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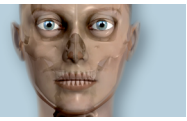

ORIGINAL ARTICLE

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Mandibular distraction osteogenesis assisted by cell-based tissue engineering: a systematic review

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Structured Abstract

Objectives – To review the advances and limitations of recent investigations on mandibular distraction osteogenesis (MDO) assisted by mesenchymal stem cell (MSC) transplantation.

Materials and Methods – Following the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines, the PubMed, Scopus, and Cochrane electronic databases were systematically searched and screened from their inception through August 2014. Searching terms included the following: 'distraction osteogenesis', 'mandible OR mandibular OR jaw', and 'cells', without any other limitations.

Results – Nineteen studies meeting the eligibility criteria were selected from 227 published articles and used for qualitative synthesis. Fifteen of the studies used small animal models (rats or rabbits), while the other four used large animal models (dogs, pigs or sheep). Among these studies, large variations exist in MDO protocol, cell transplantation time, route and quantity, as well as methodology of outcome assessment. Additionally, all studies had certain biases. Nevertheless, the majority of studies found that MSC transplantation enhanced MDO bone regeneration.

Conclusion – Evidence from animal studies indicates that MDO may be enhanced by mesenchymal stem cell transplantation, but many questions related to animal models, MDO protocols, and cell transplantation remain to be investigated.

Key words: mandibular distraction osteogenesis; stem cell; systematic review; tissue engineering

Introduction

Large craniofacial defects or growth deficiencies severely compromise patients' vital functions and quality of life (1), and yet their treatments remain challenging. Autogenous bone grafting, the long-standing gold standard treatment, has several major

limitations (2, 3). Synthetic graft materials were used as a treatment alternative, but currently they are still inferior to native bone in biocompatibility and osteoconductivity. A third option called distraction osteogenesis (DO) is a mechanically induced endogenous tissue engineering technique and is graft-free. First invented by Codivilla (4), then redeveloped by Ilizarov (5), this technique has become increasingly popular in clinical craniofacial orthopedics since the first clinical mandibular distraction osteogenesis (MDO) in the 1990s (6). Two decades later, the advantages and biological mechanisms of MDO are reasonably clear (7), and so are its limitations (8). Essentially, a standard MDO involves multiple phases and requires an extended treatment time, which increases risks of complications or failure (8).

This limitation is partly because the current MDO heavily relies on mechanical stimulation of bone regeneration. More specifically, the recruitment and activation of mesenchymal stem cells (MSC), which are indispensable for new bone regeneration, result primarily from tensile strain caused by distraction (9) besides an initial stimulation by surgical trauma (10). To address this limitation, researchers have supplemented MDO with electrical stimulation (11), low-intensity pulsed ultrasound (12), or used distractors delivering automated and continuous force (13). Growth factors (14), hormonal proteins (15), and pharmacological agents (16) have also been added to the distraction site. Although these measures can potentially enhance MSC recruitment and activation, multiple recent studies started investigating a more direct and efficient approach by combining cell-based tissue engineering with MDO. To thoroughly understand current status of this approach and better orchestrate future investigations, this systematic review was conducted.

Materials and methods

The PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines (17) were used to conduct this systematic review.

Information sources and searches

Without imposing any restriction on languages, publication date, or publication status, a thorough literature search was performed against three databases: PubMed, Scopus, and the Cochrane Library from inception until August 25, 2014, using the combinations of keywords 'distraction osteogenesis', 'mandibular OR jaw OR mandible', and 'cells'.

Eligibility criteria and process for study selection and data collection

The eligibility criteria include the following: *in vivo* studies on mammals, completion of a multiphase MDO protocol, and involving cell transplantation to enhance MDO site bone regeneration. The titles and abstracts of all entries retrieved after each literature search were screened. Then, the full texts of the studies meeting the eligibility criteria were obtained, and the characteristics of each study including animal models, MDO protocols, and detailed information of cell transplantation were extracted.

Assessment of study quality and risk of bias

The SYRCLE's risk of bias tool developed by the Cochrane Collaboration for animal intervention studies was modified and applied for this systematic review (18). Randomization of treatments, blinding of investigators and outcome assessor, exclusion of animals (incomplete data outcome), and the consistency between the reported protocols and outcomes (selective outcome reporting) were used to assess study quality. When the criteria were reported and elaborated in the paper, the study was indicated as low risk of bias ('Yes'); otherwise, the study was indicated as high risk of bias ('No').

