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Review Article

The Potential Applications of Stem Cell Conditioned Medium Secretome in Knee Cartilage Regeneration: A Systematic Review

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ABSTRACT

Background: Articular cartilage injuries often result from trauma, genetic predisposition, and degeneration. These injuries lack inherent regeneration mechanisms due to the absence of blood vessels and limited progenitor cell entry. Osteoarthritis is characterized by gradual cartilage deterioration. Mesenchymal stem cells (MSCs), particularly their secretome including exosomes, hold promise as a regenerative therapy. This review explores the application of MSCs and their secretome to address cartilage defects.
Methods: This review was conducted based on PRISMA guidelines. Animal model studies focusing on the use of stem cell secretomes for cartilage regeneration were explored. The search, encompassing PubMed, MEDLINE, the Cochrane Library, Scopus, Web of Science, and Science Direct from January 10, 2023, to July 27, 2023, was conducted utilizing Google Chrome as the search engine. Studies with outcomes based on OARSI or ICRS scores, as well as any additional outcomes related to MSC secretome utilization, cartilage regeneration, and proliferation, were included.
Results: Our systematic review identified six studies using MSCs in vivo and in vitro. Synovial membrane-derived MSCs significantly enhanced cartilage regeneration by elevating chondrogenic capabilities. Hydrogel-based systems techniques and 3D-printed scaffolds have emerged for innovative delivery. Specific microRNAs, such as miR-92a-3p, have been recognized for enhancing cartilage regeneration. Strategies for the effective targeting of MSC exosomes to the precise cartilage damage site have been explored.
Conclusions: The studies demonstrate the potential of MSC-derived secretomes and exosomes for knee cartilage regeneration in animal models. Further research and clinical trials are needed to refine these approaches for practical application.

Keywords: Human and medicine; Knee cartilage; Mesenchymal stem cells; Osteoarthritis; Secretome

INTRODUCTION

Various factors, including trauma, genetic predisposition, and degenerative alterations, contribute to the high prevalence of articular cartilage injuries. Articular cartilage, a type of hyaline cartilage, comprises chondrocytes embedded within a dense extracellular matrix (ECM). The ECM's specific composition provides the cartilage with distinct viscoelastic properties that enable smooth and frictionless movement. Chondrocytes, which arise from mesenchymal progenitor cells, constitute approximately 2% of the total cartilage volume.[1]

Following traumatic or pathological injury, hyaline articular cartilage, the weight-bearing tissue in joints, exhibits minimal or no inherent capacity for self-repair. Consequently, even small injuries can initiate a process of progressive deterioration and degeneration of the joint.[2] This lack

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of repair capacity is attributed to the absence of blood vessels within the damaged cartilage, restricting the entry of progenitor cells necessary for tissue regeneration.[3]

Osteoarthritis (OA) is a condition of progressive erosion of articular cartilage. This leads to a gradual thinning of the cartilage, causing pain, inflammation, and noticeable changes on X-rays, such as hardening of the bone (sclerosis) and the formation of bony growths (osteophytes). Recent findings suggest that OA should not be considered solely a cartilage disorder but rather a dynamic pathological condition affecting all tissues within the entire joint. OA can manifest in any synovial joint, but the knees, hips, and small joints of the hand are the most commonly affected areas.[4]

Recently, stem cell-based therapies have emerged as promising approaches for cartilage regeneration due to stem cells' capacity to differentiate into chondrocytes, the cartilage-forming cells. Clinical trials have demonstrated that stem cell therapies yield similar regeneration outcomes to autologous chondrocyte implantation (ACI), a well-accepted tissue engineering method for treating moderate-sized osteochondral defects. Notably, the stem cell treatment procedure is simpler and more cost-effective.[4,5] Mesenchymal stem cells (MSCs) can differentiate into numerous cell types, including adipose cells, chondrogenic cells, and osteocytes, under specific environmental conditions.[6] MSCs secrete active agents in response to various stimuli. This collection of secreted factors, known as the secretome, is commonly found in the medium in which these cells are cultivated, termed the conditioned medium (MSC-CM). Extensive evidence supports the beneficial impact of MSC-CM on bone and tissue regeneration, as the secretome plays a pivotal role in stimulating various cellular functions.[7] In light of their therapeutic potential, studies have explored the efficacy of MSC-CM for cartilage protection and regeneration. Research indicates that exosomes derived from human embryonic mesenchymal stem cells can improve cartilage regeneration. Similarly, exosomes derived from bone marrow MSCs have exhibited protective effects against cartilage degeneration in various in vivo and in vitro investigations. Furthermore, MSCs demonstrate lower levels of the major histocompatibility complex, indicating reduced immunogenicity.[8] While embryonic stem cells (ESCs) exhibit a greater degree of differentiation potential compared to MSCs, MSCs derived from adult somatic tissues are preferred for clinical applications due to fewer ethical and safety concerns.[9] This review explores the potential of utilizing MSCs in cell therapy aimed at addressing articular cartilage defects and associated issues.

