

Systematic Review

The Role of Exosome-Loaded Hydrogels in Improving Intervertebral Disc Degeneration: A Systematic Review and Meta-Analysis of Preclinical Animal Studies

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Abstract

Objective: Intervertebral disc degeneration (IDD) is a major cause of chronic lower back pain, with current treatment options offering limited efficacy. Exosome-loaded hydrogels have emerged as a promising therapeutic approach due to their biocompatibility and regenerative potential, making them a focus of research for IDD treatment. This study systematically evaluates and performs a meta-analysis of the effectiveness of exosome-loaded hydrogels in preclinical models of IDD. **Methods:** A comprehensive literature search was conducted across four major databases (PubMed, Embase, Cochrane, Web of Science), including animal studies that met predefined criteria. Data extraction and quality assessment were independently performed by two authors. Treatment effects were quantified using standardized mean differences (SMD) with 95% confidence intervals (CI). Outcome measures included disc height index (DHI), magnetic resonance imaging (MRI) grade, histological grade, IDD-related immunohistochemical (IHC) markers (e.g., collagen type II (COL2), matrix metalloproteinase 13 (MMP13)), and aging-related markers (e.g., p16Ink4a-positive cells, p21CIP1A-positive cells). **Results:** Treatment with exosome-loaded hydrogels significantly enhanced DHI scores at 4 ($p = 0.002$) and 8 weeks ($p < 0.0001$), and decreased MRI scores at 8 ($p < 0.00001$) and 12 weeks ($p < 0.0001$), and histological assessments. Furthermore, the treatment group exhibited increased COL2 expression at 8 ($p = 0.0002$) and 12 weeks ($p = 0.002$), decreased MMP13 levels at 8 ($p = 0.0001$) and 12 weeks ($p = 0.0009$), and a reduction in aging markers (p16Ink4a, p21CIP1A, all $p < 0.05$), suggesting that exosome-loaded hydrogels facilitate intervertebral disc repair through the modulation of molecular pathways. Sensitivity analysis confirmed the robustness of the findings. **Conclusions:** Exosome-loaded hydrogels show potential for improving the structure and function of intervertebral discs in IDD treatment, potentially slowing degeneration by inhibiting matrix degradation and cellular aging. Further investigation is required to elucidate the underlying mechanisms and to assess the safety and efficacy of these hydrogels for clinical application. **The PROSPERO Registration:** CRD420250649970 (<https://www.crd.york.ac.uk/PROSPERO/view/CRD420250649970>).

Keywords: exosome; hydrogel; intervertebral disc degeneration; animal studies; meta-analysis

1. Introduction

Intervertebral disc degeneration (IDD) is a primary cause of chronic low back pain and spinal disorders [1]. The intervertebral disc plays a critical role in the spine by acting as a cushion between vertebrae, absorbing and distributing spinal pressure. It consists of the outer annulus fibrosus, inner nucleus pulposus, and transitional zone cells, with the nucleus pulposus containing significant water content to maintain disc elasticity and height [2]. Degeneration leads to reduced hydration of the nucleus pulposus and structural compromise of the annulus fibrosus, ultimately impairing the disc's cushioning and support functions. This may result in conditions such as disc herniation, spinal instability, and nerve root compression [3,4]. IDD development is influenced by multiple factors, including aging, genetics, lifestyle, and mechanical injury [5,6]. As aging pro-

gresses, the hydration and metabolic functions of intervertebral discs decline, resulting in structural degeneration [7]. Clinically, IDD manifests as chronic back pain, radiating leg pain, and reduced mobility, severely impacting patients' quality of life. One study indicates that approximately 80% of adults will experience some form of back pain in their lifetime, with a portion progressing to IDD [8]. Thus, effective treatment options are urgently needed on a global scale.

Current treatments for IDD include conservative management, pharmacological interventions, physical therapy, and surgical procedures. Conservative approaches, such as physical therapy, massage, and rehabilitation exercises, aim to alleviate pain and improve disc function [9]. However, these strategies primarily focus on symptom management and do not address the root cause of disc degeneration [10]. Pharmacological treatments, including non-steroidal



anti-inflammatory drugs (NSAIDs) and analgesics, provide temporary pain relief but may lead to side effects with prolonged use, such as gastrointestinal discomfort and kidney dysfunction [11]. In more severe cases, surgical treatments like discectomy and spinal fusion are employed as last-resort options. While these procedures relieve symptoms, they do not restore disc structure or function and carry the risk of postoperative complications, such as spinal instability and degeneration of adjacent segments [12].

In recent years, exosomes and hydrogels have emerged as promising biological therapeutic strategies [13, 14]. Exosomes, small vesicles secreted by cells, are rich in bioactive molecules such as proteins, lipids, and RNA. They exert a range of biological functions *in vivo*, including promoting cell repair, exerting anti-inflammatory effects, and offering anti-aging properties [15,16]. Hydrogels, a type of polymeric material, exhibit excellent biocompatibility, tunable mechanical properties, and the ability to mimic the extracellular matrix (ECM). These features make hydrogels highly suitable for drug delivery and tissue repair applications [17,18]. Combining exosomes with hydrogels leverages the three-dimensional structure and controlled release properties of hydrogels, facilitating efficient exosome delivery to the target area [19]. This combination promotes the repair of intervertebral disc cells and slows degeneration, holding significant potential for clinical application.

While some preliminary studies have investigated the individual effects of exosomes and hydrogels [15,17], there is a notable lack of evidence-based research on their combined use for treating IDD. This meta-analysis aims to systematically evaluate the effects of exosome-loaded hydrogels in animal models of IDD, with a focus on their potential to improve IDD. Specifically, the analysis assessed the impact of exosome-loaded hydrogels on disc height index (DHI), magnetic resonance imaging (MRI) scores, histological evaluations, and aging-related molecular markers. Additionally, an in-depth risk-of-bias analysis was conducted using quality assessment tools, providing robust scientific evidence to inform future clinical trials and facilitating the clinical translation of this therapeutic approach.

2. Methods

2.1 Search Strategy and Selection

Two independent authors conducted a comprehensive literature search across four major databases (PubMed, Embase, Cochrane, and Web of Science). The search strategy utilized Medical Subject Headings (MeSH) terms and corresponding free-text keywords, as follows: ((“Extracellular Vesicle” OR “Exosomes” OR “Cell-Derived Microparticles” OR “EV” OR “Exovesicle” OR “Apoptotic Body”) OR (“Hydrogel” OR “*In Situ* Hydrogel” OR “Patterned Hydrogel”)) AND (“Hydrogel” OR “*In Situ* Hydrogel” OR “Patterned Hydrogel”) AND (“Intervertebral disc degeneration” OR “IDD” OR “Disc Degeneration” OR “Degenerative Disc Disease”). Modifications to the search strat-

egy were made to align with the formatting requirements of each database. No restrictions were placed on the source of exosomes or the composition of hydrogels to ensure a thorough analysis. Following this, the authors systematically reviewed the titles, abstracts, and full texts of the identified studies to exclude irrelevant ones. The references of the full-text articles were carefully examined to ensure comprehensive inclusion of relevant studies in the meta-analysis. The study protocol was developed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [20] (**PRISMA_2020_checklist** can be found in **Supplementary material**) and registered in the PROSPERO database (registration number CRD420250649970).

2.2 Inclusion and Exclusion Criteria

Inclusion criteria were: (1) Animal models of IDD created through various methods such as needle puncture, tumour necrosis factor-alpha (TNF- α), or lipopolysaccharide (LPS); (2) Experimental groups treated with exosome-loaded hydrogel; (3) Control groups receiving placebo or no pharmacological intervention; (4) Reporting of at least one of the following outcomes: DHI grade, MRI grade, histological grade, and IDD-related immunohistochemical (IHC) markers (e.g., collagen type II (COL2), matrix metalloproteinase 13 (MMP13)); (5) Original studies published in English.

