

Application of selected scaffolds for bone tissue engineering: a systematic review

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Abstract

Purpose The current systematic review investigated the results of application of some of the most commonly used scaffolds in conjugation with stem cells and growth factors in animal and clinical studies.

Methods A comprehensive electronic search was conducted according to the PRISMA guidelines in NCBI PMC and PubMed from January 1970 to December 2015 limited to English language publications with available full texts. In vivo studies in relation to “bone healing,” “bone regeneration,” and at least one of the following items were investigated: allograft, β -tricalcium phosphate, deproteinized bovine bone mineral, hydroxyapatite/tricalcium phosphate, nanohydroxyapatite, and composite scaffolds.

Results A total of 1252 articles were reviewed, and 46 articles completely fulfilled the inclusion criteria of this study. The highest bone regeneration has been achieved when combination of all three elements, given scaffolds, mesenchymal stem cells, and growth factors, were used. Among studies being

reported in this review, bone marrow mesenchymal stem cells are the most studied mesenchymal stem cells, β -tricalcium phosphate is the most frequently used scaffold, and platelet-rich plasma is the most commonly used growth factor.

Conclusion The current review aimed to inform reconstructive surgeons of how combinations of various mesenchymal stem cells, scaffolds, and growth factors enhance bone regeneration. The highest bone regeneration has been achieved when combination of all three elements, given scaffolds, mesenchymal stem cells, and growth factors, were used.

Keywords β -TCP · Bone tissue engineering · Growth factor · HA/TCP · Progenitor/stem cells · Scaffold

Introduction

Treatment of skeletal defects has remained a challenging part of many reconstructive surgeries. Currently, autogenous bone is assumed to be the gold standard for bone grafting [1, 2]. Bone substitute materials are recommended when the quantity of autogenous bone needed is greater than available amounts of autogenous bone [3] and when there is a risk of morbidity at the donor site [4–7]. Bone tissue engineering has been proposed as an alternative therapeutic option by harvesting progenitor cells and engineering graft materials in vitro to be clinically used for reconstruction of skeletal defects in vivo [8]. Over the last few decades, advancements have been achieved in tissue and bone engineering procedures [9–11]. A combination of progenitor/stem cells, growth factors, and scaffolds has been used in bone engineering [12, 13]. Different progenitor/stem cells, including mesenchymal stem cells (MSCs), can be used in bone engineering procedures. The ability of stem cells to self-renew and to differentiate into various cell lineages turns them into a special factor in tissue engineering. Among the different MSCs, bone marrow

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mesenchymal stem cells (BMSCs) are most frequently used within the studies [14]. Their osteogenic capability as well as their proliferation have been shown by various studies [12–16]. In vivo studies also have presented higher bone regeneration in treatment with MSCs compared to acellular treatments [16–19]. Osteogenic differentiation of stem cells can be promoted by using cytokines/growth factors, osteoinductive chemical factors, and biomaterials [15]. The addition of various growth factors to cell–scaffold constructs promotes bone regeneration [20, 21]. However, chemical compounds tend to be less unstable and therefore have a longer active half-life in comparison to protein-based cytokines and growth factors. Moreover, they have proven to better promote osteogenic differentiation of stem cells in vitro [15]. An ideal scaffold, grafting material, for bone engineering provides adequate environment for progenitor/stem cells to differentiate and proliferate in an osteoconductive setting. In addition, scaffold permits migration of cells to the site of defect by creating a suitable extracellular matrix for proper vascularization, attachment, infiltration, and cell growth. Scaffold also can be used as a delivery system for biological treatments of bone defects [22]. Growth factors and cells responses to each others, as well as treatment results, depend on physical properties of scaffolds such as porosity, pore size, interconnectivity of the pores, and surface texture. Also, scaffold chemical compositions can make an impression on extracellular matrix production by releasing different ions at different times [23–25]. Numerous scaffolds are commercially available causing confusion for clinicians. Hence, the current systematic review investigated the results of application of some of the most commonly used scaffolds in conjugation with stem cells in animal and clinical studies [1, 13]. The following scaffolds were selected in this study: β -tricalcium phosphate (β -TCP), bone allograft, deproteinized bovine bone mineral (DBBM), hydroxyapatite/tricalcium phosphate (HA/TCP), nano-HA, composite scaffolds, and natural bovine bone mineral (NBBM) [26–30]. This review will help clinicians to determine the suitable scaffolds for bone reconstruction in conjunction with proper stem cells for bone tissue engineering. This review will help clinicians to compare the result of given scaffolds in conjunction with various stem cells and growth factors in bone reconstruction.

Materials and methods

Eligibility criteria

Applied inclusion criteria of this study were as follows:

- Studies in relation to “bone healing” and “bone regeneration”
- Studies using at least one of these items: HA/TCP, β -TCP, NBBM, DBBM, composite scaffolds, nano-HA, and allografts
- Studies using scaffolds on animals and clinical trials

Applied exclusion criteria for this study were as follows:

- Studies on other tissues, not involving bone tissue or bone healing
- Studies that did not use our selected scaffolds and items previously explained in inclusion criteria
- Studies that were performed only in vitro

Information sources and search

An electronic search was conducted in NCBI PMC and PubMed database from January 1970 to December 2015 limited to English language publications with available full texts. Published papers on selected scaffolds were found using the following keywords: ((nano AND [hydroxyapatite OR ha]) OR ((polycaprolactone OR PCL OR PLA or polylactic acid or PLGA or poly(lactic-co-glycolic acid)) AND (“tricalcium phosphate” OR tcp or hydroxyapatite OR HA)) OR osteoporos OR tutogen OR “particulate mineralized bone” OR (dbm AND “calcium sulphate”) OR osteoset OR “natural bone mineral” OR “bio oss” OR “bio-oss” OR “tricalcium phosphate” OR tcp OR ((hydroxyapatite OR ha) AND (“tricalcium phosphate” OR tcp)) OR katsios OR “ha/tcp” OR (biphasic AND (“bone substitutes” OR scaffold)) OR “bone allograft”) AND “stem cell”(all). Figure 1 demonstrated the flow chart diagram of the present study selection according to PRISMA guidelines [31].