METHODS

Eligibility Criteria

This systematic review was registered on PROSPERO (registration number CRD42023493064) on December 24, 2023. The systematic review applied specific inclusion criteria to select relevant studies for analysis. It included controlled animal studies conducted in vivo on animals with cartilage injuries induced either manually or surgically. Interventions considered included any use of stem cell secretome, including conditioned media (CM), exosomes (Exos), or microvesicles (MV), administered to the study groups. The main outcomes of interest were functional and histological improvements in cartilage defects. Only English-language studies were considered; duplicates, review articles, and irrelevant publications were excluded from the analysis.

Literature Search and Selection of Study

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to conduct an extensive search across various databases, including PubMed, MEDLINE, the Cochrane Library, Scopus,

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Web of Science, and Science Direct, from January 10, 2023, to July 27, 2023 (Figure 1). The search terms used were "(conditioned media OR conditioned medium)" AND "(secretome OR microvesicle OR extracellular vesicle OR exosome)" AND "(cartilage knee OR cartilage)." Duplicate and review articles were removed, and the titles and abstracts were screened independently by IGNWA and F for eligibility. The same authors thoroughly studied the full text of all selected papers to apply the inclusion and exclusion criteria.

Data Extraction and Synthesis

Data were independently extracted from all included studies by two investigators (F and NGGNU). The extracted information included: the type of MSC, outcome measurement, animal model for in vivo studies, preparation or process for animals or cells, interventions, follow-up duration, results, and conclusion.

Quality and Risk Of Bias Assessment

The rigor of the methodology of the included papers was evaluated using the Animal Research: Reporting of In Vivo Experiments (ARRIVE) criteria. The ARRIVE Essential 10 outlines the crucial minimum standards for reporting animal research, allowing reviewers and readers to assess the reliability and strength of the study outcomes. By prioritizing Essential 10, journal staff, editors, and reviewers can efficiently verify if the necessary details have been adequately presented in the manuscripts. Both authors (IGNWA and F) independently conducted all evaluations, and discrepancies were resolved through discussions with other authors.

RESULTS

The study selection process is outlined in the PRISMA flow diagram. Initially, 63 studies were identified from the available literature. After a