Statistical analyses

Due to the heterogeneity of the study protocols and outcome measures, no meta-analysis was able to be performed for this systematic review.

Results

The results of the electronic and manual searches are shown in Fig. 1. Manual selection following the inclusion criteria resulted in a total of 19 studies (19–37) as summarized in Table 1.

Animal model and sample size

Among the 19 papers selected, 15 used rodent models: rats (Sprague Dawley or Lewis strain) and rabbits (New Zealand or Japanese strain); 4 used large-size animal models: dogs (Mongrel breed), pigs (domestic or miniature), and sheep (strain unclear). For animal gender, 10 and 4 studies only used male and female animals, respectively, while five studies did not report this information. The rats used were skeletally mature with a body weight of 280–400 g. The rabbits used were 4–6 months old with a body weight of 2–3.5 kg. Mean weight of the mongrel dogs used was 22.5 kg with no report on their age. The pigs and sheep used were 3 months and 2-year-olds, respectively. Sample size of the studies ranged from 6 to 90.

MDO protocol

The MDO protocols are summarized in Table 2. Unilateral and bilateral MDO protocols were performed in 10 and 6 studies, respectively, while the remaining three studies did not report this information. Various custom-made or commercially available distractors were used. The latency phase ranged between 2 and 7 days. The distraction rate ranged from 0.4 to 2.4 mm/day, the total distraction gap ranged from 3.2 to 20 mm, and the consolidation period ranged from 4 days to 10 weeks.

Cell transplantation

As shown in Table 3, 11 studies used autologous stem cells, isolated either from bone marrow, adipose tissues, or from humeral epiphysis. Two studies used human cells (xenogenic transplantation). The remaining six studies did not clarify this information. The number of transplanted cells was in a range of 0.2–50 $\times$ 10^6 cells. Converted to cell/distraction ratio (defined as number of cells in millions (M) divided by total distraction in millimeters (mm)), the range was 0.03–5.0 M/mm. Fourteen studies injected cell

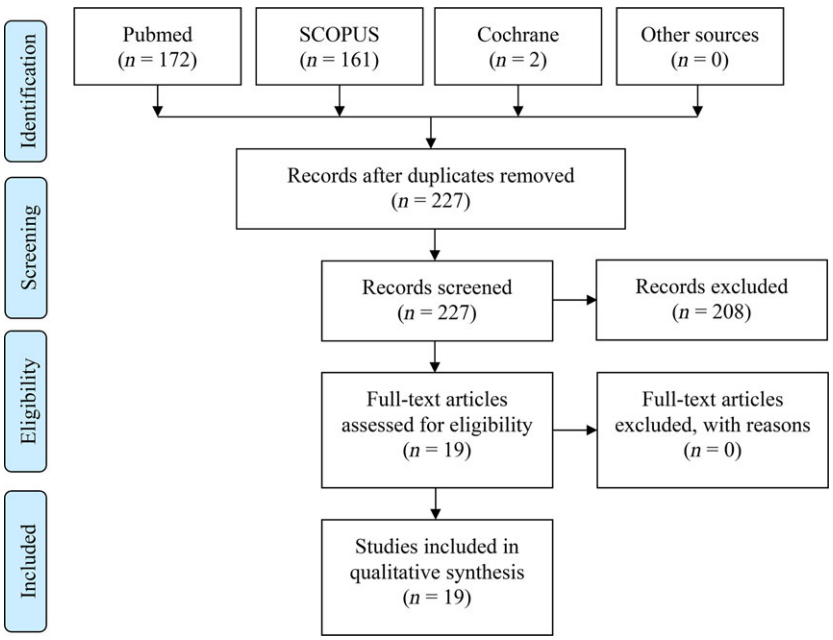

Fig. 1. Flow chart of the literature search.

