiple manufacturing centers. Marie *et al* (Marie et al., 2026) compared cryopreserved BMMSCs with fresh

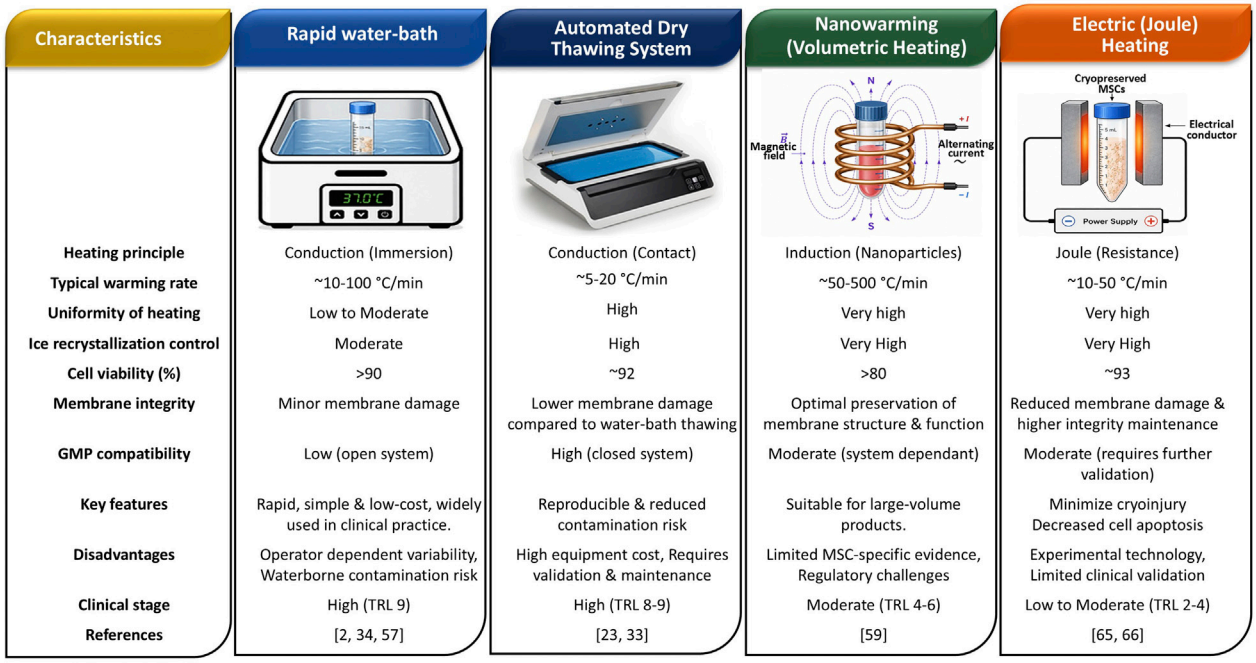


FIGURE 1
Comparative overview of thawing technologies for cryopreserved mesenchymal stem cells (MSCs). Comparison of conventional and emerging thawing approaches based on warming rate, heating uniformity, control of ice recrystallization, post-thaw cell viability, membrane integrity, good manufacturing practice (GMP) compatibility, and clinical translation stage. This figure was created by the authors using Microsoft PowerPoint and original graphical elements, incorporating selected data and visual references from the following sources: (1) Cottle et al (2022); (2) Mamo et al (2024); (3) Swanson et al (2025); (4) Aabling et al (2023); (5) Lam et al (2026); (6) VIA Thaw™ automated dry thawers (Kyodo International, Inc.); (7) Wang et al (2016); and (8) Zhan et al (2022); Han et al (2023).

MSCs upon thawing. A temporary decrease in viability was observed immediately after thawing, but no significant differences were detected after 2 h of recovery. In addition, the expression of genes involved in osteogenesis, vasculogenesis, and inflammatory signaling was not significantly altered after cryopreservation when compared to fresh MSCs, suggesting preservation of several key biological characteristics despite transient post-thaw stress. Water-bath thawing carries a risk of contamination due to exposure to water. Thawing outcomes may also vary with operator technique, variability in cryobag size, container geometry, inconsistent heat transfer, and thawing conditions. This can reduce process reproducibility in GMP-compliant manufacturing (Swanson et al., 2025).