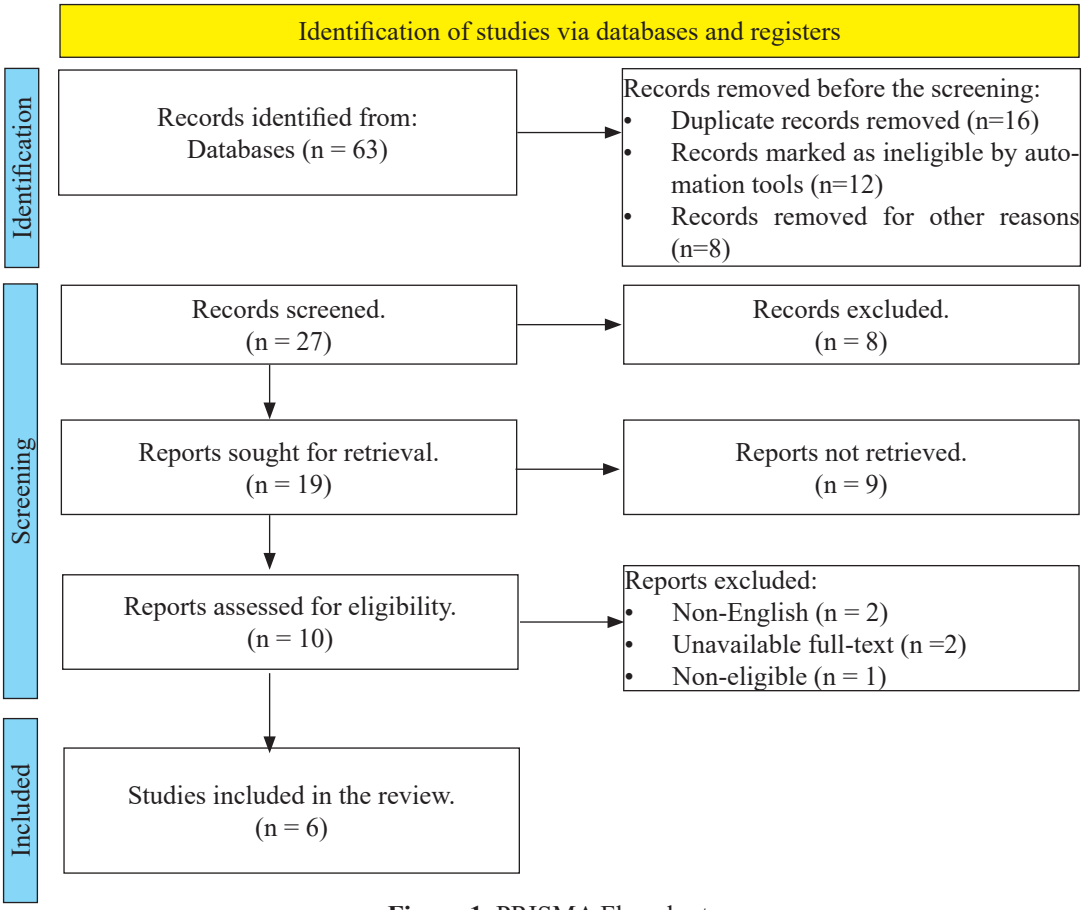

Figure 1. PRISMA Flowchart

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comprehensive review of titles and abstracts, 10 studies were selected for full-text assessment. Of these, six studies met the inclusion criteria and were included in this comprehensive review.

All included studies were assessed for methodological rigor using the ARRIVE criteria (Table 1). Two studies achieved a perfect score of 10, while the remaining four studies scored 9.5, indicating that all studies met the minimum reporting requirements.

The data extracted from the included studies are presented in Table 2.

DISCUSSION

In the six selected studies, all utilized either the OARSI score or the ICRS score to evaluate cartilage repair. Despite the diverse explanations regarding the potential mechanisms by which MSCs promote knee cartilage regeneration, the findings from all six studies collectively highlighted the promising potential of the secretome to facilitate this process.[11–15,17]

The findings from the study by Lubis et al. indicate notable variations in the total macroscopic OARSI score, score components, and knee joint surfaces among three distinct groups: hyaluronic acid, secretome, and MSCs. The group receiving the secretome exhibited the most favorable outcome, followed by the MSC group, and then the hyaluronic acid group. Previous research has shown that the secretome triggers the regeneration of chondrocytes to replace damaged cells in cartilage defects. Secretomes contain growth factors that can stimulate cartilage regeneration by binding to specific receptors in the extracellular matrix. Thrombospondin (TSP2), secreted by MSCs, plays a role in cartilage regulation and bone differentiation, supporting the positive effects of the secretome and MSCs on cartilage structure. In contrast, hyaluronic acid injection may not directly contribute to cartilage regeneration and could potentially lead to cartilage degeneration and increased chondrocyte apoptosis. The differences in osteophyte growth among the groups are likely related to their varying inhibition mechanisms. The secretome and MSC treatments resulted in significantly lower microscopic OARSI scores compared to hyaluronic acid, with similar outcomes observed between the secretome and MSC groups. This finding is consistent with studies reporting the superiority of secretome and MSC administration in maintaining cartilage structure. Factors such as IL-6, PGE2, TSG-6, and HGF in the secretome and MSC groups contribute to stimulating chondrocyte proliferation and maintaining cartilage integrity. Mechanisms of chondrocyte regeneration might explain the notable matrix differences observed between the secretome and MSC groups compared to hyaluronic acid. Integrin, a protein in the cartilage extracellular matrix, influences cartilage synthesis and degradation, affecting chondrocyte behavior. No significant difference in microscopic scores related to density and cell multiplication was found across the three groups. This supports previous research indicating the positive effects of the secretome, hyaluronic acid, and MSCs on chondrocyte regeneration in individuals with knee osteoarthritis.[11]

Another crucial aspect of developing MSC exosome therapy for articular cartilage defects is identifying the most suitable cell type for exosome isolation. Recent research has highlighted the potential of synovial membrane-derived mesenchymal stem cells (SMMSCs) in halting the progression of OA.[18] SMMSCs have distinct advantages for cartilage repair due to their shared origin with chondrocytes during synovial joint development. This shared origin suggests a closer relationship between SMMSCs and chondrocytes compared to other types of MSCs. Moreover, SMMSCs have demonstrated a greater propensity for chondrogenesis compared to MSCs from bone marrow or adipose tissue. Despite these favorable attributes, obtaining SMMSCs is challenging due to the invasive nature of synovial membrane collection.[19]

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Table 1. The ARRIVE Risk of Bias Assessment

