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# Stem cell therapy for bone repair: a systematic review and meta-analysis of preclinical studies with large animal models

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## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Studies from large animals present that stem cell therapy is a promising strategy for restoring bone damage.

## WHAT THIS STUDY ADDS

- The effects of stem cell therapy are likely dependent on both the transplanted cell number and cell transplantation mode.

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## AIM

Injury to bone is a significant clinical challenge, due to its limited regenerative capacity. The current methods of repairing bone defect are surgical, highly invasive and not always successful. A systematic review and meta-analysis of preclinical studies involving large animals with bone defects were conducted to determine the treatment outcomes with stem cell therapies.

## METHODS

A random effects meta-analysis of the available studies was conducted to assess the treatment outcomes including the rate of new bone formation and new bone mineral density (BMD). Stratified analyses were also conducted by separating studies based on each characteristic independently.

## RESULTS

Pooled analysis of 20 preclinical studies showed a significant beneficial effect of stem cell therapy in increasing new bone formation (17.79%, 95% confidence interval [CI], 10.54, 25.03; $P < 0.001$) and BMD (276.94 mg $cm^{-2}$, 95% CI, 62.71, 491.17; $P < 0.001$) for disease amelioration. Regarding new bone formation, a statistical improvement was similarly detected from randomized controlled trial groups (17.06%, 95% CI, 8.87, 25.24; $P < 0.001$) and cohort groups (17.43%, 95% CI, 10.79, 24.07; $P < 0.001$). Exploratory stratified analysis yielded significant predictors of new bone formation including cell number ($<10^7$ *vs.* $\geq 10^7$; $P = 0.048$) and the route of cell delivery (combining with matrix scaffold showed more effect than direct cell injection, $P = 0.041$). The effect of stem cell therapy diminished after 12 weeks.

## CONCLUSION

The study results suggest that stem cell therapy improves new bone formation and BMD in bone defect models. Future trials should focus on the transplantation of $\geq 10^7$ stem cells, especially using slow release biodegradable scaffolds or repetitive cell injections.

## Introduction

Human bone and articulation are vascular structures, which pose significant hurdles to repair or regeneration strategies during injury. Defective repair mechanisms result in pain, joint dysfunction, arthritis, degeneration and osteoarthritis. The damage to bone is a significant clinical problem, with huge health and socioeconomic impact.

The current methods of repairing bone defect are surgical, highly invasive and not always successful. Tissue engineering is a viable alternative with promising therapeutic advantage in restoring both the structure and function of damaged bone [1]. Although large animal studies have investigated the potential therapeutic effect of stem cell transplantation in repairing bone injuries, the results are conflicting, with some studies reporting bone regeneration when used alone [2, 3] or in combination with scaffolds [4, 5], while other studies failed to find significant differences [6, 7]. The data on bone minerals are also uncertain [8–10]. Although these preclinical studies remain controversial, the results offer important clues to unanswered clinical issues which are critical to stem cell repair of the bone including safety, feasibility, efficacy, choice of cell type, cell number, method of delivery and follow-up. The present study involved a systematic review and meta-analysis to identify qualitative and quantitative data for stem cell transplantation as an alternative in bone defects. Such studies might help in the design of future clinical studies similar to the meta-analysis of bone stem cell trials in humans. A subgroup analysis was also performed to resolve the foregoing issues.

## Methods

### *Literature search and eligibility criteria*

A meta-analysis of available preclinical data was conducted on bone defects in accordance with the Cochrane Handbook guidelines [11]. The review is reported per the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines [12].