Table 1. **Characteristics of animal models and the main outcomes of the study**

First author, Year	Animal	Strain	Sex	Age/weight	Main results: treatment (sample size)
Qi, 2006 (19)	Rat	SD	M	ND, 0.4 kg	Cell treatment (20) > Non-cell control (20)
Shao, 2007 (20)	Rabbit	NZW	ND	ND, 2 kg	Cell treatment (13) > Non-cell control (13)
Hu, 2007 (21)	Rat	SD	M	ND, 0.4 kg	Cell+BMP7 (18) > Cell treatment (18) > Non-cell control (18)
Jiang, 2010 (22)	Rabbit	NZW	M	ND, 2.5 kg	Cell+bFGF (14) > Cell treatment (14) > Non-cell control (14)
Hwang, 2010 (23)	Rabbit	NZW	M	ND, 2.75 kg	Cell+PRP (22) > PRP alone (16) > Non-cell/PRP control (38)
Kroczek, 2010 (24)	Minipig	Goettingen	F	5 weeks, 22.5 kg	Cell+BMP2/7 (8) > Other treatments (12)
Lai, 2011 (25)	Rabbit	NZW	M	ND, 3.25 kg	Cells+Osterix (18) > Cell treatment (18) > Non-cell control (18)
Long, 2011* (26)	Rabbit	Japanese	M	ND, 2.25 kg	Rapid DO+cells+rhBMP2 (12) = Normal DO+no cells (12) > Rapid DO+cells (12)
Castro-Govea, 2012 (27)	Dog	Mongrel	ND	ND, 22.5 kg	Cells+rhBMP2 (3) > Cell treatment (3) = Non-cell control (3)
Zhang, 2012* (28)	Rabbit	NZW	ND	4 months, ND	Cells+rhBMP2/7 (3) > Cell treatment (3) > Non-cell control (3) > Radiation only (3)
Huang, 2012 (29)	Rabbit	NZW	ND	2–3 months, 2.2 kg	Cells+rhBMP2 (12) > Cell treatment (12) = Non-cell control (12)
Kim, 2013 (30)	Rabbit	NZW	M	ND, 3.5 kg	Pre-distraction cell (7) > Post-distraction cell treatment (7) > Non-cell control (14)
Sun, 2013 (31)	Pig	Domestic	F	3 months, ND	Cell treatment (4) > Non-cell control (2)
Aykan, 2013 (32)	Sheep	ND	F	2 years, 55 kg	Cell treatment (8) > Non-cell control (8)
Deshpande, 2013* (33)	Rat	Lewis	M	ND, 0.4 kg	Cell treatment (10) = Non-cell control (9) > Radiation only (7)
Ma, 2013 (34)	Rabbit	NZW	M	6–8 months, 2.8 kg	Cell sheets (10) > Dissociated cells (10) = Non-cell control (10)
Alkaisi, 2013 (35)	Rabbit	NZW	ND	3–5 months, 2.7 kg	Cell treatment (9) > Non-cell control (9)
Sun, 2014* (36)	Rabbit	NZW	F	3 months, 2 kg	OVX+cells+rhRUNX2 (18) > Non-cell control (18); OVX (18) and OVX+cells (18) were excluded
Lai, 2014 (37)	Rabbit	NZW	M	6 months, ND	Cells+Osterix (18) > Cell treatment (18) = Non-cell control (18)

SD, Sprague Dawley; NZW, New Zealand White; OVX, ovariectomized; rhRUNX2, recombinant human runt-related transcription factor 2; BMP, bone morphogenetic protein; TGF, transforming growth factor; IGF, insulin growth factor; PRP, platelet-rich plasma; bFGF, basic fibroblast growth factor; L, left; R, right; F, female; M, male; ND, not defined.
*Studies involving special treatment on the animals.

suspension into the distraction site after the distraction phase and four studies loaded the cells to scaffolds and then transplanted to the distraction site during osteotomy, while the remaining study did not report this information.

Quality assessment of studies

As detailed in Table 4, all studies showed a relatively high risk of performance bias, but no reporting bias. Five studies did not mention randomization of treatments, 12 studies did not report blinding of outcome assessor, and nine studies reported attrition of samples.

Outcome synthesis

Three studies conducted pairwise comparison of sides with or without cell injection in varied animal models: rat (19), rabbit (20), and sheep (32).

