

Effects of hyaluronic acid on mesenchymal stem cells and its potential use as a cryopreservative: a systematic review

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ABSTRACT

Hyaluronic acid (HA) is a substance commonly found in the human body that is involved in various biological processes. In 2021 and 2022, Asian and European researchers employed it as a cryopreservative for dental pulp progenitor cells, reporting high viability rates. This review aims to highlight the potential of HA as a preservative agent. Scientific literature available in PubMed, Scientific Electronic Library Online, and Medical Subject Headings was reviewed since 2000, identifying 28 publications of experimental studies that support the use of HA as a cryopreservative agent.

Keywords: hyaluronic acid; stem cells; cell differentiation; cell survival.

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INTRODUCTION

The International Society for Cellular Therapy defines minimal criteria for mesenchymal stromal cells (MSCs) as stromal cells that express mesenchymal markers and lack hematopoietic markers. *In vitro*, they differentiate into osteogenic, chondrogenic, and adipogenic lineages (1). These cells can be isolated from adult and perinatal issues, although in adults they occur at lower frequency and may have reduced proliferative potential. Nevertheless, up to 150 MSCs can be harvested, and they can replicate—an attribute that makes them useful for regenerating or improving various pathologically deteriorated anatomical structures (2).

Oral MSCs sources include dental pulp and apical papilla (3), as well as periodontal ligament and gingiva (4). Furthermore, they can resist cytotoxic agents, rapidly eliminating harmful xenobiotics and generating immune responses (5).

In the scientific field, cryopreservation is emphasized as the most appropriate method to preserve MSCs, maintaining viability and potential at low temperatures through cryoprotective agents, that prevent ice-crystal damage. For decades, dimethyl sulfoxide (DMSO) has been widely used; however, it can affect cellular processes and raises safety concerns (6). Hyaluronic acid (HA), a biocompatible polymer naturally present in human tissues, has been

explored as an alternative or adjunct cryoprotectant (7, 8). Owing to its water-retention capacity and viscoelastic behavior, HA can provide high viscosity even at low concentrations and absorb mechanical impact, features of interest in medicine and cryopreservation (9).

The purpose of this systematic review is to highlight the potential of HA as a preservative agent. Preliminary findings suggest its use could simplify and reduce the costs of the preservation process.

MATERIALS AND METHODS

A systematic search of the existing literature addressing the topic was conducted, in accordance with PRISMA Statement (10) and Cochrane Collaboration (11), to promote a comprehensive and transparent reporting. Several open-access academic databases were used, including PubMed, Google Scholar, Scientific Electronic Library Online (SciELO), and Medical Subject Headings (MeSH). The search was carried out using the terms “hyaluronic acid,” “cryopreservation,” and “stem cells,” combined into the following search strings: “stem cells and hyaluronic acid,” “stem cell cryopreservation with hyaluronic acid,” and “hyaluronic acid cryopreservative for stem cells.” Study selection followed predefined inclusion criteria and was performed independently by two reviewers; disagreements were resolved by consensus. In addition, the basic data and thematic

focus were used to address the PICO question, defined as follows: population (studies referring to hyaluronic acid as a cryoprotective agent); intervention (cryopreservation of stem cells); comparison (cell viability and potential of HA compared with other cryopreservation methods or agents); outcome (effectiveness of HA as a cryoprotective agent).

The search included randomized experimental trials whose interventions involved preservation, cryopreservation, latency promotion, differentiation demonstration, cytogenic induction, or therapeutic enhancement using or involving stem cells. Books, scientific articles, and doctoral theses were also considered if they were available in full text, written in Spanish, English, or Portuguese, and published since 2000. Only publications that specifically aimed to analyze the relationship between HA and stem cells were considered, which had to be the main subjects of study. Systematic reviews and studies involving non-mesenchymal stem cells were excluded.

To assess the methodological quality, the PRISMA statement (10) was applied, complying with the items indicated for structured abstracts (Table 1).

