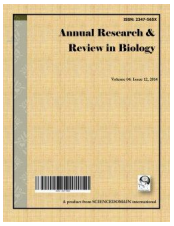

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Role of Mesenchymal Stem Cell-Conditioned Medium (MSC-CM) in the Bone Regeneration: A Systematic Review from 2007-2018

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Authors' contributions

This work was carried out in collaboration between both authors. Author IHD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JF managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Background: The therapeutic value of mesenchymal stem cells (MSCs) in tissue engineering and regenerative medicine is attributable in part to paracrine pathways triggered by several secreted factors secreted into culture media. The secreted factor here is known as the conditioned medium (CM) or secretome.
Objectives: This review is aimed to investigate and summarise the *in-vitro*, pre-clinical in-vivo studies regarding the role of CM-MSC in bone regeneration from 2007 until 2018
Data Sources: A systematic literature search on PubMed, MEDLINE, OVID, Scopus and Cochrane library was carried out by using search terms: Secretome, conditioned medium, mesenchymal stem cell, bone healing, osteogenic, osteogenesis.

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Methods: A total of 611 articles were reviewed. Ten articles were identified as relevant for this systematic literature review.
Results: Three tables of studies were constructed for in vitro studies and *in-vivo* studies.
Conclusion: All of the included in-vitro studies and in-vivo studies have shown a promoting effect of bone regeneration at various stages. Although there are no clinical studies regarding the use of CM-MSC in the human bone regeneration that have been conducted, transplantation of secretome has shown a promising result in the acceleration of bone healing process.

Keywords: Secretome; conditioned medium; bone regeneration; osteogenic; mesenchymal stem cell; tissue engineering; musculoskeletal.

1. INTRODUCTION

Bone healing is a multistage repair process that involves complex yet well-orchestrated process which depends on numerous factors including cellular, molecular, and mechanical events. The skeleton differs from other adult tissues that generate scar tissue at the site of an injury; it heals by forming new bone that is indistinguishable from an uninjured bone. However, bone healing remains challenging in musculoskeletal care [1]. Limitations with the use of autograft and allograft have led numerous studies into the exciting and evolving field of mesenchymal stem cells (MSCs) tissue engineering [2].

However, several issues with stem cells remain to be addressed, including tumorigenesis, [3] poor survival of implanted cells [4,5], transmission of infectious disease, and host-versus-graft disease. Besides, stem cell culture procedure is limited by a number of technical and ethical issues such as complicated safety and quality management issues with cell handling with the need of higher capital investment. According to recent literature from 2007-2018, the therapeutic value of mesenchymal stem cells in tissue engineering and regenerative medicine is attributable in part to paracrine pathways[6,7] triggered by several secreted factors secreted into culture media. The secreted factor here is known as the conditioned medium (CM) or secretome.

The secretome of MSCs is special, as a non-donor-specific and can be lyophilised, enabling more practical storage conditions [6]. These findings reinforce many researchers to look further into this molecule regarding their contents in the bone healing capability and to solve the existing problems with cell handling described earlier. This systematic review aims to elaborate studies that have been conducted to evaluate the osteogenic potency or the bone regeneration capability of secretome/CM-MSC.

2. METHODS

A systematic literature search using PubMed, Medline, OVID, Scopus and Cochrane library was carried out. The following keywords and search terms were used in the following order in each of the databases using a snowballing technique to cite the relevant articles: Secretome, conditioned medium, mesenchymal stem cells, osteogenic. The search was performed by an initial reviewer and subsequently checked by a second reviewer. Discussion resolved any disputes about whether an article met the inclusion criteria. The following inclusion criteria were applied:

1. Studies/reviews describing the potency of secretome or conditioned-medium mesenchymal stem cells in the acceleration of osteogenesis or chondrogenesis
2. Studies that involve osteogenicity in the maxillofacial surgery
3. Studies involving the acceleration of bone healing in the hypoxic condition
4. Studies published within 10 years prior
5. Studies in the English language
6. In-vitro and in-vivo studies

2.1 Exclusion Criteria Including

1. Effect of conditioned-medium MSC in other organs (cardiovascular, genitourinary, peripheral nerve)
2. Unpublished literature
3. Studies that evaluate osteogenic potency in other organs other than bone
4. Studies evaluating CM in the periodontal tissue

A total of 611 articles were reviewed. 10 articles were identified relevant with the aim of this systematic review. The studies included and excluded have been summarized in Fig. 1.