Study selection

Primary selection of titles and abstracts were based on the inclusion criteria. Full texts of all eligible studies were obtained and reviewed. Among different reports of one experiment, only the latest report demonstrating the most relevant information with respect to the measurements of this review was included.

Population selection

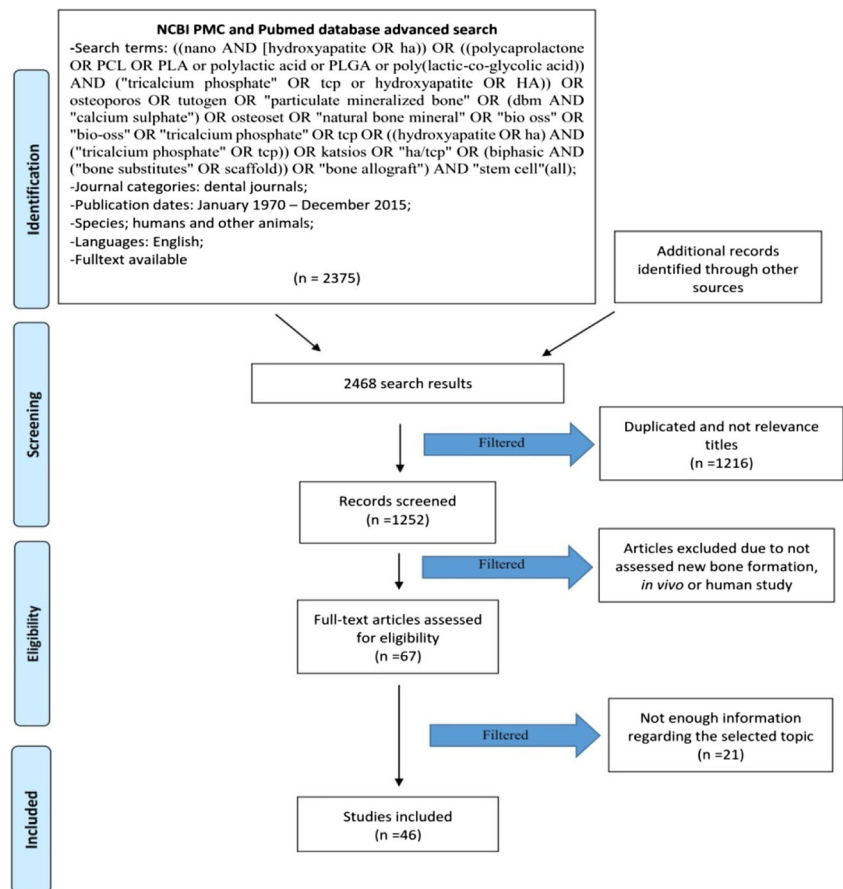
Studies of animals and human beings in which evaluated newly bone formation in specific bony defect sites.

Results

Study selection

A total of 1252 articles were reviewed. Sixty-seven articles were included as relevant for the purpose of this systematic literature review. Certain cases, showing the exclusion criteria as defined in this study, were excluded. Following the initial screening of titles and abstracts and the final screening of full texts, 46 articles completely fulfilled the inclusion criteria of this study. The qualifying data were extracted from the

Fig. 1 PRISMA flow diagram of the study



remaining cases. Since different types of study designs with a wide variation regarding the number of cases were included in this review, the results were reported based on both the number of cases and articles.

Study characteristics

Allograft

Thirty articles that have used bone allograft were evaluated, and five met the requirements for inclusion in this study [16, 28, 32–34]. Allograft was evaluated in one clinical study and two kinds of animal models, rabbit and sheep. Four of the studies used BMMSCs [16, 28, 32, 33] and only one study used stromal stem cells (SCCs) [34]. Computed tomography [35], histological evaluation (HLE), histomorphometrical analysis (HMMA), and radiographical evaluation (RGE) were used to assess the results. Follow-ups varied between 4 and 24 weeks (Table 1).

Human

Combinations of human BMMSCs and autogenous demineralized bone mineral with calcium sulfate were used

to repair alveolar cleft in two patients. After 4 months, the amount of bone regeneration was 35.4% in one patient and the second patient showed 25.6% regenerated bone, based on CT evaluation [16].

Sheep

Lucarelli et al. treated 3-cm defects in six sheep with platelet-rich plasma-based scaffold loaded with sSCCs. HMMA demonstrated 42.8 to 54.1% new bone formation (NBF) after a 4-month follow-ups [34].

Rabbit

Three studies used a rabbit model [28, 32, 33]. Autogenous BMMSCs combined with allograft were used in all of these studies. However, different methods/scoring systems were used for assessment of NBF. Lee et al. used allograft wrapped by Gelfoam containing BMMSCs for treatment of femoral defects, and they demonstrated an average Teira score of 14.4 after 12-week follow-ups in treatment groups [33]. A study by Nather et al. treating tibia bone defects, evaluated the NBF with HLE and using NBF index. They showed that the combination of allograft and autogenous BMMSCs