To identify preclinical studies investigating the use of stem cell therapy in bone defects, a literature search of PubMed and Embase was conducted for studies published until July 1 2013. The databases were searched using the following strategy: '(pig OR porcine OR swine OR canine OR dog OR sheep OR ovine OR rabbit) AND (stem cells OR progenitor cells OR bone marrow) AND (bone fracture OR bone repair OR bone defect OR bone injury)'. The inclusion criteria were as follows: (1) studies involving bone defects in large animal models, (2) randomized controlled trials (RCTs) and cohort studies investigating the effect of stem cell therapy on bone repair in terms of new bone formation and bone mineral density (BMD) and (3) articles written only in English. Trials that investigated only transfected or genetically engineered stem cells altering cell behaviour were excluded, but studies using reporter genes (solely for stem cell imaging purposes) were included. Reviews, editorials, comments, reports from scientific sessions and discussions were excluded. When two or more articles reported data from the same study, only the most recently updated data were included. References to the identified articles were also checked and principal investigators were asked whether they were aware of other trials.

### *Data extraction and quality assessment*

Two reviewers independently screened titles and abstracts and then full-text articles. Discrepancies between the two reviewers were resolved by consensus or through discussion with a third reviewer. In the meta-analysis, effect sizes of increase in new bone formation and BMD improvement between stem cell treatment and control groups were calculated to assess the therapeutic effect. To this end, the total number of animals, mean and SEM or SD pertaining to new bone volume, and BMD were extracted from the studies. Other information regarding the types of animals, defect models, treatment dose, route of delivery, follow-up duration and comorbidity were incorporated in the database. In the case of missing data, corresponding authors were contacted. Five emails were sent and three authors responded.

Standard guidelines [13] for quality assessment of clinical trials were not universally applicable to these preclinical studies. Therefore, a modified Jadad scale criteria was used to assess selection, performance and detection bias: (1) randomization, (2) description of randomization, (3) adequate allocation, (4) blinding of the operator and (5) blinding of the outcome analysis. Trials scoring 1 point were deemed as low quality and 4–5 points as high quality.

### *Statistical analysis*

Extracted data were entered into Review Manager version 5.0.2 database. The primary outcome was the difference in mean of the newly formed bone (%) between control and treated animals at follow-up. The secondary endpoint was the difference in BMD (reported as mg $m^{-2}$). In the case of multiple measurements over time, data measured at the longest duration of follow-up were used for analysis. If multiple experimental groups were next to a single control group within one study, the number of animals in the control group was divided equally by the number of experimental groups. The meta-analysis was performed using weighted mean difference with random effects model to avoid heterogeneity [14]. Heterogeneity was considered significant at $P < 0.10$ [11]. Inconsistency was estimated using the $I^2$ statistic. Values of 25, 50 and 75% were considered low, moderate and high inconsistency, respectively [15].

For a clinical perspective, a stratification analysis was also conducted to examine the impact of several factors,

such as the type of animal (pig, dog, sheep, or rabbit), cell type [bone marrow mesenchymal stem cells (BMSCs), umbilical cord blood mesenchymal stem cells (UCB-MSCs), deciduous teeth stem cells, adipose stem cells (ASCs)], number of cells injected ($<10^7$ or $\geq 10^7$), method of cell delivery (with scaffold, *in situ* injection, or intravenous administration) and follow-up after stem cell therapy (≤12 weeks, 12–24 weeks or >24 weeks).

To test the robustness of association and characterize possible sources of statistical heterogeneity, sensitivity analyses were carried out by excluding studies one by one and analyzing the homogeneity and effect size for all the remaining studies. Publication bias was assessed using the Begg adjusted rank correlation test and the Egger regression asymmetry test [16, 17].

All analyses were performed using Review Manager version 5.0.2 and Stata version 11.0 (StataCorp, College Station, TX, USA).