Swanson et al. (2025) evaluated water-bath versus refrigerator thawing of cryoprecipitate and demonstrated that the thawing method directly affects fibrinogen and factor VIII content. However, this study did not involve MSCs. A direct MSC-specific parallel exists in the coagulation domain. Moll *et al* (Moll et al., 2016) demonstrated that freeze-thawed MSCs exhibit elevated procoagulant tissue factor expression and increased susceptibility to the instant blood-mediated inflammatory reaction (IBMIR), a coagulation- and complement-driven process, upon post-thaw infusion. These findings indicate that thawing conditions influence coagulation-related activity in both blood-derived products and cellular therapeutics such as MSCs.

7.2 Automated dry thawing systems

To standardize MSC recovery and minimize operator-dependent variability during thawing, automated dry-thawing systems have been developed. These platforms achieve conductive heating via temperature-controlled metal blocks or chambers and eliminate direct contact between cryo-containers and water, unlike traditional water baths. A previously conducted study compared automated dry thawing with water-bath thawing of cryopreserved ADSCs. The mean post-thaw viability was 92.7% ± 4.5% for water-bath and 91.8% ± 4.6% for automated ($p > 0.05$). Furthermore, no significant differences were noted in cell adhesion, immunophenotype, or indoleamine 2,3-dioxygenase (IDO) activity (all $p > 0.05$), indicating comparable preservation of important MSC functional characteristics (Valero et al., 2026). Despite their benefits, automated dry-thawing systems are more expensive to implement than traditional water baths and require specific equipment. Furthermore, the evidence of effects of these systems on different MSC sources remains limited (Mello et al., 2024). An overview of the thawing systems for cryopreserved MSCs is presented (Figure 1).

8 Emerging thawing technologies

Several innovative approaches have been introduced to improve heat distribution and minimize cryoinjury associated with

TABLE 4 Comparative overview of MSC thawing technologies for clinical translation.

Thawing techniques	Protocol	Viability outcome	Advantages	Limitations
Rapid Water-Bath Thawing (37 °C)	Cryovials or cryobags are rapidly thawed in a 37 °C water bath until only a sliver of ice remains	Fresh MSC viability averaged 94.3%, with a mean post-thaw viability decrease of 4.5% using DMSO-containing cryoprotectants (p = 0.049) (Mamo et al., 2024)	Rapid, simple, and widely used for routine laboratory and clinical thawing	Risk of water-borne contamination and operator-dependent variability
Automated Dry Thawing Systems	Controlled conductive heating using temperature-regulated metal plates (e.g., VIA Thaw® and ThawSTAR®)	XT-Thrive-preserved BM-MSCs maintained ~90% viability up to 6 h post-thaw, whereas CryoStor® CS10-preserved cells declined to 60%–70% (Lam et al., 2026)	Standardized, GMP-compatible thawing with reduced operator variability and lower contamination risk	Higher equipment and implementation costs than conventional water-bath thawing; requires dedicated equipment and product-specific validation, with limited evidence across different MSC sources and processing conditions
Nanowarming	Superparamagnetic Fe_3O_4 nanoparticles generate rapid and spatially uniform heating under an alternating magnetic field during rewarming	MIH increased post-thaw hUCM-MSC viability to 71.7% compared with 18.2% using conventional rewarming (Wang et al., 2016)	Rapid and spatially uniform rewarming reduced devitrification/recrystallization and improved post-thaw cell survival, attachment efficiency, and preservation of stemness	Experimental vitrification-based technology requiring Fe_3O_4 nanoparticles and alternating magnetic field equipment
Electric (Joule) Heating	Ultra-rapid resistive rewarming using a Joule heating–based electric heating chip (EHC)	MSC viability was 93.18% versus 86.83% with conventional cryovials ($p < 0.05$), with reduced apoptotic and necrotic cell fractions (Han et al., 2023)	Ultra-rapid, uniform rewarming with markedly higher rewarming rates, improving post-thaw cell viability and structural integrity	Experimental electric heating chip platform requiring dedicated rewarming devices before broader clinical implementation

Abbreviations: MSC, mesenchymal stromal/stem cell; GMP, good manufacturing practice.

conventional recovery procedures. These include electric heating platforms, microwave-assisted warming, radiofrequency-assisted warming, and nano warming. Although these technologies show promise for reducing cryoinjury, most remain at the experimental stage and require further validation before routine clinical implementation for MSC products.