Table 1 Application of allografts in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Khojasteh et al. [31]	Rabbit	Allograft	rBMMSCs	–	5	Tibial bone	2 months	HMMA	MVL, 2.09 mm NBF, 28.5 to 64.5%
Lee et al. [33]	Rabbit	Allograft	rBMMSCs	–	30	1.5 cm femoral	4, 8, and 12 weeks	RGE	Taira score ^a : Allograft + gelfoam + rBMMSCs: 4th week, 2.4 ± 1.1 8th week, 5.4 ± 1.0 12th week, 14.4 ± 3.1 Allograft + PBS-soaked: 4th week, 0.8 ± 0.8 8th week, 3.2 ± 1.5 12th week, 9.2 ± 1.9
Nather et al. [32]	Rabbit	Allograft	rBMMSCs	–	40	1.5 cm Cortical bone segment of tibia	12, 16, and 24 weeks	HLE	NBF index ^b : Allograft: 12th week, 0.00 ± 0.00 16th week, 0.13 ± 0.26 24th week, 1.16 ± 0.00 Allograft + rBMMSCs: 12th week, 4.99 ± 1.02 16th week, 5.90 ± 1.39 24th week, 6.14 ± 2.07
Behnia et al. [16]	Human	DBM + calcium sulfate	hBMMSCs	–	2	Unilateral alveolar cleft	2 and 4 months	CT	NBR: Patient 1, 25.6% Patient 2, 34.5%
Lucarelli et al. [34]	Sheep	Allograft	sSSCs	Platelet-rich plasma	10	3 cm wide Metatarsal bone	1, 2, and 4 months	RGE	Platelet-rich plasma-based allograft + sSSCs: 4th month, 5/6 complete healing Allograft: 1/4 distal osteotomy line completely healed but the proximal osteotomy line remained detectable NBF: Platelet-rich plasma-based allograft + sSSCs, 42.8 to 54.1% Allograft, 26.7 to 42%

CT computed tomography, DBM demineralized bone matrix, hBMMSCs human bone marrow mesenchymal stem cells, HMMA histomorphometrical analysis, HLE histological evaluation, MVL mean amount of vertical bone length, NBF new bone formation, PBS phosphate-buffered saline, rBMMSCs rabbit bone marrow mesenchymal stem cells, RGE radiographical evaluation, sSSCs sheep stromal stem cells

^a Taira score: scoring system suggested by Taira et al. [36]

^b NBF index defined as the total area of all resorption cavities present in both cortices expressed as a percentage of the total area of both cortices

presented a significantly higher amount of NBF (NBF index 6.14 ± 2.07) compared to the control (allograft only). In HLE, they also observed a higher percentage of osteocyte formation compared to autograft bone treatment [32]. Khojasteh et al. used particulate bone allograft with fibrin glue in combination with BMMSCs for vertical augmentation of bone around dental implants. HMMA of the results showed the height of NBF was 2.09 mm, and the amount of new supracrestal trabecular bone formation was 28.5–64.5% [28].

NBBM

Thirty-two articles using NBBM were evaluated, and eight articles met the requirements for inclusion in this study [17, 18, 37–42]. NBBM (Bio-Oss) were evaluated in one clinical study and three kinds of animal models, mouse, rat, and dog. MSCs that have been used in these studies were BMMSC, periodontal ligament stem cells (PDLSCs), and dental pulp stem cells (DPSCs). The results were evaluated using HMMA and CT scan analysis. The follow-ups varied between 6 and 15 weeks (Table 2).

Mouse

Park et al. conducted an experiment to compare the regeneration potential of HA, coated with different ratios of TCP, and Bio-Oss as a carrier for human ABMSCs (hABMSCs). HMMA showed that bone regeneration was greater in the hABMSC-loaded HA/TCP groups. No significant difference was observed between HA/TCP groups [41].

Human

Rickert et al. assessed differences in NBF after maxillary sinus floor elevation using bovine bone mineral (Bio-Oss) in combination with either autogenous BMMSCs harvested from the posterior iliac crest (study group) or autogenous bone harvested from the retromolar area (control group). HMMA showed significantly higher NBF in the study group ($17.7 \pm 7.3\%$) compared to the control group ($12.0 \pm 6.6\%$) [37].

Dog

Three studies used dogs as their animal model [17, 39, 40]. In the study carried out by Jafarian et al., the amount of NBF was assessed by using two types of scaffold: biphasic bone substitutes (HA/TCP) and NBBM (Bio-Oss). A canine full-thickness alveolar defect was used as the model, and MSCs were isolated from dog BMMSC. The results indicated a higher NBF in the HA/TCP + dBMMSCs group ($65.78 \pm 4.94\%$) compared to the Bio-Oss + dBMMSCs group ($50.31 \pm 6.97\%$) [17]. In the study by Khorsand et al., the

effects of DPSCs on regeneration of an experimentally created defect in the periodontium of a canine model were investigated. After 8 weeks post-operation, the regeneration of the periodontal defects, including bone, periodontal ligament (PDL), and cement formation, were evaluated using HMMA. The amount of NBF, new cementum, and PDL formation were 3.60 ± 61.06 , 3.83 ± 61.32 , and 3.30 ± 61.12 mm, respectively, in study group (DPSCs + Bio-Oss) which were significantly higher than the regeneration level in control groups (Bio-Oss only 3.10 ± 60.82 , 2.42 ± 1.40 , and 1.77 ± 1.27 mm) [40]. Yu et al. also used Bio-Oss seeded with either PDLSCs or BMMSCs, and they have shown enhanced bone formation and mineralization with the maximum volume of the maxillary sinus augmentation in PDLSCs + Bio-Oss group with only 26.68 ± 2.23 mm³ of bone resorption compared to the BMMSCs + Bio-Oss group (27.34 ± 2.91 mm³) [42].

Rat

Khojaste et al. compared the effect of two different bone substitutes (Bio-Oss and β -TCP) on the rate of bone regeneration in combination with platelet-rich plasma (PRP) or rat BMMSCs. After 6 weeks, HMMA showed the highest bone regeneration in β -TCP + BMMSCs which was 2.53 mm and lowest bone regeneration was observed in the β -TCP + PRP group (1.21 mm) [18]. In the study by Yu et al., the osteogenic effects of PDLSCs and BMMSCs in combination with Bio-Oss scaffolds on 4-mm wide calvarial defects were compared. The results indicated more NBF in the defect treated with Bio-Oss and PDLSCs (28%) [39]. Raposo-Amaral et al. used NBBM loaded rat Orbicularis Oris Muscle MSCs to treat 5-mm alveolar defects in rats. The NBF was $38.35 \pm 19.59\%$ in 8 weeks after the surgery [38].