For risk of bias assessment, studies were categorized as Group A (low risk, n = 18) if they met all predefined criteria, Group B (moderate risk, n = 10) if they met at least one but not all criteria, and Group C (high risk, n = 0) if they met none of the criteria (Table 2).

Table 1. PRISMA checklist.

Topic	Checklist
Title	Effects of hyaluronic acid (HA) on mesenchymal stem cells (MSCs) and its potential use as a cryopreservative: a systematic review
Objective	To demonstrate that HA can function as a cryoprotective agent.
Methods	<i>Eligibility criteria:</i> Publications that aimed to analyze the relationship between HA and stem cells. <i>Information sources:</i> Scientific literature available in PubMed, Scientific Electronic Library Online (SciELO), and Medical Subject Headings (MeSH). <i>Risk of bias:</i> Studies meeting all criteria were assigned to Group A (low risk); those partially meeting one or more criteria were assigned to Group B (moderate risk); and those not meeting any were classified as Group C (high risk). <i>Synthesis method:</i> A summary of the risk of bias was prepared, presenting all assessments in a cross-tabulated format by domain and study (Table 2).
Results	<i>Included studies:</i> A total of 28 studies were selected—all randomized experimental trials conducted between 2000 and 2023—characterized by the use of human-derived MSCs. <i>Synthesis results:</i> In twelve trials, the intervention consisted of preservation and cryopreservation using HA; in two of these, cells were obtained from dental pulp, and in the remainder, from adipose tissue or Wharton’s jelly. Another large group comprised eleven studies that sought to demonstrate differentiation promoted by HA. Four studies showed that HA, as a cryoprotective agent, enhances the therapeutic potential of cellular material. Finally, only one study reported HA’s ability to facilitate gene transfer.
Discussion	<i>Limitations of the evidence:</i> The main limitations were the limited number of registered studies, heterogeneity in design and intervention components, differences in sample size and origin, duration of preservation or cryopreservation, handling methods of HA, and its concentrations. <i>Interpretation:</i> It can be stated that the use of HA enables the preservation of progenitor cells, including those obtained from the oral cavity.

Table 2. Assessment of risk of bias in randomized studies.

No.	Authors	Year	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of outcome assessment (detection bias)	Follow-up and attrition (exclusion bias)	Selective reporting (reporting bias)
1	Gerecht et al. (12)	2007	+	+	+	+	+
2	Chung et al. (13)	2009	+	+	+	+	+
3	Shukla et al. (14)	2010	+	+	+	?	+
4	Gojgini et al. (15)	2011	+	+	+	?	+
5	Schwartz et al. (16)	2011	?	+	+	+	+
6	Chang et al. (17)	2012	+	+	+	+	+
7	Turner et al. (18)	2012	+	+	+	+	+
8	Mohand-Kaci et al. (19)	2013	+	+	+	+	+
9	Lee et al. (20)	2014	+	+	+	+	+
10	Sawatjui et al. (21)	2015	+	+	+	+	+
11	Moreno et al. (22)	2015	+	+	+	+	+
12	Jensen et al. (23)	2015	+	+	+	+	?
13	Mineda et al. (24)	2015	+	+	+	+	+
14	Huang et al. (25)	2016	+	+	+	+	+
15	Aleksander-Konert et al. (26)	2016	+	+	+	+	+
16	Nevi et al. (27)	2017	+	+	+	+	+
17	Schmidt et al. (28)	2020	+	+	+	+	+
18	Ocampo et al. (29)	2020	+	+	+	?	+
19	Luo et al. (30)	2020	+	+	+	+	?
20	Liu et al. (31)	2020	+	+	+	+	?
21	Della Sala et al. (32)	2021	+	+	+	+	?
22	Lee et al. (33)	2021	+	+	+	+	?
23	Satin et al. (34)	2021	+	+	+	+	+
24	Shen et al. (35)	2021	+	+	+	+	+
25	Kaleka et al. (36)	2022	+	+	+	+	+
26	Pilbauerova et al. (37)	2022	+	+	+	+	+
27	Bar et al. (38)	2023	+	+	+	+	?
28	Ferroni et al. (39)	2023	+	+	+	+	+

Note: Green (+): low risk; yellow (?): moderate risk.