8.1 Nano warming

Nano warming is a new rewarming method that involves magnetic nanoparticles distributed in the cryopreserved material. The nanoparticles generate volumetric heating throughout the sample using an alternating magnetic field (AMF), thereby reducing thermal gradients and associated cryoinjury during recovery. The only known study using this technology on hMSCs was carried out in 2016 (Wang et al., 2016). The studies preceding this focused on animal models and individual organs (Ayantoye et al., 2026). Although a previously conducted study used nanowarming with cryopreserved T-cell products, it was observed that this approach enables volumetric heating throughout the specimen uniformly (Ito et al., 2020). Based on this concept, Wang *et al* (Wang et al., 2024) engineered a zwitterionic magnetic nanoparticle (ZMNP) platform for large-scale cryopreservation and nanowarming of T-cell products. The particles combined the ice recrystallization inhibition properties of the antifreeze protein with magnetic field-mediated warming and the ability to easily recover T-cell through magnetic separation. The magnetic field-mediated nano-warming resulted in post-thaw viabilities of more than 80% in 20 mL of cryopreserved cells. In an animal lymphoma model, these nanowarmed T cells exhibited a tumor suppression rate of 77.5%. The conventional DMSO-treated group achieved ~40% tumor suppression (Wang et al., 2024). Beyond single-cell suspensions, nanowarming has also shown promise in more structurally complex tissues. Lee *et al* (Lee et al., 2024) demonstrated significantly improved functional recovery and survival rates of cryopreserved cardiac organoids with Fe_3O_4 nanoparticle-based nanowarming, measured by electrophysiological signal recovery and cardiac organoid contractility after freeze–thaw. Karimi *et al* (Karimi et al., 2023) demonstrated that vitrination of ovarian tissue followed by nanowarming in a sheep model led to decreased apoptotic cell death and oxidative stress compared to conventional warming and increased follicular survival and development. Collectively, these studies suggest that nanowarming has the potential to provide more homogeneous heating during rewarming and minimize the occurrence of cryoinjury in a variety of biological materials, from single-cell suspensions to 3D tissues.

8.2 Electric (joule) heat-based thawing

Rapid recovery of cryopreserved biological materials by electric (Joule) heating is a promising method. Using this approach, the sample is heated by electrical resistance in a conductive material, enabling ultra-fast and spatially controlled warming. By rapidly traversing the critical temperature range associated with ice recrystallization, Joule heating may improve post-thaw cell recovery (Zhan et al., 2022). Han *et al* (Han et al., 2023) designed an electric heating chip. In this microscale experiment, human MSCs had higher viability (93.18%) than those thawed using conventional warming in cryovials (86.83%). Furthermore, the apoptotic and necrotic cell counts were substantially lower after electric-heating-assisted rewarming, indicating that cell integrity was better preserved after the cryopreservation procedure. However, the researchers noted limitations to this design. These included malformations of the heating chips during rapid cooling. This may result in biologics leaking. The optimization of this system is also limited by the width of heating chips and isolation layers. Further studies are needed to better understand the impact of this approach on biologics and how this method may be further enhanced. The principal MSC thawing technologies used or investigated for clinical translation, together with their reported viability outcomes, advantages, and limitations, are summarized in Table 4.

