



Comparative efficacy of stem cells and secretome in articular cartilage regeneration: a systematic review and meta-analysis

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Abstract

Articular cartilage defect remains the most challenging joint disease due to limited intrinsic healing capacity of the cartilage that most often progresses to osteoarthritis. In recent years, stem cell therapy has evolved as therapeutic strategies for articular cartilage regeneration. However, a number of studies have shown that therapeutic efficacy of stem cell transplantation is attributed to multiple secreted factors that modulate the surrounding milieu to evoke reparative processes. This systematic review and meta-analysis aim to evaluate and compare the therapeutic efficacy of stem cell and secretome in articular cartilage regeneration in animal models. We systematically searched the PubMed, CINAHL, Cochrane Library, Ovid Medline and Scopus databases until August 2017 using search terms related to stem cells, cartilage regeneration and animals. A random effect meta-analysis of the included studies was performed to assess the treatment effects on new cartilage formation on an absolute score of 0–100% scale. Subgroup analyses were also performed by sorting studies independently based on similar characteristics. The pooled analysis of 59 studies that utilized stem cells significantly improved new cartilage formation by 25.99% as compared with control. Similarly, the secretome also significantly increased cartilage regeneration by 26.08% in comparison to the control. Subgroup analyses revealed no significant difference in the effect of stem cells in new cartilage formation. However, there was a significant decline in the effect of stem cells in articular cartilage regeneration during long-term follow-up, suggesting that the duration of follow-up is a predictor of new cartilage formation. Secretome has shown a similar effect to stem cells in new cartilage formation. The risk of bias assessment showed poor reporting for most studies thereby limiting the actual risk of bias assessment. The present study suggests that both stem cells and secretome interventions improve cartilage regeneration in animal trials.

Keywords Animal trial · Cartilage regeneration · Secretome · Stem cells · Systematic review

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Introduction

Articular cartilage defects are a clinical problem due to limited intrinsic healing potential, which often leads to the development of osteoarthritis if proper treatment is not provided (Heijink et al. 2012). The connection between articular cartilage defect and osteoarthritis is pertinent, making it necessary to quest for clinical advancement in cartilage regeneration (Caldwell and Wang 2015). Cartilage injury-induced posttraumatic osteoarthritis is most common in the younger adult population for which the needed treatment strategy is to preserve the function of the affected joint by regeneration of the damaged cartilage instead of total knee arthroplasty (Caldwell and Wang 2015). Osteoarthritis is the form of arthritis that is characterized by joint pain and stiffness that accounts for the most common disability and activity limitation, especially in the elderly population (Heijink et al. 2012). Surgical options such

as bone marrow stimulation procedures, mosaicplasty, arthroscopic debridement, autologous chondrocyte implantation and arthroplasty have been introduced into clinical practice for the management of damaged articular cartilage with varying results (Bentley et al. 2012; Goldberg et al. 2017). Autologous chondrocyte implantation (ACI) and new-generation, matrix-assisted autologous chondrocyte implantation (MACI) have shown promising results in repair and restoration of cartilage defect (Kreuz et al. 2009; Zhang et al. 2016a, b). These treatments involve *in vitro* isolation and culture expansion of chondrocytes prior to implantation into the defective cartilage (Mobasheri et al. 2009). Unfortunately, limited expansion capacity of primary chondrocytes and the possibility of chondrocyte dedifferentiation into fibroblasts in a monolayer culture (Lin et al. 2008) have posed limitations to ACI and MACI. On the other hand, total knee arthroplasty is considered the last resort (Wehling et al. 2016) where postoperative complications, such as loosening, peri-prosthetic fracture and pain, may culminate in the need for revision surgery (Bhandari et al. 2012).

In recent years, stem cell therapy has emerged as promising treatment strategies for cartilage regeneration (Wolfstadt et al. 2015). The therapeutic potential of stem cells has been attributed to their capability to differentiate into several cell lineages (Ullah et al. 2015). A report has indicated that few stem cells survive or remain *in situ* after injection (Shim et al. 2014). Contrary to the initial paradigm of cell differentiation and replacement as the mechanism by which stem cells exhibit a therapeutic effect, strong evidence is emerging that the therapeutic efficacy of stem cells is attributed to the paracrine action of the secreted factors (Madrigal et al. 2014). Stem cells can secrete an array of growth factors, cytokines and extracellular vesicles in their secretome to modulate the defective tissue-surrounding milieu to evoke regenerative processes (Meirelles et al. 2009; Panagiotou et al. 2016). Secretome from adipose-derived mesenchymal stem cells has been reported to enhance the expression of collagen type 2 and down-regulate the expression of matrix metalloproteinases in osteoarthritic chondrocytes (Platas et al. 2013). Exosomes are extracellular vesicles that serve as intercellular communication vehicles for the delivery and transfer of bioactive lipid, proteins and nucleic acid cargo to the injured cells for the induction of functional and phenotypic changes to elicit regenerative processes in the recipient cells (Vishnubhatla et al. 2014; Toh et al. 2017).

Both the results of preclinical and clinical studies have provided preliminary evidence of safety and efficacy of mesenchymal stem cells (MSCs) for the treatment of osteoarthritis (Freitag et al. 2016). A systematic review of a clinical trial of stem cell in knee osteoarthritis by Pas et al. (2017) suggested a low level of evidence due to a high risk of bias of the six included trials. The results of preclinical studies offer vital evidence that would allow a robust design for clinical studies.