RESULTS

Regarding study selection process, 928 publications were identified. After excluding those that did not

meet the inclusion criteria, 28 studies were selected, all reported as randomized trials (Figure 1).

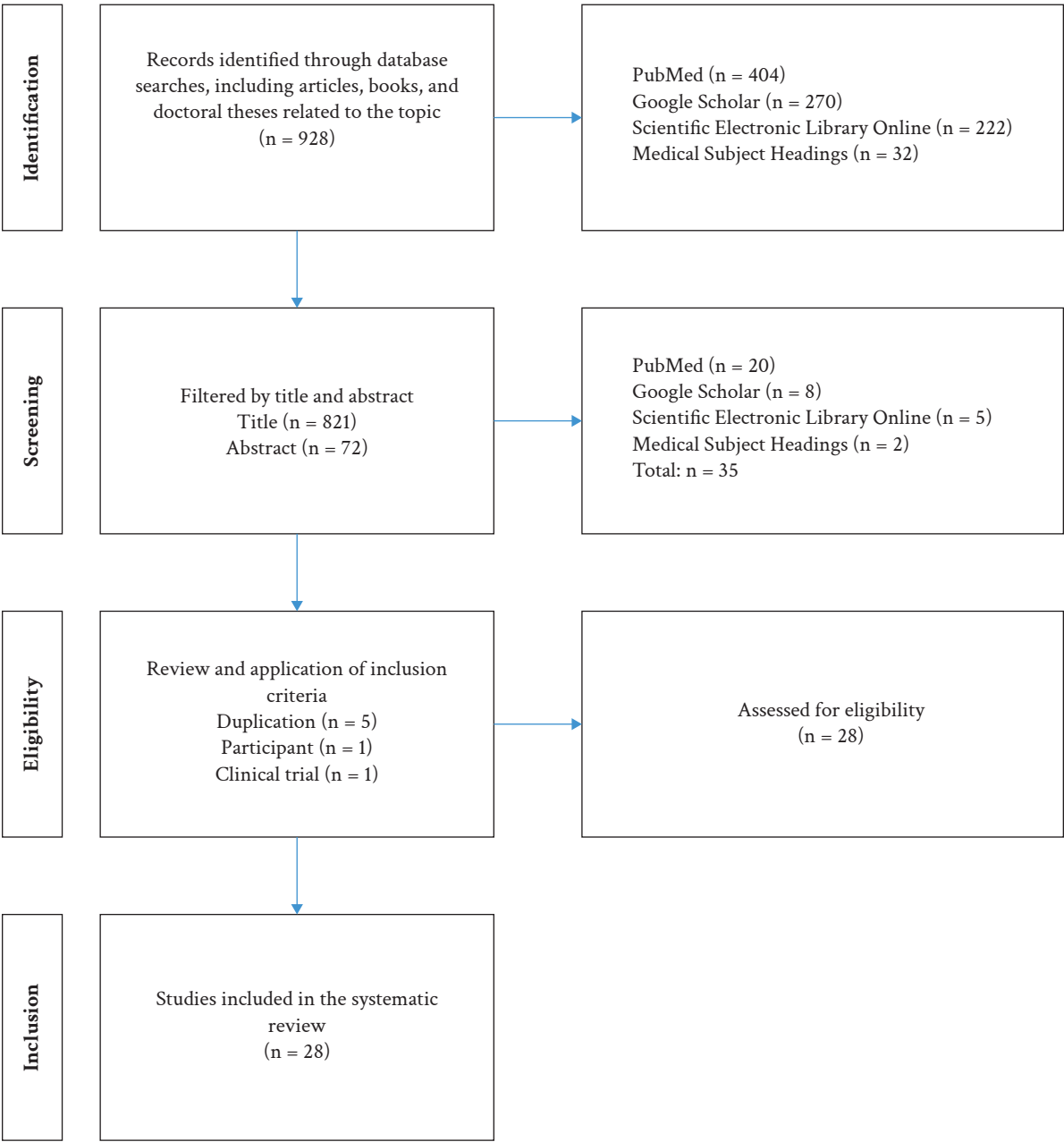


Figure 1. Flow diagram of the search and selection process of titles during the systematic review.

All included studies were experimental and conducted between 2000 and 2023, and used human-derived mesenchymal stem cells (MSCs). Across interventions, the

primary outcome was cell viability, with HA used in various formulations and concentrations (Table 2).

Table 3. Characteristics of the studies.

No.	Authors	Year	Country	Design	Intervention	Participants	Results
1	Gerecht et al. (12)	2007	USA	Experimental	Differentiation and self-renewal control of human embryonic stem cells.	Human embryonic stem cells	<i>Differentiation:</i> HA receptors CD44 (82%) and CD168 (90%).
2	Chung et al. (13)	2009	USA	Experimental	<i>Differentiation:</i> Chondrogenesis of MSCs in photocrosslinked HA hydrogels.	MSCs	<i>Differentiation:</i> In 2D-cultured MSCs, over 98% expressed CD44, and the HA receptor was present in 99.6% of the cell population, showing uniform staining.
3	Shukla et al. (14)	2010	USA	Experimental	Embryoid body differentiation: MSCs synthesize and organize hyaluronan and versican.	Differentiating embryoid body stem cells	<i>Differentiation:</i> Patterns of hyaluronan and versican accumulation and MSC organization within embryoid bodies are associated with epithelial–mesenchymal transitions occurring in differentiating cell aggregates.
4	Gojgini et al. (15)	2011	USA	Experimental	Modulation of gene transfer into stem cells within HA hydrogels.	MSCs (?)	<i>Viability:</i> 70% confluence.
5	Schwartz et al. (16)	2011	USA	Experimental	<i>Chondrogenic differentiation:</i> The effects depended on the dose of HA supplementation.	MSCs (?)	<i>Viability:</i> Similar among groups, with a mean of $79.0 \pm 15.6\%$. <i>Differentiation:</i> At 0.005% HA concentration, osteogenesis ranged from 20% to 24%.