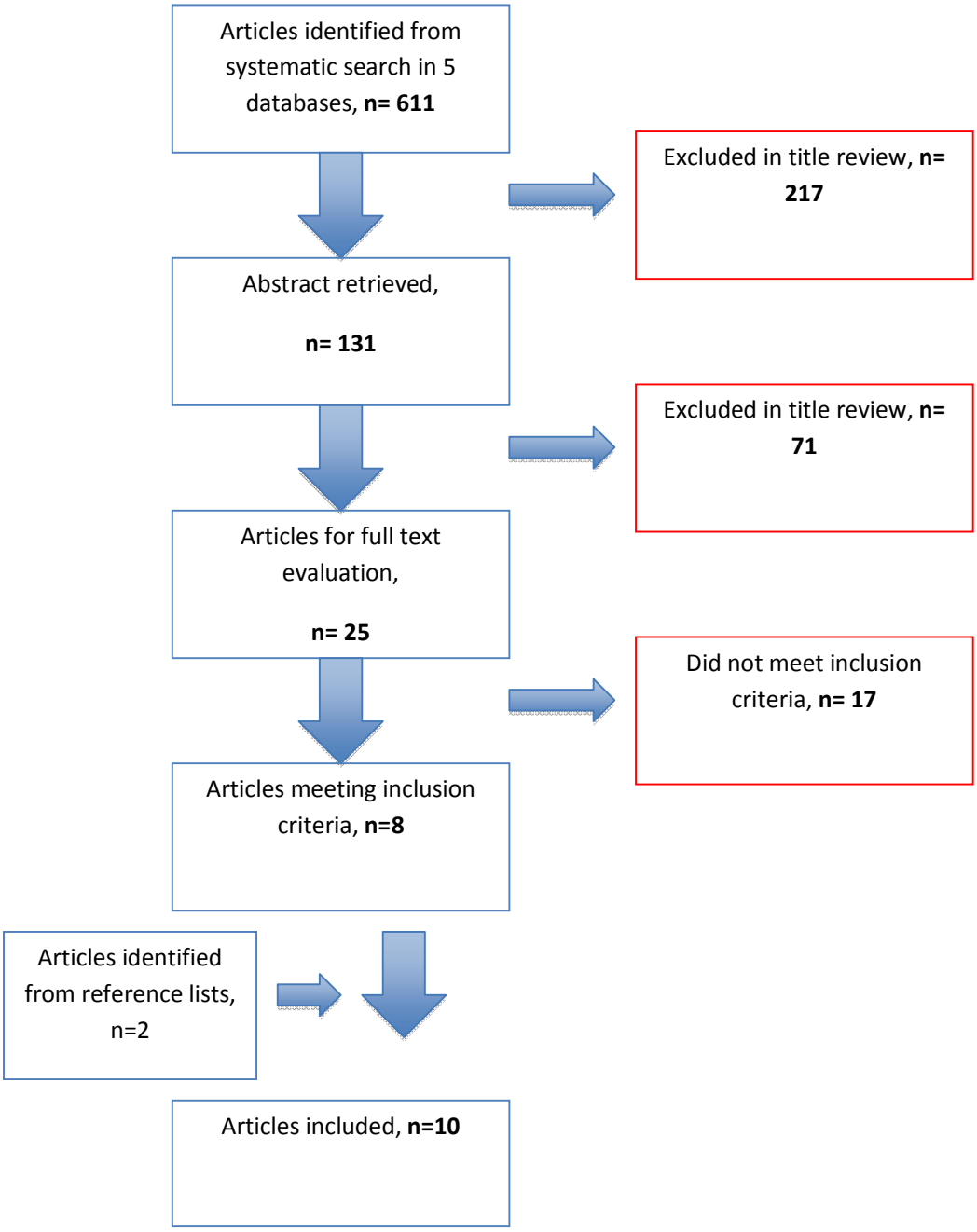

Fig. 1. Included and excluded studies

3. RESULTS

The role of CM-MSC in the in-vitro studies is summarised in Table 1. There was one study evaluating the role of MSC-CM in vitro. In 2014, Lee et al. hypothesised that embroid bodies (EBs) that were composed of either human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) with a hMSC-CM may stimulate mesodermal lineage induction. Through this induction, differentiation toward the osteogenic and chondrogenic lineage proven by quantitative reverse-transcription-polymerase chain reaction (qRT-PCR), cytochemistry, immunocytochemistry, and flow cytometry, was promoted [7].

Meanwhile, role of CM-MSC in animal studies is summarised in Table 2. 9 studies were evaluating various pathways of CM-MSC in accelerating the bone regeneration. The most critical factor for fracture union is the blood supply to the fracture site. There were two studies which evaluated the importance of angiogenesis capability hence the vascular endothelial growth factor (VEGF) in CM-MSC during acceleration of bone regeneration. In 2017, Katagiri et al. [8]. examined the value VEGF in the CM-MSC to accelerate the bone healing by comparing four groups of treatment consisting of MSC-CM, MSC-CM + anti-VEGF antibody, phosphate buffer saline (PBS), and control group with an unfilled defect. His research besides has successfully quantified the amount of IGF-1, VEGF, and TGF-β1 using ELISA, using a reconstruction computed tomography (CT) evaluation also proven a significant increase of new bone formation in the MSC-CM group compared to other groups which were verified by histological and immunohistochemistry (IHC) analysis. Meanwhile, Wang et al[9] in 2011 investigated the effects of hypoxic MSC-CM on tissue ingrowth, angiogenesis, and bone repair in the diabetic Sprague Dawley (SD) rats and proved that MSC-CM prepared under hypoxic conditions showed positive effects on angiogenesis following subcutaneous implantation and facilitated healing of segmental bone defect in a diabetic rat model.