Therefore, this study is designed to identify data for systematic review and meta-analysis of stem cell and secretome interventions and to compare the therapeutic efficacy of stem cells and secretome in animal models of cartilage defects.

Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al. 2009).

Review questions

We performed a systematic review of the literature of animal models to address the following questions: (i) Do stem cell and secretome treatments promote cartilage repair in cartilage defect as compared with control? (ii) Is there a significant difference between stem cells and secretome in articular cartilage regeneration at the site of articular cartilage defect?

Eligibility criteria

Type of studies and study population

The eligibility criteria were only preclinical controlled animal models of cartilage defect that used either stem cells or secretome as intervention. The study populations for this review were both small and large animal models of cartilage defect.

Types of control comparison

The preclinical comparison group included animals from studies that had cartilage defect that were not administered with stem cells or secretome (vehicle, scaffold, or untreated control). For studies with multiple treatments, only the stem cell or secretome group and vehicle/scaffold were selected for comparison.

Outcome measure

The primary outcome was the amount of new cartilage formation within the defect area. We concentrated on the outcomes that were relevant in cartilage regeneration such as histological score that was used as a standardized scoring system.

Literature search

A comprehensive search of five databases—PubMed, CINAHL, Cochrane Library, Ovid Medline and Scopus—was conducted to identify the studies that met our inclusion criteria until August 2017 using the following search keywords (Stem

cells OR conditioned medium OR secretome) AND (osteoarthritis OR cartilage repair OR cartilage defect) AND (animal models). We also conducted manual searches of the reference list to complement the electronic search.

Exclusion criteria

In vitro and human studies, animal studies without control group and reviews were excluded. Articles written in a language other than English were also excluded.

Data extraction and synthesis

A standardized data extraction sheet was used to extract the data from the included studies. The following data were extracted: the author, year of publication, type of animal (including age, weight and sex), stem cell type, nature of defect (chemical or surgical), interventions in the control group, mode of delivery and outcomes of interest. Data were extracted for the final time point in a situation where the outcomes of interest were assessed in sequence. In case of multiple arms in a study, the stem cell- or secretome-treated group and control group were selected. Furthermore, in a study with multiple doses or more than one type of stem cells, the most effective dose or cell type was selected for comparison. Data were extracted by two independent reviewers. Quantitative data were extracted from the studies that met the inclusion criteria. Meta-analyses were performed for outcome measure that used semiquantitative histological scoring systems. Outcome data such as mean, standard deviation (SD) and number of animals were extracted from the tables/text. Studies that presented data in graphic form were extracted using highly magnified image software (GetData Graph Digitizer, version 2.26). Standard deviation was recalculated from the data presented as mean and confidence interval (CI) per group using an online calculator (<http://www.sample-size.net/confidence-interval-mean/>) for 95% CI, whereas SE was converted to SD using GraphPad InStat software (version 3.0). Studies with a missing number of animals, SD, or SE/CI were excluded from the meta-analysis. Studies that reported macroscopic scoring without quantitative histological data were not included in the meta-analysis. One study that reported both the effects of stem cells and secretome were enrolled into the meta-analysis as two studies. Two studies that have one group with SD of 0 were adjusted to 0.01 to enable us to determine the effect estimate of the studies. In case of any discrepancies in the interpretation of data between the reviewers, it was resolved through discussion and consensus.

Risk of bias and quality assessment

The risk of bias of the included studies was performed by two independent reviewers using the Systematic Review Centre

for Laboratory Animal Experimentation (SYRCLE) risk of bias tool as described previously (Hooijmans et al. 2014). The following six domains were assessed as low, high, or unclear risk of bias.

- i. Selection bias: random sequence generation, baseline characteristics, allocation concealment
- ii. Performance bias: random housing, blinding of participants and personnel
- iii. Detection bias: random outcome assessment, blinding of outcome assessor
- iv. Attrition: incomplete outcome data
- v. Reporting bias: selective outcome reporting
- vi. Other bias (bias not covered by other domains such as power and sample size estimation, ethical criteria and conflict of interest)

Data analysis

The primary outcome was the mean differences of new cartilage formation between the treated and control animals at follow-up. The mean effect size, 95% confidence interval, forest plot and significance were examined using the inverse-variance method. For multiple measurements over time, data measured at the end of the study were used for analyses. Mean difference (MD) was used for the data analysis. Subsequently, the means and standard deviations of studies included in this review were converted to a scale of 100% (the result divided by the maximum attainable histological score and multiplied by 100) for comparison. Therefore, in this review, a higher score was adopted to indicate cartilage regeneration with a maximum score of 100%. We also conducted stratified/subgroup analyses on animal species, cell type, route of delivery, dose and duration of follow-up to examine the potential source of heterogeneity and robustness of our results using mean difference. The subgroup analyses were only performed for subgroups that consist of ≥ 2 groups. Sensitivity analyses were also conducted to determine the strength and source of heterogeneity by excluding studies one by one, and the homogeneity and effect size for the remaining studies were analyzed. Heterogeneity across individual studies was assessed using I^2 statistic. Values of 75% and above were considered high heterogeneity. A funnel plot was used to assess the possibility of publication bias. To further confirm whether publication bias existed or not, Egger regression asymmetry and Begg adjusted rank correlation tests were performed (Begg and Mazumdar 1994; Egger et al. 1997). The meta-analysis was performed using random effects model with Review Manager (version 5.3). All other analyses were performed with SPSS (version 15).