Item		Recommendation	Lubis et al. 2023	Zhu et al. 2017	Liu et al. 2017	Mao et al. 2018	Wu et al. 2019	Chen et al. 2019
Study design	1	Provide brief research design details for each experiment, including:						
		a. Specify the groups under comparison, which includes control groups. If no control group has been used, provide the reasoning for this decision.	1	1	0.5	0.5	0.5	0.5
		b. Indicate the experimental unit, such as an individual animal, a litter, or a cage of animals.						
Sample size	2	a. Clearly state the precise count of experimental units assigned to each group, along with the overall number in each experiment. Additionally, specify the total number of animals utilized.	1	1	1	1	1	1
		b. Clarify the rationale behind determining the sample size. Furnish information regarding any pre-existing sample size calculations, if conducted.						
Inclusion and exclusion criteria	3	a. Outline any criteria employed to include or exclude animals as experimental units throughout the experiment, as well as data points during the analysis. Specify whether these criteria were predetermined. If no criteria were defined, explicitly mention this.						
		b. For each group of experiments, specify any specimens, test units, or points of information that were excluded from the analysis and explain why they were excluded. Mention this explicitly if no exclusions occurred.	1	1	1	1	1	1
		c. In each analysis, provide the specific numerical value of "n" for every experimental group.						
Randomisation	4	a. State whether or not experimental units were assigned to control or treatment groups using randomization. If yes, explain how you came up with the randomization sequence.	1	1	1	1	1	1
		b. Describe the method utilized to eliminate possible confounding variables such as the order of treatments and assessments or the position of animals/cages. Mention this clearly if no efforts were made to control confounders.						

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Table 1. The ARRIVE Risk of Bias Assessment

Item		Recommendation	Lubis et al. 2023	Zhu et al. 2017	Liu et al. 2017	Mao et al. 2018	Wu et al. 2019	Chen et al. 2019
Blinding	5	Explain who knew about the group assignment at various stages of the study, such as during allocation, experiment implementation, result evaluation, and data processing.	1	1	1	1	1	1
Outcome measures	6	a. Provide precise definitions for all evaluated outcome measures, such as cell death, molecular markers, or behavioral changes.	1	1	1	1	1	1
		b. Clearly define the main result measurement—that has been used to calculate sampling size—in studies testing hypotheses.						
Statistical methods	7	a. Specify statistical methodologies and software used for each analysis.	1	1	1	1	1	1
		b. Explain any approaches used to determine if the data met the statistical approach's assumptions, and explain the steps that were taken if the presumptions were not fulfilled.						
Experimental animals	8	a. Provide species-specific information on the animals used, including species of animals, strain and substrain, gender, age or stage of growth, and weight (if necessary).	1	1	1	1	1	1
		b. Include any further essential information on the animals' origin, health/immune state, genetic alteration status, genotype, and any previous treatments.						
Experimental procedures	9	For every experimental group, controls included, elucidate the procedures with sufficient detail to enable replication by others, encompassing:						
		a. Specify the actions taken, the method employed, and the resources utilized.						
		b. Indicate the timing and frequency of the actions.	1	1	1	1	1	1
		c. Provide information on the location, including details of any acclimatization periods.						
		d. Explain the reasons behind the procedures, offering a rationale for their implementation.						

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Table 1. The ARRIVE Risk of Bias Assessment

Item		Recommendation	Lubis et al. 2023	Zhu et al. 2017	Liu et al. 2017	Mao et al. 2018	Wu et al. 2019	Chen et al. 2019
Results	10	For every performed experiment, including independent replications, document: a. Provide summary or descriptive statistical information regarding every group of experiments, including a measure of variation (e.g., mean and standard deviation, or median and range) where applicable. b. Include the effect magnitude with a confidence interval, if applicable.	1	1	1	1	1	1
		TOTAL	10	10	9.5	9.5	9.5	9.5

Table 2. Summary of included studies

Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Lubis et al. 2023[11]	in vivo	HUC-MSCs (human umbilical cord mesenchymal stem cells)	(n=18) operated on with total lateral meniscectomy on the right hind leg knee, to induce knee OA	Intra-articular injection using fluoroscopic guidance with the respective substances (secretome: 2 mL, MSC: 2x106 cells)	hyaluronic acid: 2 mL	Osteoarthritis Research Society International (OARSI) score	The secretome group showed notably better microscopic scores than the hyaluronic acid group (with an average difference of 6.0 and a 95% confidence interval of 0.15 to 12). However, the MSC group did not display a substantial distinction (average difference of 1.0 and a confidence interval of –4.8 to 6.8).	In the animal model, early-stage OA can be effectively managed through intra-articular injection of secretome, showing superior results compared to hyaluronic acid treatment. Additionally, the secretome injection demonstrates comparable efficacy to MSC injection for managing the condition.

