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REVIEW ARTICLE OPEN ACCESS

Optimal Transport Media for Maintaining Functional Mesenchymal Stem Cells during Hypothermic Preservation: A Systematic Review

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ABSTRACT

Background: Human mesenchymal stem cells (hMSCs) are a promising and cost-effective therapeutic platform in regenerative medicine. However, their clinical application depends on maintaining cell viability and function during storage and transportation, as hMSCs are highly sensitive to pH imbalance, inappropriate medium composition, and temperature fluctuations. This systematic review aimed to identify the optimal hypothermic storage and transport media for hMSC-based therapies. **Methods:** This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A literature search was conducted in PubMed, The Cochrane Library, and ScienceDirect for studies published up to September 2025 within the previous 20 years. Search terms included mesenchymal stem cells, transport or preservation media, and hypothermic storage. A total of 1,076 records were identified. After removing duplicates and ineligible studies, 340 titles and abstracts were screened, and 21 studies were included in the qualitative synthesis. **Results:** The suitability of storage or transport media varied according to the intended storage duration and the MSC quality attributes evaluated. The available evidence suggests that crystalloid solutions and selected culture-based media may be feasible for short-term storage, whereas colloid-containing and preservation-specific formulations may offer advantages during longer storage. Trehalose- or antioxidant-supplemented media also showed potential for prolonged preservation. However, these findings were derived from heterogeneous studies and should not be interpreted as universal duration-based recommendations. **Conclusions:** Selection of the hypothermic storage medium should be guided by the anticipated storage duration to maximize hMSC quality and functionality and support the clinical translation of hMSC-based therapies.

Keywords: *human mesenchymal stem cells, storage, hypothermic preservation, transport medium*

INTRODUCTION

Human mesenchymal stem cells (hMSCs) represent a promising and affordable approach in regenerative medicine, with a relatively low risk of immune rejection, making them an attractive alternative therapy for various chronic inflammatory diseases, including osteoarthritis,[1,2] inflammatory skin disorders,[3] and neurological conditions such as hemorrhagic and ischemic stroke.[4,5] The three primary sources of hMSCs are adipose tissue (Ad-MSCs), umbilical cord (UC-MSCs), and bone marrow (BM-MSCs).[5] hMSCs are multipotent cells capable of differentiating into mesodermal lineages such as chondrocytes, osteocytes, and adipocytes, and can also undergo transdifferentiation into endodermal and ectodermal lineages.[5] In addition, hMSCs secrete a wide range of bioactive molecules—including TGF-β, VEGF, growth factors, extracellular matrix components, and extracellular vesicles—that play critical

components, and extracellular vesicles—that play critical roles in maintaining tissue homeostasis and promoting regeneration.[6,7]

In recent years, interest in stem cells as regenerative medicine has grown exponentially, leading to a high demand for large-scale production.[8] However, hMSC production still faces several challenges, including the limited availability of Current Good Manufacturing Practice (CGMP)-accredited laboratories,[9] high production costs,[10] and the short storage duration of MSCs before transplantation.[11] Regulatory agencies also specify minimum viability thresholds: more than 70% viable cells according to the U.S. Food and Drug Administration (FDA) and more than 80% according to the European Medicines Agency (EMA)[12]

HMSCs are highly sensitive to suboptimal conditions such as pH imbalance, medium composition, and temperature fluctuations; therefore, to preserve their functionality and potency, the time interval between harvesting and transplantation must be minimized.[11] Hypothermic preservation is a short-term cell preservation method in which cells are stored at temperatures below 35 °C without the need for cryopreservation. This condition slows cellular metabolism and prevents cell freezing.[13-15] However, hypothermic conditions can reduce Na^+/K^+-ATPase pump activity, disrupting the intracellular Na^+/K^+ balance. The accumulation of Na^+ ions subsequently activates the Na^+/H^+ exchanger, leading to increased reactive oxygen species (ROS) production and cell swelling.[16] Therefore, identifying an optimal hypothermic preservation solution is essential to minimize Na^+/K^+ imbalance and reduce ROS generation.

According to the Indonesian Food and Drug Authority, MSC manufacturing must be carried out in cGMP-certified laboratories, most of which are located in Greater Jakarta.[17] Currently, the allowable storage period during transportation is restricted to 12 hours at 2–8 °C, creating significant barriers to distributing hMSCs to other provinces and islands across Indonesia. This limitation not only reduces equitable access but also constrains the scalability of production, ultimately increasing overall costs.

Thus, there is a need to identify hypothermic storage and transport conditions that can preserve not only hMSC viability but also their phenotypic characteristics and functional potency during the interval between manufacturing and clinical administration. Although several studies have evaluated individual preservation solutions, the available evidence remains fragmented because of differences in hMSC tissue sources, storage durations, medium compositions, cell concentrations, and outcome assessment methods. Previous reviews have broadly discussed hypothermic preservation and storage fluids for mesenchymal stem cells, including their effects on cell viability and therapeutic characteristics.[29] However, a focused systematic synthesis comparing different transport and preservation media for hMSCs at 2-8^{o}C, particularly according to storage duration and a comprehensive range of clinically relevant quality attributes, remains limited. Therefore, this review specifically addresses this gap by systematically comparing the available preservation media and examining their effects across different storage durations and hMSC quality attributes.

Therefore, this systematic review aimed to identify and compare hypothermic storage and transport media for hMSCs at 2-8^{o}C and to determined their ability to preserve cell viability and key quality attributes, including immunophenotype, differentiation potential, immunomodulatory activity, proliferation, secretory function, and mitochondrial characteristics. In addition, we sought to synthesize the available evidence according to storage duration to provide a clinically relevant framework for selecting preservation media during hMSC transportation. This approach may help address practical challenges in the distribution of hMSC-based therapies, particularly in settings where manufacturing facilities and treatment centers are geographically separated.

MATERIALS AND METHODS

Study Design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[18] The review protocol was not prospectively registered.

Search Strategy

The literature search was conducted in three electronic databases: PubMed, The Cochrane Library, and ScienceDirect. Studies published up to September 2025, within the last 20 years, were considered. The search strategy employed the following keywords: *("mesenchymal stem cells" OR MSC) AND ("cold solution" OR "transport medium" OR "preservation solution") AND ("hypothermic preservation" OR "hypothermic storage" OR "cold storage").* These terms were applied to titles and abstracts only. Full texts of potentially relevant studies were retrieved and assessed for eligibility.

Study selection

Eligibility criteria were established a priori. Articles were included if they met the following requirements: (1) described the hypothermic preservation medium and its control in hMSC storage; (2) involved hMSC storage at temperatures between 2–8 °C; (3) evaluated various storage durations; (4) reported outcomes related to functionality, viability, vitality, or potency of hMSCs after storage; (5) were published in English; and (5) were available in full text. Exclusion criteria were: (1) absence of a control group; (2) use of cryopreservation media; (3) use of non-human cell sources; and (4) publication types such as conference abstracts, reviews, preprints, or duplicate data.

The selection process was conducted in two steps. First, titles and abstracts were screened for relevance. Second, full texts of the selected articles were reviewed according to the predefined inclusion and exclusion criteria. Two investigators independently evaluated all articles.

Data Extraction

Data extraction was performed independently. For each included study, relevant information was collected from the text, tables, and figures, and subsequently summarized for analysis in relation to the objectives of this review. Specifically, the following data were extracted: (1) hMSC source (BM-MSC, UC-MSC, or AD-MSC); (2) storage medium; (3) storage duration; (4) cells container type; (5) cells concentration, and (6) functional or potency-related outcomes, including (a) cell viability; (b) population doubling time or proliferation; (c) pluripotency markers; (d) immunophenotypic profile; (e) mesenchymal differentiation potential; (f) secretory activity; (g) immunomodulatory properties; or (h) mitochondrial function. For all the analyzed records, only those experiments meeting the inclusion criteria were maintained.