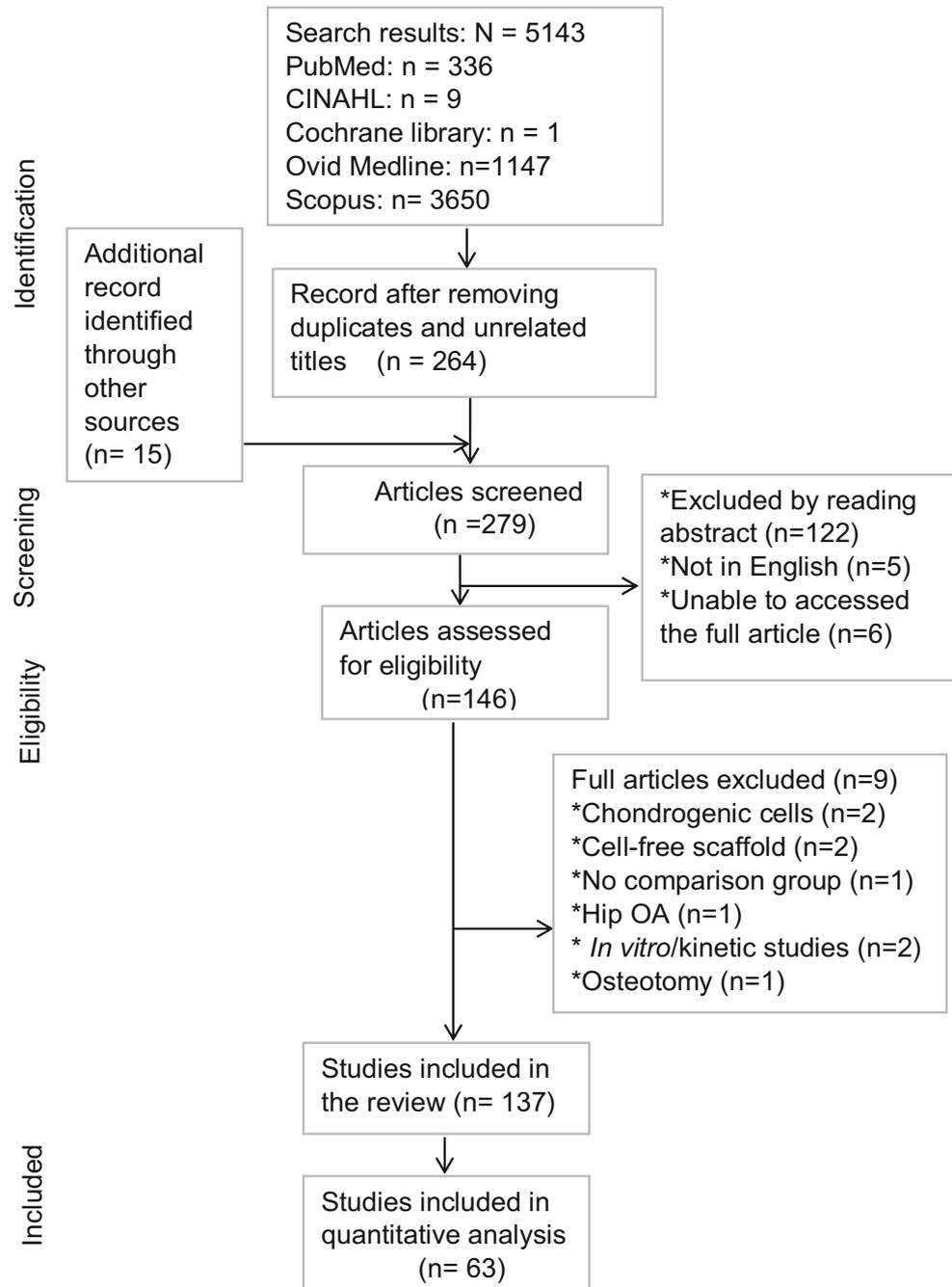
Results

Search results

Articles identified through database search were 5143 from five databases (CINAHL, Ovid Medline, PubMed and Scopus). Of the 5143 articles, 3650 were from Scopus, 1147 from Ovid Medline, 336 from PubMed, 9 from CINAHL and 1 from Cochrane Library. An additional 15 articles were identified by a manual search of other sources. After removing duplicates and unrelated articles, a total of 279 articles were

screened for eligibility. Five articles (Chinese, $n = 3$; German and Japanese one each) and six articles that cannot be accessed were excluded from this study. Of the 146 full articles assessed, 137 studies were included for qualitative analysis (Supplementary File 1). Nine studies were excluded based on the following reasons: chondrogenic cells (two studies), cell-free scaffold (two studies), no comparison group (one study), hip OA (one study), osteotomy (one study) and *in vitro*/kinetic studies (two studies). For the quantitative analysis, only 63 studies were enrolled. A summary of the literature search is depicted in Fig. 1.