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Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Zhu et al. 2017[12]	in vivo, in vitro		This study used four groups of six-week-old female C57B/L10 mice: A control group: (n = 5) An iMSC-Exos treatment group: (n = 10) An SMMSC-Exos treatment group: (n = 10) An osteoarthritis (OA) group: (n = 10) The mice were randomly assigned to each group.	Begin with administering an intra-articular injection of 12 units of collagenase VII (derived from Clostridium histolyticum) dissolved in 8 µl of saline. Inject this solution through the patellar ligament into the knee joint. Subsequently, on days 7, 14, and 21, administer 8 µl of iMSC-Exos (at a concentration of 1.0 × 1010/ml) and 8 µl of SMMSC-Exos in PBS (at a concentration of 1.0 × 1010/ml) via intra-articular injections.	N/A	The International Cartilage Research Society (ICRS) scoring system for evaluating cartilage repair	While no significant differences in ICRS scores were observed among the three experimental groups, each group demonstrated higher ICRS scores compared to the OA group. Microscopic evaluation revealed lower OARSI scores in the two groups receiving exosome treatments (iMSC-Exos and SMMSC-Exos) compared to the OA group. Interestingly, the iMSC-Exos group exhibited lower OARSI scores than the SMMSC-Exos group, while the standard treatment group showed no significant difference in OARSI scores compared to the OA group	In an experimental mouse model of OA, the therapeutic effectiveness of SMMSC-Exos was inferior to that of iMSC-Exos. iMSC-Exos demonstrated a more remarkable ability to enhance chondrocyte migration and proliferation than SMMSC-Exos.

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Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Liu et al. 2017[13]	in vivo	human induced pluripotent stem cells (hiPSCs) derived MSCs	This study used six New Zealand white rabbits weighing 2.5 to 3.0 kg. The rabbits were randomly assigned to one of two experimental groups. Researchers surgically created a cartilage defect in each rabbit's knee. This involved making an incision beside the kneecap (patella), dislocating the patella, and then using a drill to create a small, cylindrical hole (4 mm in diameter and 3 mm deep) in the cartilage and bone of the patellar groove. This standardized defect allows for the evaluation of cartilage repair strategies.	• EHG on Cell Deposition at Cartilage Defect: (n=6) two groups of rabbits were then treated with either 20 µL of EHG tissue patch precursor solution or HG. The treated areas were exposed to 395 nm LED light for 1 minute at 20 mW/cm2 • Cartilage Defect Repair and Regeneration: (n=20) randomized into 5 groups treated with: - A gel formed in the location with 20 µL volume, consisting of 1 × 1011/mL exosomes derived from hiPSC-MSCs (EHG). - A gel formed in the location with 20 µL volume (HG). - Implantation of a gel formed in vitro with 20 µL volume, containing 1 × 1011/mL exosomes (Pre-EHG). - Injection of 20 µL exosome suspension directly into the joint, with an exosome concentration of 1 × 1011/mL (Inj-Exos). Flushing the area with saline solution (saline rinsing).	N/A	International Cartilage Research Society (ICRS) score for cartilage repair	A clear and continuous link between the hydrogel and lateral cartilage. The cell population of faulty areas treated with EHG was higher than that of those treated with HG. Deposited cells are likely comprised of chondrocytes, inflammatory cells, fibroblasts, and blood cells, which are common during regeneration.	The EHG tissue patch integrates smoothly with natural cartilage, effectively retaining exosomes at the injured site. Also, it demonstrates favorable cellular regulation in vivo and in vitro settings, promoting the improvement of cartilage repair and rejuvenation.

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Table 2. Summary of included studies

Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Mao et al. 2018[14]	in vivo, in vitro	Exosomal miR-92a-3p	Degraded joint cartilage samples were procured from a group of six patients (3 male, 3 female) with an average age of 60.24 years. These individuals were undergoing total knee arthroplasty due to osteoarthritis. For comparison, healthy cartilage samples were obtained from a separate group of six individuals (3 male, 3 female) with a mean age of 54.46 years. These individuals had no prior history of OA or rheumatoid arthritis and were undergoing total hip arthroplasty as a result of femoral neck fractures.	• Exosomal miR-92a-3p expression using human MSCs undergoing chondrogenesis and in normal and OA PHCs. • MSCs received exosomes from MSC-miR-92a-3p, while PHCs were treated with exosomes containing its antisense inhibitor. • The research investigated the molecular functions of miR-92a-3p and WNT5A in chondrogenesis exosomes using siRNAs and luciferase reporter assay.	N/A	• Exosomal miR-92a-3p expression • WNT5A expression	Exosomal miR-92a-3p levels were higher in chondrogenic exosomes derived from MSCs, while they were lower in exosomes from OA chondrocytes compared to normal cartilage. The administration of MSC-miR-92a-3p-Exos stimulated cartilage proliferation and the expression of matrix genes in MSCs and PHCs. Conversely, MSC-anti-miR-92a-3p-Exos increased WNT5A expression, hindering chondrogenic differentiation and cartilage matrix synthesis. Luciferase reporter experiments unveiled that miR-92a-3p reduced WNT5A expression and 3'-UTR activity in MSCs and PHCs. MSC-miR-92a-3p-Exos demonstrated the ability to alleviate cartilage breakdown in OA mice.	Both MSC chondrogenic differentiation and OA conditions express exosomal miR-92a-3p in different patterns. It downregulates WNT5A in chondrogenesis and OA. The work suggests that regulating exosomal miR-92a-3p may prevent and treat OA in a novel way.

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Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Wu et al. 2019[15]	in vivo, in vitro	Exosomes abundant in miR-100-5p were derived from mesenchymal stem cells located in the infrapatellar fat pad.	OA was induced in nine-week-old male C57BL/6 mice by surgically destabilizing the medial meniscus (DMM) in their right knee. This DMM surgery involved cutting the medial meniscotibial ligament. A sham operation was also performed on a control group, which consisted of an incision through the skin and muscle layers.	All mice were randomly assigned to sham, PBS, or PBS-ExoIPFP groups after DMM or sham surgery. The mice in these groups received intra-articular injections of 10 µl of PBS or 10 µl of exosomes derived from mesenchymal stem cells isolated from the infrapatellar fat pad (MSCIPFP-Exos) (1010 particles/ml) for 4 or 6 weeks. The injections were given twice a week using a Hamilton micro syringe and a 30 gauge needle.	Sham	Osteoarthritis Research Society International (OARSI) score	MSCIPFP-Exos-cultured chondrocytes exhibited a reduction in mTOR protein levels. MSCIPFP-Exos' downregulation of mTOR is substantially dependent on miR-100-5p, as miR-100-5p inhibition restored the decline. The application of Antagomir-100-5p reduced the protective effect of MSCIPFP-Exos on cartilage in DMM-induced OA mice. These findings indicate that miR-100-5p plays a critical role in the therapeutic effect of MSCIPFP-Exos, specifically in delaying cartilage degradation.	MSCIPFP-derived exosomes can protect cartilage and enhance walking patterns in mice with DMM-induced OA by avoiding chondrocyte death and restoring anabolic-catabolic balance. This mechanism appears to include miR100-5p inhibiting the mTOR-autophagy pathway. Our findings imply that MSCIPFP-Exos may be a potential novel OA treatment due to the ease of arthroscopically extracting human infrapatellar fat pad (IPFP) from OA patients.

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Authors	In Vivo/ In Vitro	Type Of MSC	Population	Intervention	Control	Outcome	Results	Conclusion
Chen et al. 2019[16]	in vivo, in vitro	MSC-derived exosomes	The tenocytes were obtained under sterile conditions from the Achilles tendons of Sprague–Dawley rats (150–200 g)	Tenocytes were seeded in a 6-well plate at a density of 5 x 10^4 cells per well and cultured in a serum-free environment using either DMEM (Dulbecco's Modified Eagle Medium) or MSC-CM. This culture process continued for up to 120 minutes, allowing the cells to reach near-confluence.	0 minute	International Cartilage Research Society (ICRS) score for cartilage repair	The ECM/GelMA/ exosome scaffold effectively addressed mitochondrial dysfunction in chondrocytes, promoting their migration and influencing synovial macrophages to adopt the M2 phenotype (associated with tissue repair). This 3D-printed scaffold significantly enhanced cartilage regeneration in animal models.	• The exosomes from MSCs reduce deteriorating cartilage by addressing mitochondrial dysfunction and countering damage from oxidative stress by providing mitochondria-related proteins. • MSC-derived exosomes play a vital role in restoring equilibrium to energy metabolism and fostering cartilage regeneration. • The scaffolds made with ECM/GelMA/ exosomes successfully correct cartilage's mitochondrial issues, augment chondrocyte migration, and influence synovial macrophages. • The effectiveness of the 3D-printed scaffold in the regeneration of osteochondral lesions has been promising