When normal bone healing process is insufficient, supportive therapeutic strategies can be used to stimulate and augment bone regeneration (Walsh et al., 2008) [10]. Sun et al., 2012 [11] has reported that to enhance the effect of MSC in proliferation and differentiation of osteoblasts, a sensitive microenvironment can be formed. Chang et al. in 2015 proved that CM-rMSC collected under hypoxic condition could effectively influence bone regeneration through enhanced migration and adhesion of endogenous MSC in the SD rats with calvaria bone defect [12].

There were four studies including the works of Feng in 2012 [13], Katagiri 2013 [14], Linero 2014 [15], and Brudette 2017 [6], which evaluated the efficacy of CM-MSC from many sources (murine, human, human, human and amnion respectively) in rats' (except for Linero in rabbit) calvaria and mandible. All studies were evaluated using conventional radiograph, reconstruction CT scan, histological analysis, IHC, western blot, and RT-PCR. All studies have shown positive results where secretome enhanced the proliferation and migration of MSC osteogenic differentiation significantly.

A novel route of CM-MSC administration had been introduced by Ando et al in 2014 [16] who administered serum-free CM-hMSC locally in high-speed distraction osteogenesis (DO) in a mouse model. The CM-MSC was proved to promoted the recruitment of murine CMSCs and of endothelial cells/endothelial progenitor cells (EC/EPCs), and the establishment of a neoangiogenic network hence accelerating neo callus formation in the DO gap.

Implant fixation is commonly used in nonunion or bone defect cases, and its integration with CM-MSC should be understood. One of the material commonly used is titanium (Ti). In 2013, Tsuchiya investigated methods to enhance the stability of Ti implants using CM-BMSCs. Rat BMSC-CM was successfully immobilised on Ti implants [17]. The immobilised CM contained about 2000 proteins, including collagen type I, bone sialoprotein, fibronectin, and VEGF, which are essential for new bone formation. CM promoted cell adhesion and osteocalcin gene expression of rat BMSCs. The labeled CM remained associated with the Ti implant at 1, 7, 14, and 28 days postimplantation. Compared to controls, the removal torque value and BIC of Ti implants with immobilised CM were higher than on days 1, 7, and 14 after implantation.