Exclusion criteria included: (1) Studies involving animal models unrelated to IDD; (2) *In vitro* studies only; (3) Experimental groups treated with hydrogels loaded with substances other than exosomes, such as nanoparticles; (4) Studies lacking a control group; (5) Studies that did not report primary outcome measures; (6) Non-original studies, including reviews, meta-analyses, commentaries, and letters to the editor.

2.3 Data Extraction

Data extraction was performed by two independent authors, followed by summarization and verification. Any discrepancies were resolved through discussion with a third author to ensure accuracy. The extracted data included: (1) Study characteristics (first author, publication year, country of origin); (2) Animal baseline characteristics (species, weight, quantity, age, and model creation methods); (3) Exosome and hydrogel characteristics (source and particle size of exosomes, hydrogel composition); (4) Treatment methods (administration routes, dosage, frequency, and duration); (5) Outcome measures (DHI grade, MRI grade, histological grade, IDD-related IHC markers such as COL2 and MMP13, and aging-related markers such as p16Ink4a-positive cells and p21CIP1A-positive cells). For studies presenting results in graphical format, data extraction was performed using Origin 2023 software (OriginLab Corporation, Northampton, MA, USA).

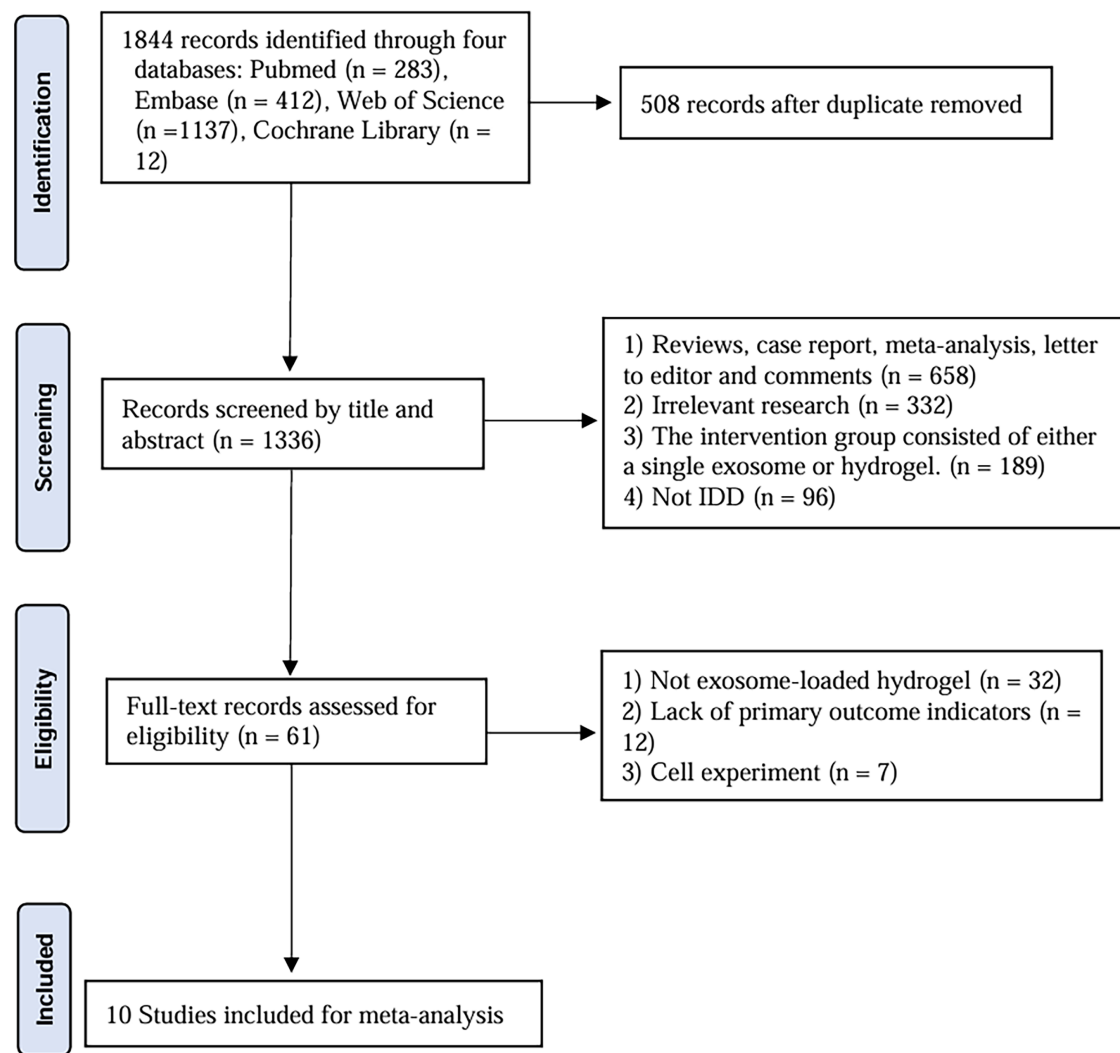


Fig. 1. Flow diagram of the study selection. IDD, intervertebral disc degeneration.

2.4 Primary and Secondary Outcome Measures

Primary outcomes included direct evidence of IDD progression or alleviation, specifically DHI grade, MRI grade, and histological grade. Secondary outcomes encompassed relevant IHC markers, including COL2, MMP13, p16Ink4a-positive cells, and p21CIP1A-positive cells.

2.5 Quality Assessment

The methodological quality of the included animal studies was assessed using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) risk of bias tool [21], which covers 10 assessment criteria: (1) Random sequence generation (selection bias); (2) Baseline characteristics (selection bias); (3) Allocation concealment (selection bias); (4) Random housing (performance bias); (5) Blinding (performance bias); (6) Random outcome assessment (detection bias); (7) Blinding (detection bias); (8) Incomplete outcome data (attrition bias); (9) Selective reporting (reporting bias); (10) Other bias. Two authors inde-

pendently evaluated each study. Positive responses to these items were classified as low risk, while unsatisfactory responses were marked as high risk. Items without a clear response were categorized as unclear risk. Any discrepancies in evaluation were resolved through discussion with a third author.

2.6 Statistical Methods

For the meta-analysis, mean values and standard deviations of reported indicators were extracted from each study, and a summary analysis of the overall effect size was performed. The combined effect size was assessed using the standardized mean difference (SMD) and 95% confidence interval (CI). Heterogeneity was evaluated using the I^2 statistic and χ^2 test, with $I^2 > 50\%$ indicating significant heterogeneity, in which case a random-effects model was applied. Otherwise, a fixed-effects model was used. For studies with significant heterogeneity, a “leave-one-out” sensitivity analysis was conducted to assess re-

Table 1. Main characteristics of animal study.

Author	Year	Country	Specie	Gender	Age	Weight	Number	Model of osteoporosis	Ref.
Liao <i>et al.</i>	2021	China	SD rats	male	8-week-old	300 g	20	TNF- α (50 ng/mL)	[22]
Liu <i>et al.</i>	2023	China	SD rats	NA	NA	250–300 g	20	needle puncture	[23]
Liao <i>et al.</i>	2022	China	SD rats	male	8-week-old	NA	30	needle puncture	[24]
Luo <i>et al.</i>	2021	China	Rats	male	2–3-week-old	NA	20	needle puncture	[25]
Xing <i>et al.</i>	2021	China	SD rats	male	NA	250–270 g	48	needle puncture	[26]
Shi <i>et al.</i>	2024	China	SD rats	NA	NA	NA	20	needle puncture	[27]
Zhan <i>et al.</i>	2025	China	SD rats	male	12-week-old	300–350 g	30	subendplate was injected with 20 μ L of lipopolysaccharide	[28]
Guan <i>et al.</i>	2023	China	SD rats	NA	12-week-old	250–280 g	NA	needle puncture	[29]
Liu <i>et al.</i>	2023	China	SD rats	male	12-week-old	NA	80	needle puncture	[30]
Peng <i>et al.</i>	2023	China	SD rats	male	6-week-old	NA	NA	needle puncture	[31]

SD rats, Sprague-Dawley rats; NA, not applicable.

sult robustness. Statistical significance was set at $p < 0.05$ for all analyses. RevMan 5.3.4 (Cochrane Collaboration, Northampton, MA, USA) and Stata 17.0 (StataCorp LLC, College Station, TX, USA) were used for the meta-analysis and sensitivity analysis.