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In a study by Zhu et al., the authors conducted a comparative investigation to assess the efficacy of exosomes derived from SMMSCs (SMMSC-Exos) and exosomes obtained from mesenchymal stem cells derived from induced pluripotent stem cells (iMSC-Exos) in the context of OA treatment. The researchers observed a significant decrease in OA severity when iMSC-Exos were administered to a mouse model with collagenase-induced OA. Histological examination of the regenerated cartilage revealed characteristics resembling healthy hyaline cartilage. Additionally, immunohistochemical analysis showed similar expression levels of collagen II, a marker of hyaline cartilage, in both the iMSC-Exos group and a healthy control group.[12] Furthermore, laboratory tests demonstrated a significant increase in chondrocyte migration and proliferation due to extracellular vesicles from iMSC-Exos. These findings indicate that iMSC-Exos can facilitate cartilage regeneration and the development of hyaline cartilage, which is crucial for treating OA.[12]

While earlier research emphasized the importance of synovial membrane-derived mesenchymal stem cells in OA, their precise role in cartilage repair remains unclear. The findings of Zhu et al. revealed that injecting SMMSC-derived exosomes significantly reduced OA progression in a collagenase-induced mouse model.[12] Although the SMMSC-Exos group showed some cartilage repair, immunohistochemical analysis revealed limited collagen II in the newly formed tissue compared to healthy cartilage. While in vitro studies confirmed that both iMSC-Exos and SMMSC-Exos enhanced chondrocyte migration and proliferation, the iMSC-Exos demonstrated superior therapeutic effects in the OA mouse model. Cartilage treated with SMMSC-Exos showed significant damage, including surface irregularities, fibrillation, proteoglycan loss, and erosion of the outer layer, none of which were observed in the iMSC-Exos treated cartilage.[1,20]

Further studies have also found that intra-articular injection of stem cell-derived exosomes (SC-Exos) is ineffective due to challenges in maintaining their presence at the cartilage defect site, resulting in rapid clearance. Developing strategies to ensure the sustained retention of SC-Exos at the injury site is crucial for their long-term efficacy, especially given the extended cartilage regeneration process. Additionally, the conventional approach of administering weekly localized injections throughout the recovery period may increase the risk of infection and patient discomfort.[1,20]

To address this issue, Liu et al. proposed an innovative solution by incorporating exosomes into a hydrogel tissue patch. Hydrogel materials are widely used in cartilage regeneration due to their distinctive characteristics, such as high water content, biocompatibility, and similarity to the cartilage matrix. However, research on hydrogel tissue patches as scaffolds for exosome-based cartilage regeneration is limited. An optimal hydrogel tissue patch for this purpose should fulfill three primary objectives: (1) conform to irregular cartilage defect shapes, (2) effectively retain SC-Exos at the defect site, and (3) seamlessly integrate with the surrounding cartilage to promote cell migration and facilitate the therapeutic action of SC-Exos.[13]

Recently, researchers developed a novel photoinduced imine crosslinking (PIC) hydrogel adhesive. This adhesive allows for in situ gel formation and strong adhesion to tissue surfaces. The PIC hydrogel possesses favorable characteristics for use as a scaffold in cartilage defect regeneration with SC-Exos, including ease of use, biocompatibility, tissue adhesion, and integration capacity. Liu et al. utilized the PIC hydrogel to encapsulate SC-Exos, creating a complex hydrogel tissue patch. The combination of SC-Exos' reparative capabilities with the advanced features of PIC hydrogel shows promise in developing a more effective scaffold for cartilage lesion healing and regeneration.[13]

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This study introduces a new method for repairing cartilage defects using an innovative acellular hydrogel tissue patch called EHG, which combines SC-Exos with PIC hydrogel glue. EHG offers several advantages: seamless integration with natural cartilage, secure bonding, prolonged exosome retention at the injury site, and positive effects on cell regulation in vitro and in vivo, significantly promoting cartilage repair and regeneration. This promising approach has the potential to advance cartilage tissue engineering and enhance treatment options for cartilage injuries.[20,21]

Previous studies have highlighted the importance of MSC exosomes in cell communication, particularly in various injuries and diseases, including OA. Mao et al. validated the presence of distinct microRNAs (miRNAs) within exosomes derived from MSC cultures. Notably, certain miRNAs exhibited increased expression during chondrogenesis. The influence of exosomal miR-92a-3p on cartilage growth and breakdown has been observed, with regulation of WNT5A playing a significant role in this process. The study showed that miR-92a-3p can upregulate SOX9 and COL2A1 expression while downregulating HDAC2 and inhibiting ADAMTS4 and ADAMTS5 expression. These molecular mechanisms have the potential to impede the progression of OA. Subsequent experimental investigations provided additional evidence supporting the binding of miR-92a-3p to the 3'-UTR region of WNT5A mRNA, resulting in the suppression of its expression. These findings also implied the involvement of miR-92a-3p in the process of cartilage breakdown. In vivo, research using a collagenase-induced OA mouse model demonstrated that extracellular vesicles derived from mesenchymal stem cells (MSC-miR-92a-3p-Exos) exhibited inhibitory effects on OA initiation and protected knee articular cartilage from extensive damage. This study elucidates the potential of exosomal miR-92a-3p as a therapeutic target for cartilage repair and OA treatment.[14]