Outcomes

The primary outcomes were cell viability and recovery after hypothermic storage, together with indicators of proliferative capacity, including population doubling time. Secondary outcomes included the preservation of MSC identity and function, as assessed by immunophenotypic characteristics, MSC-associated or stemness marker expression, adherence and proliferative recovery, mesenchymal differentiation potential, secretory activity, immunomodulatory capacity, mitochondrial function, and apoptosis-related parameters. Outcomes were evaluated at the end of hypothermic storage and, when reported, after post-storage recovery or culture.

For this review, an “optimal” transport medium was defined as a medium that maintained acceptable cell viability while also preserving one or more clinically relevant functional properties over the intended

storage duration. Therefore, viability alone was not considered sufficient to establish overall MSC quality or therapeutic potency. The interpretation also considered storage duration, MSC source, cell concentration, medium composition, and the availability of functional or potency-related assessments.

Quality Assessment

The methodological quality of each included study was assessed across three domains: (1) study design and biological material, including MSC source, donor information, passage number, ethical approval or informed consent where applicable, comparator groups, and replication; (2) preservation procedures, including medium composition, storage temperature and duration, cell concentration, container, agitation or transportation conditions, and temperature monitoring; and (3) outcome assessment, including the use of standardized assays, appropriate controls, replication, statistical analysis, and functional outcomes sufficient to support the authors' conclusions. Each domain was judged as low concern, some concern, or high concern. An overall low-concern judgment required all domains to be rated as low concern; some concern indicated at least one domain with some concern and no high-concern domain; and high concern indicated at least one high-concern domain. Studies were not excluded based on this assessment; the judgments were used to guide the interpretation of the evidence.

RESULTS

Study Selection

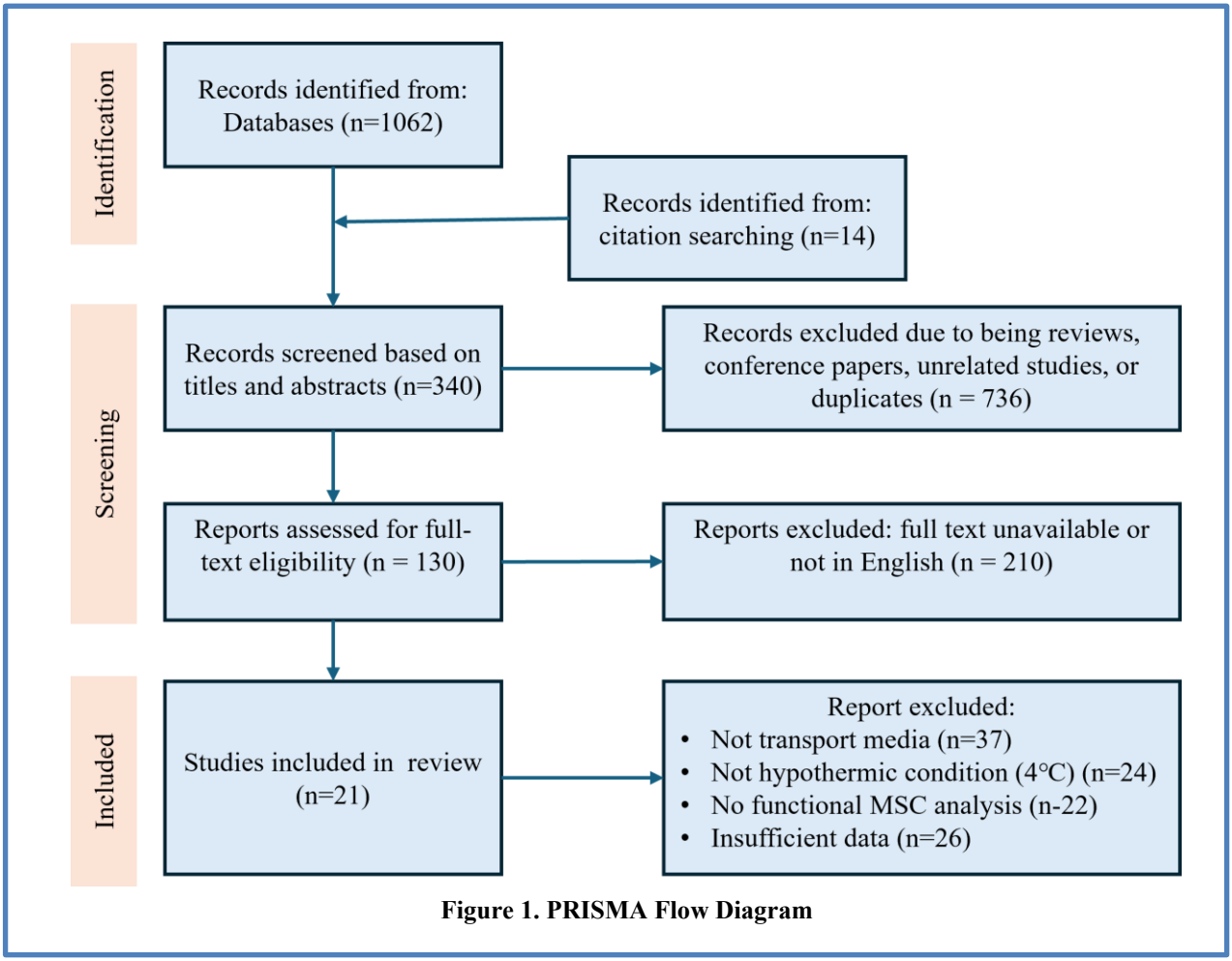


Figure 1. PRISMA Flow Diagram

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A total of 1,076 records were identified, including 1,062 records from electronic database searches and 14 records identified through citation searching. Following duplicate detection, publication-type filtering, and preliminary eligibility assessment, 736 records were removed, leaving 340 records for formal title and abstract screening. At this stage, 210 records did not meet the predefined language, publication availability, or eligibility criteria. The full texts of the remaining 130 reports were assessed for eligibility. Of these, 109 reports were excluded because they did not evaluate transport media (n = 37), were conducted under non-hypothermic conditions (n = 24), did not assess MSC functional outcomes (n = 22), or contained insufficient methodological information (n = 26). Ultimately, 21 studies were included in the qualitative synthesis (**Figure 1**).

Methodological Quality of the Included Studies

Among the 21 included studies, nine were rated as having low concern of the methodology, eleven as having some concern and one as having high concern (Table 1). The most common limitations were lack of reporting of biological replication at the level of the donor, wide or inconsistent passage reporting, limited temperature monitoring, and use of adherent monolayer or encapsulated-cell models instead of clinically realistic suspension transport. Several studies also mainly relied on viability or phenotypic markers, instead of directly assessing clinically relevant functional potency. Core outcome assays, nominal storage temperatures and durations and preservation media were mostly adequately described. Therefore, the results of studies using surrogate outcomes or non-transport models were interpreted with caution when developing practical recommendations.

Characteristics of Included Studies

A total of 21 studies met the inclusion and exclusion criteria and were included in this review. The sources of MSCs varied among the studies, with bone marrow-derived MSCs reported 8 studies, adipose tissue-derived MSCs in 8 studies, umbilical cord-derived MSCs in 6 studies, and amniotic fluid-derived MSCs in 1 study. Because one study investigating more than one MSC source, these categories were not mutually exclusive. Most studies employed MSCs at passages 2–5, while only one study used passage 6 and another used passage 8 (**table 2**).

Considerable heterogeneity was observed among the included studies in terms of MSC source, preservation duration, and outcome assessment methods. Differences in MSC tissue sources may influence the baseline characteristics and responses of cells to hypothermic preservation, as tissue-specific MSC populations have been reported to differ in their differentiation, immunomodulatory, and paracrine properties.[19] In addition, variations in preservation duration may affect the degree of cellular stress and consequently influence cell viability and functional characteristics.[20] The outcome assessment methods also varied across studies, with some studies focusing primarily on viability, whereas other evaluated additional quality attributes such as immunophenotype, differentiation potential, immunomodulatory activity, proliferation, secretory function, or mitochondrial characteristics. Therefore, these differences should be considered when interpreting the consistency and comparability of findings across studies.

