Efficacy of stem cells on bone consolidation of distraction osteogenesis in animal models: a systematic review

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Abstract: Distraction osteogenesis (DO) relies on the recruitment and proliferation of mesenchymal stem cells (MSC) to the target site, where they differentiate into osteoblasts to promote bone formation. Nevertheless, MSC recruitment appears to be slow and limits bone formation in DO defects. Thus, this systematic review aims to evaluate the ability of locally applied MSC to enhance bone formation in DO preclinical models. Databases were searched for quantitative pre-clinical controlled studies that evaluated the effect of local administration of MSC on DO bone formation. Eligible studies were identified and data regarding study characteristics, outcome measures and quality were extracted. Nine studies met the inclusion criteria. Autogenous and xenogenous MSC were used to promote DO bone formation. These included bone marrow-derived MSC, adipose tissue-derived MSC and MSC derived from human exfoliated deciduous teeth. Meta-analysis was not possible due to heterogeneities in study designs. Local MSC implantation was not associated with adverse effects. In 4 out of the 5 studies, locally delivered undifferentiated bone-marrow MSC had a positive effect on DO bone formation. Few studies evaluated the therapeutic effects of MSC from other sources. The adjunct use of biologically active molecules or forced expression of key genes involved in osteogenesis further boosted the ability of bone-marrow MSC to promote DO bone formation. While risk of bias and heterogeneity limited the strength of this systematic review, our results suggest that the use of MSC is safe and may provide beneficial effects on DO bone formation.

Keywords: Mesenchymal Stromal Cells; Osteogeneses, Distraction; Osteogenesis; Review.

Introduction

Distraction osteogenesis (DO) is a method that induces osseous neoformation between two bone segments surgically separated in response to the application of graduated and controlled traction force throughout the bony gap.[1,2] This technique have been used in treatment of congenital and acquired craniofacial deformations, as it provides some advantages over traditional autogenic bone grafts, including no need for a second surgical site, reduced operating time and post-operative

morbidity.[2] Craniofacial DO outcome depends on multiple factors, including patient´s age, the surgical technique (corticotomy or osteotomy), distraction rate and rhythm, latency period, contention period, and the type of the distraction device used (*i.e.* intraoral, subcutaneous or extraoral).[1,2,3,4]

DO relies on the recruitment and proliferation of mesenchymal stem cells (MSC) to the target site, where they differentiate into osteoblasts to promote bone formation/mineralization.[5] MSC recruitment into DO defects is stimulated endogenously by the fracture healing process and exogenously by mechanical distraction.[6] Nevertheless, under standard circumstances, MSC recruitment appears to be slow and limits the amount of DO bone formation.[7] Moreover, MSC migration may be further compromised in elderly and under conditions such as poor vascularity, severe trauma and radiotherapy. Along with these lines, DO animal models were developed to evaluate the effect of locally applied MSC on DO bone formation.[8,9,10,11,12,13,14,15,16] Thus, this systematic review aims to evaluate the following PICO question: "In animals submitted to DO (Participant), how does local MSC administration (Intervention), compared to no MSC administration (Comparison), influence bone consolidation (Outcome)?"

Methodology

Focused question

We conducted a systematic review of the literature to address the following PICO question: "In animals submitted to DO (Participant), how does local MSC administration (Intervention), compared to no MSC administration (Comparison), influence bone consolidation (Outcome)?" This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[17]

Eligibility criteria

Type of studies

Only pre-clinical controlled animal model studies using MSC locally in association with DO were eligible.

Study population

The population of interest included animals that underwent DO.

Type of intervention and comparison

DO sites treated with MSC were compared to control DO sites that did not receive cells. The protocol of control groups varied according to the type of intervention in each study.

Outcome measures

The primary outcome was the amount of new bone formation, measured histologically, radiographically or by micro-computed tomography (micro-CT) in DO sites.

Search strategy

Search strategies were developed for MEDLINE, EMBASE, LILACS, SCIELO, SCOPUS, WEB OF SCIENCE databases, as well as the grey literature. Medical subject headings (MesH) terms were combined with Boolean operators and used to search databases. All searches were performed up to August 2017. The following MeSH terms were used: (((("stem cells" OR "stem cell" OR "progenitor cells" OR "progenitor cell" OR "induced pluripotent stem cells" OR "IPS cells" OR "IPS cell" OR "adult stem cells" OR "pluripotent stem cells" OR "pluripotent stem cell" OR "multipotent stem cells" OR "multipotent stem cell" OR "totipotent stem cells" OR "totipotent stem cell" OR "hematopoietic stem cells" OR "hematopoietic stem cell" OR "mesenchymal stromal cells" OR "mesenchymal stromal cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal progenitor cells" OR "mesenchymal progenitor cell" OR "bone marrow stromal cells" OR "bone marrow stromal cell" OR "stromal cells" OR "stromal cell" OR "bone marrow cells" OR "bone marrow cell" OR "epithelial mesenchymal transition" OR "cell transplantation" OR "stem cell transplantation" OR "mesenchymal stem cell transplantation" OR "hematopoietic stem cell transplantation" OR "peripheral blood stem cell transplantation")) AND "bone regeneration") AND ("distraction osteogenesis" OR "osteogenic distraction ")) AND (oral OR intraoral), NOT review. Manual searches of reference lists from

selected full articles complemented the electronic search. Potentially eligible studies were limited to articles published in English, Spanish, and Portuguese.

Exclusion criteria

Reviews, in vitro, human studies, animal studies without controls were excluded.