3. Results

3.1 Study Selection

Fig. 1 illustrates the literature screening process. A total of 1844 potential articles were initially retrieved from four databases using a fixed search strategy. After removing duplicates with Endnote 20 (Thomson Corporation, Stamford, CT, USA), 1336 articles remained. Subsequently, 1275 articles were excluded based on title and abstract reviews. Of the remaining 61 articles, 51 were further excluded according to the inclusion and exclusion criteria. Finally, 10 articles [22–31] were included in the meta-analysis and quality assessment.

3.2 Characteristics of Included Studies

All included studies were published within the past four years and conducted in China. The characteristics of the animal models used are detailed in Table 1 (Ref. [22–31]). Notably, except for one study that did not specify rat species, all other studies employed Sprague-Dawley (SD) rats. Seven studies used male rats, with ages ranging from 2 to 12 weeks and weights ranging from 250 to 350 g. IDD was induced in eight studies via needle puncture, while the remaining two studies used TNF- α and LPS for induction. Table 2 (Ref. [22–31]) outlines the characteristics of the exosomes and hydrogels used. Of the studies on exosomes, two reported sourcing them from cartilage endplate stem cells and M2c macrophages, while the remaining studies used mesenchymal stem cells (MSCs) as the source. For hydrogels, three studies used ECM-based hydrogels, two used gelatin, and two used chitosan. Other hydrogel components included hyaluronic acid, alginate, and decellularized nucleus pulposus. The treatment details for exosome-loaded

hydrogels are also presented in Table 2. Treatment administration varied: two studies administered the treatment intravenously, while the other eight studies used local disc administration. Regarding dosing frequency, five studies administered a single dose, two studies administered treatment once a week, one study on the first and fourth weeks, and one study on the first and fifth weeks. One study did not provide specific dosing information. Treatment duration ranged from a single session to eight weeks. Given the variability in assessment time points across studies, a phased analysis was conducted for specific outcome measures based on consistent assessment intervals.

3.3 Methodological Quality Assessment

The methodological quality of the included studies was assessed using the SYRCLE RoB tool. One study reported allocation concealment for experimental animals, which was deemed to have a low risk of bias. However, no study described the random allocation method for animals, nor did any study report blinding methods for implementation or measurement. One study lacked a description of the baseline characteristics of the experimental animals, which was considered to carry a high risk of bias regarding baseline characteristics. Additionally, two studies did not report the number of experimental animals, leading to a higher risk of bias (Fig. 2A,B). Overall, most studies displayed an uncertain risk of bias.

3.4 DHI Grade

The DHI grade, reflecting changes in disc height, is a critical indicator for assessing recovery following IDD treatment. Given the varying treatment time points, changes in DHI grade were analyzed separately at 4 weeks and 8 weeks. At 4 weeks, the meta-analysis revealed that the exosome-loaded hydrogel group significantly increased the DHI grade compared to the control group (SMD = 2.07, 95% CI: 0.76 to 3.38, $p = 0.002$) (Fig. 3A). Similarly, at 8 weeks, the exosome-loaded hydrogel group demonstrated

Table 2. Characteristics of exosome and hydrogel and administration methods.

Author	Year	Characteristics of exosomes		Characteristics of hydrogel		Therapeutic method				Ref.
		Source	Diameter (nm)	Source	Thickness (nm)	Route	Dose	Time	Duration	
Liao <i>et al.</i>	2021	MSCs	NA	3% w/v solution of alginate, nucleus pulposus cells and 0.1 M calcium chloride	2 mm	discs	100 µg/mL, 2 µL	once	once	[22]
Liu <i>et al.</i>	2023	MSCs	NA	dopamine-functionalized gelatin	NA	discs	4 µL	once	once	[23]
Liao <i>et al.</i>	2022	MSCs	30–150 nm	decellularized ECM	10–20 nm	intravenously	2 µL with 10 µg exosomes	one a week	8 weeks	[24]
Luo <i>et al.</i>	2021	Cartilage endplate stem cells	NA	costal cartilage ECM	NA	discs	10 µL	once	once	[25]
Xing <i>et al.</i>	2021	ADMSCs	30–150 nm	thermosensitive acellular ECM	NA	discs	5 mL	once	once	[26]
Shi <i>et al.</i>	2024	MSCs	141 nm	gelatin methacrylate	20–50 µm	intravenously	2 µL	one a week	6 weeks	[27]
Zhan <i>et al.</i>	2025	MSCs	130 nm	CaCO ₃ /chitosan composite hydrogel	NA	discs	50 µL	week 1 and week 5	twice	[28]
Guan <i>et al.</i>	2023	MSCs	NA	quaternized chitosan and oxidized starch	NA	discs	3 µL	NA	NA	[29]
Liu <i>et al.</i>	2023	M2c macrophages	NA	1 mM hydrochloric acid and hyaluronic acid	NA	discs	5 µL	once	once	[30]
Peng <i>et al.</i>	2023	MSCs	50–200 nm	DNP hydrogels	NA	discs	2 µL	week 1 and week 4	twice	[31]

MSCs, mesenchymal stem cells; ADMSCs, adipose-derived MSCs; NPSCs, nucleus pulposus stem cells; ECM, extracellular matrix; DNP, decellularized nucleus pulposus; NA, not applicable.

a significant increase in the DHI grade (SMD = 3.15, 95% CI: 1.69 to 4.61, $p < 0.0001$) (Fig. 3B). These results suggest that exosome-loaded hydrogels show potential for improving intervertebral disc condition in IDD models, particularly in the early and middle stages of treatment.

3.5 MRI Grade

The MRI grade is an essential imaging tool for assessing structural, morphological, and pathological changes in the intervertebral disc. The meta-analysis showed that, compared to the control group, the exosome-loaded hydrogel group significantly reduced the MRI grade at 8 weeks (SMD = -3.16, 95% CI: -4.40 to -1.92, $p < 0.00001$) (Fig. 4A). Additionally, the exosome-loaded hydrogel group also significantly reduced the MRI grade at 12 weeks (SMD = -3.57, 95% CI: -5.52 to -1.89, $p < 0.0001$) (Fig. 4B). In conclusion, exosome-loaded hydrogels significantly influence MRI grading, helping slow the progression of degeneration.

3.6 Histological Grade

Additionally, the impact of the treatment on the histological grade of the intervertebral disc was assessed. Due to significant heterogeneity ($I^2 = 61\%$, $p = 0.03$), a random-effects model was applied. The meta-analysis results revealed that the exosome-loaded hydrogel group significantly reduced the histological grade of the intervertebral disc (SMD = -4.49, 95% CI: -6.72 to -2.27, $p < 0.0001$) (Fig. 5). These findings suggest that exosome-loaded hydrogels may play a pivotal role in slowing the degeneration of the intervertebral disc and improving its function.

3.7 Exosome-Loaded Hydrogel Improves Histological Evaluation of IDD

COL2 and MMP13 are key biomarkers involved in the degeneration, repair, and regeneration of the intervertebral disc. Meta-analysis results showed that the exosome-loaded hydrogel significantly upregulated the expression of COL2 protein in the IDD model at both 8 weeks (SMD = 4.66, 95% CI: 2.17 to 7.15, $p = 0.0002$) and 12 weeks (SMD = 5.10, 95% CI: 1.92 to 8.27, $p = 0.002$) (Fig. 6A). Conversely, the exosome-loaded hydrogel significantly downregulated the expression of MMP13 protein in the IDD model at 8 weeks (SMD = -6.09, 95% CI: -9.23 to -2.95, $p = 0.0001$) and 12 weeks (SMD = -14.16, 95% CI: -22.48 to -5.84, $p = 0.0009$) (Fig. 6B). These results suggest that exosome-loaded hydrogels may facilitate intervertebral disc repair and inhibit matrix degradation by upregulating COL2 and downregulating MMP13.