9 Clinical implications

In a meta-analysis of seven RCTs investigating MSC therapy for cardiovascular disease, the overall effect was a significant improvement in LVEF of 2.11% (95% CI 0.66–3.56; $p = 0.004$). Interestingly, MSC products with reported viabilities >80% were associated with greater improvements in LVEF by 3.44% (95% CI 1.46–5.43; $p = 0.0007$), suggesting that preserving viable cells may enhance clinical efficacy. However, viability does not necessarily correspond to the potency of MSCs. The immunomodulatory, metabolic, and secretory functions can be impaired while the MSCs still remain viable (Safwan et al., 2025). Multiple studies have shown that MSC injury persists after thawing. This phenomenon is known as delayed-onset cell death (Bahsoun et al., 2020). Clinically, MSC infusions have demonstrated a favorable safety profile across a wide range of indications, with rare serious infusion-related side effects (Česnik and Švajger, 2024; Farge et al., 2022). However, preclinical research indicates that high local cell densities may interfere with microvascular perfusion. In a porcine kidney model, the intrarenal delivery of 10 million MSCs was well tolerated, whereas 100 million cells caused microthrombus formation, decreased renal perfusion, and renal damage (Munk et al., 2022). Although derived from a combination of preclinical and clinical models, these results underscore the need for a controlled dose regimen, standardized infusion protocols, and monitoring adverse events (embolus/thrombosis) during cell infusion (Baranovskii et al., 2022; Terai et al., 2025). Another important factor affecting the quality of the MSC is the composition of the post-thaw suspension vehicle. The amount of DMSO used with MSC products is much lower than that used in HSC (hematopoietic stem cell) transplantation, but prolonged exposure after thawing may negatively impact cell viability and function. Therefore, it is common to recommend dilution, washing, and controlled infusion to avoid stressing the cells and exposing the patient (Niebergall-Roth and Kluth, 2025; Aabling et al., 2023). MSC recovery can be significantly affected by post-thaw storage conditions before administration. Protein-free suspension media have been associated with significant early cell loss, including immediate loss of more than 40% and viability below 80% when cells were diluted to below 1×10^6 cells/mL. HSA supplementation reduced these effects and enhanced cell stability (Niebergall-Roth and Kluth, 2025). Aabling *et al* (Aabling et al., 2023) also demonstrated that the formulation with saline maintained >90% MSC viability for more than 4 h at room temperature, whereas PBS and culture medium resulted in >40% loss of MSCs and viability of <80% within 1 h. These results highlight the need to choose clinically compatible suspension vehicles that provide osmotic and protein support during the holding time after thawing. A PBS formulation with 5% HSA maintained cells at viabilities above 90% for extended periods, while the same formulation in Lactated Ringer's solution caused progressive cell viability loss, thus making PBS a suitable cell-carrying solution for infusion (Nguyen et al., 2024). Comparative studies of cryopreservation formulations by Putra et al. (2021) have also revealed that post-thaw viability and recovery are generally ~88–89% for 10% DMSO-based formulations, but lower DMSO formulations have resulted in reduced post-thaw viability (82.7% ± 1.5%) and recovery rates. However, thawed MSCs remained >75% non-apoptotic throughout the observation period, indicating that preservation and handling can maintain cell integrity during preparation for clinical use. Another factor to consider is the mechanical stress during administration. Preclinical data on MSC delivery using fine-gauge needles showed that cell viability remained at 93%–95% and did not differ significantly between injected and non-injected cells ($p = 0.51$–0.65) (Tan et al., 2024). Collectively, these findings indicate that the clinical efficacy of MSC therapy is governed not by any single variable but by a chain of interdependent handling factors. Each of these may independently reduce therapeutic activity if not controlled properly. The gap between viability and function helps explain the variable clinical outcomes seen across MSC trials, including cases where inadequate post-thaw handling has been implicated in reduced efficacy. Taken together, the evidence argues for a shift from viability-based release criteria toward matched potency and functional assays, standardized post-thaw handling windows (dilution, washing, choice of suspension vehicle, and time-to-infusion), and dose-density limits informed by preclinical thrombosis/perfusion data. Until such standards are harmonized across manufacturers and trial sites, heterogeneity in these "downstream" handling variables will remain a confound that is difficult to disentangle from the biological efficacy of MSCs and will continue to limit cross-trial comparability and regulatory clarity. The overall clinical workflow linking MSC thawing, post-thaw processing, quality assessment, infusion, and therapeutic outcomes is illustrated in Figure 2.