Table 2. **Characteristics of MDO protocols**

First author, Year	MDO – Side	Latency (days)	Distraction rate (mm/day)	Distraction gap (mm)	Consolidation	Cell/distraction ratio (M/mm)	Infection rate (%)
Qi, 2006 (19)	Uni-R	5	0.4	3.2	2, 6 weeks	0.16	15.0
Shao, 2007 (20)	Bi	7	2.0	10.0	2, 4, 6 weeks	0.5	13.3
Hu, 2007 (21)	Uni-R	5	0.4	3.2	2, 6 weeks	0.3	5.6
Jiang, 2010 (22)	Uni-R	3	2.0	10.0	1, 8 weeks	1.0	0.0
Hwang, 2010 (23)	Bi	5	2.1	6.3	1, 2, 3, 4 weeks	1.6	15.6
Kroczek, 2010 (24)	Uni-R	5	1.5	9.2	1, 2, 3, 4 weeks	ND	ND
Lai, 2011 (25)	Uni-L	6	0.8	4.8	2, 6 weeks	2.0	0.0
Long, 2011 (26)	Uni-ND	7	0.8 or 2.4	15.0	2, 4, 8 weeks	0.7	ND
Castro-Govea, 2012 (27)	ND	ND	1.0	10.0	10 weeks	1.5	0.0
Zhang, 2012 (28)	ND	7	0.9	9.9	4 weeks	ND	ND
Huang, 2012 (29)	Uni-L	5	1.0	7.0	2, 6 weeks	0.03	0.0
Kim, 2013 (30)	Bi	7	1.0	7.0	4, 8, 14 days	0.3	0.0
Sun, 2013 (31)	Bi	2	1.0	10.0	5 weeks	5.0	33.3
Aykan, 2013 (32)	Bi	5	2.0	20.0	3, 6 weeks	0.4	18.8
Deshpande, 2013 (33)	ND	4	0.6	5.1	4 weeks	0.4	Unclear
Ma, 2013 (34)	Bi	5	1.5	9.0	3, 6 weeks	0.7	0.0
Alkaisi, 2013 (35)	Uni-R	4	1.0	6.0	2, 4, 6 weeks	1.0	18.0
Sun, 2014 (36)	Uni-R	7	0.8	8.0	3, 6, 9 weeks	1.25	40.0
Lai, 2014 (37)	Uni-R	6	0.8	8.0	2, 6 weeks	1.25	7.4

Uni, unilateral; Bi, bilateral; L, left; R, right; wks, weeks; ND, not defined.

All studies used a low cell/distraction ratio (<1 M/mm) and found some improvement in bone regeneration at the cell-treated side, although some improvement did not last (20) or was not confirmed by mechanical testing (32).

Ten studies investigated the effects of transplanting genetically modified stem cells to the DO site. Specifically, several genes encoding growth factors including bone morphogenetic proteins-7 (BMP-7) (21, 28), basic fibroblast growth factor (22), BMP-2 (26, 27, 29), transforming growth factor-β, and insulin-like growth factor-1 (24) as well as genes encoding transcription factors such as osterix (25, 37) and Runx2 (36), were introduced to MSCs before they were injected into varied MDO models. Interestingly, four studies (21, 22, 25, 28) reported that cell transplantation improved bone regeneration compared to non-cell control, with transfected cells being better than non-transfected cells, while the other five studies (26, 27, 29, 36, 37) found only transplantation of transfected cells was beneficial.

Four studies transplanted stem cells during the osteotomy by integrating the cells into scaffolds prior to transplantation and found the treatments improved DO site osteogenesis (27, 31, 33, 35). Gelatin sponge (31, 33) and demineralized human bone matrix (27) were used as scaffolds, in which two of them (27, 31) used a large cell/distraction ratio (≥1.5 M/mm). In contrast, among 15 studies using the injection approach, only two exceeded the 1.5 M/mm ratio (23, 25).

Two studies transplanted xenogenic instead of autologous MSCs to the distraction site (30, 35). Both studies found that human stem cells were effective in stimulating DO site osteogenesis without provoking an immune response in rabbit MDO models. Additionally, three groups used rodent models that had received radiation in the mandibles (28, 33) or ovariectomy (36) to simulate the clinical scenarios of radiotherapy and menopause, respectively. The results of one study showed that transplanting stem cells significantly improved bone mineral density and