Screening methods and data extraction

Two calibrated reviewers (CMR and FG) screened independently the titles and abstracts. Studies appearing to meet the inclusion criteria, or those with insufficient information in the title and abstract to make a clear decision, were selected for evaluation of the full manuscript, which was done independently by the same reviewers to determine study eligibility. Disagreements were solved by discussion and agreement. Reasons for rejecting studies were recorded for each study. Agreement between reviewers was described by kappa coefficient. The following data were extracted independently by the same reviewers and recorded: citation, year of the publication, MSC origin, characterization, state of differentiation at application, time of transplantation, number of cells transplanted, animal model, number of animals, number of defects, defect type, size, location, scaffold experimental groups, length of follow-up, healing period and results.

Quality assessment

Quality assessment of included studies was performed independently by two reviewers (CMR and FG), blinded to the name of the authors. Studies were categorized according to the SYRCLE's risk of bias tool for animal studies.[18]

Results

Study selection

The computerized search strategy yielded 29 citations, of which 14 were screened for potentially

meeting the inclusion criteria (κ = 0.791; Figure 1). Independent screening of abstracts led to the rejection of 4 articles (κ = 0.837; Figure 1). Full texts of the remaining 10 publications were reviewed for possible inclusion. Of these, one article[19] was further excluded for reasons indicated in Table 1 (κ = 1.00) reference. A manual search of reference lists of selected studies yielded no additional studies (Figure 1). Characteristics of the final nine retained studies are reported in Tables 2 and 3.

Study characteristics

Experimental animals

Most studies (6 of 9) used New Zealand white rabbits,[8,9,10,11,12,14] one study use Japanese white rabbits.[15] Finally, two use Sprague-Dawley rats.[13,16]
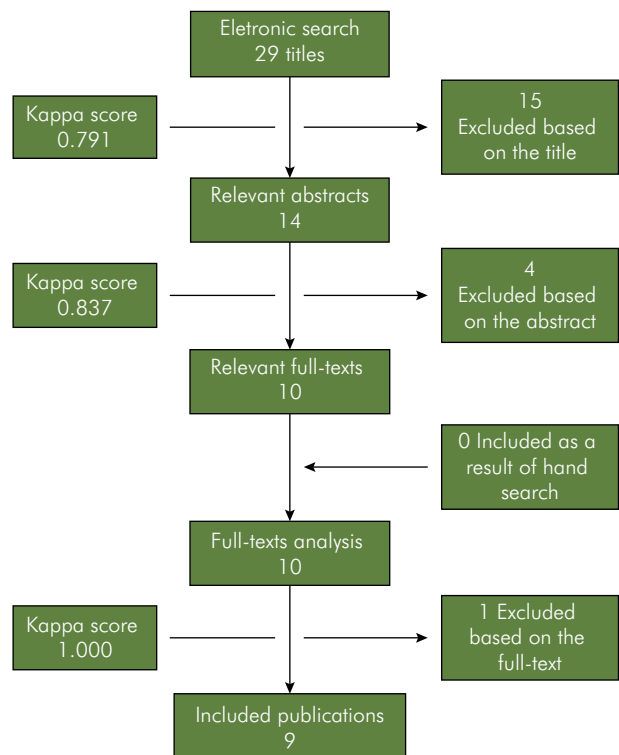

Figure 1. Simplified search strategy outline.

Table 1. Excluded study.

Study	Reason for exclusion
Zeng et al. (2016)[19]	Control group did not receive the scaffold used in MSC-based therapy.

Table 2. Description of the models used in the included studies.