4. DISCUSSIONS

It is clear that the included studies have demonstrated that the therapeutic effects of transplanted stem cells are considered to be effective for tissue regeneration. In addition, they play an essential role as cellular modulators, apart from their multi-potent differentiation ability [18-20]. Stem cells, including MSCs, are attracted to damaged tissue site where they produce the secretome that enhances angiogenesis, reduces inflammation, promotes tissue repair, and inhibits fibrosis and cell apoptosis [21-23]. The application of cell-free secretome may avoid the limitations associated with cell therapy, including higher costs for cell preparation, longer waiting time, and immune incompatibility [24,25]. Although it has been proven that the effect of MSC is mainly due to its paracrine effect, reviews regarding the osteogenic potency of this paracrine effect concerning bone regeneration promotion are scarce.

Table 1. *In-vitro* studies of secretome MSC role in bone regeneration

Study	Secretome source	Method	Evaluation	Results
Lee, 2014 Tissue eng part A[7]	hMSC	Hypothesis**:** treatment of embryoid bodies (EBs) composed of either human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) with a hMSC-conditioned medium (CM) may stimulate mesodermal lineage induction and subsequent differentiation toward the osteogenic and chondrogenic lineage. Group I: EB culture + hMSC-CM (each cultured in osteogenic & chondrogenic medium) Group II: EB culture + EB medium (each cultured in osteogenic & chondrogenic medium)	Quantitative RT-PCR for osteogenic (ALP, COL I, OP, ON, OC genes) & chondrogenic (COL II, AGG genes) Chemical staining with osteogenic (Alizarin red S & von Kossa) and chondrogenic (Alcian Blue & Safranin O staining) then evaluated using a light microscope. IHC osteogenic (human OC antibody) and chondrogenic (anti-human COL II antibody) Flowcytometry analysis for osteogenic with rhodamine-conjugated OC antibody then analysis by FACS Aria I using CellQuest software	qRT-PCR: expressions of osteogenic marker genes were significantly enhanced in the hMSC-CM treatment group compared with the untreated control group. OL II and AGG expressions were significantly enhanced in the hMSC-CM treatment group compared with the untreated control group. Calcium deposition was enhanced in hMSC-CM hESCs compared with untreated hESCs. hMSC-CM hESCs were more positively stained with Alcian Blue and Safranin O staining compared with untreated hESCs IHC: hMSC-CM-treated hESCs showed more positive signals for OC (red) and COL II (green) compared with the untreated hESCs Flow cytometric: 21.3% of the hMSC-CM hESCs cultured in the osteogenic medium were OC positive, whereas only 14.8% of the untreated hESCs were positive Conclusion: enhanced osteogenic and chondrogenic differentiation compared with untreated EBs, as evaluated using qRT-PCR, cytochemistry, immunocytochemistry, and flow cytometry

Table 2. *In-vivo* animal studies of secretome MSC role in bone regeneration

Study	Secretome source	Trial	Evaluation	Results
Wang, 2011 J Tissue Eng Med9	hMSC	The hypothesis investigates the effects of MSC-CM on tissue ingrowth, angiogenesis and bone repair in diabetes mellitus (DM) SD rats. Group 1(subcutaneous model): - normal control - DM control - DM MSC-CM - DM 293-CM - DM MEM Group II (bone defect model): - normal control - DM control - DM MSC-CM - DM 293-CM - DM MEM	- Xray at 3, 5, 8 wk - Histology exam at 3, 8 wk: CD 31 stain for endothelial cell count - Micro CT at 8 wk: measure BMD and bone volume	MSC-CM addition by subcutaneous implantation could facilitate healing of segmental bone defects in a diabetic rat model by positive effects on angiogenesis mechanism
Katagiri, 2017[8] Maxillofacial Plastic and Recon Surg	hMSCs	Hypothesis: angiogenesis is an important step for bone regeneration, and VEGF is one of the crucial factors in MSC-CM that would enhance its osteogenic potential 24 Wistar/ST rats with 5 mm diameter calvaria bone defect Group I: MSC-CM Group II: MSC-CM + anti-VEGF antibody Group III: PBS Group IV: unfilled defect	Human IGF-1, VEGF, TGF-β identification by ELISA 3D-CT evaluation IHC analysis by CD31-, CD105-, or FLK-1	In MSC-CM, the concentrations of IGF-1, VEGF, and TGF-β1 were 1515.6 ± 211.8 pg/mL, 465.8 ± 108.8 pg/ mL, and 339.8 ± 14.4 pg/mL, Newly formed bone area in the MSC-CM group (72.3 ± 17.1%) increased significantly compared to those in the Defect (22.2 ± 8.0%), PBS (30.9 ± 6.2%), and MSC-CM + anti-VEGF (33.1 ± 12.4%) groups ($p < 0.05$). No statistically significant differences between the MSC-CM + anti-VEGF group and other controls were found.