Table 3. **Characteristics of transplanted cells**

First author, Year	Cell type	Cell source	Cell number	Transplantation time/route
Qi, 2006 (19)	Auto or Allo	Tibia	0.5 M	Consolidation/injection
Shao, 2007 (20)	Auto	Ilium	5.0 M	Consolidation/injection
Hu, 2007 (21)	Auto or Allo	Tibia	1.0 M	Consolidation/injection
Jiang, 2010 (22)	Auto	Tibia	10.0 M	Consolidation/injection
Hwang, 2010 (23)	Auto	Iliac crest	10.0 M	Consolidation/injection
Kroczek, 2010 (24)	ND	ND	ND	Consolidation/injection
Lai, 2011 (25)	Auto	Tibia	10.0 M	Consolidation/injection
Long, 2011 (26)	Auto or Allo	Femur	10.0 M	Consolidation/injection
Castro-Govea, 2012 (27)	Auto	Humeral epiphyses	15.0 M	Osteotomy/scaffold
Zhang, 2012 (28)	Auto or Allo	Tibia	ND	ND
Huang, 2012 (29)	Auto	Tibia	0.2 M	Consolidation/injection
Kim, 2013 (30)	Xeno	Human MSC	2.0 M	Latency or Consolidation/injection
Sun, 2013 (31)	Auto	Tibia	50.0 M	Osteotomy/scaffold
Aykan, 2013 (32)	Auto	Ilium	8.0 M	Consolidation/injection
Deshpande, 2013 (33)	Auto or Allo	Femoral/humeral cavities	2.0 M	Osteotomy/scaffold
Ma, 2013 (34)	Auto	Ilium	6.5 M	Consolidation/injection
Alkaisi, 2013 (35)	Xeno	Human teeth	6.0 M	Osteotomy/ND
Sun, 2014 (36)	Auto	Adipose	10.0 M	Consolidation/injection
Lai, 2014 (37)	Auto	Adipose	10.0 M	Consolidation/injection

M, number of cells in million; MDO, mandibular distraction osteogenesis; Auto/Allo/Xeno, autogenic/allogeneic/xenogeneic transplantation; ND, not defined.

mechanical strength of the radiated DO site (33), while the other two studies demonstrated that enhanced expression of growth factor genes through gene modification is also necessary for the enhancement (28, 36).

Among all studies, infection was the most commonly reported complication, which resulted in exclusion of a small number of animals/sites for analysis (20, 23, 31, 32, 36). A few studies reported infection but did not exclude the animals based on the strength of regenerated bone (19, 21, 37). Appliance breakage (31) and device dislodgement (26) were additional complications reported.

Discussion

Among the 19 studies, large variations exist in animal models, DO protocols, and approaches of cell transplantation, all of which are important factors to be considered in evaluating the feasibility and significance of cell-assisted MDO.

For animal models, currently small rodents – rats and rabbits, have been extensively used, while studies based on large animal models are relatively scarce. Clearly, small animal models are easier for a large sample size and in-depth molecular and genetic analysis, but they are limited in their clinical relevance to human MDO patients due to their large discrepancy in mandibular size, morphology, and function from the human counterparts (38). Therefore, more studies based on large preclinical animal models are desirable.

The gender of animals may affect the outcome of cell-assisted MDO, but no studies have directly compared cell-assisted MDO between male and female animals of a particular species. Additionally, most rodent-model studies used male animals, while most large animal-model studies used female animals, which precludes the possibility of conducting a meta-analysis to shed some light on this issue. With an increasing demand of eliminating sex bias in animal studies (39), future efforts are warranted

Table 4. **Quality assessment of studies**

Author, Year	Selection bias Randomization	Performance bias Blinding of investigators	Detection bias Blinding of outcome assessor	Attrition bias Incomplete outcome data	Reporting bias Selective outcome reporting
Qi, 2006 (19)	Yes	No	Yes	Yes	Yes
Shao, 2007 (20)	Yes	No	No	No	Yes
Hu, 2007 (21)	Yes	No	Yes	Yes	Yes
Jiang, 2010 (22)	Yes	No	Yes	Yes	Yes
Hwang, 2010 (23)	No	No	No	No	Yes
Kroczek, 2010 (24)	No	No	No	No	Yes
Lai, 2011 (25)	Yes	No	Yes	Yes	Yes
Long, 2011 (26)	Yes	No	No	No	Yes
Castro-Govea, 2012 (27)	No	No	No	Yes	Yes
Zhang, 2012 (28)	Yes	No	Yes	No	Yes
Huang, 2012 (29)	Yes	No	No	Yes	Yes
Kim, 2013 (30)	No	No	No	Yes	Yes
Sun, 2013 (31)	Yes	No	Yes	No	Yes
Aykan, 2013 (32)	No	No	No	No	Yes
Deshpande, 2013 (33)	Yes	No	No	No	Yes
Ma, 2013 (34)	Yes	No	No	Yes	Yes
Alkaisi, 2013 (35)	Yes	No	No	Yes	Yes
Sun, 2014 (36)	Yes	No	No	No	Yes
Lai, 2014 (37)	Yes	No	Yes	Yes	Yes