β -TCP

Forty articles using β -TCP were evaluated, and ten articles met the requirements for inclusion in this study [18, 43–51]. Two of them were done in a rabbit model, two in humans, one in a dog model, four in a rat, and one in a pig model. Growth factors such as bone morphogenetic protein-2 (BMP-2) and PRP were used in these studies. BMMSCs, adipose-derived stem cells (ASCs), and urine stem cells (USCs) were used as MSCs. The follow-up periods varied between 1 day and 12 months (Table 3).

Human

Two studies were conducted on humans. In a study by Thesleff et al., the results were compared using CT Hounsfield units (HUs). They evaluated the effect of ASCs loaded on β -TCP in reconstruction of large cranial defects in

Table 2 Application of natural bovine bone minerals in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Park et al. [41]	Mouse	NBBM + HA/TCP	hABMSCs	–	24	Dorsal surfaces	8 weeks	HMMMA	MTA: HA/TCP ceramics: 60% HA/40% TCP, 0.12 mm ² /slide 20% HA/80% TCP, 1.23 mm ² /slide Bio-Oss, 0.02 mm ² /slide
Yu et al. [42]	Dog	NBBM	dBMMSCs dPDLSCs	–	12	Maxillary sinus floor	12 weeks	CT HLE	CT: The mean changes in sinus volume: Bio-Oss, -34.70 ± 3.62 mm ³ Bio-Oss + dBMMSCs, -27.34 ± 2.91 mm ³ Bio-Oss + dPDLSCs, -26.68 ± 2.23 mm ³ (the minus sign indicates bone resorption)
Yu et al. [39]	Rat	NBBM	rPDLSCs rBMMSCs	–	24	4 mm Calvaria	8 weeks	μ -CT	HLE: NBF: Bio-Oss, 26.5% Bio-Oss + dBMMSCs, 28.5% Bio-Oss + dPDLSCs, 30% NBF: Bio-Oss, 22.6% Bio-Oss + rBMMSC, 26.9% Bio-Oss + rPDLSCs, 28.0% BVR: Bio-Oss, 41.6% Bio-Oss + rBMMSC, 46.9% Bio-Oss + rPDLSCs, 48.6% Trabecular thickness: Bio-Oss, 4.45 mm ⁻¹ Bio-Oss + rBMMSC, 0.145 mm Bio-Oss + rPDLSCs, 0.165 mm Trabecular number: Bio-Oss, 0.168 mm ⁻¹ Bio-Oss + rBMMSC, 4.88 mm ⁻¹ Bio-Oss + rPDLSCs, 5.13 mm ⁻¹ Trabecular separation: Bio-Oss, 0.168 mm Bio-Oss + rBMMSC, 0.158 mm Bio-Oss + rPDLSCs, 0.154 mm NBF: 38.35 \pm 19.59%
Raposo-Amaral et al. [38]	Rat	NBBM	Rat orbicularis oris muscle MSCs	–	5	5 mm Alveolar	8 weeks	HMMMA	NBF: 38.35 \pm 19.59%
Khorsand et al. [40]	Dog	NBBM	dDPSCs	–	10	BL 3 mm AC 5 mm MD 8 mm	8 weeks	HMMMA	NBF: Bio-Oss + dDPSCs, 3.60 \pm 61.06 mm Bio-Oss, 3.10 \pm 60.82 mm New cementum:

Table 2 (continued)

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Rickert et al. [37]	Human	NBBM	hBMMSCs	–	12	Maxillary sinus floor	15 weeks	HMMA	Bio-Oss + dDPSCs, 3.83 ± 61.32 mm Bio-Oss, 2.42 ± 1.40 mm PDL: Bio-Oss + dDPSCs, 3.30 ± 61.12 mm Bio-Oss, 1.77 ± 1.27 mm NBF: Bio-Oss + hBMMSCs, 17.7 ± 7% Bio-Oss + autogenous bone, 12.0 ± 6.6%
Khojasteh et al. [18]	Rat	NBBM + β-TCP	rBMMSCs	Platelet-rich plasma	22	5 mm Parietal bone	6 weeks	HMMA	NBF: Bio-Oss + platelet-rich plasma, 1.27 mm Bio-Oss + rBMMSCs, 1.44 mm β-TCP + platelet-rich plasma, 1.21 mm β-TCP + rBMMSCs, 2.53 mm
Jafarian et al. [17]	Dog	NBBM + HA/TCP	dBMMSCs	–	4	10 mm TAT Mandible	6 weeks	HMMA	NBF: HA/TCP, 44.9 ± 13.45% Bio-Oss, 36.84 ± 8.73% HA/TCP + dBMMSCs, 65.78 ± 4.94% Bio-Oss + dBMMSCs, 50.31 ± 6.97%

AC apicororally, BL buccolingually, BVR bone volume ratio, CT computed tomography, dBMMSCs dog bone marrow mesenchymal stem cells, dPDLSCs dog periodontal ligament stem cells, dDPSCs dog dental pulp stem cells, MSCs mesenchymal stem cells, HA hydroxyapatite, hBMMSCs human bone marrow mesenchymal stem cells, HLE histological evaluation, HMMA histomorphometrical analysis, MD mesiodistally, MTA mineralized tissue area, NBBM natural bovine bone mineral, NBF new bone formation, PDL periodontal ligament, rBMMSCs rat bone marrow mesenchymal stem cells, TAT through and through, TCP tricalcium phosphate