Study	Secretome source	Trial	Evaluation	Results
				Histological analysis also showed well-regenerated bone in the MSC-CM group compared with the other groups. IHC staining showed that numerous CD31-, CD105-, or FLK-1-positive cells were present throughout the specimen in the MSC-CM group. In MSC-CM + anti-VEGF, PBS, and Defect groups, fewer CD31-, CD105-, or FLK-1-positive cells were seen MSC-CM addition could enhance bone regeneration compared to MSC-CM + anti VEGF, PBS and unfilled defect hence showing the possibility that MSC-CM bone healing acceleration is via the effect of VEGF.
Chang, 2015[12] Mol Cells	rMSC	Hypothesis: conditioned medium collected under hypoxic condition could effectively influence bone regeneration through enhanced migration and adhesion of endogenous MSC. 21 SD rats with calvarial bone defect model Group 1: 1 ml SFM (serum-free medium) + greenplast Group II: 1 ml NCM (normoxic conditioned medium) + greenplast Group III: 1 ml HCM (Hypoxic conditioned medium) + greenplast	Defect evaluation: 3D reconstruction CT Staining with calcein, fluorescent images by confocal microscope	After 24 h, the migration rate of rMSC-HCM was 30- and 4.3-fold higher than that of rMSC-SFM and rMSC-NCM, respectively (*$p < 0.05$ vs. SFM and NCM) The spreadability of HCM-treated rMSCs was 75% greater than that of SFM-treated rMSCs and 25% greater than that of NCM- treated rMSCs. Percentage of new bone formation in NCM and HCM significantly higher than in SFM from 28-56 days after treatment

Study	Secretome source	Trial	Evaluation	Results
				Calcein density in HCM group was 5.6- and 2.3- fold higher than in SFM and NCM groups ($p<0.05$ vs. SFM and NCM) Under hypoxic conditions, behavioral changes of endogenous MSC through microRNA221 targeted- ICAM-1 expression may be a potential treatment for those with bone defects.
Feng Li, 2012[13] Biochem and Biophys Res	Murine BMSC	Aim: to investigate whether factors secreted by MSCs undergoing osteogenic differentiation induce expression of osteoblast markers in exogenous MSCs as well as their migration One million MSCs were cultured in osteogenic medium and the medium conditioned by the cells was collected at day 0, 5, 10, 15 and 20. Prepare medium supplemented with neutralizing antibodies, BMP-2 (R&D, Minneapolis, MN) and VEGF (Santa Cruz, Santa Cruz, CA) neutralizing antibodies were added in the conditioned medium at a concentration of 2.5 ug/ ml and 5 ug/ml, respectively. The media were then used for assessing activities of these factors. A tunnel was created within five mice femurs cavities via the femoral condyle using a 26 gauge needle. Subsequently,a smaller gauge needle (30 gauge) attached to a syringe	ALP assay every day 7 Western Blot to evaluate protein anti-mouse VEGF, PEDF, Col1, GAPDH, goat anti-BMP2 (R&D, Minneapolis, MN), rabbit anti-osteocalcin (Millipore Corp, Billerica, MA) followed by HRP-conjugated secondary antibodies. The cells retrieved from marrow and bone of the recipient mice at four consecutive weeks were expanded in culture in the	ALP activity was elicited by the CM at day 15 and 20 following osteogenic differentiation The BMP-2 synthesis was maximal beginning at day 5 and continued up to day 15 and then began to show a decline. VEGF appeared to be synthesized throughout the differentiation period with a slight increase at day 10. PEDF synthesis appeared maximal at day 10 and showed a steady decline to day 25. BMP-2 expression was detected beginning at day 3 in bone marrow (BM) following cell infusion into femurs. There was no expression of BMP-2 by donor cells in bone (B) at all the time periods assessed. VEGF was expressed by donor cells in bone marrow beginning at day 1 through day 14. Expression of VEGF by donor cells