## Results

### *Search results and characteristics of studies included in the meta-analysis*

The electronic database search identified 202 articles, of which 20 articles were eligible for review (10 RCTs and 10 cohort studies; Figure 1). Characteristics of the enrolled studies are depicted in Table 1. In 15 of the included studies, stem cells were seeded with matrix scaffolds, four studies with cells directly injected into the injury site and one administered by tail vein. Four different cell types were studied. All cases involved single frequency of stem cell therapy. Seventeen studies reported data on the rate of new bone formation and four studies were based on

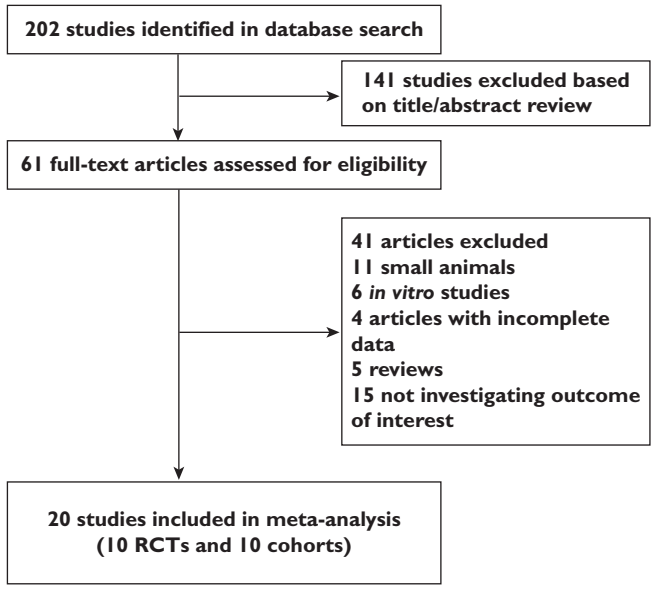
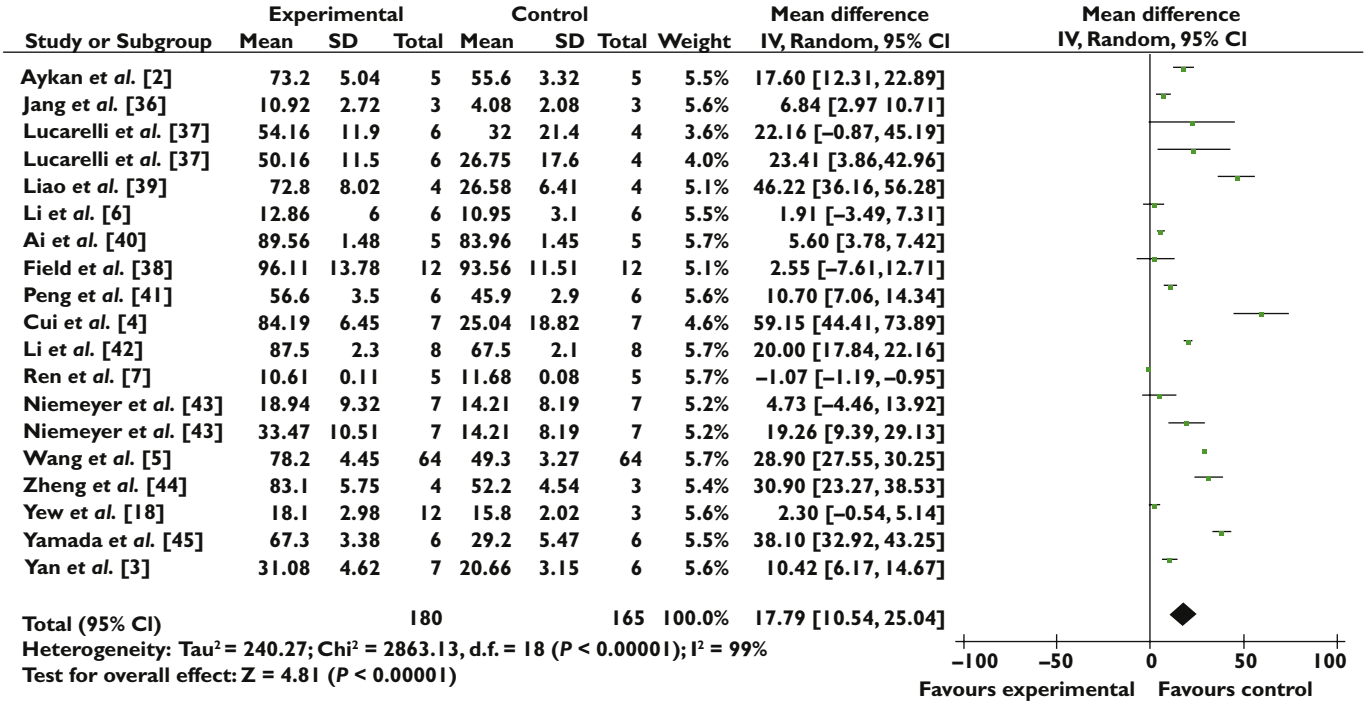