Study	Animal model	Cell origin (n)/ characterization/ number of transplanted cells/ state of cell differentiation at application/ time of transplantation	Defect description/ location/ size	Scaffold
Ma et al. (2017)[8]	24 New Zealand white rabbits aged 3 months and weighing 2.5 - 3.0 kg.	BM-MSC, donor origin was not stated (n: not informed); ability to adhere; positive for CD29 and CD44 and negative or low expression of CD34 and CD45; ability to undergo osteogenic and adipogenic differentiation; aifferentiated into an osteogenic lineage at application; 5×10^6 cells/defect; injected into the distraction gap at the end of the DO period.	An osteotomy line was made between the first premolar and the mental foramen of the left mandible. An external distractor was fixed across both cortices. Left jaw underwent osteotomy by a fissured bur. After three days, the distractor was activated at a rate of 10 mm/twice a day, for five days, resulting in a 10 mm distraction gap	HA
Sun et al. (2014)[9]	54 OVX + 18 sham operated female New Zealand white rabbits, aged approximately 3 months and weighing approximately 2 kg.	Autogenous ADSC (n = 36); fibroblast-like cell morphology; 1×10^7 cells/defect; undifferentiated at application; injected into the distraction gap at the end of the DO period.	The corticotomy was performed immediately anterior to the first premolar. The distractor was fixed and distraction started after seven days, with an activation rate of 0.8 mm/day for ten days, resulting in 8 mm distraction gap.	None
Alkaisi et al. (2013)[10]	22 skeletally immature New Zealand white rabbits aged 3 to 5 months and weighing approximately 2.7 kg.	Xenogenous SHED (n = 4); fibroblast-like cell morphology; ability to adhere; colony forming efficacy; positive for CD105 and CD166; 6×10^6 cells/defect; undifferentiated at application; cells were transplanted at the surgical appointment.	A longitudinal incision was made on the inferior border of the right mandible. An incomplete osteotomy cut was placed between the first premolar and the mental foramen. A 12 X 12 mm distraction device was fixed to either side of the osteotomy cut.After four days, the distraction device was activated at a rate of 1.0 mm/day for six days, resulting in a 6 mm distraction gap.	None
Zhang et al. (2012)[11]	12 New Zealand white rabbits aged approximately 4 months.	BM-MSC, donor origin was not stated (n= not informed); ability to undergo osteogenic, adipogenic, and chondrogenic differentiation; ability to adhere and colony forming efficiency; number of implanted cells was not informed; undifferentiated at application; applied into the distraction gap at the end of the DO period.	A straight unilateral osteotomy was performed from the first premolar to the mental foramen. A custom-made distractor was placed and fixed perpendicularly to the plane of the osteotomy. After one week, the distraction device was activated at a rate of 0.9 mm/day for 11 days, resulting in a 10 mm distraction gap.	None
Lai et al. (2011)[12]	54 skeletally mature male New Zealand white rabbits weighing 3.0 - 3.5 kg.	Autogenous BM-MSC; ability to adhere; 1×10^7 cells/defect; undifferentiated at application; injected into the distraction gap at the end of the DO period.	The osteotomy was performed on the bucal aspect of the left anterior mandible, anterior to the first molar. A custom-made distractor was fixed with 4 self-tapping screws. After six days, the distraction device was activated at a rate of 0.4 mm/12 hours for 6 days, resulting in a 4.8 mm distraction gap.	None
Jiang et al. (2010)[14]	42 skeletally mature male New Zealand white rabbits weighing 2.0 – 3.0 kg	Autogenous BM-MSC; ability to adhere; 1×10^7 cells/defect; undifferentiated at application; applied into the distraction gap at the end of the DO period.	An osteotomy line was made on the right mandibles, between the first premolar and the mental foramen. A custom-made titanium external distractor was fixed across both cortices. After three days, the distraction device was activated at a rate of 2.0 mm /day for 5 days, resulting in a 10 mm distraction gap.	None
Kinoshita et al. (2008)[15]	12 adult male Japanese white rabbits weighing 3.0 - 3.4 kg.	Autogenous BM-MSC Ability to adherence; ability to undergo osteogenic differentiation; 1×10^7 cells/defect; differentiated into osteogenic lineage at application; applied into the distraction gap at the end of the DO period.	Osteotomies were made bilaterally on the zygomatic process of the maxilla, and custom-made distractor devices were fixed. After five days, the distraction device was activated at a rate of 2.0 mm/day for 4 days, resulting in an 8 mm distraction gap.	PRP
Hu et al. (2007)[13]	54 adult male Sprague-Dawley rats weighing rats weighing 350–400 g.	Autogenous BM-MSC; Fibroblast-like cell morphology; ability to adhere; colony forming efficacy; 1×10^6 cells/defect; undifferentiated at application; applied into the distraction gap at the end of the DO period.	The osteotomy was performed to the middle of the anterior ramus to the inferior border of the right mandible; After five days, the distraction device was activated at a rate of 0.4 mm/day for 8 days, resulting in a 3.2 mm distraction gap.	None
Qi et al. (2006)[16]	40 adult male Sprague–Dawley rats weighing 350–400 g.	Autogenous BM-MSC; fibroblast-like cell morphology, ability to adhere, colony forming efficacy; 5×10^5 cells/defect undifferentiated at application; applied into the distraction gap at the end of the DO period.	Osteotomy was performed from the upper border of the anterior ramus to the inferior border of the mandible. A custom made distractor was fixed to osteotomy site. After five days, the distraction device was activated at a rate of 0.4 mm/day, for 8 days, resulting in a 3.2 mm distraction gap.	None

Table 3. Summarized outcomes of the included studies.

Study	Groups/ n of defects	Healing period	Outcomes
Ma et al. (2017)[8]	Group 1: BM-MSC+HA (n=8). Group 2: BM-MSC-CSF + HA (n=8). Group 3: oBM-MSC-CSF + HA (n=8).	6 weeks	*Micro-CT* *New bone (mm³):* BM-MSC + HA: ~ 360 (A) BM-MSC-CSF + HA: 445 ± 29.6 (B) oBM-MSC-CSF + HA: 510 ± 26.3 (C) oBM-MSC group exhibited a more mature cortical bone.
Sun et al. (2014)[9]	Group 1: OVX rabbits + saline solution (n=6 /time point). Group 2: OVX rabbits + Adv-GFP-ADSC (n=6 /time point). Group 3: OVX rabbits + Adv-Runx2-GFP-ADSC (n=6 /time point). Group 4: sham rabbits + saline solution (n=6 /time point).	3, 6, and 9 weeks	i) *Micro-CT* *Bone mineral density (mg/cc) at:* 3 weeks: OVX control: ~ 70 (A) OVX + ADSC: ~ 85 (A) OVX + Runx2-ADSC: ~ 145 (B) Sham control: ~ 250 (C) 6 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~ 610 (A) Sham control: ~ 460 (B) 9 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~ 800 (A) Sham control: ~ 825 (A) *Bone volume to total volume ratio (%) at:* 3 weeks: OVX control: ~ 6 (A) OVX + ADSC: ~ 6 (A) OVX + Runx2-ADSC: ~ 11 (B) Sham control: ~ 24 (C) 6 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~ 55 (A) Sham control: ~ 42 (A) 9 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~62 (A) Sham control: ~ 64 (A) *Trabecular number (1/mm) at:* 3 weeks: OVX control: ~ 0.8 (A) OVX + ADSC: ~ 0.8 (A) OVX + Runx2-ADSC: ~1.6 (B) Sham control: ~ 0.9 (A) 6 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~2.4 (A) Sham control: ~ 1.4 (B) 9 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~3 (A) Sham control: ~ 2.2 (B) *Trabecular thickness (um) at:* 3 weeks: OVX control: ~ 50 (A) OVX + ADSC: ~50 (A) OVX + Runx2-ADSC: ~70 (B) Sham control: ~ 80 (C) 6 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~150 (A) Sham control: ~ 190 (B) 9 weeks: (data restricted to 2 groups) OVX + Runx2-ADSC: ~240 (A) Sham control: ~ 240 (A) ii) *Histological analysis: (data restricted to 2 groups)* No differences between OVX + Runx2-ADSC and sham control.