Wu et al. studied the effects and mechanisms of exosomes from infrapatellar fat pad mesenchymal stem cells (MSCIPFP-Exos) in treating OA. This investigation employed in vitro and in vivo approaches to examine these research objectives. The results demonstrated that MSCIPFPs produced exosomes with typical characteristics. MSCIPFP-Exos exhibited advantageous outcomes in vivo by reducing the severity of osteoarthritis and suppressing chondrocyte apoptosis. In vitro, they facilitated extracellular matrix production and reduced the expression of catabolic factors. Notably, MSCIPFP-Exos have been observed to promote autophagy in chondrocytes, primarily through the inhibition of the mTOR pathway. Analysis of exosomal RNA revealed a significant microRNA, miR-100-5p, which targeted the 3'-UTR of mTOR. Inhibition of the mTOR signaling pathway by MSCIPFP-Exos was reversed by silencing miR-100-5p. Furthermore, the protective effect of MSCIPFP-Exos on articular cartilage in vivo was compromised by the intra-articular injection of antagomir-miR-100-5p. In summary, exosomes derived from MSCIPFPs demonstrated considerable promise in preserving cartilage homeostasis and ameliorating gait impairments in mice with OA. This effect was attributed to the regulation of the mTOR-autophagy pathway by miR-100-5p. Given the relative ease of obtaining human infrapatellar fat pad (IPFP) tissue from patients with OA, it is plausible that exosomes derived from mesenchymal stem cells in the infrapatellar fat pad could potentially serve as a therapeutic intervention for OA in the coming years.[15]

Chen et al. conducted research to explore the therapeutic potential of exosomes derived from MSCs in addressing two key characteristics of osteoarthritis: mitochondrial dysfunction and oxidative stress damage. Despite the critical role of MSC exosomes in intercellular mitochondrial communication, their specific application in managing mitochondrial function in OA has not been thoroughly investigated. In their research,

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the team utilized advanced desktop-stereolithography (SLA) technology to develop a highly efficient one-step system for cartilage tissue engineering. The scaffold they created consisted of GelMa hydrogel, MSC-derived exosomes, and an extracellular matrix with decellularized cartilage. Compared to traditional freeze-dried collagen scaffolds, this 3D-printed scaffold demonstrated superior cell recruitment ability. The decellularized ECM preserved crucial peptides, which promoted cell adhesion and migration. Furthermore, the scaffold facilitated chondrocyte migration within defect regions and sustained the release of exosomes, including MSC-derived exosomes that were effectively internalized by chondrocytes. Consequently, this resulted in improved production and repair of damaged mitochondria in chondrocytes, supported by the presence of mitochondria-related proteins in the exosomes. The innovative SLA-based approach presented by the researchers offers a promising solution for regenerating osteochondral defects through a single efficient procedure. The combination of GelMa hydrogel, MSC-derived exosomes, and ECM with decellularized cartilage represents a significant advancement in cartilage tissue engineering, holding potential for improving therapeutic outcomes in cartilage repair and regeneration.[17]

A limitation of this systematic review is the lack of diversity in measurement tools, as all six studies used either the OARSI or ICRS score to evaluate cartilage repair, which may not capture the full range of regeneration outcomes. Additionally, all included studies involved only experimental animals; further research is needed on a larger number of experimental animals before clinical trials in humans can be considered.

CONCLUSION

The collective findings from the six selected studies strongly support the promising potential of the secretome in facilitating knee cartilage regeneration. The secretome group exhibited the most favorable outcomes compared to other treatment groups, indicating its superiority in promoting cartilage repair. Additionally, recent research has highlighted the importance of SMMSCs in cartilage repair due to their close relationship to chondrocytes and enhanced chondrogenic potential. To enhance the therapeutic impact of MSC exosomes, researchers have explored methods for effectively retaining them at the cartilage defect site. Overall, the cumulative evidence from these studies underscores the importance of MSC exosomes and the secretome in cartilage repair and presents exciting opportunities for advancing treatment options for cartilage injuries and osteoarthritis. Further research and clinical trials are warranted to validate and refine these promising therapeutic approaches.

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