**Figure 1**
Study flow diagram

**Table 1**
Study characteristics

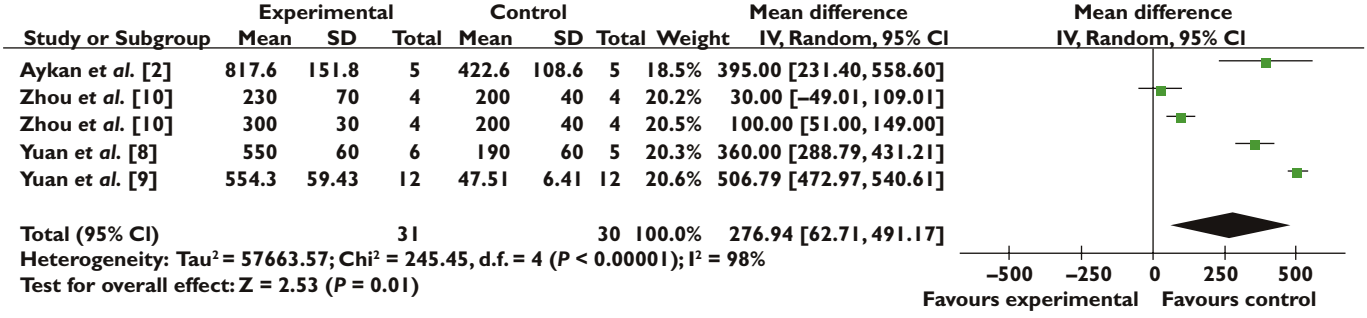
| Author | *n* | Type of animal | Study design | Type of defect | Cell type | Number of cells | Route of delivery | Follow-up (weeks) |
|---|---|---|---|---|---|---|---|---|
| Aykan *et al.* [2] | 20 | Sheep | RCT | Mandibular | BMSCs | $8 \times 10^6$ | DI | 6 |
| Jang *et al.* [36] | 6 | Dog | RCT | Radiul | UCB-MSCs | $2 \times 10^6$ | CS | 12 |
| Lucarelli *et al.* [37] | 20 | Sheep | RCT | Metatarsal | BMSCs | $4 \times 10^6$ | CS | 16 |
| Field *et al.* [38] | 24 | Sheep | RCT | Tibial | BMSCs | $2.25 \times 10^8$ | CS | 36 |
| Liao *et al.* [39] | 8 | Dog | RCT | Mandibular | BMSCs | $1 \times 10^8$ | DI | 16 |
| Li *et al.* [6] | 12 | Dog | RCT | Ulna | ASCs | $2 \times 10^7$ | CS | 16 |
| Ai *et al.* [40] | 10 | Rabbit | Cohort | Tibial | BMSCs | $1 \times 10^6$ | CS | 8 |
| Peng *et al.* [41] | 12 | Dog | RCT | Femoral head | BMSCs | $1 \times 10^7$ | CS | 30 |
| Cui *et al.* [4] | 14 | Dog | Cohort | Parietal bones | ASCs | $2 \times 10^7$ | CS | 24 |
| Li *et al.* [42] | 16 | Sheep | RCT | Metatarsus | BMSCs | $2 \times 10^8$ | CS | 24 |
| Ren *et al.* [7] | 10 | Pig | Cohort | Ulna | ASCs | $1 \times 10^6$ | TVI | 12 |
| Niemeyer *et al.* [43] | 28 | Sheep | Cohort | Tibia | BMSCs | $2 \times 10^7$ | CS | 26 |
| Wang *et al.* [5] | 128 | Rabbit | RCT | Femurs | BMSCs | $5 \times 10^6$ | CS | 12 |
| Zheng *et al.* [44] | 7 | Pig | Cohort | Mandibular | SPDs | $2 \times 10^7$–$4 \times 10^8$ | CS | 24 |
| Yan *et al.* [3] | 13 | Dog | Cohort | ONFH | BMSCs | $2 \times 10^7$ | DI | 12 |
| Yew *et al.* [18] | 15 | Rabbit | Cohort | Cranial bone | BMSCs | $1 \times 10^6$ | CS | 12 |
| Yamada *et al.* [45] | 12 | Dog | Cohort | Mandible | BMSCs | $1 \times 10^7$ | DI | 8 |
| Yuan *et al.* [8] | 11 | Dog | Cohort | Mandibular | BMSCs | $2 \times 10^7$ | CS | 32 |
| Yuan *et al.* [9] | 24 | Sheep | Cohort | Mandibular | BMSCs | $2 \times 10^7$ | CS | 32 |
| Zhou *et al.* [10] | 16 | Dog | RCT | Inferior orbital rim bone | BMSCs | $2 \times 10^7$ | CS | 12 |