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| Alkaisi et al. (2013)[10] | Group 1: negative control (n=9).
Group 2: SHED (n=9). | 2, 4, and 6 weeks | *i) Radiographic analysis*
2 weeks: SHED group had more bone radiopacity bridging the DO gap.
 4 weeks: SHED group demonstrate bony continuity with greater radiodensity than control group.
6 weeks: SHED group demonstrated greater radiodensity in the distraction gap than that the control group.
ii) Histomorphometric analysis.
New Bone (%) at:
2 weeks:
Control: 18.41 (A)
SHED: 35.97 (B)
4 weeks:
Control: 31.45 (A)
SHED: 56.69 (B)
6 weeks:
Control: 52.61 (A)
SHED: 65.28 (B)
Bone union at:
2 weeks:
Control: 0.96 (A)
SHED: 1.75 (B)
4 weeks:
Control: 1.58 (A)
SHED: 2.96 (B)
6 weeks:
Control: 2.5 (A)
SHED: 3.25 (B)
Stage of bone maturity at:
2 weeks:
Control: 2.25 (A)
SHED: 3.16 (B)
4 weeks:
Control: 3.25 (A)
SHED: 4.5 (B)
 6 weeks:
Control: 3.5 (A)
SHED: 4.66 (B) |
| Zhang et al. (2012)[11] | Group 1: Sham-RT (n=3).
Group 2: RT (n=3).
Group 3: RT + BM-MSC (n=3).
Group 4: RT +BMP2/7_BM-MSC (n=3). | 4 weeks | *i) Radiographic analysis*
RT + MSC and RT + MSC_BMP-2/7_BM-MSC groups showed more cortical bone formation compared with sham-RT and RT control groups.
ii) Micro-CT
Trabecular separation (mm):
Sham-RT: 0.99 ± 0.16 (A)
RT: 1.00 ± 0.07 (A)
RT + BM-MSC: 0.99 ± 0.17 (A)
RT + BMP2/7_BM-MSC: 1.32 ± 0.17 (B)
Trabecular thickness (mm):
Sham-RT: 0.12 ± 0.01 (A)
RT: 0.12 ± 0.03 (A)
RT + BM-MSC: 0.15 ± 0.01 (A)
RT + BMP2/7_BM-MSC: 0.22 ± 0.02 (A)
Number of trabeculae (1/mm):
Sham-RT: 0.91 ± 0.12 (A)
RT: 0.90 ± 0.04 (A)
RT + BM-MSC: 0.83 ± 0.04 (A)
RT + BMP2/7_BM-MSC: 1.08 ± 0.1 (A)
Bone volume fraction:
Sham-RT: 0.12 ± 0.03 (A)
RT: 0.11 ± 0.03 (A)
RT + BM-MSC: 0.08 ± 0.02 (A)
RT + BMP2/7_BM-MSC: 0.12 ± 0.04 (A)
iii) Histological analysis
BM-MSC and BMP2/7_BM-MSC exhibited more mature medullary and cortical bones than the control groups. |

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| Lai et al. (2011)[12] | Group 1: Saline solution (n = 9 /time point). Group 2: BM-MSC (n = 9 /time point). Group 3: OSX_BM-MSC (n = 9 /time point). | 2 and 6 weeks | *i) Radiographic analysis* *Radiodensity at:* 2 weeks: Control: ~54 (A) BM-MSC: ~75 (B) OSX_BM-MSC: ~95 (C) 6 weeks: Control: ~105 (A) BM-MSC: ~121 (B) OSX_BM-MSC: ~143 (C) *ii) Histological analysis.* *Trabecular thickness (mm) at:* 2 weeks: Control: 22.89 ± 1.96 (A) BM-MSC: 26.96 ± 3.23 (B) OSX_BM-MSC: 39.39 ± 3.54 (C) 6 weeks: Control: 34.69 ±3.73 (A) BM-MSC: 42.41 ± 2.34 (B) OSX_BM-MSC: 54.21 ± 2.38 (C) *Newly formed cortical bone area (%)at:* 2 weeks: Control: 44.53 ± 5.43 (A) BM-MSC: 52.43 ± 2.74 (B) OSX_BM-MSC: 61.62 ± 3.10 (C) 6 weeks: Control: 84.42 ±3.06 (A) BM-MSC: 90.26 ± 2.12 (B) OSX_BM-MSC: 97.32 ± 2.65 (C) *Cancellous bone area (%) at:* 2 weeks: Control: 24.54 ± 3.22 (A) BM-MSC: 33.85 ± 3.49 (B) OSX_BM-MSC: 42.45 ± 2.98 (C) 6 weeks: Control: 42.05 ±3.58 (A) BM-MSC: 53.80 ± 2.91 (B) OSX_BM-MSC: 67.75 ± 4.04 (C) |
| Jiang et al. (2010)[14] | Group 1: Saline solution (n = 14). Group 2: Ad5_BM-MSC (n = 14). Group 3: Ad5_bFGF BM-MSC (n =1 4). | 8 weeks | *i) DXA analysis* *Bone mineral density (g/cm^2):* Control: ~0.23 (A) Ad5_BM-MSC: ~0.25 (B) Ad5_bFGF BM-MSC: ~ 0.27 (C) Bone mineral content (g): Control: ~0.21 (A) Adv5_BM-MSC: ~0.23 (B) Ad5_bFGF BM-MSC: ~0.25 (C) *ii) Micro-CT* *Bone volume to total volume ratio (%):* Control: 56.00 ± 3.72 (A) Adv5_BM-MSC: ~ 61.6 (B) Ad5_bFGF BM-MSC: ~67.2 (C) *Connectivity density (mm^3):* Control: 11.05 ± 1.73 (A) Adv5_BM-MSC: ~ 13.26 (B) Ad5_bFGF BM-MSC: ~ 21.00 (C) *Trabecular thickness (mm):* Control: 0.21 ± 0.04 (A) Adv5 BM-MSC: ~ 0.30 (B) Ad5-bFGF BM-MSC: ~ 0,38 (C) *Trabecular separation (mm):* Control: 0.32 ± 0.03 (A) Adv5_BM-MSC: ~ 0.32 (AB) Ad5_bFGF BM-MSC: ~ 0,36 (B) *Trabecular number:* Control: 2.01 ± 0.39 (A) Adv5_BM-MSC: ~ 2.60 (B) Ad5_bFGF BM-MSC: 3.28 (C) *iii) Histological analysis (data restricted to half of the samples).* While only disordered tiny trabeculae were sporadically found in the distraction gaps of group 1, the distraction gaps in group 3 were completely bridged with mature and regular trabecular bone. Bone trabeculae only partially filled distracted gaps in group 2. |