10 A recommended thawing protocol for clinical MSC use

Based on the synthesis above, the following protocol may be used for routine clinical use of cryopreserved MSCs across a multi-source program. Institutional validation is required before adoption.

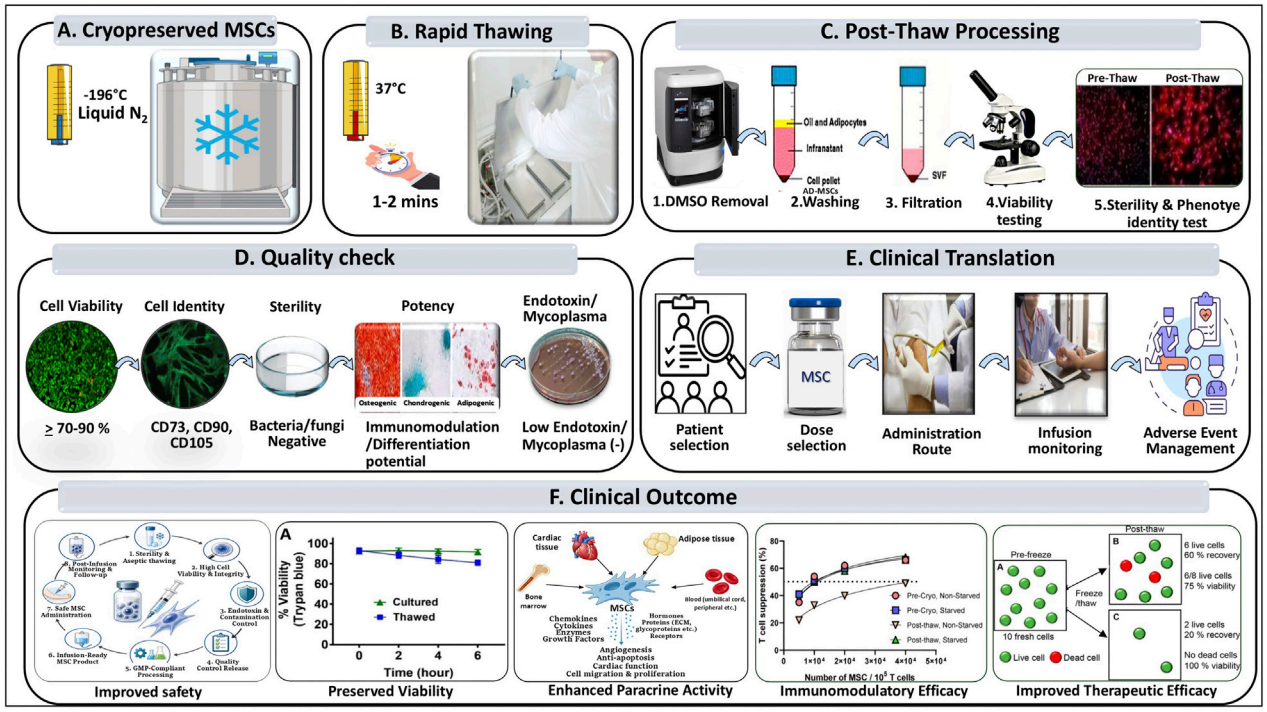


FIGURE 2
Schematic workflow from cryopreservation of mesenchymal stem cell (MSC) products to post-thaw processing, quality assessment, clinical administration, and therapeutic outcomes. **(A)** Cryopreservation of MSC products in liquid nitrogen at −196°C. **(B)** Rapid thawing at 37°C for approximately 1–2 min. **(C)** Post-thaw processing, including dimethyl sulfoxide removal, washing, filtration, and assessment of cell viability and MSC phenotype. **(D)** Quality-control assessment of thawed MSC products, including cell viability, MSC identity, sterility, potency, and endotoxin/mycoplasma testing. **(E)** Clinical translation steps, including patient selection, dose selection, administration route, infusion monitoring, and adverse-event management. **(F)** Representative post-thaw and therapeutic outcomes, including preserved cell viability, enhanced paracrine activity, immunomodulatory efficacy, and improved therapeutic efficacy. Arrows indicate the sequential progression from cryostorage and thawing through post-thaw processing, quality assessment, clinical administration, and therapeutic outcomes. This figure was created by the authors using Microsoft PowerPoint SmartArt and original graphical elements, incorporating selected visual elements adapted from the following sources: (1) Valentini et al (2024), CC BY 4.0; (2) Fischer (2022); (3) Asch et al (2025), CC BY 4.0; (4) Bharti et al (2020); (5) Murray and Gibson (2020), CC BY 4.0; (6) Kosinski et al (2020), CC BY 4.0; (7) Ding et al (2022), CC BY 4.0; (8) Lechanteur et al (2016), CC BY 4.0; (9) Mabotuwana et al (2022), CC BY 4.0; and (10) Tan et al (2019), CC BY 4.0. Modifications included cropping, resizing, rearrangement, integration of individual visual elements into a composite layout, and addition of labels and schematic annotations.

10.1 Pre-thaw preparation

- Confirm patient identity, product identity, expiration date, lot history.
- Verify continuous-temperature shipment log; preferably maintained at −135 °C.
- Prepare a dilution solution (e.g., Plasma-Lyte A with 5% HSA, USP) at room temperature, 5× the volume of the product, in a sterile transfer bag (Niebergall-Roth and Kluth, 2025; Aabling et al., 2023).
- Prepare and prime the infusion line with an in-line 170 µm filter preferably to remove aggregates (*In-line filtration at 170–260 µm follows standard blood-component administration practice) (Lotterman et al., 2026).