ASCs, adipose stem cells; BMSCs, bone marrow mesenchymal stem cells; CS, cell-seeded scaffold; DI, directly inject into the defect; SPDs, stem cells of pig deciduous teeth; TVI, tail vein injection; UCB-MSCs, umbilical cord blood-derived MSCs.




## Figure 2

Forest plot showing the impact of stem cell therapy on new bone formation, compared with controls. 95% CI, 95% confidence interval




## Figure 3

Forest plot showing the impact of stem cell therapy on histologic score improvement, compared with controls. 95% CI, 95% confidence interval

BMD outcomes. The rates of new bone formation were assessed by computed tomography (six studies), single-photon emission computed tomography (one study), histomorphology (nine studies) and X-ray (one study). No study used the animal model with comorbidity.

### *Meta-analysis*

The 20 identified studies involved 406 animals (211 control and 195 treated groups, respectively) to assess the effects of stem cell therapy on the rate of new bone formation and BMD. There was a significant beneficial effect of stem cell treatment on the new bone formation increase (17.79%, 95% CI 10.54, 25.03; $P < 0.001$), with significant heterogeneity ($P < 0.001$) and inconsistency ($I^2$ 99%; Figure 2). Regarding BMD, a statistical improvement by 276.94 mg cm$^{-2}$ (95% CI 62.71, 491.17; $P < 0.001$) was similarly detected with significant heterogeneity ($P < 0.001$) and inconsistency ($I^2$ 98%; Figure 3).

### *Stratified analysis*

The stratified analysis showed that cell injection dose ($P = 0.048$) and route of cell delivery ($P = 0.041$) are the two significant predictors of enhanced new bone formation, although the heterogeneity among studies was significant (Table 2). No significant difference ($P = 0.951$) was observed regarding study design type: 17.06% in the RCT