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| Kinoshita et al. (2008)[15] | Group 1: PRP (n = 6 defects). Group 2: Saline solution -contralateral internal control for group 1 (n = 6 defects). Group 3: oBM-MSC + PRP (n = 6 defects). Group 4: Saline solution - contralateral internal control for group 3 (n = 6 defects). | 2,3 and 4 weeks | *i) Radiographic analysis* *Radiodensity (mm Al) at:* 2 weeks: Ratio PRP/saline control: ~ 0.8 (A) Ratio BM-MSC + PRP/saline control: ~ 1.07 (B) 3 weeks: Ratio PRP/saline control: ~0.7 (A) Ratio BM-MSC + PRP/saline control: ~1.09 (B) 4 weeks: Ratio PRP/saline control: ~0.6 (A) Ratio BM-MSC + PRP/saline control ~1.1 (B) *ii) Histological analysis (data restricted to week 4)* New bone (%): Ratio PRP/saline control: ~1.2 (A) Ratio BM-MSC + PRP/saline control: ~1.6 (B) Bony content in the newly formed bone (%): Ratio PRP/saline control: ~0.95 (A) Ratio BM-MSC + PRP/saline control: ~1.2 (B) |
| Hu et al. (2007)[13] | Group 1: Saline solution (n = 9 /time point). Group 2: pEGFP-BM-MSC (n = 9 /time point). Group 3: pEGFP-BMP-2_BM-MSC (n = 9 /time point). | 2 and 6 weeks | *i) Radiographic analysis* *Radiodensity* 2 weeks: Control: ~52 (A) BM-MSC: ~58 (B) BMP-7 BM-MSC: ~70 (C) 6 weeks: Control: ~99 (A) BM-MSC: ~113 (B) BMP-2_BM-MSC: ~135 (C) *ii) Histomorphometric analysis* *Newly formed cortical bone area (%) at:* 2 weeks Control: 43.52 ± 6.11 (A) BM-MSC: 51.28 ± 3.85 (B) BMP-2_BM-MSC: 60.59 ± 4.00 (C) 6 weeks Control: 82.66 ± 2.06 (A) BM-MSC: 89.30 ± 1.95 (B) BMP-2: 95.54 ± 2.07 (C) *Cancellous bone area (%) at:* 2 weeks Control: 23.69 ± 2.60 (A) BM-MSC: 32.22 ± 4.22 (B) BMP-2_BM-MSC: 41.54 ± 3.01 (C) 6 weeks Control: 41.46 ± 4.01 (A) BM-MSC: 52.47 ± 1.93 (B) BMP-2_BM-MSC: 66.33 ± 4.00 (C) *Trabecular thickness (mm) at:* 2 weeks: Control: 21.13 ± 1.05 (A) BM-MSC: 25.43 ± 2.10 (B) BMP-2_BM-MSC: 37.32 ± 3.39 (C) 6 weeks: Control: 32.45 ± 4.33 (A) BM-MSC: 40.97 ± 2.52 (B) BMP-2_BM-MSC: 52.32 ± 3.12 (C) |

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Qi et al. (2006)[16]	Group 1: Saline solution (n = 10/time point). Group 2: BM-MSC (n = 10/time point).	Day 27 and 55	i) Radiographic analysis *Radiodensity at:* Day 27: Control: 169 (A) BM-MSC: 203 (B) Day 55: Control: 221 (A) BM-MSC: 255 (B) ii) Histomorphometric analysis *Newly formed bone volume in the cortical bone area (%) at:* Day 27: Control: 38.52 ± 3.25 (A) BM-MSC: 54.28 ± 3.50 (B) Day 55: Control: 85.48 ± 4.69 (A) BM-MSC: 92.30 ± 1.95 (B) *Newly formed bone volume at the cancelous bone area (%) at:* Day 27: Control: 23.29 ± 3.60 (A) BM-MSC: 34.02 ± 3.21 (B) Day 55: Control: 41.46 ± 4.01 (A) BM-MSC: 53.47 ± 1.83 (B) *Trabecular thickness (mm) at:* Day 27: Control: 23.13 ± 4.31 (A) BM-MSC: 30.40 ± 2.17 (B) Day 55: Control: 33.31 ± 3.55 (A) BM-MSC: 45.97 ± 3.52 (B)

Experimental models

DO defects were surgically created in all studies. Nonetheless, differences in defect location and morphology were identified among the studies. In five studies, the osteotomy line was made between the first premolar and the mental foramen.[8,10,11,12,14] In other two studies, the osteotomy line was made from the upper border of the anterior ramus to the inferior border of the mandible.[13,16] Finally, in the remaining two studies, the osteotomy cut was made anterior to the first molar[9] and bilaterally on the zygomatic process of the maxilla.[15]

The activation rate also varied among the studies. More specifically, activation rates of 0.4 mm/day,[13,16] 0.8 mm/day,[9,12] 0.9 mm/day,[11] 1 mm/day[8,10] and 2 mm/day[14,15] were reported. The resulting DO gap defect measured 10 mm,[8,11,14] 8 mm,[9,15] 6 mm,[10] 4.8 mm,[12] and 3.2 mm[13,16] in the included studies.