'Yes', if criteria were met, indicating a low risk of bias; 'No', if criteria were not met, indicating a high risk of bias.

to clarify sex-related differences of cell-assisted MDO.

Another issue related to animal models is animal age and skeletal maturity, which was highly variable among the studies. More specifically, all rats used were skeletally mature, while for rabbits, contradictory information was presented between studies (22, 35) regarding their skeletal maturity. For large animals, Sun et al. (31) studied pigs at an age comparable to adolescent humans in skeletal maturity and Aykan et al. (32) studied skeletally mature sheep, but information about the dogs is not available (27). Clinically, as MDO may be performed on patients at all ages, it is important to interpret data obtained from animals relevant to human skeletal age. With well-recognized differences in stem cell properties between growing and mature humans (40) and animals (41), future studies are needed to compare age-related differences in cell-assisted MDO of a particular species.

Through extensive research in recent decades, it has become clear that different animal models may have a different optimal MDO protocol. For example, the optimal distraction rate in small and large animals (including humans) is commonly thought to be 0.5 and 1 mm/day, respectively. Clearly, to test the efficacy of cell-assisted MDO, the optimal MDO protocol without cell transplantation should be used as the baseline control, which, however, was not the case in some studies (20, 22). More specifically, using controls known to regenerate bone poorly for the purpose of demonstrating a relatively better outcome in cell-assisted MDO may produce flawed conclusions. On the other hand, given that the ultimate goal of cell-assisted MDO is to shorten the treatment time, it is desirable to include faster distraction rates and shorter consolidation durations for cell-assisted MDO groups. Unfortunately, few studies have carried out that so far (26). Because of these limitations,

it is still too early to conclude that cell-assisted MDO saves treatment time.

The total size of the distraction gap is another important factor to consider for cell-assisted MDO. At present, most studies conducted ≤10 mm of distraction, which is below common clinical levels. Conceivably, the larger the distraction gap, the more stem cells may be needed. Therefore, whether the benefits of MSC transplantation seen in most of the animal studies can remain with a larger distraction is an open question. Based on clinical cell-assisted DO studies performed on long bones (42), larger quantities of cells and longer consolidation time are clearly needed to maintain the benefits of cell transplantation, which further warrants investigation of MDO with large distraction gaps.

For cell-assisted MDO, undoubtedly the transplanted stem cells play the most critical role. Unfortunately, however, cell-related parameters appear to be the most variable factor among the 19 studies. First, the number of cells reflected by the cell/distraction ratio ranged widely from 0.16 to 5 M/mm, and yet all studies found some improvement in bone regeneration with cell transplantation. While these results indicate that transplanting as few as 0.2×10^6 or as many as 50×10^6 MSC may improve MDO bone regeneration, given the heterogeneity of animal models and methodology among the studies, such an interpretation is clearly liable to oversimplification. On the other hand, to maximize the effects of cell enhancement without wasting excessive time and resources in preparing the cells, optimal cell quantity range for common animal models needs to be established in the future.

Second, among the 19 studies, four different cell sources were used: autologous bone marrow from long bone (19, 21, 22, 25, 27, 28, 31, 33) and ilium/iliac crest (20, 23, 32, 34), autologous adipose stem cells (36, 37), and xenogenic human stem cells from bone marrow (location not specified) (30) and exfoliated deciduous teeth (35). Allogeneic cell transplantation may have also been used in some studies but the description was unclear (Table 3). As reported, no immune rejection was triggered by xenogeneic translation. Although MSCs have been suggested to be immunologically privileged (43), whether allogeneic or xenogenic transplantation compromises clinical enhancement in MDO or whether autologous transplantation is absolutely better needs to be further clarified. All studies used undifferentiated cells but one (31) which found transplantation of osteogenic differentiated and undifferentiated cells into MDO site produced similar enhancing effects. Due to a small sample size (31) and lack of confirmation from other studies, whether cells should be induced toward osteogenic differentiation before transplantation is another unanswered question.