## Table 2

New bone formation rate: Stratified analysis of stem cell-treated *vs*. control

| | Number of studies | Mean difference (IV, random, 95% CI) | $I^2$ value (%) | $P^*_h$ | $P^{**}$ |
|---|---|---|---|---|---|
| **Type of animal** | | | | | |
| **Dog** | 78 | 23.52 (12.20, 34.84) | 97 | <0.001 | 0.8144 |
| **Pig** | 17 | 14.68 (–16.65, 46) | 75 | <0.001 | |
| **Sheep** | 88 | 14.24 (6.42, 22.06) | 99 | <0.001 | |
| **Rabbit** | 153 | 12.29 (–5.79, 30.37) | 96 | <0.001 | |
| **Cell injection dose** | | | | | |
| **<$10^7$** | 249 | 12.37 (1.08, 23.67) | 97 | <0.001 | 0.048 |
| **≥$10^7$** | 146 | 21.35 (13.82, 28.88) | 95 | <0.001 | |
| **Follow-up after cell therapy (weeeks)** | | | | | |
| **≤12** | 194 | 28.64 (16.74, 40.55) | 98 | <0.001 | 0.129 |
| **12–24** | 77 | 12.95 (1.11, 24.79) | 94 | <0.001 | |
| **>24** | 64 | 9.54 (3.66, 15.42) | 56 | 0.08 | |
| **Type of cell** | | | | | |
| **BMSCs** | 296 | 17.62 (10.67, 24.57) | 96 | <0.001 | 0.966 |
| **Other types** | 49 | 17.24 (5.87, 28.61) | 99 | <0.001 | |
| **Route of delivery** | | | | | |
| **CS** | 295 | 19.50 (15.74, 23.26) | 98 | <0.001 | 0.041 |
| **DI** | 95 | 12.22 (8.26, 16.149) | 97 | <0.001 | |
| **Type of study** | | | | | |
| **RCT** | 216 | 17.06 (8.87, 25.24) | 95 | <0.001 | 0.951 |
| **Cohort** | 129 | 17.43 (10.79, 24.07) | 98 | <0.001 | |

*$P$ value for heterogeneity within each subgroup. **$P$ value for heterogeneity between subgroups with meta-regression analysis. BMSCs, bone marrow mesenchymal stem cells; CI, confidence interval; CS, cell seeded scaffold; DI, directly inject into the defect.

group (95% CI 8.87, 25.24; $P < 0.001$) *vs*. 17.43% in the cohorts group (95% CI 10.79, 24.07; $P < 0.001$). A similar effectiveness was observed between BMSCs and other stem cell types. During follow-up, the effect of stem cell therapy appeared to decline over time. Considering the sources of heterogeneity, we also separated the studies by animal type and found no difference ($P = 0.814$).

Data from BMD outcome were obtained only from four studies with a total of 61 animals, 30 of which were controls and 31 stem cell-treated animals (Figure 3). Therefore, the effect of stem cell therapy on BMD was not assessed in the stratified meta-analysis because of the limited data on each group.

### *Quality of the included trials*

Methodological quality of the included trials was modest (2.6). Two trials were of low quality, with a score of 1 on the modified Jadad scale, four were judged high in quality with a score of 4. Blinded outcome analysis was performed on 13 studies. The operator was blinded in nine studies. No article reported the method of randomization. Further details on the scores for each trial are presented in Table 3.

### *Sensitivity analysis and publication bias*

In the sensitivity analysis of stem cell therapy on new bone formation outcomes, we sequentially removed one study

## Table 3

Quality of the included trials

| Study ID | Modity Jadad scale a | b | c | d | e | Total |
|---|---|---|---|---|---|---|
| **Aykan *et al.* [2]** | 1 | 0 | 1 | 1 | 0 | 3 |
| **Jang *et al.* [36]** | 1 | 0 | 1 | 0 | 0 | 2 |
| **Lucarelli *et al.* [37]** | 1 | 0 | 1 | 1 | 1 | 4 |
| **Field *et al.* [38]** | 1 | 0 | 1 | 1 | 1 | 4 |
| **Liao *et al.* [39]** | 1 | 0 | 1 | 0 | 0 | 2 |
| **Li *et al.* [6]** | 1 | 0 | 1 | 0 | 0 | 2 |
| **Ai *et al.* [40]** | 0 | 0 | 1 | 0 | 1 | 2 |
| **Peng *et al.* [41]** | 1 | 0 | 1 | 0 | 0 | 2 |
| **Cui *et al.* [4]** | 0 | 0 | 1 | 1 | 1 | 3 |
| **Li *et al.* [42]** | 1 | 0 | 1 | 0 | 1 | 3 |
| **Ren *et al.* [7]** | 0 | 0 | 1 | 1 | 1 | 3 |
| **Niemeyer *et al.* [43]** | 0 | 0 | 1 | 0 | 1 | 2 |
| **Wang *et al.* [5]** | 1 | 0 | 1 | 1 | 1 | 4 |
| **Zheng *et al.* [44]** | 0 | 0 | 1 | 0 | 0 | 1 |
| **Yan *et al.*[3]** | 0 | 0 | 1 | 0 | 0 | 1 |
| **Yew *et al.* [18]** | 0 | 0 | 1 | 1 | 1 | 3 |
| **Yamada *et al.* [45]** | 0 | 0 | 1 | 1 | 1 | 3 |
| **Yuan *et al.* [8]** | 0 | 0 | 1 | 0 | 1 | 2 |
| **Yuan *et al.* [9]** | 0 | 0 | 1 | 0 | 1 | 2 |
| **Zhou *et al.* [10]** | 1 | 0 | 1 | 1 | 1 | 4 |