3.8 Exosome-Loaded Hydrogel Improves Cellular Aging in IDD

Regarding cellular aging, p16Ink4a and p21CIP1A proteins are critical markers for assessing the aging, proliferation, and repair of intervertebral disc cells. Two studies

evaluated the effects of exosome-loaded hydrogels on cellular aging in the IDD model. Meta-analysis results showed that, compared to the control group, the exosome-loaded hydrogel significantly reduced the number of p16Ink4a-positive cells and p21CIP1A-positive cells at both 8 weeks and 12 weeks (Fig. 7A,B). These findings suggest that exosome-loaded hydrogels may promote disc repair by slowing or reversing cellular aging during the later stages of treatment.

3.9 Subgroup Analysis

Four studies reported MRI grading of intervertebral discs at 8 weeks following treatment with exosome-loaded hydrogels, including two studies using gelatin-based hydrogels and two using composite hydrogels. Subgroup analysis revealed that both types of exosome-loaded hydrogels effectively reduced MRI grading of intervertebral discs (Supplementary Fig. 1), with no significant heterogeneity observed ($I^2 = 0\%$).

Due to significant heterogeneity in the pooled analysis of histological grade ($I^2 = 61\%$), subgroup analyses were conducted based on hydrogel type, exosome source, and animal model characteristics to identify potential sources of heterogeneity. Regarding hydrogel type, studies were classified into ECM-based hydrogels and composite hydrogels. Both types of exosome-loaded hydrogels significantly reduced histological grading of intervertebral discs (Supplementary Fig. 2). However, significant heterogeneity persisted in the composite hydrogel subgroup ($I^2 = 60\%$), suggesting that hydrogel type might not be the primary source of heterogeneity. When analyzed by exosome source, subgroups were defined as MSC-derived and M2c macrophage-derived exosome. The results showed that MSC-derived exosome-loaded hydrogels significantly reduced histological grade (SMD = -5.45, 95% CI: -7.13 to -3.77, $p < 0.00001$) (Supplementary Fig. 3), indicating their potential efficacy in mitigating IDD. Subgroup analysis based on administration route revealed that the intradiscal injection group significantly reduced histological grade (SMD = -3.89, 95% CI: -6.12 to -1.66, $p = 0.0006$) (Supplementary Fig. 4). Among the six included studies, five reported on intervention frequency. Subgroup analysis demonstrated that, regardless of whether a single or multiple injection protocol was used, exosome-loaded hydrogels significantly reduced histological grade (Supplementary Fig. 5).

Subgroup analyses based on animal model characteristics also revealed valuable insights. The modeling method-based subgroup analysis showed that exosome-loaded hydrogels reduced histological scores in both the needle puncture subgroup and the LPS/TNF- α subgroup (Supplementary Fig. 6). In the age-based subgroup analysis, the six included studies were divided into two groups: 8-week-old and 12-week-old animals. Pooled results indicated that exosome-loaded hydrogels reduced histological

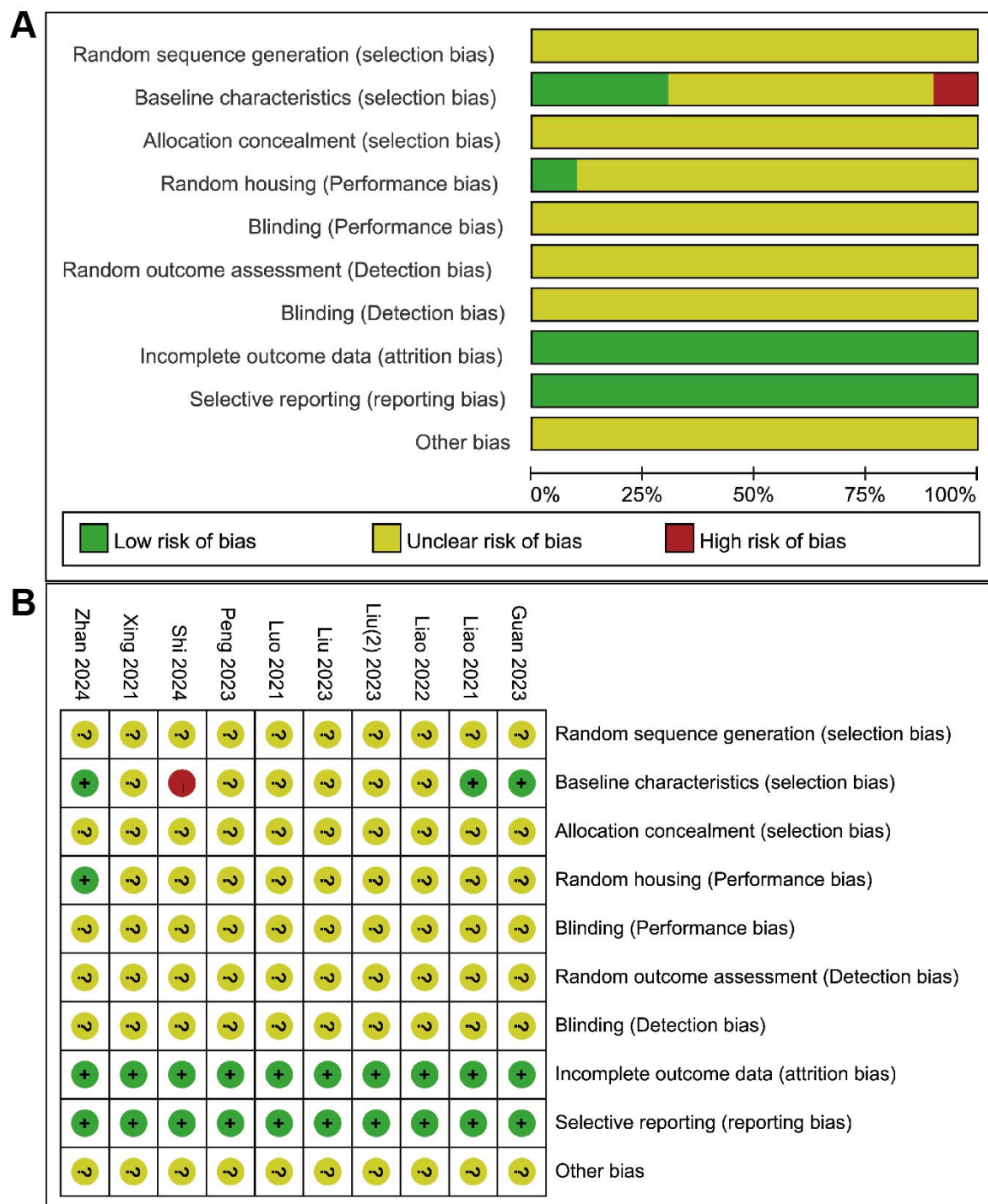


Fig. 2. Risk of bias assessment based on the SYRCLE's RoB tool. (A) Risk of bias graph for the included studies. (B) Risk of bias summary for each included study.

grade in both age subgroups, demonstrating a beneficial effect in ameliorating IDD (**Supplementary Fig. 7**). Despite the observed effects across all subgroups, factors such as hydrogel type, exosome source, administration route, injection frequency, modeling method, and animal age may not be the primary contributors to the observed heterogeneity.

3.10 Sensitivity Analysis

Given the significant heterogeneity in the combined effect size for histological grade ($I^2 = 61\%$), a sensitivity analysis was conducted to assess the robustness of the results. The sensitivity analysis revealed that, excluding individual studies, the size and direction of the combined effect size remained consistent (Fig. 8), suggesting that the summary analysis results for histological grade are robust.