Table 3 Application of β -TCP in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Eftekhari et al. [51]	Rat	HA TCP	hBMSCs	–	27	5 × 3 × 3 mm Femur	45 days	HLE	NBF: The quantity of newly formed lamellar bone in the healing site in β -TCP group was better than onward compared to HA. BV/TV: USCs/ β -TCP, 16.3 ± 4.2% β -TCP, 8.2 ± 3.7% Empty control, 6.2 ± 2.4% BMD (mg/cm ³): USCs/ β -TCP, 953.0 ± 120.1 β -TCP, 834.5 ± 67.7 Empty control, 693.1 ± 38.6 BV/TV: β -TCP-NS, 34.29 ± 1.35% β -TCP-ADSC-NS, 49.24 ± 3.54% β -TCP-VD, 56.13 ± 2.28% β -TCP-ADSC-VD, 64.96 ± 5.17%
Guan et al. [50]	Rat	β -TCP	hUSCs	–	11	6 mm Femur	12 weeks	Micro-CT	
Feng et al. [43]	Rat	β -TCP	rADSCs	–	36	3 × 5 × 5 mm Femur	28 days	HLE	
Damlar et al. [48]	Pig	β -TCP	–	–	8	10 × 4 mm Temporal	8 weeks	HMMA	NBF: β -TCP (Cerasorb®), 42% β -TCP (Kasios®), 44% β -TCP (Poresorb®), 48% Autogenous bone graft, 65% Blood clot, 40% CBCT and HLE More new bone, bone marrow, and native cells were detected in β -TCP/CPC + rBMSCs The mean Hu ⁺ : 1–3 days, 91.5 3 months, 1025 1 year, 1031
Wang et al. [47]	Rabbit	β -TCP/CPC	rBMSCs	–	18	4 × 5 mm Femoral condylar regions	8, 16, 24 weeks	CBCT HLE	
Thesleff et al. [45]	Human	β -TCP	hASCs	–	4	78.3 × 61.0 mm cranial	1–8 days 3 months 1 year	CT	NBF: β -TCP + rBMSCs: 4th week, 0.0 ± 0.0 8th week, 2.22 ± 0.39% 12th week, 5.42 ± 0.43% 16th week, 7.68 ± 0.84% β -TCP + rBMSCs + EC-derived MSCs: 4th week, 5.02 ± 0.39% 8th week, 11.58 ± 0.67% 12th week, 17.12 ± 0.97% 16th week, 23.31 ± 1.41%
Zhou et al. [46]	Rabbit	β -TCP	rBMSCs rBMSCs + EC-derived MSCs	–	32	15 mm Ulnar	4, 8, 12, and 16 weeks	HLE	
Khojasteh et al. [18]	Rat	Bio-Oss + β -TCP	rBMSCs	Platelet-rich plasma	22	5 mm Parietal bone	6 weeks	HMMA	NBF:

Table 3 (continued)

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Li et al. [49]	Dog	β -TCP/CPC	dASCs	BMP-2	14	Ulnar	16 weeks	HMMA	Bio-Oss + platelet-rich plasma, 1.27 mm Bio-Oss + rBMMSCs, 1.44 mm β -TCP + platelet-rich plasma, 1.21 mm β -TCP + rBMMSCs, 2.53 mm BSA/TSA: β -TCP; 10.95 \pm 3.10% β -TCP + dASCs, 12.86 \pm 6.0% β -TCP + osteoinduced dASCs, 25.38 \pm 8.24% β -TCP + transduced dASCs, 39.95 \pm 8.55% RSA/TSA: β -TCP; 2.28 \pm 0.68% β -TCP + dASCs, 3.83 \pm 2.23% β -TCP + osteoinduced dASCs, 2.79 \pm 1.06% β -TCP + transduced dASCs, 0.99 \pm 0.46% MTH: increased 7.3 \pm 4.6 mm
Ueda et al. [44]	Human	β -TCP	hBMMSCs	Platelet-rich plasma	6	3 \times 5 mm Alveolar augmentation	12 M	RGE	

BMD bone mineral density, *BMP-2* bone morphogenic protein-2, *BSA* newly formed bone surface area, *BV* bone volume, *CBCT* cone beam computed tomography, *CPC* calcium phosphate cement, *CT* computed tomography, *dASCs* dog adipose-derived stem cells, *EC* endothelial cell, *hASCs* human adipose-derived mesenchymal stem cells, *hBMMSCs* human bone marrow mesenchymal stem cells, *HLE* histological evaluation, *HMMA* histomorphometrical analysis, *HU* Hounsfield unit, *hLSCs* human urine stem cells, *MSCs* mesenchymal stem cells, *MTH* mineralized tissue height, *NBF* new bone formation, *NS* normal saline, *rBMMSCs* rabbit bone marrow mesenchymal stem cells, *TCP* tricalcium phosphate, *TSA* total surface area of bone defect, *TV* tissue volume, *RGE* radiographic evaluation, *RSA*: residual TCP surface area, *VD* vitamin D

^a Hu is the numeric information in a CT image. It is related to the composition and nature of the tissue imaged used to represent the density of tissue. Air has a value of -1000, water is 0, and compact bone is considered +1000

humans. The mean HU in 1 to 3 days was 791.5, in 3 months was 1025, and in 1 year was 1031 [45]. In the second study by Uede et al., the mean increase in tissue height of 7.3 ± 4.6 mm was evident when β -TCP and hBMMSCs were used in combination with PRP for maxillary sinus floor augmentation [44].

Rabbit

Wang et al. evaluated the bone regeneration capability of alginate microencapsulated rabbit BMMSCs (rBMMSCs) with β -TCP/calcium phosphate cement (CPC). Evaluation of bone substitute degradation and NBF using cone beam computed tomography (CBCT) showed more new bone, bone marrow, and native cells in the group treated with rBMMSCs/ β -TCP/CPC than those of control group (β -TCP/CPC) [47]. In a study by Zhou et al., two types of MSCs including BMMSCs and BMMSCs + endothelial cell (EC)-derived MSCs were used on β -TCP scaffold. The amount of NBF on 1.5-cm ulnar defect was assessed. HLE after a 16-week follow-up indicated more NBF in β -TCP + rBMMSCs + EC-derived MSCs group ($23.31 \pm 1.41\%$) than β -TCP + rBMMSCs ($7.68 \pm 0.84\%$) [46].