Points were awarded as follows: a = the study was described as randomized, 1 point; b = the randomization scheme was described and appropriate, 1 point; c = adequate allocation, 1 point; d = blinding of the operator, 1 point; e = blinding of the outcome analysis, 1 point.

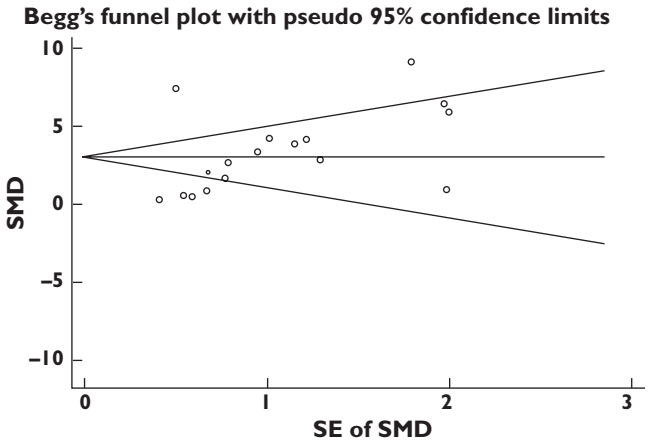



**Figure 4**

Funnel graph for the assessment of potential publication bias
SMD, Standardized Mean Difference

at a time and re-analyzed the data. The 15 study-specific mean differences ranged from a low of 15.78 (95% CI 8.42, 23.14) after omitting the study by Cui *et al*. [4] to a high of 18.75 (95% CI 11.00, 26.50) after omitting the study by Yew *et al*. [18], but they were generally similar. For BMD outcomes, similar sensitivity analyses were carried out without a significant impact on the results (data not shown).

The funnel plots (Figure 4) revealed that no significant publication bias existed in new bone formation in the present analysis and Egger's test showed $P = 0.838$ and Begg's test showed $P = 0.086$.

## Discussion

Twenty published preclinical studies involving large animals treated with stem cells were analyzed to investigate the treatment-related effects on bone injury. In brief, the study findings suggest that (1) stem cell therapy promoted new bone formation by 17.79% accompanied with BMD increase of 276.94 mg $cm^{-2}$, (2) cell injection dose and the route of cell delivery were important predictors of new bone formation in the bone defect model, and (3) no differences in animal and stem cell types were found.

The meta-analysis results reinforced the evidence supporting stem cell therapy in experimental bone defects, especially in increasing the new bone formation. For BMD outcome, the present analysis showed consistent benefit of stem cell therapy, although the availability of limited data available and decreased number of experiments reporting this endpoint could render these findings less robust. Further evidence is required to assess BMD improvement in experimental bone injury models.

Transplantation of a higher number of cells ($\geq 10^7$) appeared to have a stronger impact on new bone formation. This could be due to the increasing stimulation of the endogenous regenerative capacity of the bone by release of growth factors, cytokines and other paracrine molecules from the transplanted and host cells, enhancing angiogenesis and reducing apoptosis [19–21]. Stratified analysis showed that combining stem cell therapy with matrix scaffold had significantly larger benefit compared with direct cell injection. The use of appropriate matrix scaffolds as stem cell delivery vehicles or as a three dimensional support for the repair tissue has advantages including enhanced osteogenic property, improved cell loading, prevention against leakage of transplanted cells, increased bone differentiation and tissue repair support [22, 23]. The proposed design is an effective approach for future clinical trials.