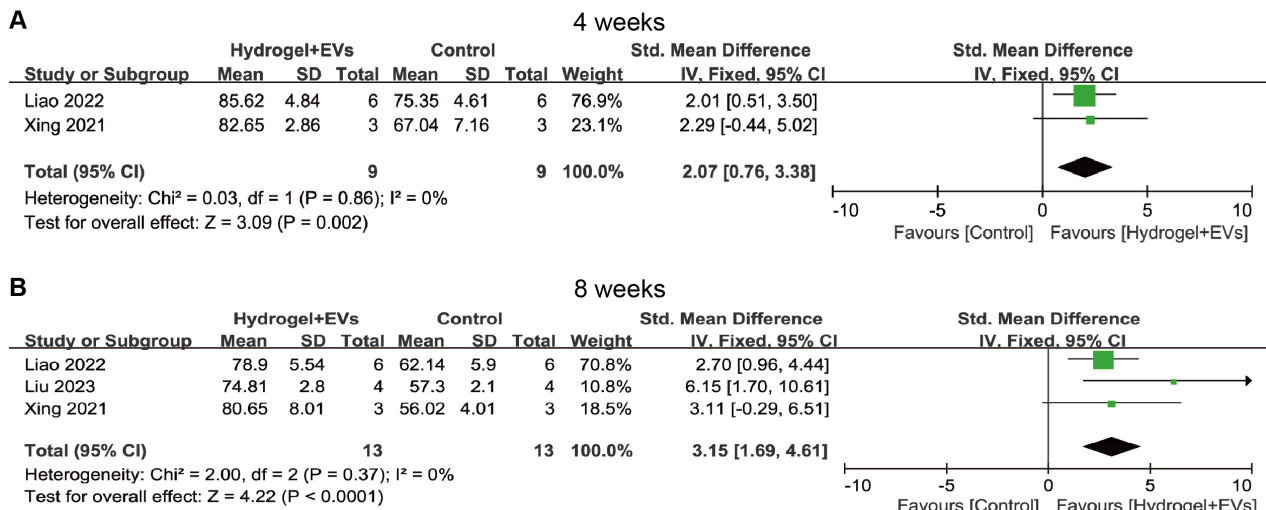


Fig. 3. Forest plot showing the effect of exosome-loaded hydrogel on disc height index (DHI) grade at 4 weeks (A) and 8 weeks (B). EVs, extracellular vesicles; CI, confidence intervals.

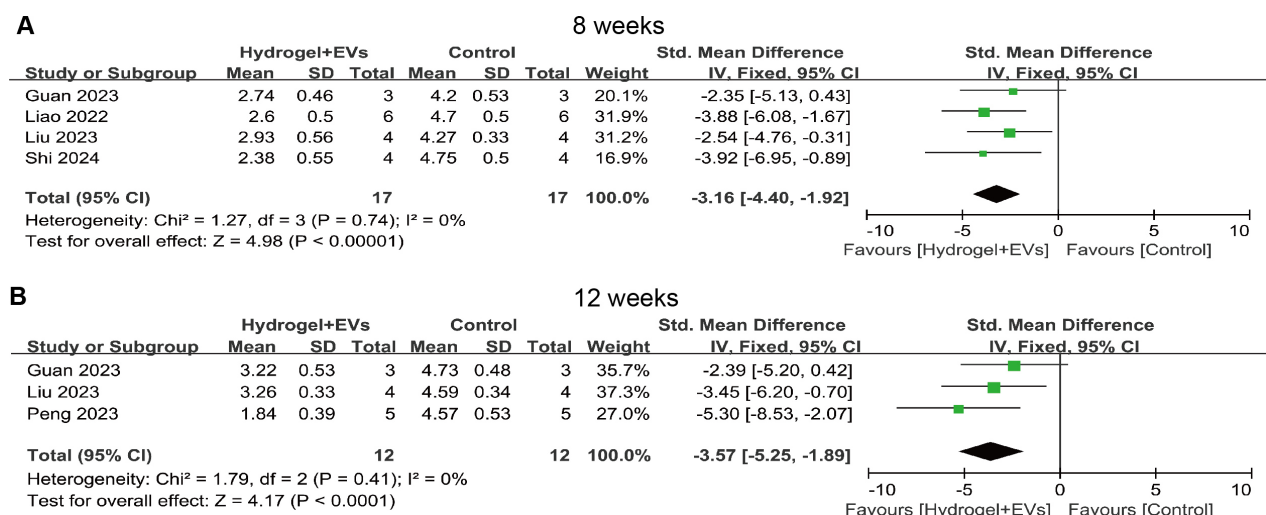


Fig. 4. Forest plot showing the effect of exosome-loaded hydrogel on magnetic resonance imaging (MRI) grade at 8 weeks (A) and 12 weeks (B).

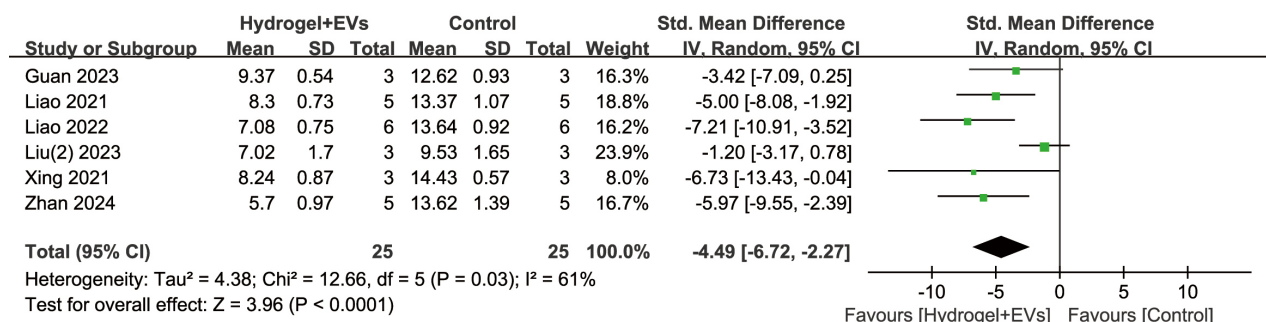


Fig. 5. Forest plot showing the effect of exosome-loaded hydrogel on histological grade.

4. Discussion

4.1 Main Findings

This meta-analysis demonstrated that, compared to the control group, exosome-loaded hydrogels significantly

improved the DHI scores, reduced MRI and histological scores, and enhanced the structure of intervertebral discs in IDD models, thereby slowing the degeneration process. Additionally, at the microscopic level, exosome-loaded hy-