Fig. 1 Flowchart of systematic search of the literature



Study characteristics

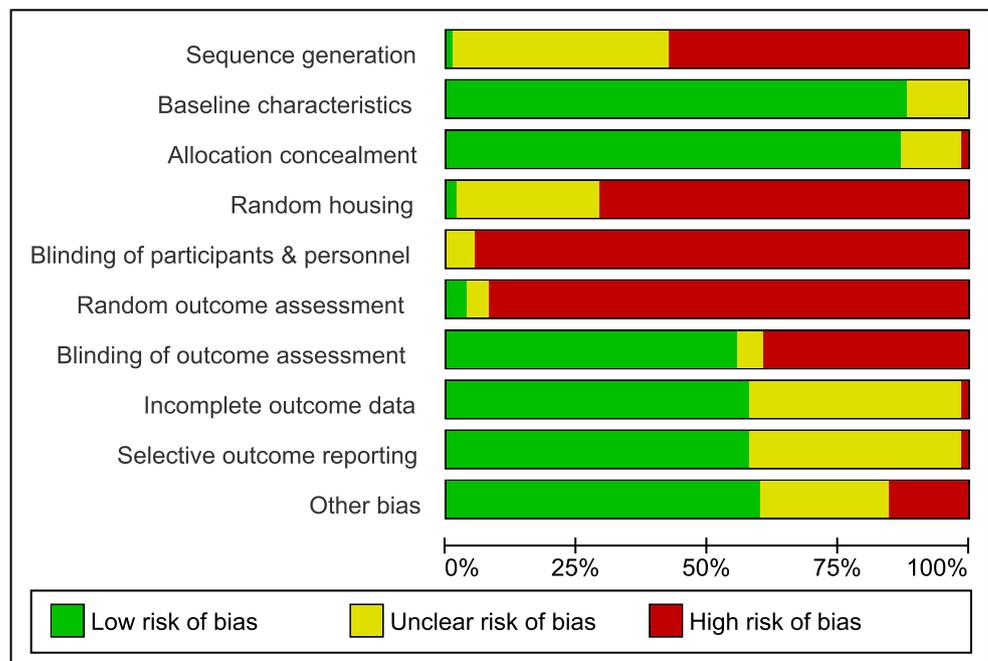
The study characteristics of the included studies showed clear substantial variations (Supplementary File 1). Different animal species were used, ranging from small (mouse, rat, rabbit and guinea pig) to larger animals (pig, dog, sheep, goat, monkey, horse and donkey). A total of 3500 animals were used in 131 studies, whereas 6 studies did not provide detailed information on the total number of animals utilized. Rabbits were the most studied animals. Of the 137 studies, 60 studies used rabbit models, followed by rats with 27 studies, sheep (14 studies), pig (13 studies), mouse (11 studies), goat (4 studies), horse (3 studies), dog (2 studies) and monkey, guinea pig and donkey (1 study each), respectively. Furthermore, 15 different types of cells were used with bone marrow mesenchymal stem cells having the highest number of 75 studies, adipose-derived mesenchymal stem cells (25 studies), synovial-derived MSC (15 studies), umbilical blood derived MSC (7 studies), embryonic stem cells/embryonic-MSC (6 studies), induced pluripotent stem cells/induced pluripotent-MSC (4 studies) and dental pulp stem cells, stem cells from human exfoliated deciduous teeth, Wharton's jelly MSC, induced neural crest MSC, turbinates-derived MSC, bone marrow mononuclear cells, periosteum-derived MSC, muscle-derived mesenchymal stem cells and peripheral blood mononuclear cells (1 study each). There were also variations in the age and weight of the animals. Duration of the studies also varied significantly among the studies, ranging from a minimum duration of 2 weeks to a maximum of 24 months. Different approaches were used to create/induce cartilage defect ranging from anterior cruciate ligament transection, full or partial meniscectomy to chemical

(monosodium iodoacetate, collagenase and amphotericin B) induced cartilage defects. Spontaneous osteoarthritis was reported in one study (Sato et al. 2012). A significant variation was also observed between the doses of the cells administered to the animals, ranging from 1×10^4 to 1.5×10^8 cells per animal. The dose used for secretome intervention was between 5 and 100 μ l. Cell-seeded scaffolds (52 studies), intraarticular injection (57 studies) and direct implantation (29 studies) were used as the route of delivery of the cells. One study reported intravenous as the transfusion method (Leonard et al. 2015). The histological scoring systems adopted in the included studies consist of scoring parameters such as cell morphology, surface area filled with cells, toluidine blue staining, safranin O staining, alcian blue staining, chondrocyte clustering, degenerative changes, restoration of the subchondral bone and integrity, thickness and structural integrity.

Risk of bias assessment

The methodological rigor of the included studies was assessed using SYRCLE. Overall risk of bias assessment is depicted in Fig. 2. For risk of bias in individual studies, refer to Supplementary File 2. The overview of the result shows a lack of some key information required for experiments involving animals. In the included studies, details describing the method of randomization were largely lacking and, as such, studies were scored unclear risk of bias if animals were randomized without giving the detail. Allocation concealment was scored low if a study allocated animals to the treatment and control groups. For baseline characteristics, there was a low risk of bias as most studies provided information regarding the

Fig. 2 Risk of bias assessment



weight, age and sex of the animals as well as the size of the defect at the start of the experiment. Random housing and blinding of personnel that measure performance bias were scored high risk of bias as most of the included studies did not provide detailed information. Most of the studies provided information on the blinding of the outcome assessor in scoring the cartilage repair and were scored low risk of bias. Selective outcome reporting and incomplete outcome data were scored unclear if clear details were not provided in both the methodology and results section on the number of animals used at the start and end of the experiment. Other risk of bias is scored low if the following details were provided: ethics, funding/conflict of interest and/or power calculation. Another key important factor that was missing in the experimental designs of the included studies was sample size and power calculations. It was only in 11 studies that a priori sample size determination was described (Dashtdar et al. 2015; Horie et al. 2012b; Lam et al. 2014; McIlwraith et al. 2011; Pei et al. 2013; Proffen et al. 2015; Tay et al. 2012; Toratani et al. 2017; van Buul et al. 2014; Xu et al. 2016; Zscharnack et al. 2010). Overall, some of the key factors that posed threats to internal validity of a study were not reported in some of the studies.