The hypothermic preservation solutions used across the studies were categorized into four groups: (1) crystalloid solutions, including Ringer's lactate, normal saline, plasma-lyte, and 5% dextrose; (2) colloid solutions, such as human serum albumin, human albumin, and hydroxyethyl starch; (3) preservation-specific solutions, including Celsior, HypoThermosol, ViaSpan, trehalose, and Rokepie; and (4) conditioned media, such as complete growth medium, Dulbecco's Modified Eagle Medium (DMEM), Hanks' Balanced Salt Solution (HBSS), Phosphate Buffered Saline (PBS), Roswell Park Memorial Institute (RPMI) 1640, Keratinocyte Serum-Free Medium (K-SFM), and Dulbecco's Phosphate-Buffered Saline (DPBS). Additionally, several studies incorporated antioxidant

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supplements—such as resveratrol, salubrinal, alginate, ascorbic acid, Sul-109, alpha-tocopherol, Trolox, and quercetin—to enhance MSC stability during hypothermic storage (**table 2**).

Table 1. Methodological quality assessment of the included studies

References	Study design and biological material	Preservation procedure	Outcome assessment	Overall concern
Ngo et al., 2021	Low	Low	Low	Low
Petrenko et al., 2019	Low	Low	Low	Low
Corwin et al., 2014	Some concern	Low	Low	Some concern
Swioklo et al., 2016	Low	Low	Low	Low
Gálvez-Martín et al., 2014	Some concern	Low	Some concern	Some concern
Chen et al., 2013	Some concern	Low	Low	Some concern
Abazari et al., 2017	Some concern	Some concern	Low	Some concern
Celikkan et al., 2019	Low	Low	Low	Low
Muraki et al., 2006	Some concern	Some concern	Low	Some concern
Di et al., 2012	Low	Low	Low	Low
Sohn et al., 2013	Low	Some concern	Low	Some concern
Ścieżyńska et al., 2021	Low	Some concern	Some concern	Some concern
Wicaksono et al., 2023	High concern	Some concern	High concern	High concern
Yu et al., 2018	Low	Low	Low	Low
Wada et al., 2023	Low	Low	Low	Low
Veronesi et al., 2014	Low	Low	Low	Low
Pal et al., 2008	Low	Low	Low	Low
Hajmousa et al., 2017	Some concern	Some concern	Low	Some concern
Branco et al., 2022	Low	Some concern	Low	Some concern
Freitas-Ribeiro et al., 2019	Low	Some concern	Low	Some concern
Wang et al., 2025	Low	Some concern	Low	Some concern

Abbreviations: Low, low methodological concern; Some concern, incomplete reporting or a methodological limitation that was not considered sufficient to invalidate the findings; High concern, a major limitation likely to materially affect interpretation. Overall concern was classified as low when all domains were rated low, as some concern when at least one domain had some concern without any high-concern domain, and as high when at least one domain was rated high.

Storage temperatures varied across the included studies. Most experiments evaluated refrigerated preservation at approximately 2–8 °C, particularly at 4 °C. However, several studies also evaluated broader controlled non-freezing conditions, including comparisons between refrigerated and room-temperature storage. These temperature differences may influence cellular metabolism, viability, recovery, and functional outcomes and should therefore be considered when comparing preservation

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media across studies. Because storage temperature was not uniform, favorable findings reported for a particular medium should be interpreted within the temperature condition evaluated in the corresponding study.

Regarding preservation duration, 6 (28%) studies evaluated storage for ≤ 24 hours, 9 (42%) studies assessed preservation for 1–6 days, and 6 (28%) studies examined durations of 7–21 days. Concentration used for these studies mostly in range of $1x10^4$ to $1x10^7$ cells/mL medium. The containers used for hypothermic preservation varied and included syringes, culture plates, and cryotubes. For viability assessment, most studies employed trypan blue exclusion and Annexin V/PI staining to determine apoptosis and necrosis. To evaluate the differentiation potential of MSCs, adipogenic, osteogenic, and chondrogenic assays were commonly performed. Additionally, one study examined the ability of MSCs to differentiate into myogenic, neurogenic, and epithelial lineages (**table 2**). Table 2 summarizes the principal characteristics and outcome-assessment methods of the included studies. Storage-temperature variations are described narratively below, whereas study-level methodological limitations are summarized separately in Table 1 to maintain the readability of Table 2.

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Table 2. Characteristics of included studies

No	References	Type of MSC	Passage	Hypothermic Solutions	Storage durations	Containers	Concentration	Viability	Differentiation	Other parameters
1	Petrenko et al, 2019[21]	Human Bone Marrow	2-4	1. Buffer trehalose solution 2. Ringer's solution 3. Plasma-Lyte 4. HypoThermosol RFS	72 hours	cryovials	$1x10^7$ cells/mL	1. **Viability**: Trypan blue	1. **Adipogenic**: nile red fluorescence 2. **Osteogenic**: alizarin red	1. **MSC characteristics**: flow cytometry 2. **Immunomodulatory:** coculture with PBMCs, followed by Alamar Blue assay to assess the PBMC proliferation index. 3. **mRNA expressions**: IDO-1, PD-L1, and IL-6 4. **Recovery cells**: Alamar blue test
2	Corwin et al, 2014[22]	Human Bone Marrow	8	1. Complete Growth medium (GCM) 2. HBSS with Ca^{2+} and Mg^{2+} 3. Viaspan 4. CGM + 11 uM resveratol 5. HBSS + 11 uM resveratol 6. CGM + 25 uM salubrinal 7. HBSS + 25 uM salubrinal 8. Viaspan + 25 uM Salubrinal	72 hours	Culture plates	5.500 cells/well in 100 uL solution	1. **Viability:** Alamar blue 2. **Apoptosis:** propidium iodine (necrotic) and Yo-Pro-1 (apoptotic)	n/a	1. **Immunomodulatory**: caspase9, caspase3, caspase7, Bax, Bcl-2, Bid, Calnexin, PD1, GAPDH (western blot)
3	Swioklo et al, 2016[23]	Human Adipose	n/a	1. 1.2 % Alginate encapsulated with growth medium	72 hours	cryovials	2×10^6 cells/mL	1. **Viability:** Calcein-AM 2. **Proliferation:** methylene blue assay	1. **Adipogenic:** oil red O 2. **Osteogenic:** alizarin red	1. **MSC characteristics:** flowcytometry

				2. Without encapsulation				3. **Metabolic activity:** alamar blue 4. **Apoptosis:** Annexin/Propidium iodine	3. **Chondrogenic**: Alcian blue	
4.	Galvez-Martin et al, 2014[24]	Human adipose tissue	4	1. RL+5% glucose+20% albumin 2. RL+ 5% glucose 3. RL 4. 0.9% NaCl	60 hours	syringe	$1x10^6$ cells/mL	1. **Viability:** trypan blue	1. **Osteogenic:** Alizarin Red S 2. **Adipogenic:** Oil Red O	1. **hMSC karyotyping** 2. **Morphology characteristics**
5	Chen et al, 2013[25]	Human umbilical cord	3	1. 0.9% saline 2. 5% dextrose 3. Dextrose and NaCl 4. Plasma Lyte A 5. 1% HSA 6. 5% HSA	6 hours	n/a	2×10^6 cells/ml	1. **Viability:** trypan blue 2. **Apoptosis:** Annexin V/Propidium iodine	1. **Osteogenic**: Alizarin Red 2. **Adipogenic:** Oil Red O	1. **Cell Adhesion:** crystal violet 2. **Population double time**: replanted in 3 days → trypan blue 3. **Immunomodulatory:** coculture with PBMC → lymphocyte proliferation 4. **MSC Characteristics:** flow cytometry
6.	Abazari et al, 2018[26]	Human Bone marrow	2-10	1. Culture medium 2. Normosol-R 3. Plasma-Lyte A, 4. AQIX 5. Celsior 6. ViaSpan 7. HypoThermosol RFS	7 days	Culture plate	$1x10^4$ cells/well	1. **Viability:** Alamar Blue		1. **Mitochondrial assessment:** MitoTracker Red CMXRos
7.	Celikkan et al,2019[27]	Human umbilical cord	4	1. PBS + 1% HSA 2. PBS + 1% HSA + 10% HES	36 hours	n/a	$2x10^6$ cells/mL	1. **Viability:** trypan blue		1. **Proliferation doubling time:** trypan blue