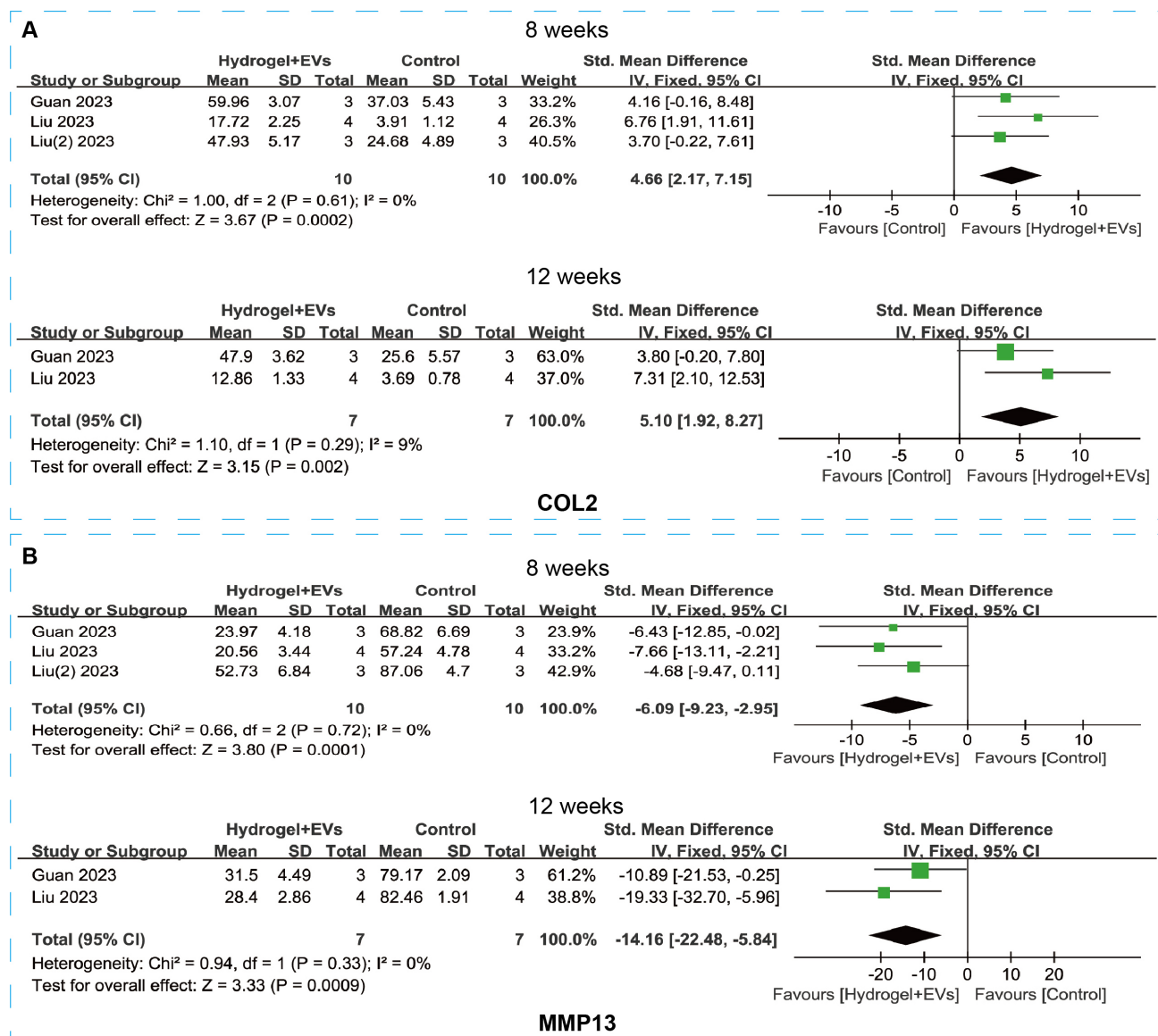


Fig. 6. Forest plot showing the difference in relative expression of Collagen Type II (COL2) (A) and Matrix Metalloproteinase 13 (MMP13) proteins (B) between the exosome-loaded hydrogel group and the control group at 8 weeks and 12 weeks post-treatment for intervertebral disc degeneration (IDD).

drogels promoted the expression of COL2 protein, while decreasing MMP13 protein levels and reducing the number of p16Ink4a and p21CIP1A-positive cells. These results suggest that exosome-loaded hydrogels may improve IDD by promoting the synthesis of the intervertebral disc matrix, inhibiting its degradation, and slowing or reversing the aging process of disc cells.

The study found that exosome-loaded hydrogels significantly improved the DHI grade at both 4 weeks and 8 weeks, and significantly reduced the MRI grade at 8 weeks and 12 weeks, indicating a consistent positive effect on the recovery of intervertebral disc structure in the early stages of IDD. These findings align with previous research on exosome treatments for IDD. For instance, Liao *et al.* [32] reported that MSC-derived exosome significantly increased

DHI scores and reduced MRI scores in the IDD model at 4 and 8 weeks, demonstrating a beneficial effect in delaying and improving IDD. Similarly, another study showed that hypoxia-preconditioned bone marrow mesenchymal stem cell (BMSC)-derived exosome upregulated DHI scores and downregulated MRI scores at 4 and 8 weeks, alleviating the progression of the rat IDD model [33]. These results further support the notion that exosome-loaded hydrogels can effectively improve disc height and morphology in IDD. However, while exosome-loaded hydrogels exhibit significant efficacy in improving disc degeneration in the short term, their therapeutic effects may be limited in later stages due to the delivery efficiency of exosome and the degradation rate of the hydrogel [34,35]. This insight offers valuable context for our study, suggesting that precise control

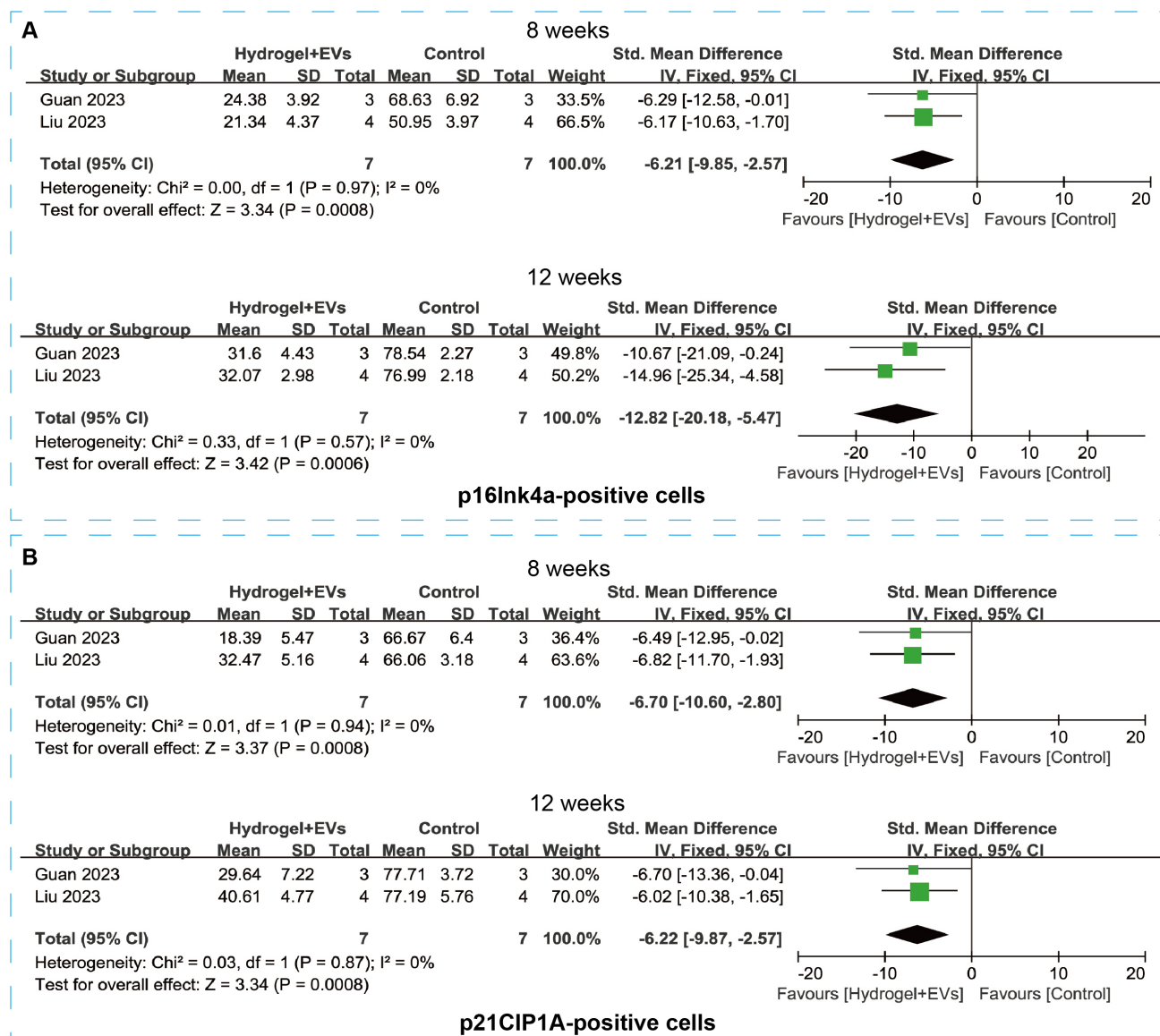


Fig. 7. Forest plot showing the difference in p16Ink4a-positive cells (A) and p21CIP1A-positive cells (B) between the exosome-loaded hydrogel group and the control group at 8 weeks and 12 weeks post-treatment for IDD.

over exosome release may be crucial for exploring the long-term efficacy of exosome-loaded hydrogels in treating IDD.