Meta-analysis

The 63 studies included in the meta-analysis utilized 712 animals. Of these 63 studies, 59 studies were on stem cells, whereas the remaining 4 were on the secretome. A study by Wang et al. (2017) was considered two studies in the quantitative analysis because the study utilized both stem cells and secretome with a different set of animals. The stem cell studies utilized 660 animals comprising 329 animals for the treatment groups and 331 for the control groups. In the secretome studies, a total of 52 animals were used consisting of 26 animals each for the secretome and control groups, respectively. The results indicated a significant therapeutic effect of stem cells in cartilage regeneration (25.99%, 95% CI 22.16, 29.82; $p < 0.00001$) with significant heterogeneity ($I^2 = 90%$, $p = 0.00001$) (Fig. 3). Treatment with secretome (Fig. 4) showed a significant increase in cartilage formation (26.08%, 95% CI 14.74, 37.42; $p < 0.00001$) with significant heterogeneity ($I^2 = 80%$, $p = 0.002$). There was no significant difference ($p = 0.648$) between the effect of stem cells and secretome in articular cartilage regeneration.

Stratified analysis

The result of subgroup analyses is depicted in Table 1. Stratified analysis indicated significant cartilage regeneration in the seven subgroups of animals with the exception of the horse subgroup ($p = 0.32$). The effect of stem cells was better in pig (40.46%, 95% CI 24.67, 56.25), followed by dog (33.94%, 95% CI 9.44, 58.44), whereas a similar effect was observed in rat (27.51%, 95% CI 21.31, 33.71), mouse (27.15%, 95% CI 15.06, 39.23)

and rabbit (27.05%, 95% CI 21.34, 32.76), respectively. Furthermore, the lowest effect was observed in sheep (14.08%, 95% CI 6.12, 22.04) and horse (4.15%, 95% CI -4.05, 12.35) subgroups. However, the result of meta-regression showed no significant difference ($p = 0.407$) in the effect of stem cells in cartilage regeneration between the animals. There was no significant heterogeneity in the sheep ($I^2 = 0%$, $p = 0.95$), horse ($I^2 = 0%$, $p = 0.37$) and mouse ($I^2 = 43%$, $p = 0.17$) subgroups, whereas a significant heterogeneity was observed in the remaining subgroups. Cell-type subgroup analysis showed significant new cartilage formation with significant heterogeneity in the bone marrow mesenchymal stem cell (BMSC), adipose-derived mesenchymal stem cell (ADSC) and synovial mesenchymal stem cell (SMSC) subgroups. In the ESC subgroup, there was no heterogeneity ($I^2 = 0%$, $p = 0.72$). SMSC showed the highest effect of 40.06% (95% CI 27.05, 53.07) in new cartilage formation, though there was no significant difference ($p = 0.657$) between the cell types in new cartilage formation. The subgroup analysis for the route of delivery showed significant cartilage regeneration with the intraarticular route having the highest effect (31.34%, 95% CI 26.16, 36.52). There was no significant difference ($p = 0.546$) between the three routes of delivery in new cartilage formation. Significant heterogeneity was observed across the three routes of delivery. Cartilage regeneration was not dose dependent as shown by the result of subgroup analyses. Furthermore, the subgroup analysis of the dose administered also showed significant heterogeneity across the groups. The subgroup analysis showed that the duration of follow-up is a significant predictor ($p = 0.007$) of enhanced cartilage regeneration. The effect of stem cell treatment seemed to decline after 6 months of follow-up (8.51%, 95% CI 2.58, 14.44). However, there was no significant heterogeneity ($I^2 = 0%$, $p = 0.53$) in the repair of cartilage at > 6 months after intervention.

Sensitivity analysis and publication bias

Sensitivity analyses of the effect of stem cell and secretome on cartilage regeneration were performed by sequentially removing one study at a time and reanalyzing the data. In the secretome studies, removing the study of Zhang et al. (2016a, b) resulted in nonsignificant heterogeneity ($I^2 = 0%$, $p = 0.66$) with treatment effect (20.32%, 95% CI 19.26, 21.38). For stem cell therapy, the sensitivity analyses showed no significant impact on the new cartilage formation (data not shown). The funnel plot (Fig. 5) indicated that no significant publication bias existed in the present study. Egger's and Begg's tests showed no significant publication bias with $p = 0.948$ and $p = 0.996$, respectively. Publication bias was not performed for secretome studies because of few studies involved. There should be no fewer than 10 studies to test for funnel plot asymmetry due to low test power to distinguish chance from real asymmetry (Sterne et al. 2011).