6	Chang et al. (17)	2012	USA	Experimental	<i>Preservation:</i> Physical characterization and survival/proliferation measurement of stem cells encapsulated in hydrogels.	Cardiosphere-derived cells (CDCs)	<i>Viability:</i> In blood hydrogels with HA, $82.2 \pm 3.7\%$ on day 1, $54.5 \pm 5.5\%$ on day 3, and $32.4 \pm 12.4\%$ on day 7, compared with 0% for HA-PEG hydrogels ($p < 0.001$).
7	Turner et al. (18)	2012	USA	Experimental	Cryopreservation of serum-free cells.	Human MSCs	<i>Viability:</i> It remained between 79% and 87% across all buffers; HA buffers yielded higher CD44 expression in human hepatic progenitor and hepatoblast stem cells.
8	Mohand-Kaci et al. (19)	2013	France	Experimental	<i>Preservation:</i> Maintaining MSC characteristics via encapsulation in HA-based hydrogel.	MSCs	<i>Viability:</i> 100% ($n = 3$) on day 0; 80% in static and 120% in dynamic conditions after 21 days. <i>Differentiation:</i> Markers CD44 (89.31%), CD73 (95.03%), and CD90 (99.77%), characteristic of MSCs.
9	Lee et al. (20)	2014	South Korea	Experimental	<i>Differentiation:</i> Nodule and duct stem cells (NDSCs) differentiated into neural cells with an efficiency similar to that observed in very small embryonic-like stem cells (VSELs) under <i>in vitro</i> conditions.	NDSCs rich in HA and VSELs	<i>Viability:</i> NDSCs and VSELs have similar properties <i>Differentiation:</i> NDSCs express both mRNA and protein corresponding to the three markers at levels similar to those found in a murine embryonic stem cell line (ES D3).
10	Sawatjui et al. (21)	2015	Thailand	Experimental	<i>Differentiation:</i> Chondrogenic induction of bone marrow stem cells compared to a silk fibroin scaffold and conventional sediment.	Bone marrow stem cells	After 21 days, cells had a morphology similar to spherical and scattered chondrocytes. DNA content was significantly higher in silk fibroin with gelatin/chondroitin sulfate/hyaluronate cultures.