The timing of cell transplantation also varied among studies. Overall, three different time points: during osteotomy (27, 31, 33, 35), latency (30), or consolidation (19–23, 25, 28, 32, 34, 36, 37) were chosen. Among them, only Kim et al. (30) directly compared the effects of cell transplantation at the latency phase versus at the consolidation phase and found the former was better. Indirectly, Deshpande et al. (33) also showed that transplanting cells during osteotomy tends to be more preferable than after active distraction as performed in two similar studies (28, 36). So far, none of the studies has investigated transplanting cells at multiple time points. In addition to cell delivery time, two routes of cell delivery have been adopted: directly through injection (19–23, 25, 30, 32, 36, 37) and integration with scaffolds before transplantation (27, 31, 33, 34). No study has compared the efficacy of these two routes. Indirectly, Ma et al. (34) found injection of the dissociated cells has inferior bone regeneration capacity than injection of cell sheets, which presumably provides better cell retention in the DO site. Hwang et al. (23) also improved cell retention using platelet-rich plasma during injection. Scaffolds would provide even better retention, which also allows transplantation of large quantities of cells at once, but it does require an open surgery for this approach. Conceivably, cell delivery to the MDO site may be optimized through a scaffold-based cell transplantation during osteotomy and injection-based transplantation during or after distraction. Combined, these studies demonstrate the importance of cell retention in the DO site, and therefore, the

choice of scaffold and transplantation time point should be further optimized.

Furthermore, several studies that investigated the effects of transplanting genetically modified stem cells found that non-transfected cells resulted in no improvement. This finding, however, is in disagreement with several other studies that also involved genetically modified cells and with many studies that only used MSC without genetic modification (26, 27, 29, 36, 37). Combined, these studies suggest that transfecting stem cells with pro-osteogenic growth factors or transcription factors may enhance the potency of transplanted MSCs, but future studies need to further characterize the differences between genetically modified cells and plain MSCs.

Lastly, all *in vivo* studies included in this review had certain biases in research design and execution. The restriction of sample size in large animal studies and attrition of samples due to complications further reduced the quality of several studies.

Overall, current investigations on MSC-assisted mandibular distraction osteogenesis (MDO) are still at a preliminary stage. This is first determined by the status quo of research on stem cell-based approach for bone regeneration. Despite much progress in bench studies in the last decade, only a few small clinical trials have been conducted worldwide so far to test the efficacy of cell-based therapy (44, 45). For MSC-assisted DO in particular, clinical studies have been attempted on long bones (42, 46) but not on craniofacial bones. Without confirmation from clinical applications, the promises of cell-based therapy demonstrated by *in vitro* and preclinical animal studies remain disputable. Next, it also reflects the unique challenges involved in integrating cell-based therapy to MDO sites vs. static bone defect sites. Because of the interaction between mechanical stimuli and cell-based treatments at MDO sites, common factors involved in bone defect regeneration such as cell source, type, and amount (44) have to be considered in conjunction with unique factors pertaining to the DO processes as reviewed above.

Conclusion

This systematic review found current cell-assisted MDO studies are widely variable in methodology, limited in completeness, and prone to certain biases. Although all studies demonstrated that transplanting MSC has a strong potential to enhance osteogenesis of MDO, many questions related to animal models, DO protocols, and details of cell transplantation, which are necessary for designing optimal stem cell-assisted strategy for subsequent clinical trials, require further investigation.

Clinical relevance

The last several decades have witnessed extensive bench and animal investigations on mesenchymal stem cells (MSCs) for their ability to enhance bone regeneration, which is now further tested in many clinical trials. The potential of using MSCs in mandibular distraction osteogenesis, a commonly used clinical treatment, to improve its efficacy in bone regeneration and shorten its treatment time, has also been investigated recently in varied animal models. To better prepare for future translation of MSC-assisted mandibular distraction osteogenesis to human patients, here, we reviewed current animal studies and discussed some questions yet to be addressed in subsequent studies.

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