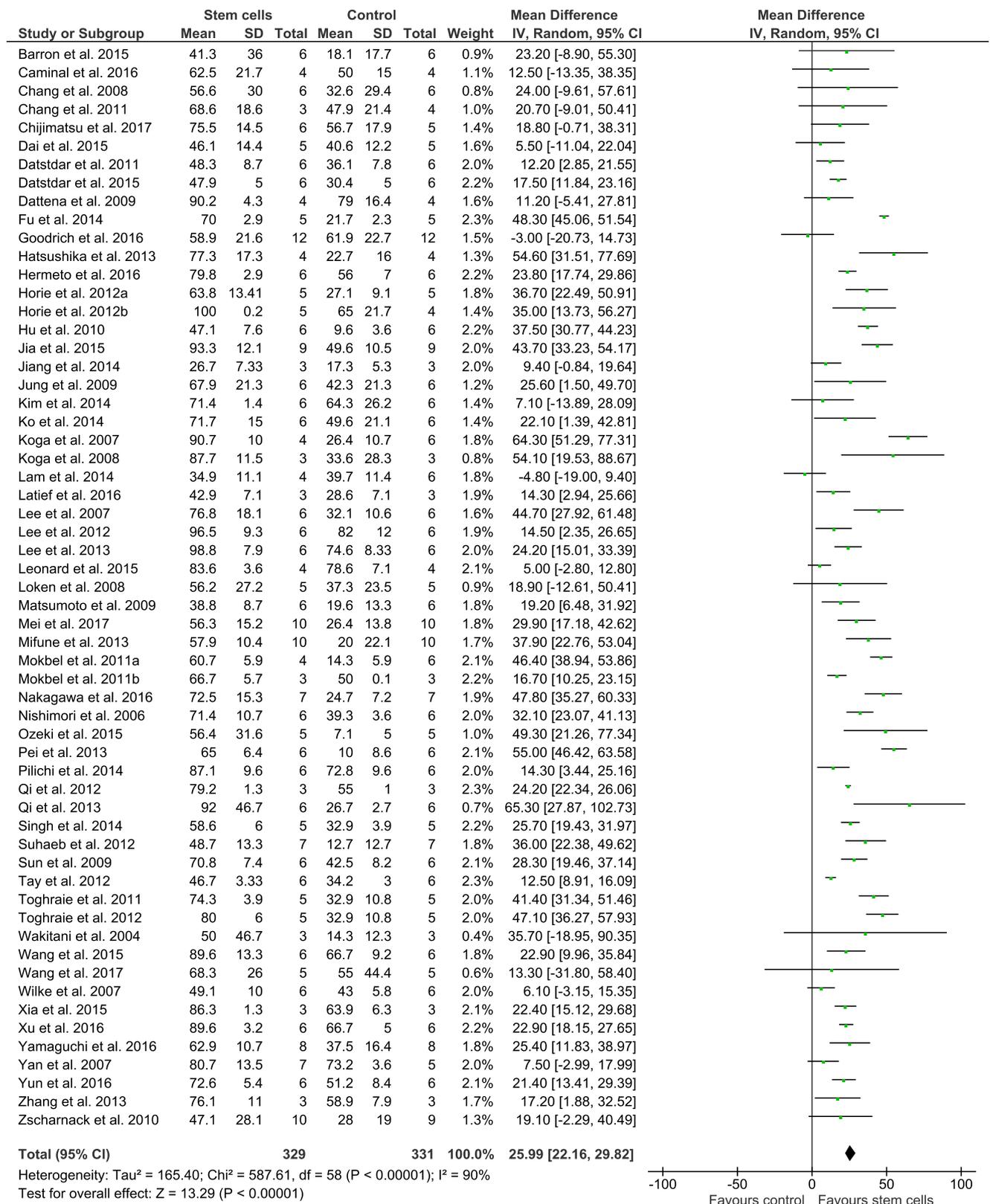


Fig. 3 Forest plot showing the effect of stem cells in cartilage regeneration. *df*—degrees of freedom, CI—confidence interval, SD—standard deviation

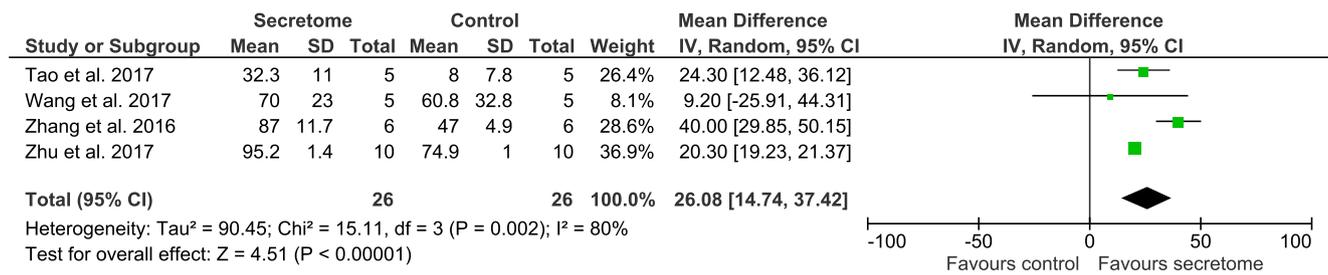


Fig. 4 Forest plot showing the effect of secretome in cartilage regeneration. *df*—degrees of freedom, CI—confidence interval, SD—standard deviation

Discussion

Stem cells have been widely investigated in the regeneration of damaged articular cartilage in preclinical models and in some clinical trials. However, the therapeutic efficacy of stem cell implantation has revealed mixed results (Ceylan et al. 2016; Davatchi et al. 2016; Jo et al. 2014). These may be attributed in part to differences in the actual dose of cells with some studies reporting that a higher number of cells lead to a superior repair (Hui et al. 2008; Jo et al. 2014). Furthermore,

the use of scaffold as a substrate to support cell implantation may also contribute to the nonsatisfactory results because of its own limitations, which include cell leakages, poor cell survival and differentiation, poor integration into the tissue of the host, inappropriate cell distribution as well as dedifferentiation of the normal chondrocyte (Rai et al. 2017). Recent studies suggested that stem cells secrete multiple factors that allow them to exert a therapeutic effect via the paracrine mechanism (Baraniak and McDevitt 2010; Madrigal et al. 2014; Muhammad et al. 2018; Wakayama et al. 2015;