				3. RL + 1% HSA 4. RL + 1% HSA + 10% HES						2. **MSC characteristics:** flow cytometry 3. **Actin quantity:** in-cell western assay **4. Karyotyping assay**
8.	Muraki et al, 2006[28]	Human bone marrow	n/a	1. PBS	24 hours	Culture plate	1x10^6 cells/mL	1. **Viability:** trypan blue	1. **Osteogenic:** alkaline phosphatase, 2. **In vivo osteogenic:** new bone formation in mice	1. **MSC characteristics**: Flow cytometry
9.	Di et al, 2012[29]	Human umbilical cord	4	1. Parental buffer: 140 mM NaCl, 5 mM KCl, 5 mM, glucose mixed 1:1 with the whole growth medium 2. Parental buffer + 20 mM trehalose 3. Parental buffer + 40 mM trehalose 4. Parental buffer + 60 mM trehalose 5. Parental buffer + 80 mM trehalose	3 weeks	n/a	2x10^6 cells/mL	1. **Viability:** trypan blue 2. **Apoptosis:** V-annexin-Propidium iodine	1. **Osteogenic:** alkaline phospahate 2. **Adipogenic:** Oil red O	
10	Sohn et al, 2013[30]	Human Bone marrow	2-3	1. 5% Dextrose 2. 0.9% NaCl	8 hours	n/a	5×10^5 cells/mL	1. **Viability:** Annexin V/propidium iodide	1. **Osteogenic:** von Kossa staining 2. **Chondrogenic:** safranin O	1. **MSC characteristics:** flow cytometry 2. **CFU assay:** culture

									3. **Adipogenic:** Oil Red O	
11	Ścieżyńska et al, 2021[31]	Human adipose tissue	3	1. 0.9% NaCl, 2. 0.9% NaCl + 5% glucose 3. lactated Ringer's solution 4. Plasmalyte 5. Hypothermosol RFS	120 hours	n/a	n/a	1. **Viability:** propidium iodine	n/a	n/a
12	Wicaksono et al,2023[32]	Human umbilical cord	6	1. 0.9% NaCl	24 hours	n/a	n/a	n/a	n/a	1. **MSC Characteristics:** flow cytometry
13	Yu et al, 2018[33]	Human amnion fluid	5	1. DMEM (H) 2. DMEM/F-12 3. K-SFM 4. RPMI 1640 5. α-MEM 6. DMEM(L)	48 hours	Plastic syringe and glass bottle	$1x10^8$ cells/mL	**1. Viability:** trypan blue	**1. Neurogenic:** nestin **2. Myogenic:** a-SMA **3. Epithelial:** pancytokeratine	**1. Stemness markers:** RT-PCR
14	Wada et al, 2023[34]	Human adipose tissue	3 and 4	1. RL solution 2. RL+ 1000 mg ASA 3. Cellstor-W 4. Cellstor-W + 1000 mg ASA 5. Cellstor-S 6. Cellstor-S + 1000mg ASA	7 days	1.5 mL microtubes	7.5×10^5 cells /1.5 mL	1. **Viability:** trypan blue	n/a	**1. MSC characteristic** → flow cytometry
15	Ngo et al, 2021[20]	Human bone marrow, adipose tissue, and umbilical cord	4	1. NaCl, 2. RL 3. NaCl+0.4% HA 4. RL+0.4% HA	72 hours	cryovial	$2×10^6$ cells/mL	**1. Viability:** trypan blue and 7-AAD	n/a	1. **Cell recovery assessment:** microplate reader 2. **Mitochondrial stress and glycolytic stress:** Seahorse 3. **Growth factor and cytokine assay** → multiplex immunoassay

16	Veronesi et al, 2013[35]	Human bone marrow	2	1. Maintenance media 2. 4% HSA 3. Normal saline	18 hours	syringe	2 x 10^7 cells/mL	1. **Viability:** Propidium iodine	1. **Osteogenic:** alkaline phosphatase and in vivo bone formation	n/a
17	Pal et al, 2008[36]	Human bone marrow	4	1. normal saline 2. 5% dextrose 3. plasmalyte A 4. DPBS	8 hours	n/a	1 × 10^8 cells/ml	1. **Viability:** 7-AAD	1. **Osteogenic:** calcium Von Kossa Staining 2. **Adipogenic:** Oil Red O staining	n/a
18	Hajmousa et al, 2017[37]	Human adipose tissue	3	**1.** Culture medium with Alpha-tocopherol **2.** Culture medium with Trolox **3.** Culture medium with SUL-109	48 hours	n/a	2 x 10^6 cells/ml	**1.** Viability: V Annexin/Ethidium	**1. Osteogenic:** Alizarin Red **2. Adipogenic:** Oil Red O **3. Myogenic:** SM22 Alpha	**1. Proliferation:** Ki67 **2. Metabolic status:** OCR **3. Extracellular acidification rate:** Seahorse **4. Mitocondrial morphology:** mitocondrial membrane potential independent dye **5. Mitocondrial membran potential:** JC-1 mitoprobe **6. ATP production:** ATP Determination kit **7. ROS production**: fluorescent ROS-indicator DHE
19	Branko et al, 2022[38]	Human adipose tissue	n/a	**1.** Encapsulation with alginate bead **2.** Non encapsulated	12 days	cryotubes	6 × 10^6 cells	1. **Viability:** trypan blue	**1. Osteogenic:** alkaline phosphate and von kossa staining **2. Adipogenic**: oil red O	**1. Hematopoietic support assay** **2. Glucose and lactate profile:** membrane-bound immobilized

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									3. **Chondrogenic**: alcian blue	enzyme quantification
20	Freitas-Ribeiro et al, 2019[39]	Human adipose tissue	4	1. Culture medium 2. Culture medium with rokepie 3. Hypothermosol	7 days	n/a	n/a	1. **Viability:** 7AAD	2. **Osteogenic** Alizarin Red S 3. **Adipogenic**: Oil Red O	1. **MSC characteristics:** flow cytometry 2. **Metabolic activity:** Alamar blue 3. **Extracellular matrix**: western blot and immunohistochemistry
21	Wang et al, 2025[40]	Human umbilical cord	3-5	1. Celsior (CS) 2. CS + Quercetin 5 uM 3. CS + Quercetin 10 uM 4. CS + Quercetin 20 uM	7 days	Culture plate	1×10^5 cells/mL	1. **Viability:** CCK8 kit assay 2. **Viability:** Calcein AM and propidium iodine staining	**Osteogenic**: safranin S staining	1. **Intracellular ATP assay**: Enhanced ATP Assay Kit 2. **MSC characteristics**: flow cytometry 3. **Oxidative stress**: Lipid Peroxidation MDA Assay Kit