Dog

In a study by Li et al., the combination of β -TCP with ASCs genetically modified by BMP-2 (transduced ADSCs) was used to treat a critical-sized canine ulnar bone defect. After 16 weeks, HMMA indicated that β -TCP with transduced ASCs showed a significant increase of NBF. Newly formed bone surface area/total surface area of bone defect was $39.95 \pm 8.55\%$ [49].

Rat

Three studies evaluated the NBF in rat femoral defects. In a study by Feng et al., combination of β -TCP, rat ADSCs, and vitamin D (VD) were used. The results showed a significant amount of bone volume when both cells and VD were used ($64.96 \pm 5.17\%$) [43]. Guan et al. evaluated bone regeneration potential of β -TCP loaded with USCs. Micro-CT analysis showed higher bone volume in β -TCP loaded with USCs ($16.3 \pm 4.2\%$) compared to β -TCP alone ($8.2 \pm 3.7\%$) [50]. Eftekhari et al. compared the regeneration potential of TCP and HA in combination with human BMMSCs, and they showed better bone formation in TCP group [51].

Khojasteh et al. carried out a study to compare BMMSCs and PRP loaded to Bio-Oss and β -TCP for rat calvarial bone repair. The defects were treated either with Bio-Oss plus PRP or Bio-Oss loaded with BMMSCs. The results revealed higher percentage of bone formation in the group treated with BMMSCs groups compared with the PRP group [18].

Pig

Damlar et al. compared three types of commercially viable β -TCP including Cerasorb®, Kasios®, and Poresorb®. No stem cells were used in combination with these bone substitute materials. For the control group, autogenous bone and blood clot were used. HMMA indicated that no significant difference was observed between the three types of β -TCP and the control groups [48].

Nano-HA

Thirteen studies using nano-HA were reviewed, and only five studies were relevant according to inclusion criteria of this study [9, 52–55]. Nano-HA was evaluated in two kinds of animal models, rabbit and goat. Three studies used BMMSCs and two studies used DPSCs. Three of the studies used nano-HA particles in combination with collagen in their studies [52–54]. The results were reported using HLE, RGE, and HMMA. The follow-ups varied between 4 and 12 weeks (Table 4).

Goat

Liu et al. treated 25-mm tibia defect with nanoHA/collagen/poly (L-lactic acid) (PLLA)/chitin fibers (nHACP/CF) loaded with goat BMMSCs. After 8 week follow-up, HMMA demonstrated the highest bone regeneration in treatment groups ($16.56 \pm 6.5\%$) compared to other treatments (scaffold, autogenous bone, and control without any treatment) [54].

Rabbit

Four studies used a rabbit model. Three studies used HMMA and one study used synchrotron radiography (SRGE) for result analysis. A study by Liu et al. indicated that the combination of nano-HA + PLA with DPSCs and rhBMP-2 result in bone mineral apposition (BMA) of $1.77 \pm 0.11\%$, whereas in the group without rhBMP-2, BMA was $2.52 \pm 0.33\%$ [53]. In another study that evaluated NBF using SRGE after 6, 8, and 12 weeks, the imatology grading rates of nano-HA group were 4.2, 6.6, and 9.6, respectively, which was significantly higher than rates in control groups [52]. Behnia et al. studied the effect nanocrystalline HA silica gel matrix in combination with BMMSCs and PRGF on bone regeneration of parietal bone defects. HMMA after 12 months showed that the amount of NBF was significantly higher in treatment groups with both autogenous BMMSCs and PRGF [9]. Ling et al. suggested higher bone formation in DPSCs loaded on β -TCP group ($15 \pm 1.7\%$) compared to DPSCs loaded on nHAC/PLA group ($11.15 \pm 1\%$) [55].

Table 4 Application of nano-HA in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Ling E et al. [55]	Rabbit	nHAC + PLA	rDPSCs	–	10	10 × 4 × 3 mm Alveolar bone	12 weeks	HMMMA	MBF: DPSCs + b-TCP, 15 ± 1.7% DPSCs + nHAC/PLA, 11.15 ± 1%
Behnia et al. [9]	Rabbit	ncHASGM	rBMMSCs	PRGF	8	8 mm Parietal	6 and 12 weeks	HMMMA	NBF: ncHASGM: 6th week, 11.35% 12th week, 32.53% ncHASGM + autologous PRGF: 6th week, 29.10% 12th week, 39.74% ncHASGM + rBMMSCs: 6th week, 25.82% 12th week, 39.11% ncHASGM + rBMMSCs + autologous PRGF: 6th week, 29.45% 12th week, 44.55%
Sun et al. [52]	Rabbit	nHAC	rBMMSCs	–	45	5 mm Femur	4, 8, and 12 weeks	SRGE ^a	NBF(IG): nHAC + rBMMSCs: 4th week, 6.5 ± 0.707 8th week, 10.2 ± 0.422 12th week, 11.9 ± 0.316 nHAC: 4th week, 4.3 ± 0.949 8th week, 7.4 ± 0.516 12th week, 11.0 ± 0.667 Negative control: 4th week, 0.1 ± .316 8th week, 0.8 ± 1.033 12th week, 1.4 ± 1.265
Liu et al. [53]	Rabbit	nHAC + PLA	rDPSCs	rhBMP-2	36	10 × 4 × 3 mm Alveolar bone	12 week	HMMMA	BMA: nHAC + PLA + rDPSCs, 2.52 ± 0.33% nHAC + PLA + rDPSCs + rhBMP-2, 1.77 ± 0.11%
Liu et al. [54]	Goat	nHACP + CF	gBMMSCs	–	32	25 mm Tibia	4 and 8 weeks	HLE RGE	HLE NBF: Negative control: 8th week, 0.46 ± 0.3% Autograft bone: 8th week, 6.96 ± 3.3% nHACP + CF:

Table 4 (continued)