Table 1 Stratified analysis of stem cells in cartilage regeneration

| Subgroups | Number of studies | Mean difference IV, random, 95% CI | Value of I ² (%) | p ^h | p ^a |
|---|-------------------|------------------------------------|-----------------------------|----------------|----------------|
| Type of animal | | | | | |
| Rabbit | 27 | 27.05 (21.34, 32.76) | 94 | < 0.00001 | 0.407 |
| Sheep | 4 | 14.08 (6.12, 22.04) | 0 | = 0.95 | |
| Rat | 14 | 27.51 (21.31, 33.71) | 57 | = 0.005 | |
| Pig | 4 | 40.46 (24.67, 56.25) | 67 | = 0.03 | |
| Mouse | 3 | 27.15 (15.06, 39.23) | 43 | = 0.17 | |
| Dog | 2 | 33.94 (9.44, 58.44) | 95 | < 0.00001 | |
| Horse | 2 | 4.15 (- 4.05, 12.35) | 0 | = 0.37 | |
| Type of cell | | | | | |
| BMSCs | 34 | 24.17 (18.95, 29.39) | 93 | = 0.00001 | 0.657 |
| ADSCs | 8 | 26.35 (18.14, 34.55) | 80 | = 0.00001 | |
| SMSCs | 9 | 40.06 (27.05, 53.07) | 90 | = 0.00001 | |
| ESCs | 6 | 18.76 (13.49, 24.02) | 0 | = 0.72 | |
| Cell dose | | | | | |
| ≤ 10 ⁶ | 28 | 25.36 (20.17, 30.55) | 86 | < 0.00001 | 0.645 |
| > 10 ⁶ ≤ 2 × 10 ⁶ | 6 | 28.27 (9.41, 47.13) | 92 | < 0.00001 | |
| > 2 × 10 ⁶ | 22 | 25.90 (19.26, 32.55) | 93 | < 0.00001 | |
| Route of delivery | | | | | |
| CS | 20 | 20.67 (12.76, 28.58) | 93 | < 0.00001 | 0.546 |
| DT | 16 | 23.47 (16.44, 30.51) | 88 | < 0.00001 | |
| IA | 23 | 31.34 (26.16, 36.52) | 83 | < 0.00001 | |
| Follow-up after intervention | | | | | |
| ≤ 6 months | 54 | 27.59 (23.63, 31.55) | 90 | < 0.00001 | 0.007 |
| > 6 months | 5 | 8.51 (2.58, 14.44) | 0 | = 0.53 | |

BMSCs—bone marrow mesenchymal stem cells, ADSCs—adipose-derived mesenchymal stem cells, SMSCs—synovial mesenchymal stem cells, CS—cell-seeded scaffold, DT—direct implantation to the defect site, IA—intraarticular injection, CI—confidence interval, p^h—value for heterogeneity, p^a—value for meta-regression between each subgroup

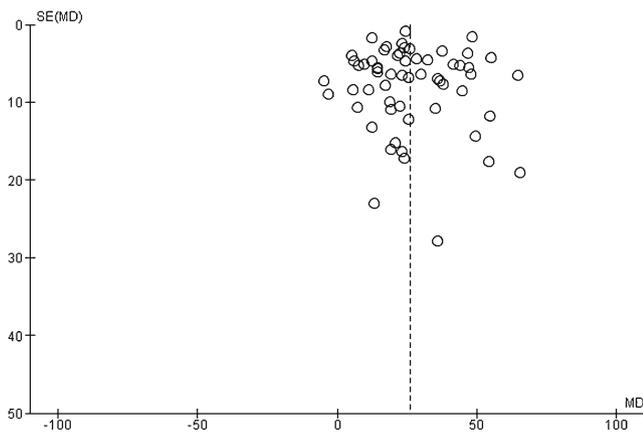


Fig. 5 Funnel plot for the assessment of potential publication bias. There was no evidence of publication bias for stem cell studies. MD—mean difference, SE—standard error

Yamagata et al. 2013; Yang et al. 2013). Therefore, this systematic review aimed to provide a current overview of the existing knowledge as well as to identify the gaps on the use of stem cells and secretome in articular cartilage regeneration that could help to design future preclinical animal models and clinical trials. We also compared the efficacy of stem cell and secretome in articular cartilage regeneration.

The overall result of this systematic review and meta-analysis indicated that treatment of articular cartilage defect with stem cells and secretome significantly improved cartilage regeneration as compared with control by 25.99 and 26.08%, respectively. A large number of studies that met our inclusion criteria were excluded in the quantitative analysis due to lack of information, ranging from the number of animals that contributed to the results, to missing SD, SE and/or CI, whereas in some studies, no histological quantitative data were reported. It is important to emphasize that reporting the number of animals at the start and the actual animals that contributed to the result, as well as detailed data analysis in a study, is crucial for good systematic review and meta-analysis. There was high heterogeneity of the effect of stem cells and secretome in articular cartilage regeneration. Due to high heterogeneity, subgroup analyses of cell types, types of animals, dose, route of delivery and duration of the study were performed. We also performed a sensitivity test to determine the potential source of heterogeneity. For the subgroup analyses, no significant differences were observed between each subgroup with the exception of duration of follow-up. The result showed that duration of follow-up is a significant predictor of stem cell intervention in this study. High heterogeneity was also observed across the subgroup analyses with few subgroups showing nonsignificant heterogeneity. A report indicated that higher metabolic activity and increased density of chondrocytes in rabbits may confer greater healing capacity in models of articular cartilage defect than other species (Moran et al. 2016). However, the result of stem cell intervention in this meta-analysis did not support this assertion because rabbits, rats and

mice displayed a similar effect, whereas pigs and dogs showed superior effects in new cartilage formation. Similarly, SMSC was the cell type that demonstrated superior new cartilage formation.