Abbreviations: MSC, mesenchymal stem cells; PBMC, peripheral blood mononuclear cells; mRNA, messenger ribonucleic acid; IDO-1, indoleamine 2,3 dioxygenase-1; PDL-1, programed death ligand-1; IL-6, interleukin-6; HBSS, Hanks' Balanced Salt Solution; PD-1, programmed death-1; GADPH, glyceraldehyde-3-phosphate dehydrogenase; Calcein-AM, calcein acetoxymethyl ester; RL, ringer lactate; NaCl, sodium chloride; HAS, human serum albumin; HES, Hydroxyethyl Starch; KCl, Kalium chloride; RFS, Free Radical Scavenging; DMEM (H), Dulbecco's Modified Eagle Medium high glucose; K-SFM, Keratinocyte Serum-Free Medium; RPMI, Roswell Park Memorial Institute; α-MEM, Alpha modified Minimum Essential Medium; DMEM (L), Dulbecco's Modified Eagle Medium low glucose; PBS, phosphate buffer solutions; RT-PCR, Real time polymerase chain reaction; ASA, Ascorbic acid; 7-AAD, 7-amino-actinomycin D; HA, human albumin; D-PBS, Dulbecco's Phosphate Buffered Saline; SUL-109, 6-hydroxychromanol derivative compound; ATP, Adenosine triphosphate; OCR, Oxygen Consumption Rate; and MDA, Malondialdehyde.

hMSC viability in Crystalloid Based Solution

According to FDA guidelines, a viability threshold of >70% is generally required for clinical application of hMSCs. Crystalloids are affordable preservation media due to their low cost and wide availability. In the included studies, normal saline, Ringer's Lactate, and Plasma-Lyte were the crystalloid solutions most commonly used for MSC preservation. hMSC viability in crystalloid solutions was evaluated in 10 studies. Normal saline (0.9% NaCl, NS) is an isotonic solution with pH and osmolarity similar to human body fluids and does not contain additional potassium or calcium. Chen et al.[25] and Ścieżyńska et al.[31] demonstrated that hMSCs stored in NS maintained acceptable viability for more than 6 hours (83.02%) and up to 12 hours (82.7% ± 2.5%). Additionally, Ngo et al.[20] reported different viability patterns among AD-MSCs, BM-MSCs, and UC-MSCs stored in NaCl. AD-MSCs and BM-MSCs retained >70% viability during 24 hours of storage, whereas UC-MSCs dropped below 70% within the same period.

Ringer's Lactate (RL), a crystalloid solution used for fluid resuscitation, contains electrolytes such as sodium, chloride, lactate, potassium, and calcium. For Ringer's Lactate (RL)-based media, hMSCs showed viability of 81.3% ± 3.3% at 30 hours, but this declined to below 70% by 36 hours.[24] According to Ngo et al.,[20] AD-MSCs maintained 84.2% viability at cold temperatures in RL for up to 72 hours; however, BM-MSCs and UC-MSCs demonstrated viability below 70% at 72 hours.

Similar to other crystalloids, Plasma-Lyte is an isotonic electrolyte solution that also contains dextrose as an energy source. Its use as a preservation medium was reported by Petrenko et al[21], Chen et al,[25] Abazari et al,[26] Scienzynska et al,[31] and Pal et al.[36] Consistent with other crystalloid solutions, Plasma-Lyte maintained MSC viability above 70% for up to 24 hours. However, Scienzynska et al[31] observed a significant decline thereafter, with hMSC viability decreasing to approximately 50% at 48 hours and dropping to <15% by 72 hours.

hMSC viability in Colloid Solution

For preservation media, colloids were used as supplements to crystalloid solutions, typically at concentrations of 0.4–20% human serum albumin (HSA) or 10% hydroxyethyl starch (HES). Martín-Galvez et al.[24] reported that a combination of Ringer's Lactate, 5% glucose, and 20% albumin at 4 °C sustained hMSC viability at 82.2% ± 2.7% for 48 hours, which was higher than crystalloid solutions alone. In contrast, Chen et al.[25] found that adding 1% or 5% HSA to crystalloid media did not improve viability compared with crystalloid alone.

Celikkan et al.[27] demonstrated that crystalloid solutions supplemented with 1% HSA and 10% HES preserved MSC viability at 93.4% ± 1.5% at 6 hours, with viability remaining as high as 78% at 36 hours. Ngo et al.[20] further showed that the effects of HSA supplementation varied depending on the MSC source: in AD-MSCs, adding 0.4% HSA to normal saline did not enhance viability, whereas in BM-MSCs and UC-MSCs, 0.4% HSA provided a slight increase in viability during 72 hours of cold storage. Similarly, Veronesi et al.[35] reported that normal saline supplemented with 4% HSA maintained cell viability above 80% after 24 hours of cold storage, although the improvement was not significantly different from normal saline alone.

hMSC viability in preservation-specific solutions

Preservation-specific solutions are designed to maintain tissues or organs under hypothermic conditions by providing osmotic stabilization, supporting ion balance, reducing oxidative damage, and maintaining pH stability. This review evaluates the effectiveness of HypoThermosol®-RFS, ViaSpan®, Celsior®, and SUL109 (6-hydroxycromanol, Rokepie®)as preservation media for hMSCs. HypoThermosol® is an optimized hypothermic (2–8 °C), serum-free and xeno-free solution formulated

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to limit reactive oxygen species (ROS) formation during storage. Its use in hepatocyte preservation has been shown to improve cell viability and metabolic function.[41]Celsior® is another widely used preservation solution, originally developed for heart and lung transplantation. Its formulation is based on glutathione, lactobionate, and a low potassium concentration, providing effective buffering and antioxidant protection.[42] Meanwhile, ViaSpan® (University of Wisconsin solution), commonly used for pancreas, kidney, and liver transplantation, mimics the intracellular environment and helps maintain cell membrane integrity under cold storage conditions.[42] The used of rokepie was described by Haj Mosa dan Freitas Ribeiro. Rokepie is a 6-hydroxychromanol derivate SUL-109 that work as preservation that reduces ROS during cold preservation and during heating to 37 C. SUL109 can maintain in 48h viability ~84%, and still survive in 4 days, through blocking apoptosis and necrosis following the hypothermia.[37] However, these solutions are not intended for direct clinical infusion. Cells preserved in these media must be thoroughly washed prior to administration.

Some studies have reported inconsistent findings regarding the performance of preservation solutions. Petrenko et al.[21] and Abazari et al.[26] demonstrated that HypoThermosol® maintained >90% cell viability during 72 hours and up to 5 days of storage, with metabolic activity remaining at 97 ± 3% after 3 days.[31] In another study, Corwin et al.[22] reported that hMSCs preserved in ViaSpan® for 72 hours retained >80% viability, and Wang et al.[40] showed that viability after 5 days of cold storage remained >70%. In contrast, Abazari et al.[26] found that 3-day storage using ViaSpan® and Celsior® resulted in much lower viable cell recovery, at only 32 ± 4% and 16 ± 8%, respectively.

hMSC viability in Conditioned Medium

Conditioned media used for MSC preservation included complete growth medium (CGM), DMEM, HBSS, PBS, RPMI 1640, K-SFM, and DPBS. The CGM formulations varied across studies, but typically consisted of α-MEM supplemented with 10% FBS or human serum and 1% antibiotic–antimycotic, with or without additional glutamine. Abazari et al.[26] using the Alamar Blue assay to assess metabolic activity, reported a significant decline in MSC viability after 24 hours in CGM. This decrease was comparable to viability losses observed in other preservation-specific solutions (ViaSpan, Celsior), except for HypoThermosol-RFS, which performed better.

Yu et al.[33] found that DMEM (high glucose) provided the highest viability compared with K-SFM, DMEM/F-12, RPMI 1640, DMEM (low glucose), and α-MEM during 12 hours of storage at 4 °C. Veronesi et al.[35] reported that serum-free CGM maintained viability above 85% for up to 18 hours under cold transportation conditions. However, Hajmousa et al.[37] showed that AD-MSC stored in culture medium at 4 °C for 48 hours experienced a marked viability drop to 7%. Similarly, Di et al.[29] observed a significant decline to 29 ± 7% viability in UC-MSC preserved in culture medium for 7 days, and Freitas Ribeiro et al.[39] reported only 10.16% viability in AD-MSC after 7 days in culture medium.

hMSC Viability in Antioxidant-Supplemented Media

To enhance MSC stability during storage, antioxidant supplements have been added to preservation media to mitigate reactive oxygen species (ROS) accumulation and endoplasmic reticulum (ER) stress. Various antioxidants—including resveratrol, salubrinal, trehalose, ascorbic acid, Sul-109 (Rokepie), α-tocopherol, trolox, and quercetin—have been investigated for their ability to improve MSC stability during hypothermic storage. Studies by Corwin et al.,[22] Di et al.,[29] Hajmousa et al.,[37] and Wada et al.,[34] have reported varying degrees of effectiveness depending on the antioxidant and storage conditions.