Mesenchymal stem cells and scaffolds

Both autogenous[9,12,13,14,15,16] and xenogenous[10] MSC were used in the studies. Two trials failed to inform the donor origin of the cells.[8,11] In regard to the source of the cells, bone marrow-derived MSC (BM-MSC) were utilized in seven trials,[8,11,12,13,14,15,16] while adipocyte-derived MSC (ADSC)[9] and stem cells derived from human exfoliated deciduous teeth (SHED)[10] were used in one trial each. In most studies, MSC were characterized by plastic adherent capacity,[8,10,11,12,13,14,15,16] fibroblast-like morphology,[9,10,13,16] colony forming[10,11,13,16] and multipotency[8,11,13,16] abilities. In only two studies, MSC were phenotypically characterized using flow cytometry to detect expression of mesenchymal markers. MSC were characterized as positive for CD29,[8] CD44,[8] CD115,[10] CD166,[10] and negative for CD34[8] and CD45.[8]

Table 4. Risk of bias in individual studies, assessed using the SYRCLE tool.

Study	1 Selection bias 1	2 Selection bias 2	3 Selection bias 3	4 Performance bias 1	5 Performance bias 2	6 Detection bias 1	7 Detection bias 2	8 Attrition bias	9 Reporting bias	10 Other bias
Ma et al. (2017)[8]	?	✓	x	?	x	x	x	✓	✓	✓
Sun et al. (2014)[9]	x	✓	x	?	x	x	x	x	✓	✓
Alkaisi et al. (2013)[10]	?	✓	x	?	x	x	x	x	✓	✓
Zhang et al. (2012)[11]	?	✓	x	?	x	x	✓	x	✓	✓
Lai et al. (2011)[12]	?	✓	x	?	x	x	x	x	✓	✓
Jiang et al. (2010)[14]	?	✓	x	?	x	x	x	x	✓	✓
Kinoshita et al. (2008)[15]	?	✓	x	?	x	x	x	x	✓	✓
Hu et al. (2007)[13]	?	✓	x	?	x	x	x	✓	✓	?
Qi et al. (2006)[16]	?	✓	x	?	x	x	x	✓	✓	✓

IRB, institutional review board; 1 ✓ = Adequate randomization; ? = randomized but no details; x = no evidence of randomization; 2 ✓ = Baseline characteristics given; x = baseline characteristics not given; 3 ✓ = Evidence of adequate concealment of groups; x = no evidence of adequate concealment of groups; 4 ✓ = Evidence of random housing of animals; ? = unknown housing arrangement; 5 ✓ = Evidence of examiners blinded to intervention; x = no evidence of examiners blinded to intervention; 6 ✓ = Evidence of random selection for assessment; x = no evidence of random selection for assessment; 7 ✓ = Evidence of researchers blinded; x = no evidence of assessor blinded; 8 ✓ = Explanation of missing animal data; x = no explanation of missing animal data; 9 ✓ = Free of selective reporting based on methods/results; x = selective reporting; 10 ✓ = Evidence of random housing of animals; ? = unknown housing arrangement.
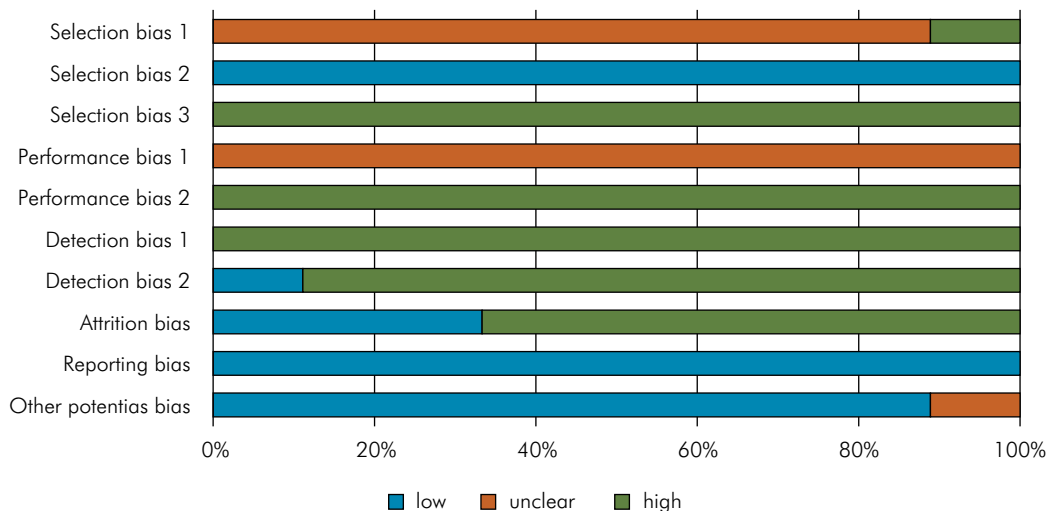

Figure 2. Risk of bias score for each risk item in animal studies, as assessed using the SYRCLE tool.

In all but one study, MSC were locally injected at the end of DO period.[9,11,12,13,14,15,16] The exception was the study from Alkaisi et al.[10] in which SHED cells were locally delivered at the surgical appointment. Scaffolds were only employed in two studies.[8,15]

Owing to the high degree of methodological heterogeneity among the included studies no meta-analysis was performed.