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
									8th week, $5.16 \pm 3.2\%$ nHACP + CF + gBMMSCs: 8th week, $16.56 \pm 6.5\%$ RGE Negative control: 4th week, 0/4 D 8th week, 0/4 D nHACP + CF + gBMMSCs: 4th week, 0/4 D 8th week, 4/4 D Autograft bone: 4th week, 0/4 D 8th week, 4/4 D nHACP + CF: 4th week, 0/4 D 8th week, 4/4 D

BMA bone mineral apposition, *CF* chitin fibers, *D* density, *gBMMSCs* goat bone mesenchymal stem cells, *HMMMA* histomorphometrical analysis, *HLE* histological evaluation, *IG* imagology grading, *MBF* mature bone formation, *nHA* nanocrystalline hydroxyapatite, *nHAC* nanohydroxyapatite collagen, *nHACP* nanohydroxyapatite collagen poly(L-lactide), *ncHASGM* nanocrystalline hydroxyapatite silica gel matrix, *NBF* new bone formation, *PRGF* platelet-rich growth factor, *PLA* poly(L-lactide), *rBMMSCs* rabbit bone mesenchymal stem cells, *rDPSCs* rabbit dental pulp stem cells, *RGE* radiographic evaluation, *rhBMP-2* recombinant human bone morphogenetic protein-2, *SRGE* synchrotron radiographic evaluation

^a SRGE: synchrotron radiation light is the fourth artificial light source, which previously include electric light, X-ray, and laser. It can record different structural information in weakly absorptive substances and clearly displays X-ray two-dimensional photography or three-dimensional computed tomography, which consists of the unrecorded information by the traditional absorption image

HA/TCP

Among 31 articles using HA/TCP scaffold, seven studies were included according to our inclusion criteria [17, 19, 20, 35, 56–58]. Two of the studies compared NBF of HA/TCP with Bio-Oss. The studies were conducted on human and dog models. All of the studies used BMMSCs, and only in one study were BMMSCs and PDLSCs used [57]. The results were reported using HEL, HMMA, RGE, and CBCT. The follow-ups varied between 1 and 12 months (Table 5).

Human

In these studies, HA/TCP loaded with BMMSCs were used. One of the studies conducted the bone augmentation on the maxillary sinus floor, and the other study was performed in the anterior cleft of the maxilla. PRGF was used as growth factor in the second study. The control group was not reported in following studies, and results were based on bone formation mean. Combination of HA/TCP, BMMSCs, and PRGF showed 51.3% mean bone formation after 3-month follow-ups. Interestingly, the combination of HA/TCP and BMMSCs showed similar bone formation after 12 months 41.34% [19, 20].

Dog

Five studies used a dog model. Three studies were conducted on mandibular defects and two were performed on femur defects. Studies carried out by Jafarian et al. and Vahabi et al. were the same in terms of defect size, cells, scaffold, and follow-up periods. In both studies after 6-month follow-ups with HMMA, the highest bone formations were observed in the groups treated with bone formation HA/TCP + BMMSCs [17, 56]. A study by Kim et al. showed enhanced bone formation in the treatment with BMMSCs compared to PDLSCs [57]. In two studies on femur bone defects, BMMSCs using the same follow-up period were conducted. In both studies, similar amounts of NBF were reported using both histological and radiological analysis [35, 58].

Composite

Among 22 articles that used composite scaffolds, ten studies were included according to our inclusion criteria [27, 28, 59–66]. Composite was evaluated in five types of animal models: dogs, pigs, rabbits, rats, and mice. Different composite scaffolds including PLGA/CPC, PEG/PCL/HA, PLGA/ β -TCP/COL I, PCL/TCP, and PLGA/HA were evaluated. Eight of the studies used autogenous BMMSCs and ASCs. Two other studies used human BMMSCs and ASCs in animal models [62, 63]. The results were mostly reported by using

HMMA. In three studies, μ -CT were used. The follow-ups varied between 2 and 36 weeks (Table 6).

Dog

Two studies used dogs as their animal model. Kim et al. investigated the combination of PCL/TCP, auto-fibrin glue (aFG), rhBMP-2, PCL membrane, and BMMSCs for bone regeneration. The results showed the highest NBF in the treatment group [66]. Khojasteh et al. used the combination of TCP/PCL and BMMSCs in the dog mandible. NBF was approximately 48%, and the amounts of remaining scaffold were minor in the cell-seeded group after 2 months [28].

Pig

Liao et al. treated temporal bone defects in two pigs with PEG/PCL/HA scaffold containing BMMSCs. HMMA after 6 months demonstrated a higher NBF (64%) in the PEG/PCL/HA + BMMSCs group than the PEG/PCL + BMMSCs group (22%) [60]. Konopnicki et al. showed higher bone surface area in PCL/TCP + pBMMSCs ($22.11 \pm 22.45\%$) than PCL/TCP ($1.87 \pm 3.66\%$) [64].

Rabbit

Four studies used rabbit as their animal model. Two of the studies used the same composite scaffold PLGA/ β -TCP/COL I and the same experimental defect, 15 mm on radial bone. Two of them used autogenous BMMSCs, and the other used autogenous ASCs. In the study by Pang et al., using BMMSCs, HLE showed $97.27 \pm 2.65\%$ bone formation after 36-week follow-ups [61]. The results of the other study by Hao et al., using ASCs, showed similar bone formation ($96.4 \pm 2.3\%$) after 24-week follow-ups [27]. He et al. used PRP in combination with PLGA/CPC and BMMSCs. Application of PRP indicated better bone formation 12 weeks after operation [59].

Rat

Rai et al. treated femur defects in rats with PCL/TCP scaffold containing BMMSCs. μ -CT analysis demonstrated more new bone volume ($1.0 \pm 0.7 \text{ mm}^3$) in BMMSC-seeded PCL/TCP group compared to treatment of the defect with scaffold only [63].