Corwin et al.[22] demonstrated that resveratrol, a polyphenol with strong antioxidant and antitumor properties, when added to complete growth medium (CGM), increased MSC viability to 64.2 ± 6.1%

after 24 h of storage, compared with only 10% viability in the absence of resveratrol. In contrast, salubrinal—a selective inhibitor of protein phosphatase-1 that reduces ER stress–induced apoptosis—did not significantly improve cell viability.

Trehalose, a disaccharide known to stabilize cell membranes and proteins and to reduce dehydration, has shown more consistent benefits. Di et al.[29] reported that supplementation with 40 mM trehalose in complete growth medium containing electrolytes and glucose maintained hUC-MSC viability at 91.5 ± 3.8% after 1 week, 84.3 ± 5.0% after 2 weeks, and 71.1 ± 11.1% after 3 weeks. Similarly, Wada et al.[34] demonstrated that 3% trehalose in Ringer's lactate (RL) with 5% dextrose preserved adipose-derived MSC viability above 70% after 7 days. Furthermore, the addition of 1000 mg ascorbic acid to RL + 5% dextrose + 3% trehalose maintained cell viability of up to 90% for 21 days, with an optimal population doubling time of less than 24 h.

Stability of HMSC characterization after hypothermic storage

Human mesenchymal stem cell characteristics was assessed across studies using standard immunophenotyping criteria, defined by positive expression of CD73, CD90, and CD105, and negative expression of CD34, CD45, CD19, CD14, and HLA-DR.[43] Several studies reported preservation of MSC surface marker expression following cold storage in various media. Storage in normal saline, 5% dextrose, dextrose–NaCl, Plasma-Lyte supplemented with 1% human serum albumin (HSA), and 5% HSA maintained the expression of MSC-associated antigens, including CD29, CD44, and CD105.[21,24,25] Petrenko *et al.*[21] demonstrated that hMSCs stored for 72 h in trehalose-based solutions, Plasma-Lyte, Ringer's lactate, or HypoThermosol continued to meet standard immunophenotyping criteria. Comparable findings were reported by Galvez-Martin *et al.*,[24] who showed that storage in Ringer's lactate supplemented with 5% glucose and 20% albumin for 48 h resulted in immunophenotypic profiles comparable to unstored controls. In longer storage conditions, Freitas-Ribeiro *et al.*[39] found no differences in the expression of CD73, CD90, or CD105 following 7 days of storage in culture medium supplemented with Rokepie. Consistently, Wang *et al.*[40] reported that quercetin supplementation in Celsior solution did not affect hMSC surface marker expression. Similarly, Di *et al*.[29] reported that trehalose supplementation during extended storage (up to 3 weeks) did not alter MSC surface marker expression.

In contrast, limited evidence indicated potential marker modulation under specific conditions. Swiokło *et al.*[23] observed reduced expression of CD44 and CD166 after 72 h of storage in growth medium, although negative MSC markers (CD14, CD45, and HLA-DR) remained unchanged. Additionally, Wicaksono *et al*.[32] reported that prolonged storage in normal saline for up to 24 h was associated with decreased expression of CD73 (approximately 75%) and CD90 (below 90%).

HMSC Differentiation after hypothermic storage

According to the International Society for Cell and Gene Therapy (ISCT) criteria, human mesenchymal stem cells (hMSCs) must retain the ability to differentiate into three mesodermal lineages: chondrocytes, osteoblasts, and adipocytes. Overall, the available evidence indicates that prolonged cold storage during hMSC distribution does not substantially impair their differentiation capacity. Petrenko et al.[21] and Galvez-Martin et al.[24] demonstrated that storage at 4 °C for 48–72 h in trehalose-based buffer, Ringer's lactate (RL), HypoThermosol, or Plasma-Lyte preserved adipogenic and osteogenic differentiation. However, chondrogenic differentiation was adversely affected when RL and Plasma-Lyte were used as storage media. Swiokło et al.[23] reported that alginate encapsulation for 72 h did not compromise osteogenic or adipogenic differentiation, while Muraki et al.[28] similarly showed that cold storage in phosphate-buffered saline (PBS) for 24 h had no significant impact on multilineage

differentiation potential. Yu et al.[33] further demonstrated that hMSCs stored in culture medium for up to 48 h maintained their ability to differentiate toward myogenic, neurogenic, and endothelial lineages.

For extended storage durations, Di et al.[29] showed that hMSCs stored for up to three weeks in trehalose-supplemented medium retained the capacity to differentiate into mature adipocytes and osteocytes. Freitas Ribeiro et al.[16,39] reported that storage in Rokepie or HypoThermosol for seven days did not alter osteogenic or adipogenic differentiation, with lipid droplet formation comparable to fresh controls, whereas cells stored in unsupplemented media exhibited reduced lipid deposition. Additionally, Celsior media, supplemented with 20 uM quercetin did not impair osteogenic or chondrogenic differentiation, as evidenced by Alizarin Red S and Safranin O staining. Hajmousa et al.[37] also demonstrated preserved osteogenic, myogenic, and adipogenic differentiation of adipose-derived stem cells following 48 h of storage in culture media supplemented with α-tocopherol, Trolox, or SUL-109.

In contrast, Sohn et al.[11] reported that short-term storage in 5% dextrose or normal saline for as little as six hours was associated with a slight reduction in osteogenic and chondrogenic differentiation potential. Nevertheless, the underlying mechanisms and clinical relevance of this observation require further elucidation.

hMSC immunomodulatory effect after hypothermic storage

The immunomodulatory capacity of hMSCs is commonly assessed by their ability to inhibit phytohemagglutinin (PHA)–stimulated lymphocyte proliferation. Chen et al.[25] demonstrated that hMSCs stored for 6 h in 5% dextrose, normal saline, or human serum albumin (HSA) retained lymphocyte proliferation–inhibitory activity comparable to that of freshly cultured (unstored) hMSCs. Consistently, Petrenko et al.[21] reported that hMSCs preserved for up to 72 h in Ringer's lactate (RL), Plasma-Lyte, HypoThermosol, or trehalose-based buffered solutions maintained the ability to suppress PHA-induced lymphocyte proliferation. However, hMSCs stored in RL exhibited the weakest inhibitory capacity relative to the other storage media. Furthermore, hMSCs stored for 72 h continued to express key immunomodulatory molecules, including indoleamine 2,3-dioxygenase-1 (IDO-1), programmed death-ligand 1 (PD-L1), and interleukin-6 (IL-6), following stimulation with interferon-γ and tumor necrosis factor-α, indicating preservation of their immunomodulatory function after hypothermic storage.[21]

DISCUSSION

This review demonstrates that different hypothermic storage media have distinct capacities to maintain hMSC viability, and functional characteristics during storage at 2-8ºC. Overall, the findings suggest that the suitability of a preservation medium depends on the storage duration, composition of the preservation solution, MSC source, and quality attributes evaluated. Importantly, no single preservation medium consistently demonstrated superiority across all storage durations and functional outcomes. This heterogeneity should be considered when interpreting the findings and when selecting a preservation strategy for clinical transportation.