4.2 Risk of Bias Assessment

As with previous meta-analyses, a thorough assessment of the potential risk of bias in the included studies is essential. The SYRCLE risk of bias tool, consisting of 10 items, was employed to evaluate the methodological quality of the preclinical studies. The assessment revealed that most studies exhibited an unclear risk of bias. Regarding selection bias, 90% of the studies did not report the method of random sequence generation (e.g., computer-based randomization or sealed envelope methods), and only one study mentioned allocation concealment. The absence of these measures may lead to imbalances in baseline characteristics, such as uncontrolled variations in animal age or

weight, affecting the comparability of effect sizes. Three studies provided detailed descriptions of key baseline characteristics, such as animal age and weight, and were thus considered to have a low risk of bias in this domain. Conversely, one study lacked sufficient information on baseline characteristics and was rated as having a high risk of bias.

For selective reporting and incomplete outcome data, all included studies were judged to have a low risk of bias. However, in terms of performance and detection bias, none of the studies reported whether blinding was applied to operators or animal caretakers, which could lead to non-standardized administration of interventions, such as variations in exosome-loaded hydrogel dosage and frequency. Additionally, blinding during outcome assessment was not clearly described, raising concerns about potential bias, especially for subjective endpoints like histological scoring.

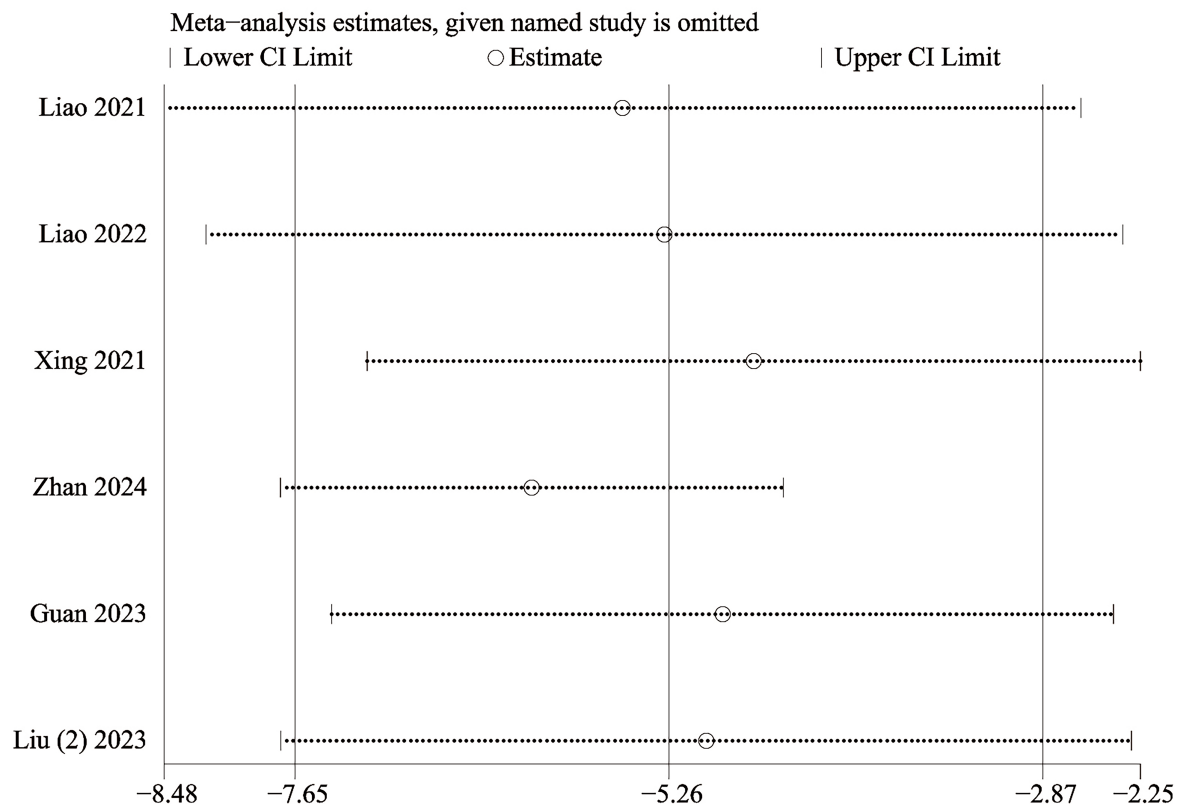


Fig. 8. Sensitivity analysis of the studies included in histological grade. CI, confidence intervals.

Despite these uncertainties, the consistency in the direction of key outcome measures across the studies strengthens the overall stability of the meta-analysis results. Moving forward, future animal studies on the therapeutic effects of exosome-loaded hydrogels for IDD should adhere to the ARRIVE 2.0 guidelines [36] for standardized reporting, with particular emphasis on clear descriptions of randomization methods and the implementation of blinding procedures, to enhance the rigor and reproducibility of the evidence base.

4.3 Subgroup Analysis and Heterogeneity

Variations in animal models, hydrogel types, and exosome characteristics across studies may introduce potential biases that could affect the outcomes of this meta-analysis. The pooled analysis of histological scores for intervertebral discs revealed significant heterogeneity ($I^2 = 61\%$). To address this, subgroup analyses were conducted based on hydrogel type, exosome source, administration route, intervention frequency, modeling method, and animal age. The results showed that ECM-based hydrogels, composite hydrogels, stem cell-derived exosome-loaded hydrogels, intradiscal administration, single-dose administration, alternative injection frequency subgroups, needle puncture models, and LPS/TNF- α -induced models were all associated with reductions in intervertebral disc histological scores.

In patients with intervertebral disc IDD, the balance between ECM degradation and synthesis is disrupted, often accompanied by abnormal MMP expression and a reduction in COL2 [37,38]. Restoring the ECM metabolic balance is critical in treating IDD. Recent interest has focused on decellularized ECM, which, due to its reduced immunogenicity, may enhance the therapeutic efficacy of exosomes [39]. Subgroup analyses suggest that ECM-based hydrogels may play a beneficial role in reducing histological scores associated with IDD. exosome derived from various MSC sources are pivotal in slowing IDD progression, including exosome from BMSCs, umbilical cord-derived MSCs (UCMSCs), adipose-derived MSCs (ADSCs), induced pluripotent stem cells (iPSCs), and nucleus pulposus stem cells [40,41]. Compared to the limitations of intravenous MSC infusion, MSC-derived exosomes overcome this barrier and demonstrate therapeutic efficacy in IDD treatment. Subgroup analysis indicates that MSC-derived exosomes significantly reduce histological scores, highlighting their potential in ameliorating IDD. However, research on exosomes derived from M2c macrophages for IDD treatment remains limited [30], emphasizing the need for further investigation to assess their therapeutic value in this context.