The route of delivery showed intraarticular injection with a superior effect in cartilage regeneration. Cell dose seems not to serve as a predictor of cartilage regeneration as there was no significant difference in the effect between low, medium and high doses of cells administered. Sensitivity analysis showed an impact in identifying the source of heterogeneity in the effect of secretome in new cartilage formation because no heterogeneity resulted after removing one study.

Davatchi et al. (2016) reported that transplantation of mesenchymal stem cells in the knee of OA patients in a 5-year follow-up improved walking time and pain at 6 months but gradually deteriorated, though the improvement was still better than at baseline. They concluded that early transplantation of MSC may give a superior effect than in a long-term follow-up. The finding of this review corroborated their result because subgroup analysis at follow-up showed better cartilage regeneration within the first 6 months of intervention. However, the result for subgroup analyses should be interpreted with caution, particularly for those with a limited number of studies.

Mesenchymal stem cells are capable of producing cartilage-like tissue with a matrix based on aggrecan and collagen type 2 under appropriate culture conditions (Akpancar et al. 2016; Murdoch et al. 2007). Macroscopic, magnetic resonance imaging and histological evaluations are the various outcome measures that were used to investigate cartilage regeneration. In this review, only data from semiquantitative histological examination were used as outcome measure because they allow for a quantitative comparison between treatment and control groups.

In this systematic review, secretome showed a comparable effect to stem cells in articular cartilage regeneration. Intraarticular injection of adipose-derived MSC into the knee of an OA patient showed improved function and pain of the knee joint as well as regeneration of hyaline-like cartilage without any report of adverse effect (Jo et al. 2014).

The methodological rigor and risk of bias assessment of the included studies were performed to assess the quality of experimental designs and the reliability of the results. Several threats to internal validity were identified in the preclinical studies (Henderson et al. 2013) that delay the translation of most animal studies to clinical trials. A low methodological rigor may lead to underestimation or overestimation of the effect size. Overall, the prospect of assessing the actual risk of bias was limited due to the lack of key details regarding the experimental design in most studies. Furthermore, detail regarding the method of randomization was not described in the included studies. It was only a few studies that described a priori sample size and power estimation. The risk of bias in individual studies varies across the studies with some studies having high risk of bias, whereas in some studies, it was moderate. To minimize the risk of bias in preclinical studies, it is therefore important to

introduce a golden standardized publication checklist (Pot et al. 2016), or strict adherence to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines could improve reporting of studies involving animals.

A funnel plot is a visual aid of treatment effect against a measure of study precision that is used to detect bias or systematic heterogeneity. A symmetrical funnel shape indicates no publication bias. Furthermore, the funnel plot in this systematic review did not indicate the existence of publication bias as shown by the symmetry of the graphical representation. To further confirm this, Egger's and Begg's tests were performed, which showed no significant publication bias.

It is evident from this systematic review and meta-analysis that the therapeutic effect of stem cells is mediated via a paracrine mechanism because treatment using cell-free secretome has shown a similar effect in articular cartilage regeneration in animal models. This present study is, to our knowledge, the first systematic review and meta-analysis to compare the therapeutic efficacy of stem cells and secretome in cartilage regeneration.

This study has several limitations. The experimental design of the included studies varied in terms of the age and type of animals that received the intervention, the cell type, the cell dose and the route of delivery of the cells as well as the duration of follow-up. This caused significant levels of heterogeneity while pooling histological outcome and, as such, limits the conclusion that can be drawn from this review. We limited the analysis to semiquantitative histological outcomes. In addition to histological outcome, biochemical and biomechanical data and functional outcome such as pain may be just as important in relation to assessing the potential of stem cells and secretome intervention in cartilage regeneration. A large number of studies that were included in the qualitative analysis failed short to be included in the quantitative analysis due to methodological shortcomings. Furthermore, five studies were excluded from this study based on language restriction. Taken together, this might introduce publication bias; however, the result of this review showed no significant publication bias as indicated by the funnel plot as well as Egger's and Begg's tests. Different histological scoring systems were used in the included studies, limiting the ability to effectively pool the data. However, for consistency, the data were converted to indicate that a high score favors cartilage regeneration. Therefore, the result of this systematic review and meta-analysis should be interpreted in the light of these limitations.

Conclusion

This study presents new insight regarding the therapeutic efficacy of stem cells and secretome to treat cartilage defect in animal models. The result of this systematic review and meta-analysis showed a similar therapeutic benefit of secretome to stem cell transplantation in preclinical animal studies. A standardized study

design with validated outcome measures and long-term safety studies is a key factor for possible translation to clinical trials.

Recommendations

Meta-analyses of studies involving animals are important to guide a robust future design of preclinical studies and clinical trials. On this basis, we recommended that well-designed studies with robust methodological quality on preclinical studies and clinical trials should focus on the use of a cell-free product such as secretome because it will allow easy standardization of the dose to be administered. It can also be prepared and stored as off-the-shelf products for easy transportation and accessibility. Also, a golden standard for histological scoring system that will use a similar scale of measurement should be adopted as it will allow for robust systematic review and meta-analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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