Study	Secretome source	Trial	Evaluation	Results
		containing cells for injection was inserted. MSCs for injection were suspended in 20 ll of PBS (2 10^6 cells) and delivered within the bone cavity by slowly retracting the needle while depositing cells. Donor GFP+ cells were retrieved from recipient femur bones and marrow at 1, 3, 7, 14 and 28 days following cell infusion. Retrieved cells were expanded in culture in a medium supplemented with Zeocin for selection of GFP+ donor cells.	presence of Zeocin for seven days. The expanded cells were sorted by FACS for the GFP+ donor cells prior to their use for gene expression analysis. RNA isolation and RT-PCR to detect BMP-2, VEGF, SDF-1 genes	in bone appeared to increase for an extended period. SDF-1 was equally expressed at all time periods by donor cells in bone marrow and bone. MSCs marked with GFP and Zeocin resistant genes were infused into femurs and retrieved from bone marrow and bone at specified days.
Katagiri, 2013[14] Int J Oral Maxillofac Implants	hMSCs	Aim: to investigate the effects of hBM-MSC-CM on bone regeneration and its ability to induce endogeneous stem cell mobilization and bone regeneration 24 Wistar/ST rats with 2 circular full-thickness bone defects (5 mm diameter) Group I: MSC-CM Group II: PBS Group III: unfilled defect Rats were sacrificed at 2 or 4 wk after transplantation (n=4 per group)	Cellular migration by HE staining and light microscope cell count RT-PCR analysis of ALP, type I alpha 2 collagen, OCN, Runx2, GAPDH Micro –CT analysis, analyzed using OsiriX imaging software Histological analysis using HE stain and light microscope	MSC-CM increased rMSC migration more than seven-fold compared to DMEM(–) The ALP, OCN, and Runx2 genes expression levels were significantly upregulated in rMSCs cultured in MSC-CM compared to rMSCs cultured in control medium After two weeks, the mean area of newly regenerated bone in the MSC-CM defects was significantly increased compared to that of the unfilled defects and the PBS-treated sites (81.50% ± 2.7%, 8.63% ± 1.78%, and 60.63% ± 5.8%, respectively). After four weeks, the defect areas were almost completely filled by newly formed bone in the MSC-CM and PBS groups (93.07% ± 6.6% and 84.04% ± 4.9%, respectively).

Study	Secretome source	Trial	Evaluation	Results
				At 2 weeks, the bone defect in the MSC-CM group was almost covered with newly regenerated bone. Whereas in the PBS group, the defect was covered with a large amount of connective tissue. At 4 weeks, newly regenerated bone was partially noticeable within the defect of the PBS group. However, in the MSC-CM group, the defect was almost completely replaced by mature bone tissue
Linero, 2014[15] PLoS ONE	hAdMSC	Aim: to evaluate the ability of hAd-MSC and their CM by radiographic, morphometric and histological analysis, and to repair surgical bone lesions using an in vivo model (rabbit mandibles) delivered with human blood plasma hydrogels (HBPH) Group I: 12 rabbits with HBPHs + Ad-MSC + HBPHs w/o cells on contralateral side (control) Each 4 animal sacrificed at 15, 30, 45 days after surgery Group II: 4 rabbits with HBPHs + Ad-MSC both sides and sacrificed 3, 6, 9, 12 days after treatment Group III: 3 rabbits with a hydrogel containing CM-1 on one side and CM-2 on contralateral side; sacrificed 45 days after surgery.	Radiograph analysis by Image J Histological analysis Immunohistochemistry analysis	Ad-MSC enhances bone regeneration process more through paracrine mechanism (conditioned medium). When paracrine factors collected and applied as CM are used instead of Ad-MSC itself which is undetected after 12 days of implantation, the amount and quality of regenerated bone is similar.
Burdette, 2017[6] J Craniofac Surg	Amnion-derived secretome	Aim: Evaluate the efficacy of this secretome biotherapeutic in vitro on the proliferation and migration of MSC and osteoprogenitor cells as	Micro CT and histology evaluation	The secretome biotherapeutic enhanced the proliferation and migration of MSC and proliferation of