Safety

Overall, the majority of experimental procedures were well tolerated by most animals. Nonetheless, some authors refer to develop of infections,[9,13,16] mobility of the distraction devices,[9,10] changes in occlusal relationships[10] and overgrowth of the incisors.[12] Most of these reported adverse effects appeared to be

related exclusively to the DO procedure. Two studies failed to provide information on adverse effects.[8,11]

Quality Assessment

The use of the SYRCLE risk of bias tool to assess quality of animal studies indicated a high risk of bias for most studies in the majority of categories (Table 4 and Figure 2). Only two categories, baseline characteristics and reporting bias, were assessed as having a low risk of bias for the majority of studies.

Effect of mesenchymal stem cells on DO bone formation

Effect of undifferentiated mesenchymal stem cells on bone formation following DO

Most of the studies included in this systematic review report the effect of undifferentiated MSC on DO bone formation.[9,10,11,12,13,14,16] More specifically, five studies evaluated the effect of undifferentiated BM-MSC on DO bone formation, one study used undifferentiated ADSM and another used undifferentiated SHED cells.

Out of the five studies that evaluated undifferentiated BM-MSC, four showed a positive effect of these cells on DO bone formation.[12,13,14,16] The positive effect of undifferentiated BM-MSC on DO bone formation was demonstrated by radiographic determination of cortical bone formation,[11] bone mineral content[14] and bone density.[12,14,16] Moreover, histological demonstration of increased bone formation by undifferentiated BM-MSC was shown by increased a. cortical bone formation;[12,3,16] b. cancellous bone formation;[12,13,16] and c. trabecular thickness.[12,13,16] Corroborating these results, micro-CT analysis also showed that the use of undifferentiated BM-MSC was associated with increased new bone, bone volume ratio, connectivity density, trabecular thickness and trabecular number[14] in DO defects. In sharp contrast, in one study, BM-MSC failed to promote increased bone formation in DO gaps, as compared to the control treatment.[11]

Only one study evaluated the effect of undifferentiated SHED cells on new bone formation in DO gap defects. Histological and radiographic analysis demonstrated that the use of these cells resulted in increased bone formation and bone density, respectively.[10] Finally, a micro-CT study failed to demonstrated a positive effect of undifferentiated ADSC on DO bone formation, as compared to a control group.[9]

Effect of pre-differentiated MSC on bone formation following DO

Only one study reported on the use of osteogenically differentiated BM-MSC for DO bone formation. In this study, micro-CT analysis demonstrated that osteogenically pre-differentiated BM-MSC arranged in cell sheets promoted greater bone formation than undifferentiated BM-MSC sheets and negative control treatment.[8] Moreover, according to histological evaluations, the use of osteogenically differentiated BM-MSC cell sheets induced the formation of a more mature cortical bone.[8]

The combined effect of undifferentiated MSC and biologically active factors

Biologically active factors used in association with undifferentiated MSC included: transcription factors Runt-related transcription factor 2 (Runx2)[9] and Osterix (OSX),[12] bone morphogenetic protein (BMP)[11,13] and basic fibroblast growth factor (bFGF).[14]

A micro-CT study demonstrated that Runx2 transfected ADSC promoted increased bone mineral density, bone volume, trabecular number and trabecular thickness in DO defects, as compared to control treatment with no adjunct use of cells.[9] Moreover, the same study showed that forced expression of Runx2 increased the ability of ADSC to promote bone formation.[9] Likewise, OSX transfected BM-MSC promoted increased cortical and cancellous bone formation, trabecular thickness and radiographic bone density in DO defects, as compared to control treatment.[12] Forced OSX expression also increased the ability of BM-MSC to promote bone formation in DO defects.[12]

Conflicting results were reported on the use of BMP. While one micro-CT study showed that undifferentiated BM-MSC transfected with BMP-2 and BMP-7 failed to promote increased bone formation in DO defects in a rabbit model,[11] another demonstrated that forced BMP-7 expression improved the ability of BM-MSC to promote bone formation in a rat model of DO, as demonstrated by radiographic and histomorphometric analyses.[13]

Finally, bFGF transfected BM-MSC promoted greater bone mineral content and bone volume, higher bone density and increased trabecular number and thickness as compared to non-transfected BM-MCS and negative control treatment.[14]

The combined effect of pre-differentiated MSC and biologically active molecules

A combination of osteogenically differentiated MSC and platelet rich plasma (PRP) has been tested for the treatment of DO defects in only one study. In a rabbit model, the adjunctive use osteogenically differentiated BM-MSC significantly enhanced new bone formation and radiographic bone density in DO defects treated with PRP.[15]

Discussion

Tissue engineering has been proposed as an adjunct therapy to boost and overcome limitations associated with DO. Along with these lines, the use of MSC has been evaluated in several studies in an attempt to accelerate ossification and consolidation processes, and therefore, increase bone formation.[20] Thus, this systematic review provides evidence on the efficacy of locally applied MSC in preclinical models of maxillary and mandibular DO. The included studies, however, exhibited an overall high risk of bias. This in turn, seriously weakens confidence in the results and may curtail potential clinical applications of stem cell-based therapies in DO.

Cell source is expected to impact the ability of MSC to efficiently differentiate in bone forming cells.[21] Because of their ability to differentiate into multiple different cell types, BM-MSCs are frequently employed as a source of regenerative cells in various tissues, including bone.[22] Seven trials evaluated the effect of locally applied BM-MSC on DO outcomes.[10,13,14,15,16,17,18] Among those, five used undifferentiated BM-MSC.[11,14,16] Four of these studies demonstrated that local application of undifferentiated BM-MSC resulted in increased bone formation in DO gap defects.[12,14,16] One micro-CT study, however, concluded that undifferentiated BM-MSC failed to promote increased DO bone formation, as compared to the control treatment.[11]

Although the basis for this difference remains unknown, it is important to highlight that the conclusions of the last study were solely based on microtomographic data.[11] This is of relevance, as the correlation between microtomographic and histomorphometry data for assessment of new bone formation has been reported as weak.[23] Interestingly, a gross description of the histological findings from the above mentioned study revealed that BM-MSC-treated sites exhibited more mature medullary and cortical bones than control defects.[11] Further supporting the notion that micro CT still needs to improve to differentiate woven from lamellar bone.[24] Finally, the number of transplanted cells and the origin of BM-MSCs (autogenous or allogenous) used in the micro-CT study were not informed.[11] Thus, it is not possible to exclude the possibility that the lower results reported in the micro-CT study might be explained by differences related to cell numbers and cell populations.