HA: hyaluronic acid; MSC: human mesenchymal stem cell; DPSC: dental pulp stem cell; (?): origin of MSCs not specified.

Tabla 3. (Continuation).

No.	Authors	Year	Country	Design	Intervention	Participants	Results
11	Moreno et al. (22)	2015	Spain	Experimental	Knee osteoarthritis therapy: To evaluate <i>in vitro</i> effects of HA on adipose-derived MSCs.	Adipose-derived stem cells (ASCs)	<i>Viability:</i> The number of ASCs increased with HA ($158 \pm 39\%$; $p < 0.05$). <i>Differentiation:</i> No change in CD44 expression or chondrogenic differentiation was observed.
12	Jensen et al. (23)	2015	Denmark	Experimental	<i>In vitro</i> osteogenic differentiation of dental pulp cells on polycaprolactone scaffolds with HA and β -tricalcium phosphate facilitates cell migration.	Dental pulp MSCs	<i>Differentiation:</i> More than 95% of total cells expressed osteogenic MSC markers CD90+, CD73+, and CD105+.
13	Mineda et al. (24)	2015	Japan	Experimental	<i>Therapy:</i> Derivatives of human adipose tissue, in the form of stem cell/stromal microspheres, have therapeutic potential.	Human adipose-derived stem cells	<i>Differentiation:</i> Approximately 40% of ASC spheroids tested positive for SSEA-3, a marker of pluripotent stem cells (muse cells).
14	Huang et al. (25)	2016	China	Experimental	<i>Preservation:</i> To determine the effects of a hydrogel on the growth and differentiation of human adipose-derived stem cells.	Human adipose-derived stem cells	<i>Viability:</i> Good (35,000 cells/mm ²). <i>Differentiation:</i> More than 90% of the cells expressed CD105 (99.4%) and CD90 (99.7%), and most showed that the cells were negative for CD34 (16.4%) and CD45 (0.1%), which were the hematopoietic stem cell surface markers.
15	Aleksander-Konert et al. (26)	2016	Poland	Experimental	<i>Preservation:</i> To evaluate two commercial HA-based hydrogels for Wharton's jelly MSC culture and chondrogenic differentiation.	Wharton's jelly MSCs	<i>Viability:</i> at an initial density of 15,000 cells per well, was 81.33% after 24 hours of culture. <i>Differentiation:</i> The cells showed positive expression for CD90, CD105, and CD73 (specific markers of mesenchymal cells) and negative expression for CD34, CD11b, CD19, CD45, and HLA-DR.
16	Nevi et al. (27)	2017	Italy	Experimental	<i>Preservation:</i> To develop a rapid, simple method to coat human biliary tree stem cells (hBTSCs) with HA.	Human stem cells coated and uncoated with hyaluronan (HA) and hBTSC	<i>Viability:</i> It was higher in HA-coated cells (70-90%) compared to uncoated hBTSC (69% to 85%). <i>Differentiation:</i> Positive expression of CD45, CD31, CD34, CD90, and α -SMA was observed (data not shown).
17	Schmidt et al. (28)	2020	Czech Republic	Experimental	<i>Preservation:</i> Assess the impact of low/high molecular weight HA on dental pulp stem cells (DPSCs) <i>in vitro</i> .	DPSC	<i>Viability:</i> It was higher than 94% in contrast to the control group. The latter showed greater positivity for surface markers CD29, CD44, CD73, and CD90.
18	Ocampo et al. (29)	2020	Brazil	Experimental	Chondrogenic differentiation <i>in vitro</i> of MSCs. The effect of HA and triamcinolone acetonide (TA), administered individually or in combination, was compared.	MSCs	Cell viability was $\geq 80\%$. Adhesion to the flask was achieved after 48 hours. <i>Differentiation:</i> HA stimulates the cells to adopt a morphology similar to that of chondrocytes (?).