Study	Secretome source	Trial	Evaluation	Results
		well as in vivo using a critical size rat calvarial defect model. Ten male Fischer 344 (CDF) rats with 8 mm diameter calvarial defect Group I: saline + collagen scaffold Group II: secretome + collagen scaffold Sacrificed at four weeks or 12 weeks		osteoprogenitor cells. The secretome improved new bone volume and connectivity by 12 weeks and significantly improved angiogenesis at four weeks and bone density at 4 and 12 weeks with no deleterious effects. The improvement in new bone volume, connectivity, and angiogenesis suggests that the secretome biotherapeutic has beneficial effects on bone healing, and a higher dose of the secretome biotherapeutic may further improve bone regeneration.
Ando, 2014[16] Bone	hBM-MSC-CM	Hypothesis: local administration of serum-free conditioned medium from human mesenchymal stem cells (MSC-CM) accelerated callus formation in the mouse H-DO model female ICR mice DO model Control group: performed distraction after three days latency period and continued for eight days at 0.2 mm/12 h which was sacrificed at 15 days after surgery MSC Treatment group: 3×10^5 MSCs or FBs transplanted with distraction rate of 0.4 mm/12 h, length of increase 3.2 mm in 4 days which was sacrificed 5, 7, or 11 days after surgery MSC-CM group: 20 µl serum-free DMEM (control) or FB-CM or MSC-CM injected	Histology analysis using HE stain Histomorphometric analysis IHC analysis cytokine antibody assay Osteoblast differentiation using ALP assay	The secretomic analysis identified factors contained in MSC-CM that recruit murine bone marrow stromal cells (mBMSCs) and endothelial cells/endothelial progenitor cells (EC/EPCs), inhibit inflammation and apoptosis, and promote osteoblast differentiation, angiogenesis, and cell proliferation. Functional assays identified MCP-1/-3 and IL-3/-6 as essential factors in recruiting mBMSCs and EC/EPCs. Moreover, IL-3/-6 enhanced the osteogenic differentiation of mBMSCs. MSC-CM that had been depleted of MCP-1/-3 failed to recruit mBMSCs, and consequently failed to promote callus formation.

Study	Secretome source	Trial	Evaluation	Results
		transcutaneously into the center of distraction zone using a 29-gauge needle on days 3, 5, and 7; mice were sacrificed at 7 and 11 days after surgery.		
Tsuchiya, 2013[17] Int J Oral Maxillofac Implants	BMSC from rat femur	Purpose: To improve the stability of titanium (Ti) implants using the conditioned medium (CM) derived from rat bone marrow stromal cell (BMSC). Rat BMSC-CM was immobilized on the surface of Ti implants with calcifying solution	Ti implants topology observed by SEM microscopy Ti-immobilized CM analyzed by liquid chromatography with tandem mass spectrometry. Adhesiveness & osteogenic differentiation: rt-PCR Localization of CM by in vivo imaging at day 1, 7, 14 after implant Removal torque test and histologic bone-implant contact (BIC)	Immobilized CM contained about 2000 proteins (collagen type I, bone sialoprotein, fibronectin, and VEGF) CM promoted cell adhesion and osteocalcin gene expression of rat BMSCs. Compared to controls, removal torque value and BIC of Ti implants with immobilized CM were higher on days 1, 7, and 14 post-implantation. During an initial stage, immobilized CM components on the surface of Ti implants promoted integration into bone.