10.2 Thaw

- Transfer the cryobag or cryovial from liquid nitrogen storage directly into the validated dry warming device (Plasmatherm or ThawSTAR) using cryogloves and protective face shield.
- Initiate the validated thaw program for the relevant product geometry. Do not interrupt the cycle.
- Time the thaw from initiation. Record cycle completion time and device temperature trace; archive the electronic record.
- If a 37 °C water bath is the only option, use freshly disinfected water, immerse in a sterile over pouch, and remove at the "small ice nubbin" endpoint (~2–3 min for vials, ~5–8 min for bags).

10.3 Dilution and quality control

- Transfer thawed product into the dilution bag using closed-system connectors; perform stepwise dilution (1:1, then 1:5) over ~2 min.
- Mix gently; do not vortex.
- Withdraw a 0.5 mL QC sample for viability, total cell count, and (if release-eligible) a potency assay.
- The viability should preferably be above 70% (target ≥80%, a clinically validated threshold) (Safwan et al., 2025), or if visible aggregates persist after gentle resuspension.

10.4 Optional acclimation step

- For systemic infusions and high-stakes indications, thawed cells may be plated in a closed culture device for 12–24 h in clinical-grade expansion medium before infusion.
- Re-suspend, re-test viability, and proceed to infusion as a fresh-thawed product.

10.5 Infusion

- Complete infusion at the earliest when thaw is complete.
- Premedicate per institutional standard for DMSO-containing infusions.
- Monitor vital signs at baseline, 5, 15, 30, 60 min, and every 30 min thereafter for 4 h.
- Document any infusion-related events using the validated form recommended by the ISCT and FACT–JACIE standards.

Following thaw, quality control should include assessment of cell recovery and viability, identity/immunophenotype, sterility, and, where applicable, a validated functional potency assay. Although post-thaw viability of ≥70–80% is commonly reported as a practical target in clinical MSC studies, this value should not be interpreted as a universal release criterion (Renesme et al., 2025). The acceptable viability, cell recovery, potency, and other quality attributes should be defined according to the specific MSC product, manufacturing process, cryopreservation formulation, thawing system, dose, route of administration, and intended clinical application. Therefore, the thawing procedure described here should be considered a general framework rather than a universal clinical protocol, and product-specific validation is required before clinical implementation.