For short-term storage (<24 hours), conditioned medium and crystalloid solutions, such as normal saline (NS) and Ringer's lactate (RL), appeared to provide adequate preservation of hMSC viability. These findings are consistent with previous evidence demonstrating that relatively simple preservation conditions can maintain MSC viability and functionality during clinically relevant storage periods. Ngo *êt al*,[20] reported that RL was able to maintain high viability in bone marrow-derived MSCs for up to 72 hours at room temperature, while adipose- and umbilical cord-derived MSCs showed favorable viability under cold condition of 4-8ºC. Importantly, preserved cells also maintained adherence capacity, metabolic profiles, and the secretion of several growth factors and cytokines. These

findings support the potential practicality of simple preservation solutions for relatively short transportation periods. However, their favourable effect should be interpreted primarily in the context of short storage durations, as their ability to maintain more complex functional attributes during prolonged hypothermic exposure remains less certain.[20] These solutions help reduce ionic imbalance, preserve Na^+/K^+-ATPase activity, and maintain mitochondrial function, thereby supporting cell viability during hypothermic storage (**figure 2**).[44]

For intermediate storage (up to 3 days), colloid solutions including 20% human serum albumin (HSA) and 10% hydroxyethyl starch (HES) provide superior preservation. Their high-molecular-weight components maintain colloid osmotic pressure by limiting intracellular fluid loss and preventing excessive movement of small molecules into the extracellular space, thereby improving cellular stability (**figure 2**).[45] However, the apparent advantage of colloid-based formulations should not be interpreted as evidence that they are universally superior to other assessments across studies, make direct comparisons are difficult. In particular, preservation of cell viability does not necessarily indicate equivalent preservation of therapeutic functionality.

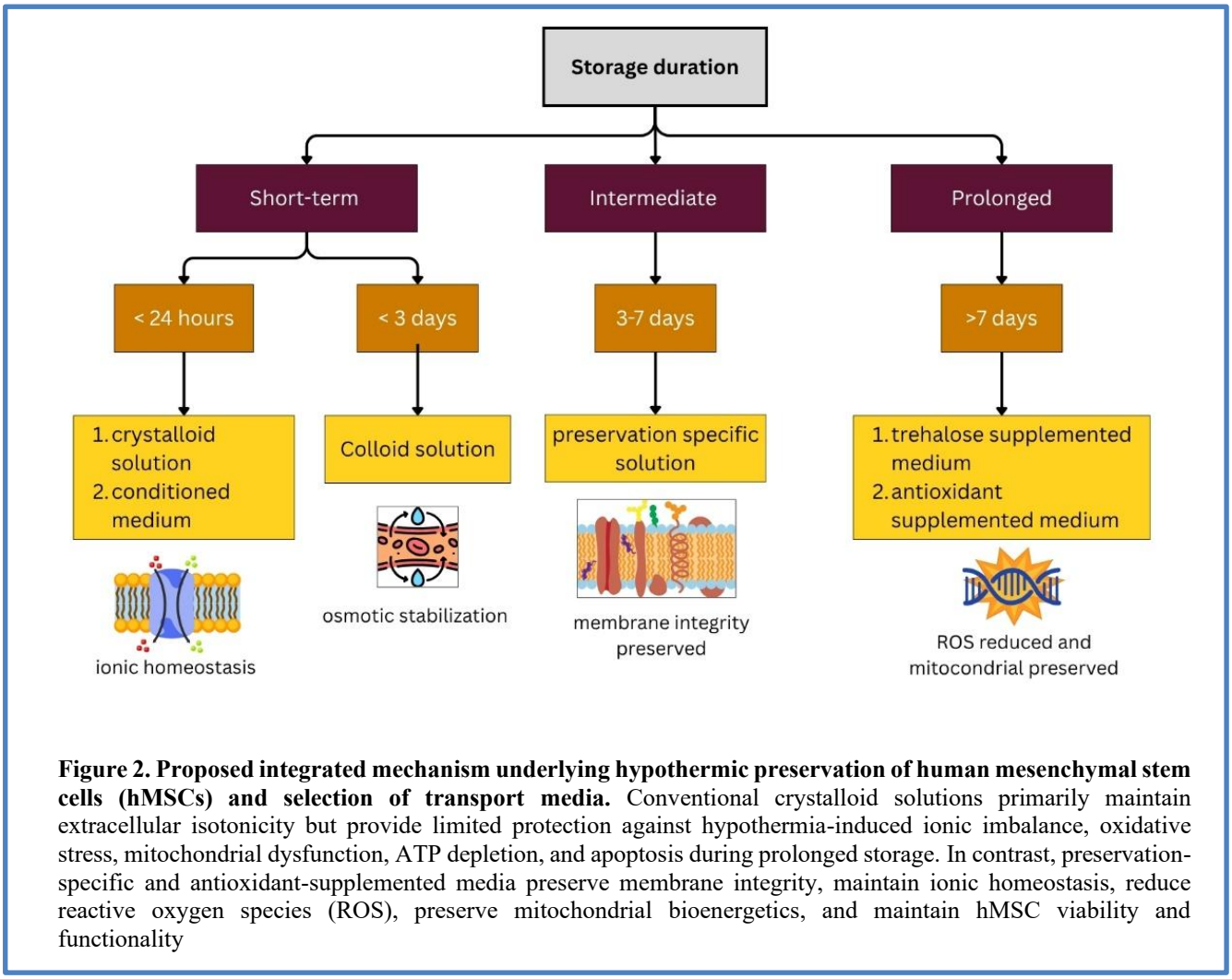


Figure 2. Proposed integrated mechanism underlying hypothermic preservation of human mesenchymal stem cells (hMSCs) and selection of transport media. Conventional crystalloid solutions primarily maintain extracellular isotonicity but provide limited protection against hypothermia-induced ionic imbalance, oxidative stress, mitochondrial dysfunction, ATP depletion, and apoptosis during prolonged storage. In contrast, preservation-specific and antioxidant-supplemented media preserve membrane integrity, maintain ionic homeostasis, reduce reactive oxygen species (ROS), preserve mitochondrial bioenergetics, and maintain hMSC viability and functionality

For longer storage periods of approximately 3–7 days, preservation-specific solutions, including HypoThermosol, ViaSpan®, and Celsior®, demonstrated potential advantages. These solutions are specifically formulated to mimic the intracellular environment, minimize metabolic activity, preserve membrane integrity, and reduce cold-induced cellular injury during hypothermic storage.[42]

Nevertheless, the evidence for prolonged storage remains heterogeneous, and the benefits observed for viability were not always accompanied by equivalent evidence for all functional characteristics. Therefore, preservation-specific solutions may be particularly promising for extended transportation, but their superiority should be evaluated using multiple potency-related outcomes rather than viability alone.

For storage exceeding 7 days, several studies investigated whether membrane-stabilizing and antioxidant supplements may further improve cell preservation. Trehalose, a water-soluble disaccharide widely used in cryopreservation, stabilizes cell membranes and proteins while preventing osmotic damage during storage.[46] However, the role of trehalose in hypothermic (non-freezing) storage remains insufficiently understood. Current evidence is limited, and its underlying mechanisms under cold storage conditions require further investigation through well-designed experimental studies, as most existing data are derived from cryopreservation models.

Meanwhile, the addition of antioxidants also used as supplemental in cold storage medium. Antioxidants, including SUL-109 (Rokepie), ascorbic acid, and α-tocopherol, help neutralize reactive oxygen species, reduce oxidative stress, preserve membrane integrity, and inhibit apoptosis-related signaling pathways, including p53 activation.[47] By mitigating oxidative damage induced during prolonged cold storage, these agents may contribute to improved cell survival and functional preservation (**figure 2**). Regarding phenotypic characteristics, differentiation potential, and immunomodulatory function.