HA: hyaluronic acid; MSC: human mesenchymal stem cell; DPSC: dental pulp stem cell; (?): origin of MSCs not specified.

Tabla 3. (Continuation).

No.	Authors	Year	Country	Design	Intervention	Participants	Results
19	Luo et al. (30)	2020	China	Experimental	HA-induced chondrogenic differentiation.	Human amniotic mesenchymal stem cells (hAMSCs).	<i>Differentiation:</i> hAMSCs highly expressed chondrogenic markers: CD90 ($91.30\% \pm 5.31\%$), CD73 ($91.22\% \pm 5.19\%$), CD105 ($96.88\% \pm 3.28\%$), and CD44 ($98.12\% \pm 3.49\%$).
20	Liu et al. (31)	2020	China	Experimental	Differentiation of stem cells into muscle cells through the action of myogenic growth factors present in a heparin-HA gel (hp-HA gel).	Human urine-derived stem cells.	<i>Differentiation:</i> After 28 days, the proportion of early myogenic markers relative to the human nuclear antigen ranged between 30% and 50% of total cells.
21	Della Sala et al. (32)	2021	Italy	Experimental	<i>Therapy:</i> Cell therapy based on MSCs to achieve lung tissue regeneration.	Mesenchymal cells (?)	<i>Viability:</i> Compared to DMEM® and SAGM® controls, viability exceeded 100% for samples containing LMWH and reached approximately 120% in samples with medium-molecular weight hyaluronic acid and secretion. <i>Differentiation:</i> After 21 days, positive expression of CD73 (red) was detected in cells maintained with DMEM®, indicating the presence of undifferentiated cells.
22	Lee et al. (33)	2021	China	Experimental	Cryopreservation of stem cells with HA.	Mesenchymal cells (?)	<i>Viability:</i> Between 80% and 87%. HA concentrations may have induced a cytotoxic effect during cell freezing, possibly due to CD44 receptor damage.
23	Satin et al. (34)	2021	USA	Experimental	<i>Preservation:</i> To evaluate the in vitro effect of platelet-rich plasma (PRP) and HA combination on cellular metabolism.	Bone marrow-derived MSCs.	<i>Viability:</i> Up to 600,000 cells using 5% PRP + 0.5 mg HA. <i>Differentiation:</i> Greater than 400,000 chondrocytes with 1% PRP + 0.25 mg HA.
24	Shen et al. (35)	2021	China	Experimental	<i>Preservation:</i> To evaluate colony formation and biocompatibility of MSCs cultured on a chitosan (Chi)-HA film combined with varying amounts of gold.	MSCs (?)	<i>Viability:</i> After 7 days, the number of colonies was higher in the Chi-HA-Au 50 ppm group ($\sim 553.2\%$; $p < 0.05$), compared to the Chi-HA-Au 25 ppm ($\sim 359.1\%$; $p < 0.01$) and Chi-HA ($\sim 252.4\%$) groups. <i>Differentiation:</i> Cells expressed surface markers CD29, CD44, and CD90, consistent with the phenotype of human MSCs.
25	Kaleka et al. (36)	2022	Israel	Experimental	<i>Preservation:</i> To evaluate <i>in vitro</i> the viability of adipose-derived MSCs in different commercial HA solutions.	Adipose-derived MSCs.	<i>Viability:</i> At 24 hours, viability with HA was 96.2%, and at 48 hours, it was 95.8%, values comparable to phosphate-buffered saline. Positive percentage ($>80\%$) for cell adhesion-related markers (CD29 and CD90), mesenchymal markers (CD73 and CD105), and class I MHC HLA-ABC.