We have observed that all included studies in this review revealed that the CM-MSC significantly enhances the bone regeneration compared to normal medium [6,7,9,12-17]. The secretome was proven to be superior compared to MSC alone [14]. The transplanted MSC have poor differentiation and survival of engrafted stem cells suggesting that the regenerative properties of these cells are exerted primarily through paracrine mechanisms. However, there was a study stating that no superiority between CM-MSC and MSC in terms of osteogenic potency was found [15]. Ad-MSC improves bone regeneration process, and that the amount and quality of regenerated bone is similar when paracrine factors collected and applied as CM are used instead of Ad-MSC [15].

The CM-MSC effect was also proven to be increased under stress condition, including hypoxia [9,13]. MSCs express significantly higher levels of several arteriogenic cytokines when subjected to hypoxic stress [26]. When they are deprived of serum, starvation stress induces them to secrete angiogenic factors [27]. In general, severe stress causes cells to activate survival pathways and secrete factors to counteract toxic conditions. 27 Therefore, severe stress conditions may significantly increase the therapeutic efficiency of factors harvested in the MSC-CM.

The most critical factor for new bone formation is adequate blood supply [28]; impairment of angiogenesis at the fracture site usually results in non-union or delayed union [29]. MSCs also have been shown to secrete cytokines and growth factors that can inhibit hypoxia-induced endothelial apoptosis and promote angiogenesis [30]. Several studies have determined that CM-MSC, especially that prepared under hypoxic conditions, contains a higher amount of angiogenic factors [30,31]. Wang et al., [25] have proved that MSC-CM has shown significantly higher levels of angiogenesis factors (VEGF and IL-6) and that MSC-CM delivered in gelatin sponges stimulates angiogenesis and promotes fracture healing in a diabetic rat model and may be an alternative strategy for treating fracture non-union in patients with diabetes.

There was one study by Ando et al., advocating a novel administration technique of MSC-CM in a DO model. The study demonstrated that when locally administered into the H-DO gap, MSC-CM promoted new bone callus formation at the distal end of the gap by accelerating the recruitment of endogenous mBMSCs and EC/EPCs. He also elaborated the ten tissue-regenerating trophic factors that participated in the recruitment of endogenous BMSCs and EC/EPCs as well as in osteoblast differentiation, angiogenesis, cell proliferation, and inflammation suppression. This finding offers an opportunity for newer less invasive and effective method of bone healing treatment with MSC-CM.

Mechanical stabilisation is the key factor in the diamond concept of bone healing accompanied with osteoprogenitor cells, osteoinductive proteins, and osteoconductive scaffolds.[32,33] Upon the usage in patients in clinical settings, a combination of MSC-CM with fixation is crucial. There was one study by Tsuchiya that proved that immobilised CM components on the surface of Ti implants promoted integration into bone during an early stage [17].

Secretome-based approaches using CM may present osteogenic potential advantages over living cells regarding manufacturing, storage, handling, product shelf life and their potential as a ready-to-go biological therapeutic agent [34-41].

Although the use of CM is generally safe from ethical issues, inflammatory risk, tumorigenesis complication, and even host-versus-graft disease, studies that evaluate the risk, harm, safety of CM application should be performed, and comparison potency of MSC-CM and MSC are needed [42-50].

Our review also found the diverse source of MSC-CM used from murine, human, human adipose, human bone marrow, and amnion. However, there has been no study comparing the osteogenic potency of each of them. Further research might also evaluate the comparison of dosages related effect in the MSC-CM application to obtain the optimum dosage with the least side effects in the clinical setting.

Finally, more extensive trials on animal models with more extended observation period are required to answer these questions above before conduction clinical trials on human subjects.

5. CONCLUSION

All of the included in-vitro studies and in-vivo studies from 2007-2018 have shown a promoting effect of bone regeneration at various stages. Although there is no clinical study regarding the

use of CM-MSC in the human bone regeneration to this date, transplantation of secretome has shown a promising result in the acceleration of bone healing process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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