Recent evidence has highlighted that the high heterogeneity of clonally expanded MSC, characterized by differences in stages of lineage commitment, expansion capabilities and phenotypes, are expected to determine their regenerative potential and clinical efficacy.[25] The studies included in this systematic review used cells from different sources, with distinct phenotypes and isolated under various protocols, all of which may impact their bone forming efficacy. Additionally, protocols also varied according to the time of the delivery and the use of scaffolds. Thus, future studies should focus on defining ideal in vitro MSC phenotypes and clinical protocols to boost DO bone formation.

Only one study compared the use of undifferentiated and osteogenically differentiated BM-MSC on DO bone formation.[8] Results from this study showed that osteogenically differentiated BM-MSC arranged in cell sheets promoted greater bone formation and remodeling into mature cortical bone, than undifferentiated BM-MSC sheets and negative control treatment.[8] This finding suggests the hypothesis that locally delivered of osteogenically pre-differentiated MSC may have a positive effect on promoting DO bone formation; nevertheless further investigations are needed to validate this finding.

Only two studies employed MSC other than BM-MSC in DO defects.[9,10] In one of these studies, locally administered SHED had a positive effect on DO bone formation.[10] Although SHED are multipotent and highly proliferative cells,[26,27] BM-MSCs are still the mostly used cells for bone regeneration due to their greater potential for osteogenic differentiation.[28] Therefore, despite the positive outcomes of locally delivered SHED cells in the treatment of DO defects,[10] additional studies are needed to validate these preliminary results and to compare their effectiveness to the one of BM-MSC for DO bone formation. The second study failed to demonstrate a positive effect of locally delivered ADSC on DO bone formation as compared to the control group.[9] Although ADSC undergo osteogenic differentiation and have been used for bone regeneration due to their wide availability and easy to obtain,[29,30] these cells have been reported to have a lower osteogenic potential as compared to BM-MSC.[31,32]

The combined effect of locally delivered MSC and biologically active molecules has been tested in six studies.[9,11,12,13,14,15] MSC gene transfection has become an exciting and promising strategy in MSC regenerative therapy.[33] Along with these lines, a few DO studies used Runx2,[9] OSX,[12] BMP-2/7,[11] BMP-2[13] and bFGF[14] genetically modified MSC to favor their differentiation into osteogenic cell lineages, and further improve bone formation.[9,11,12,13,14] Runx2 is an essential gene required for the osteoblastic differentiation and bone tissue formation,[34,35] whose expression can be induced both by BMP-2 and BMP-7.[36] Forced expression of Runx2 increased the ability of ADSC to promote bone formation in DO defects,[9] supporting the role of Runx2 in bone formation. OSX is a zinc finger-containing transcription factor essential for osteoblast differentiation, endochondral and intramembranous bone formation.[37,38,39,40] In line with its biological activity, OSX forced expression also increased the ability of BM-MSC to promote bone formation in DO defects.[12]

BMPs form the most extensive subgroup of the transforming growth factors-β (TGF-β) superfamily of cytokines,[41,42] whose main function is to promote bone formation by directing MSC differentiation into osteoblasts.[43] Among all the bone morphogenetic proteins, BMP-2 and BMP-7 have been tested alone or in combination with MSC in different experimental models of bone regeneration with variable results.[44] In this systematic review, two studies reported conflicting results on the efficacy of BMP to further improve the osteogenic results of undifferentiated MSC in DO defects.[11,13] While one study showed that undifferentiated BM-MSC transfected with BMP-2 and BMP-7 failed to improve bone formation in DO defects in a rabbit model,[11] another demonstrated that forced BMP-7 expression improved the ability of BM-MSC to promote DO bone formation in a rat model.[13] These conflicting results might be explained by methodological differences between the studies, such as: the animal models, DO defect sizes, DO latency period and observation time, distraction rate and rhythm. On the last matter, it has been demonstrated that distinct distraction rates differentially regulate BMP expression in DO defect, which ultimately, have a therapeutic impact on DO outcomes.[45] Lastly, these studies also differed in their method of analysis for the bone formation, with histological analysis being employed only in the study that supported the therapeutic benefits of BMP-7 gene therapy.[13]

Finally, one study evaluated the therapeutic efficacy of bFGF transfected BM-MSC in DO bone formation.[14] bFGF is a pleiotropic growth factor that normalize cell proliferation, migration, and differentiation in various organs, including bone.[46] bFGF enhances RUNX2 phosphorylation and functional activity.[37] FGF-2 is expressed in osteoblast-lineage cells,[46] and its deficiency inhibits bone formation in animal models.[47] bFGF transfected BM-MSC promoted greater DO bone formation than non-transfected BM-MCS and negative control treatment,[14] further supporting its role on bone formation.

Conclusion

It is possible to conclude that while risk of bias and heterogeneity limited the strength of this systematic review, our results suggest that the therapeutic use of MSC is safe and may provide beneficial effects on DO bone formation. Moreover, it is reasonable to propose that genetic modification

of these cells increase their ability to further induce DO bone formation. Finally, findings of this systematic review revealed that identification of ideal MSC populations for DO bone formation remains largely unknown. Future studies should be of high methodological quality, with a particular emphasis on identifying MSC phenotypes with higher potential to boost DO bone formation.

Acknowledgements

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