HA: hyaluronic acid; MSC: human mesenchymal stem cell; DPSC: dental pulp stem cell; (?): origin of MSCs not specified.

Tabla 3. (Continuation).

No.	Authors	Year	Country	Design	Intervention	Participants	Results
26	Pilbauerova et al. (37)	2022	Czech Republic	Experimental	Cryopreservation of DPSC using an uncontrolled rate freezing method.	DPSC	Viability: Greater than 90%. Differentiation: Positive for CD29, CD44, CD73, and CD90.
27	Bar et al. (38)	2023	Switzerland	Experimental	Differentiation: To study the osteogenic potential of the DPSC.	DPSC	Differentiation: 63.6% ± 3.69%
28	Ferroni et al. (39)	2023	Italy	Experimental	Therapy: To demonstrate the potential of extracellular vesicles derived from MSCs to repair skin by modulating dermal fibroblast and endothelial cell function.	MSCs	Differentiation: MSCs showed positive expressions for surface markers CD44, CD73, CD90, and CD105, characteristic of these cells.

HA: hyaluronic acid; MSC: human mesenchymal stem cell; DPSC: dental pulp stem cell; (?): origin of MSCs not specified.

Table 3 shows the distribution of the screened trials across the databases searched. In twelve trials, the intervention involved preservation and cryopreservation with HA; in two of these, cells were derived from dental pulp, whereas in the remainder they originated from adipose tissue or Wharton’s jelly. Another eleven studies evaluated HA-induced differentiation. Additionally, four trials suggested that using HA as a preservation agent enhanced the therapeutic potential of the cells, and one study reported HA’s capacity to facilitate genetic transfer.

Table 3. Distribution of the studies.

Intervention	n	%
Cryopreservation	3	10.71
Preservation	9	32.14
Differentiation	11	39.28
Therapy	4	14.28
Genetic transfer	1	3.57
Total	28	99.98

DISCUSSION

There were several limitations, including the limited number of studies, heterogeneity in intervention design and components, sample size and origin, preservation or cryopreservation times, and how HA and its concentrations were handled. Moreover, not all studies clearly reported outcomes in terms of the percentage of viable cells or MSC markers. Notably, Gerecht et al. (12) stated that cellular self-renewal and differentiation are difficult to control when poorly defined culture systems are used. Importantly, the choice of cryoprotectant remains critical. While Schmidt et al. (28) and Pilbauerova et al. (37) concur that HA is an effective preservative agent, further evidence is required before concluding that HA could replace DMSO.

Pilbauerova et al. (37) recovered 390,000 cells approximately after thawing dental pulp progenitor cells and achieved between 2-3 million cells after one week of culture. Similarly, Lee et al. (33) reported that HA-supplemented cryopreservation media did not reduce ice-crystal size during freezing yet yielded 80-87% cell viability. Turner et al. (18) obtained comparable results using hyaluronan-supplemented buffers that preserved adhesion mechanisms and facilitated cryopreservation of human hepatic stem/progenitor cells. Kaleka et al. (36) reported 96.2% viability at 24 hours and 95.8% at 48 hours with HA.