Critical appraisal of conflicting evidence

It is important to note that the results from the various studies were not fully consistent when different preservation media or outcome measures were used. In several included studies, preservation media that maintained relatively high post-storage viability did not necessarily preserve other clinically relevant hMSC characteristics to the same extent, including proliferation, differentiation potential, immunomodulatory activity, secretory function, or mitochondrial function. This distinction is important because cell viability alone may not adequately reflect the functional quality or therapeutic potency of hMSCs after hypothermic storage. Petrenko et al, demonstrated that a trehalose-based preservation solution improved cell recovery, attachment, and subsequent proliferation compared with Plasma-Lyte® 148 and Ringer's solution, while immunophenotype, differentiation potential, and immunomodulatory properties were largely comparable among the tested solutions after 72 hours of storage.[21] Conversely, recent evidence indicates that metabolic and mitochondrial changes may occur before substantial loss of cell viability, suggesting that conventional viability measurements may underestimate cold-storage-induced cellular dysfunction. In a recent study of hMSCs stored at 4 °C, ATP content and mitochondrial membrane polarization declined substantially despite relatively modest changes in viability, particularly in PlasmaLyte A, whereas these parameters were better preserved in xeno-free medium.[20] Therefore, differences in preservation outcomes should be interpreted across multiple quality attributes rather than viability alone, particularly when considering the suitability of a medium for clinical translation .

Accordingly, the selection of a hypothermic preservation medium should be based on a composite assessment of viability and functional potency rather than on viability alone. This is particularly relevant for clinical translation, where preservation of the therapeutic properties of hMSCs is as important as maintaining an adequate number of viable cells.

The inconsistencies observed across studies may be attributable to both methodological and biological differences. MSCs derived from bone marrow, adipose tissue, umbilical cord, and other tissue sources exhibit distinct biological and functional characteristics, including differences in proliferation, differentiation capacity, immunomodulatory activity, and paracrine function.[19,48,49] These source-dependent characteristics may influence cellular responses to hypothermic stress and consequently

contribute to differences in viability and functional preservation across studies. Importantly, tissue-specific differences are increasingly recognized as relevant to MSC manufacturing and clinical translation, suggesting that preservation strategies may not necessarily have equivalent effects across different MSC sources.[19,50] Therefore, comparisons of hypothermic preservation media should consider MSC tissue origin as an important source of biological heterogeneity.

Tissue-specific heterogeneity is highly relevant to interpreting the present findings. Ngo et al. demonstrated that optimal preservation conditions vary by mesenchymal stem cell (MSC) tissue source: bone marrow-derived MSCs exhibited favorable viability under RL at room temperature, while adipose- and umbilical cord-derived MSCs were better preserved at 4–8 °C.[20] Therefore, differences observed between studies may not indicate that one preservation medium is inherently superior, but rather reflect interactions between the preservation formulation and the biological characteristics of the MSC source. In addition to MSC source, differences in preservation duration, cell concentration, passage number, medium composition, and post-storage recovery conditions may also contribute to inconsistent findings. Consequently, comparisons between preservation media should be interpreted cautiously when experimental conditions are not standardized.[51] Regarding phenotypic characteristics, differentiation potential, and immunomodulatory function, the available evidence generally suggests that several preservation approaches can maintain selected hMSC characteristics after hypothermic storage. However, the extent to which these characteristics are preserved appears to depend on the specific storage conditions and assessment methods. Therefore, the current evidence does not support the assumption that preservation of viability necessarily indicates preservation of the complete therapeutic phenotype or potency of hMSCs.

Clinical implications

From a clinical perspective, these findings have important implications for the transportation of hMSC-based products from centralized manufacturing facilities to geographically separated treatment centers. A preservation medium should ideally maintain not only cell viability but also clinically relevant quality attributes throughout the expected transportation period.[21] For short transportation periods, relatively simple crystalloid or conditioned-media formulations may be practical because of their availability, simplicity, and demonstrated ability to maintain viability under selected conditions. For longer transportation periods, preservation-specific formulations or appropriately supplemented media may provide greater protection against hypothermia-associated cellular injury.[21]

However, the findings do not support the selection of a single optimal preservation medium for all hMSC products. Instead, the choice of preservation medium should consider the intended transportation duration, MSC tissue source, cell concentration, required quality attributes, and manufacturing requirements. This consideration is particularly important because MSCs derived from different tissues exhibit distinct biological and functional characteristics, which may influence their response to preservation conditions.[52] Consistent with this concept, Ngo et al. demonstrated that bone marrow-derived MSCs showed favorable viability in Ringer's lactate at room temperature, whereas adipose- and umbilical cord-derived MSCs showed higher viability under cold conditions (4–8 °C), indicating that preservation requirements may differ according to MSC tissue source.[20]

The findings also have practical implications for centralized manufacturing models. A preservation strategy capable of maintaining acceptable viability and functionality for several days could potentially expand the geographic distribution of hMSC-based products beyond treatment centers located near manufacturing facilities. Ngo et al. demonstrated that selected preservation conditions maintained viability, adherence, metabolic profiles, and secretory activity of MSCs for up to 72 hours, supporting the feasibility of controlled hypothermic transportation for selected MSC products.[20] Similarly, Petrenko et al. reported that bone marrow-derived MSCs stored at 4 °C for up to 72 hours

retained viability, attachment and proliferation capacity, immunophenotype, differentiation potential, and immunomodulatory properties in selected preservation solutions.[21] Nevertheless, these findings should not be directly extrapolated to longer transportation periods without additional validation, as preservation performance may vary according to the cell source, formulation, and storage conditions.[20]

Clinical translation also requires consideration of factors beyond post-storage viability. Preservation media intended for clinical use should be compatible with cGMP manufacturing processes and demonstrate reproducible performance, sterility, appropriate formulation safety, and maintenance of relevant potency attributes after transportation. Petrenko et al. emphasized the need for preservation media that are nontoxic and capable of maintaining both viability and therapeutically relevant properties during hypothermic storage before clinical application.[21] In addition, evidence from hypothermic storage studies indicates that viability alone may not fully reflect preservation of cellular functionality; changes in metabolic activity, gene expression, and paracrine secretion may occur during storage and therefore warrant independent assessment.[51] Accordingly, the absence of a standardized testing framework across the included studies limits the ability to establish definitive clinical recommendations. Future studies should therefore use standardized storage durations and cell concentrations and evaluate a consistent panel of quality attributes, including viability, immunophenotype, differentiation potential, immunomodulatory activity, secretory function, and mitochondrial function.

The interpretation of the findings should consider the methodological limitations of the included studies. More than half of the studies had at least some methodological concern, most frequently because of incomplete reporting of donor-level biological replication, storage-container conditions, and actual temperature monitoring. Several investigations used adherent monolayers, culture plates, or encapsulated-cell models rather than MSC suspensions transported in clinically applicable containers. Furthermore, although viability was assessed in nearly all studies, fewer studies evaluated clinically relevant potency-related outcomes, such as immunomodulatory activity, post-storage proliferative recovery, or secretory function. Wicaksono et al. [32] was judged to have high methodological concern because donor and replication information was insufficient, passage reporting was inconsistent, statistical methods were incompletely described, and conclusions regarding functional preservation were inferred primarily from surface-marker expression. Storage temperature constituted an additional source of heterogeneity because the included studies did not uniformly apply the same refrigerated conditions. Consequently, differences attributed to preservation-medium composition may partly reflect temperature-dependent responses, and direct comparisons between media should be limited to studies conducted under reasonably comparable storage conditions. Moreover, incomplete reporting or monitoring of actual storage temperatures limited our ability to distinguish the effects of preservation-medium composition from those of thermal exposure. Therefore, preserved membrane integrity or cell viability should not be interpreted as equivalent to preserved therapeutic potency.

CONCLUSION

The available evidence suggests that crystalloid or selected culture-based media may be feasible for short-term hypothermic storage, whereas colloid-containing and preservation-specific formulations may offer advantages during longer storage. Trehalose- or antioxidant-supplemented media also showed potential for prolonged preservation. However, these duration-based patterns should not be interpreted as universal storage limits because the included studies were heterogeneous in MSC source, medium composition, cell concentration, temperature, storage duration, and potency assessment. Further standardized studies under clinically realistic transportation conditions are required before definitive recommendations can be established.

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RK and NRG were involved in concepting the topic of the manuscript, NRG and MU prepared the manuscript draft, RK and MU designed the figures and tables. All authors took parts in giving critical revision of the manuscript.

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Conflict of interest

The authors declare that there are no conflicts of interest related to this work.

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