In large animals, the effect of stem cell therapy disappears 12 weeks after cell injection, consistent with initial observations in patient studies [24]. This finding should motivate researchers to explore novel applications and strategies for stem cell therapy including slow release agents, genetic engineering of stem cells or repetitive injections over time.

Clinically, BMSCs are the most commonly used cell type in the autologous or allogeneic transplantation and can differentiate into various lineages including bone, cartilage, adipose, tendon, ligament, muscle and nerve cells *in vivo* and *vitro* [25–28]. The results from the present meta-analysis showed no added benefits with BMSCs compared with other stem cells on new bone formation. Compared with BMSCs, ASCs and UCB-MSCs have several advantages as new cell sources including ease of isolation, relative abundance, rapidity of expansion and multipotency that are independent of serum source and quality [29, 30]. A recent study reported that human UCB-MSCs have a significantly stronger osteogenic potential but less capacity in adipogenic differentiation than BMSCs *in vitro* [31]. Further evidence is required to assess the effectiveness of different sources of stem cells on experimental bone injury models. Results concerning new bone formation from RCT (17.06%) and from cohort (17.43%) studies suggested that the effect of stem cell therapy on bone injury was consistent.

### *Recommendations*

Meta-analyses of animal studies are not common, although still recommended in several settings [32] to guide research and clinical endeavours [33]. Meta-analyses of preclinical studies may also be attractive to evaluate the effect of other therapies and to design (pre-)clinical trials in the future. Over the next few years, adequately powered large animal studies and clinical trials should focus on the transplantation of $\geq 10^7$ stem cells, with matrix scaffold or slow-release biodegradable scaffolds and repetitive cell injections.

### *Limitations*

To the best of our knowledge, this analysis represents the first systematic review and meta-analysis assessing stem cell implantation in the treatment of bone defects, encompassing 20 studies and 406 large animals. Results of the meta-analysis revealed statistically significant heterogeneity for new bone formation and BMD. In the current work, the diversity in animal types, delivery methods, time to follow-up and number of cells may explain this heterogeneity and play a role in the observed outcomes. Heterogeneity may also be attributed to the extremely sensitive endpoints chosen. The risk of erroneous estimates could be minimized using random effects analysis.

Another limitation highlighted by this meta-analysis pertains to the lack of animal studies with comorbidities, unlike studies in humans [24, 34, 35]. Autologous stem cells extracted from large young animals are 'fresh', whereas cells from patients are 'aged'. Further, animal studies offer a relatively short duration of follow-up. Despite these differences, the present analysis has shown that preclinical data are highly relevant to predict outcomes for clinical trials.

## Conclusion

Based on the data included in this meta-analysis, stem cell therapy is associated with a 17.79% higher new bone formation and 276.94 mg $cm^{-2}$ more BMD increase compared with control groups. The analysis demonstrated that large animal models were able to provide valid data to design or predict outcomes in clinical trials. In view of the limitations inherent in the design of a majority of the studies included in the meta-analysis, large, multicentre, well-designed RCTs with extensive follow-up are needed to validate these findings.

## Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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## Data access and responsibility

Ming-Kang Zhong had full access to all the data in the study, takes responsibility for the integrity of the data and the accuracy of the data analysis and acts as guarantor of the paper.

## Authors' contributions

Study idea: Yun Liao, Xiao-Long Zhang

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Statistical analysis: Yun Liao, Xiao-Long Zhang, Ling Li

Data interpretation: Yun Liao, Xiao-Long Zhang, Ling Li

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Final approval of the version to be published: Yun Liao, Xiao-Long Zhang, Ling Li, Fu-Ming Shen, Ming-Kang Zhong

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