Regarding therapeutic application, Shukla et al. (14) suggested that hyaluronans are associated with epithelial-mesenchymal transitions. Moreno et al. (22) used HA with adipose-derived MSCs to treat knee osteoarthritis. Mineda et al. (24) showed potential for angiogenesis and tissue regeneration, and Huang et al. (25) reported potential use in vocal fold repair via fibroblast differentiation. Schwartz et al. (16) indicated that adding HA to the chondrogenic milieu enhances cartilage production, and Sawatjui et al. (21), reached similar conclusions using a 3D SF-GCH scaffold that promoted MSC proliferation and chondrogenic differentiation.

Della Sala et al. (32) combined HA with secretome-based MSC therapy to promote lung tissue regeneration, improving viability and differentiation into type II alveolar cells. Lee et al. (20) reported similar benefits in partial repair of ischemic brain injury. Nevi et al. (27) found that HA coating could improve outcomes in stem cell-based therapies for hepatic disease, with potential for near-term clinical translation (40). Ferroni et al. (39) demonstrated the potential of human MSCs in skin repair.

Khetan et al. (41) used HA-based hydrogels with a gradual cooling and freezing protocol and achieved 70-90% viability, compared with 69-85% in uncoated cells. According to Chung and Burdick (13), such hydrogel systems provide beneficial interactions with encapsulated cells, this aligns with Chang et al. (17), who showed that blood-based hydrogels are adhesive, biodegradable, and support survival and cardiac functional improvement post-infarction. Mohand-Kaci et al. (19) obtained similar results in aortic repair using HA-based hydrogels. Liu et al. (31) employed a heparin-HA gel to promote the myogenic potential of human urine-derived stem cells, obtaining 30-50% differentiation after 28 days.

Jensen et al. (23) evaluated the osteogenic differentiation potential of human dental pulp stem cells using HA scaffolds, achieving osteogenic marker expression in more than 95% of the MSCs, while Schmidt et al. (28) reported >94% viability of dental pulp stem cells with low and high molecular-weight HA *in vitro*. Aleksander-Konert et al. (26) reported 81.33% viability of

Wharton's jelly-derived stem cells in HA during chondrogenesis, and Ocampo et al. (29) observed ≥80% with chondrocyte differentiation. Satin et al. (34) and Shen et al. (35) likewise reported high viability and chondrogenic differentiation in bone-marrow MSCs; two years later, Bar et al. (38) demonstrated the osteogenic potential of dental pulp stem cells with differentiation rates of $63.6 \pm 3.69\%$.

Gene transfer within HA hydrogels was also explored. Gojgini et al. (15) designed an HA hydrogel scaffold loaded with DNA, suggesting feasible gene transfer and the potential for localized gene therapy. Luo et al. (30) used RASL11B to direct differentiation, enhancing HA-mediated chondrogenesis in human amniotic MSCs with high expression of chondrogenic markers (e.g., CD90 91%, CD73 91.22%, CD105 96.88%, CD44 98.12%).

Among cryoprotectants, low molecular weight agents penetrate cell membranes, mitigate dehydration, and reduce ice-crystal injury. DMSO remains widely used but has documented adverse effects on cellular processes and metabolism (6). In this scenario, HA emerges as a valid alternative due to its accessibility, affordability, and demonstrated efficiency and efficacy. This could enable the implementation of oral stem cell banks in hospitals equipped with appropriate materials and personnel. In practice, oral MSC biobanks represent the most suitable strategy to support future clinical applications. They will allow the cryopreservation of viable and healthy oral stem cells to be used in clinical trials (42).

CONCLUSION

Evidence from the past 24 years derives from a limited set of randomized trials evaluating HA in progenitor cell culture and preservation. The studies suggest that HA may enhance viability and preserve cellular potential, including in cells derived from the oral cavity. However, heterogeneity in protocols, tissues, and cell characteristics limits comparability. Future research with more robust designs and standardized methods is needed to clarify HA's role in regenerative medicine.

Conflict of interest:

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SA: research, software, visualization, writing - review & editing.

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