In pharmacological interventions for IDD, local injection at the disc site offers the advantage of rapidly delivering therapeutic agents directly to the lesion, maintaining a local therapeutic concentration, and is therefore the most

common delivery route in animal model studies. Previous research has demonstrated that the intervertebral disc primarily acquires nutrients through diffusion from blood vessels in adjacent vertebrae via the endplate [42]. Subgroup analysis based on intervention characteristics revealed that intradiscal administration significantly reduced histological scores. Whether administered as a single or multiple injections, exosome-loaded hydrogels consistently led to a reduction in histological scores post-injection. Further studies incorporating a greater number of standardized intervention protocols are necessary to validate the effects of different delivery routes and injection frequencies on therapeutic outcomes in IDD animal models. Needle puncture is recognized as the standard method for inducing IDD in animal models due to its minimally invasive nature, stability, and reliability [43]. Other modeling approaches include chondroitinase ABC, LPS, and TNF- α injections [44]. Subgroup analyses indicated that both needle puncture and chemically induced models were effective in reducing disc histological scores, underscoring the need for future large-sample studies utilizing standardized modeling techniques. Additionally, the effects of exosome-loaded hydrogels were explored in animal models of varying ages, and results showed that both 8-week-old and 12-week-old groups exhibited reduced histological scores. However, due to significant heterogeneity within the subgroups, no single factor was identified as the primary source of heterogeneity in histological scores. Notably, the small sample size and limited number of studies may affect the reliability of the meta-analysis, and therefore, the subgroup analysis results should be interpreted with caution. Despite observed heterogeneity and sample size limitations, the overall findings of this meta-analysis remain stable and reliable. Future studies, incorporating larger sample sizes in animal models, will be essential for validating and enhancing the reliability of exosome-loaded hydrogels in IDD treatment.

4.4 Mechanism of Exosome-Loaded Hydrogel Improving IDD

Exosome-loaded hydrogels significantly improved histological scores of IDD intervertebral discs and inhibited disc matrix degradation by upregulating COL2 expression and downregulating MMP13 expression. These findings align with the study by Fan *et al.* [45], who demonstrated that exosomes derived from M1 macrophages restored ECM metabolic balance in both *in vitro* and *in vivo* models by promoting COL2 expression and reducing MMP13 expression. Another study showed that exosomes derived from human adipose tissue stem cells alleviated rat IDD by downregulating MMP13 expression [46]. Additionally, this meta-analysis revealed that exosome-loaded hydrogels slowed the aging process of disc cells by reducing the number of p16Ink4a and p21CIP1A-positive cells. Peng *et al.* [31] further demonstrated that functionalized extracellular vesicles coupled with matrix hydrogels alleviate

nucleus pulposus stem cell (NPSC) aging by targeting the Homeodomain-Interacting Protein Kinase 2 (HIPK2)/p53 pathway, promoting intervertebral disc regeneration. In summary, exosome-loaded hydrogels may alleviate IDD by restoring nucleus pulposus (NP) cell aging and ECM metabolic balance, with hydrogels contributing synergistically through their regenerative and anti-aging properties [47].

From a mechanistic perspective, multiple signaling pathways are involved in the regulation of exosomes in IDD repair. MSC-derived exosomes maintain nucleus pulposus cell (NPC) homeostasis by modulating autophagy balance. For instance, Xiao *et al.* [48] observed that BMSC-exosomes promote autophagy by inhibiting the Akt-mTOR pathway, reducing NPC apoptosis and alleviating IDD. Luo *et al.* [25] developed ECM-modified hydrogels loaded with MSC-exosomes, which promote autophagy in neural precursor cells by transporting Sphk2 to activate the Phosphatidylinositol 3-Kinase (PI3K)/p-AKT pathway, improving IDD. MSC-exosomes also regulate ECM synthesis and degradation. In a study by Liao *et al.* [24], a thermo-responsive ECM hydrogel was developed to continuously release MSC-exosomes, activating the Notch signaling pathway and maintaining ECM metabolic balance. Additionally, miR-199a carried by BMSC-exosomes improved IDD by inhibiting the TGF- β pathway and upregulating COL2 protein levels [49]. MSC-exosomes can also reverse the senescence phenotype of NPSCs. For example, Ma *et al.* [50] demonstrated that Cavin2-modified BMSC-exosomes downregulated senescence markers (p16Ink4a, p21CIP1A, and p53) and restored cell proliferation by reducing age-related pathways. Furthermore, MSC-exosome-loaded hydrogels alleviated NPSC senescence by targeting the HIPK2/p53 pathway, mitigating IDD [31]. However, current research has not fully explored the specific mechanisms through which exosome-loaded hydrogels downregulate MMP13 and upregulate COL2 expression, nor has it provided in-depth insights into the mechanisms regulating senescence markers. Future studies should focus on elucidating the precise mechanisms by which exosomes promote ECM metabolic balance and regulate cellular senescence.

4.5 Limitations and Prospects

This study has several limitations. First, there are variations in the types of hydrogels, characteristics of exosomes, and animal model parameters across studies. Although subgroup analyses were performed to identify potential sources of heterogeneity, no significant sources were found. Second, the included studies exhibit an unclear risk of bias, particularly regarding randomization and blinding, which could impact the stability of the pooled analysis results. Finally, despite comprehensive literature searches, the limited number of studies and small sample sizes may affect the reliability of the summary analysis. Future re-

search should focus on standardized, large-sample controlled trials to validate the preclinical efficacy of exosome-loaded hydrogels for IDD.

Although the results suggest that exosome-loaded hydrogels have a positive therapeutic effect in IDD treatment, their clinical translation faces several challenges. First, the source and preparation methods of exosomes can influence their therapeutic efficacy. While most studies used MSC-derived exosomes, those from different sources might vary in treatment outcomes. Second, the type of hydrogel and delivery method significantly impact therapeutic outcomes. Variations in hydrogel materials, such as gelatin and hyaluronic acid, may lead to differences in biodegradation rates, structural stability, and biocompatibility, directly affecting exosome release and therapeutic effects [51]. Future research should focus on optimizing exosome sources, selecting appropriate hydrogel types, and determining the most effective delivery methods to achieve optimal therapeutic outcomes. Moreover, while preclinical studies offer insights, they may not fully predict clinical outcomes, necessitating more rigorous clinical trials to confirm the efficacy and safety of exosome-loaded hydrogels for clinical applications.

5. Conclusions

This meta-analysis suggests that exosome-loaded hydrogels improve disc height, MRI scores, and histological grading, potentially alleviating IDD progression by promoting COL2 expression, inhibiting MMP13, and slowing intervertebral disc cell aging. However, the optimization of exosome-loaded hydrogel components and precise delivery methods remains critical to exploring their long-term efficacy in treating IDD. Future research should adhere to standardized protocols to further validate their efficacy and safety in both preclinical and clinical studies.

Abbreviations

ADSCs, adipose-derived mesenchymal stem cells; ARRIVE, Animal Research: Reporting of *In Vivo* Experiments; BMSCs, Bone marrow mesenchymal stem cells; CI, confidence interval; COL2, collagen type II; DHI, disc height index; ECM, extracellular matrix; EV, extracellular vesicle; HIPK2, Homeodomain-Interacting Protein Kinase 2; IDD, intervertebral disc degeneration; IHC, immunohistochemical; iPSCs, induced pluripotent stem cells; LPS, lipopolysaccharide; MSC, mesenchymal stem cell; MMP13, matrix metalloproteinase 13; MRI, magnetic resonance imaging; MeSH, medical subject headings; NSAIDs, non-steroidal anti-inflammatory drugs; NPSCs, nucleus pulposus stem cells; NPCs, nucleus pulposus cells; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PI3K, Phosphatidylinositol 3-Kinase; SMD, standardized mean difference; SYRCLE, Systematic Review Center for Laboratory Animal Experimentation; TGF- β , transforming growth factor beta; TNF-

α , tumor necrosis factor-alpha; UCMSCs, umbilical cord mesenchymal stem cells.

Availability of Data and Materials

All data relevant to the study are included in the article. Further supplementary data be available from the first author or corresponding author upon reasonable request.

Author Contributions

BW, DX and XC designed the study and wrote the manuscript. JH and ZH conducted a literature analysis search. WW, DH and YZ extracted the data and conducted the data analysis. All authors have read and agreed to the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL38302>.

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