11 Discussion

11.1 Ethical concern

Ethical and regulatory considerations for MSC thaw extend beyond post-thaw viability to include safety, quality, biosafety, and biosecurity. Patient safety, product quality, and transparency are critical considerations for the clinical translation of cryopreserved MSC therapies. While there are established guidelines for cellular products in major regulatory bodies, there are no consistent practices for thawing in countries and clinical settings. Avoidable risks may be added through variability in handling, the control of contamination, documentation, and assessment of release. More harmonization of thawing procedures and risk-based quality control procedures is therefore required. These measures are vital for the safe, transparent and responsible use of the cryopreserved MSC product in the clinic.

11.2 Future directions

Several active research areas may meaningfully change MSC thawing practice in the near future. One of the critical areas is the development of engineered, cryoprotectants and DMSO-free formulations, such as trehalose, polyampholytes, ice-recrystallization-inhibition (IRI) peptides, and pathogen-inactivated human platelet lysate (p-IHPL) (Martín-López et al., 2023). Furthermore, cells supplemented with boron in low DMSO solutions (Demirci et al., 2014) and curcumin-primed cells (Azam et al., 2023) are advancing towards clinical validation, with the goal of minimizing DMSO toxicity while preserving the quality of thawed MSCs. These advances are accompanied by emerging interest in volumetric rewarming, which has been successfully used with iron oxide nanoparticle-based nano-warming in small-scale model tissues (Fahy and Wowk, 2021; Gangwar et al., 2022) leading to the development of nano-warming ice-free clinical MSC products in cryobag format. However, improving thaw and rewarming may not be sufficient to achieve consistent clinical results, and thus real-time post-thaw potency assays are becoming increasingly significant. The Moll *et al* critique (Moll et al., 2016) suggests using multiplexed cytokine-release assays, microfluidic immune-suppression assays, and rapid mitochondrial membrane potential readouts to aid release decisions beyond viability alone. These developments could also contribute to establishing source-specific release criteria, which needs to be further developed as more evidence, organized by source accumulates, with the release criteria for UC/WJ-MSC potentially becoming increasingly source-specific. Finally, the advances that have been made will need to be integrated into automated bedside closed systems that can further support clinical translation by combining thawing, washing, QC sampling, and infusion within a single closed pathway, which is currently being developed and is in early clinical use, and will simplify clinical workflow and GMP documentation (Santurette et al., 2022; Tostoes et al., 2017).

12 Conclusion

Thawing is the last cell-handling step before an MSC product reaches the patient, and the available evidence shows that it is also of great consequence. Rapid rewarming to 4 °C–10 °C above the freezing-medium melting point, immediate dilution or removal of DMSO, and a brief post-thaw culture acclimation together restore most of the potency deficit induced by freeze-thawing cells. Automated dry-warming devices may match or modestly exceed the 37 °C water bath in viability, eliminate microbial contamination risk, and provide GMP-grade documentation. Post-thaw albumin dilution is adequate for most small volume MSC infusions. Automated closed system washing is preferred for high volume or paediatric infusions. The most recent meta-analytic evidence ties post-thaw viability ≥70% to a clinically meaningful improvement in efficacy. This supports adoption of viability and ideally a potency assay as a release criterion for every clinical infusion. Umbilical cord and Wharton's jelly-derived MSCs have shown promising post-thaw viability, proliferative capacity, and scalability in several studies, although direct comparisons across MSC sources remain limited. BMMSCs remain among the most extensively characterized sources for angiogenic and orthopedic indications. Adipose-derived MSCs have demonstrated robust immunomodulatory properties *in vitro* and in local/topical wound indications. Across every source, the thawing step represents a critical determinant of therapeutic

quality. Well-validated handling decisions help the cryopreserved MSC product achieve its intended therapeutic performance.

Author contributions

UEH: Project administration, Supervision, Writing – review and editing. DLG: Conceptualization, Writing – original draft, Writing – review and editing. SaS: Data curation, Validation, Writing – original draft. SuS: Data curation, Software, Visualization, Writing – original draft. RS: Data curation, Software, Visualization, Writing – original draft.

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Conflict of interest

Authors UEH, DLG, SaS, SuS, and RS were employed by R3 Stem Cell LLC.

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