Mice

James et al. treated calvarial defects in mice with PLGA/HA scaffold containing human ASCs. In this study, the effect of adding fresh stem cells and frozen stem cells to scaffolds were

Table 5 Application of HA/TCP in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample size	Defect size and location	Follow-up	Tests	Results
Vahabi et al. [56]	Dog	HA/TCP Bio-Oss	dBMMSCs	–	5	10 × 10 × 10 mm Mandible	6 weeks	HMMA	MBF: HA/TCP + dBMMSCs, 47.5 ± 20.5% Bio-Oss, 31.3 ± 14.6% HA/TCP, 31.3 ± 13.6% Negative control, 36.7 ± 15.1% MWB: HA/TCP + dBMMSCs, 46.38 ± 13.49% Bio-Oss, 40.60 ± 9.15% HA/TCP, 44.93 ± 8.81% Negative control, 60.80 ± 9.07% MBF: 51.3% (mean) HLE and HMMA NBF: HA/TCP + dBMMSCs: 8th week, 34.99% 16th week, 40.17% HA/TCP + dPDLSCs: 8th week, 31.90% 16th week, 36.51% HMMA MBF: 12th month, 41.34%. RGE MT: 12th month, 8.58 mm HMMA NBF: HA/TCP, 44.9 ± 13.45% HA/TCP + dBMMSCs, 65.78 ± 4.94% Bio-Oss, 36.84 ± 8.73% Bio-Oss + dBMMSCs, 50.31 ± 6.97% HLE MBF: 16th week, 49.0 ± 12.3% RGE MT: 16th week, 0.75 ± 0.52 mm HLE and HMMA NBF: 16th week, 39.9% RGE MT: 16th week, 1.5 mm
Behnia et al. [20] Kim et al. [57]	Human Dog	HA/TCP HA/TCP	hBMMSCs dBMMSCs dPDLSCs	PDGF –	4 4	Anterior maxillary cleft 5 × 10 mm Mandible	3 months 8 and 16 weeks	CBCT HLE HMMA	
Shayesteh et al. [19]	Human	HA/TCP	hBMMSCs	–	7	Maxillary sinus floor	3 and 12 months	HMMA RGE	
Jafarian et al. [17]	Dog	HA/TCP Bio-Oss	dBMMSCs	–	4	10 mm TAT Mandible	6 weeks	HMMA	
Arinze et al. [58]	Dog	HA/TCP	dBMMSCs	–	16	21 mm Femur	4, 8, and 16 weeks	HLE RGE	
Bruder et al. [35]	Dog	HA/TCP	dBMMSCs	–	15	21 mm Femur	4, 8, 12, and 16 weeks	HLE HMMA RGE	

CBCT cone beam computed tomography, dBMMSCs dog bone marrow mesenchymal stem cells, dPDLSCs dog periodontal ligament stem cells, HA hydroxyapatite, hBMMSCs human bone marrow mesenchymal stem cells, HLE histological evaluation, HMMA histomorphometrical analysis, MBF mean percentage of bone fill, MWB mean woven bone, NBF new bone formation, PDGF platelet-rich growth factor, rhBMP-2 recombinant human bone morphogenetic protein-2, RGE radiographic evaluation, TAT through and through, TCP tricalcium phosphate

Table 6 Application of composite scaffolds in combination with stem cells

Author(s) and year	Study model	Scaffold	Stem cell	Growth factor	Sample Size	Defect size and location	Follow-up	Tests	Results
He et al. [59]	Rabbit	PLGA/CPC	rBMMSCs	PRP	8	6 × 10 mm Femoral	3, 6, and 12 weeks	μ-CT	NBF: 12th week, more bone growth in PLGA/CPC + rBMMSCs + PRP
Konopnicki et al. [64]	Pig	PCL/TCP	pBMMSCs	–	2	20 × 20 mm Mandible	8 weeks	HMMA	BPSA: PCL/TCP + pBMMSCs, 22.11 ± 22.45% PCL/TCP, 1.87 ± 3.66% Negative control, 35.05 ± 17.62%
Proseck et al. [65]	Rabbit	PCL/HA	rBMMSCs	TRS	30	6 × 10 mm	12 weeks	HMMA	NBF: The highest volume and most uniform distribution of newly formed bone occurred in defects treated with scaffolds enriched with both BMMSCs and TRS
Liao et al. [60]	Pig	PEG/PCL/HA	pBMMSCs	–	2	20 × 20 mm Temporal bone	6 months	HLE	HU ^a : PEG/PCL/HA + pBMMSCs, 64% PEG/PCL + pBMMSCs, 22%
Pang et al. [61]	Rabbit	PLGA/β-TCP/COL I	rBMMSCs	–	46	15 mm Radial	12, 24, 36 weeks	HLE	NBF: PLGA/β-TCP/COL I + rBMMSCs 12th week, 48.56 ± 3.12% 24th week, 78.34 ± 2.85% 36th week, 97.27 ± 2.65%
Khojasteh et al. [28]	Dog	PCL/TCP	dBMMSCs	–	4	20 × 10 × 10 mm Mandible	2 months	HMMA	NBF: PCL/TCP + dBMMSCs, 48.63 ± 4.66% PCL/TCP, 63.29 ± 17.27%
James et al. [62]	Mice	PLGA/HA	hASCs	–	5	4 mm Calvaria	4 weeks	μ-CT	NBF: PLGA/HA, 25% PLGA/HA + fresh hASCs, 80% PLGA/HA + frozen hASCs, 22% Negative control, 5%
Rai et al. [63]	Rat	PCL/TCP	hBMMSCs	–	6	8 mm Femur	3 weeks	μ-CT	BV: PCL/TCP + hBMMSCs, 1.0 ± 0.7 mm ³ PCL/TCP, 0.34 ± 0.1 mm ³
Kim et al. [66]	Dog	PCL/TCP	dBMMSCs	rhBMP-2	2	5 × 5 × 8 mm Scapula	2 and 4 weeks	HLE	4th week, the highest bone formation in PCL/TCP + aFG + dBMMSCs + rhBMP-2
Hao et al. [27]	Rabbit	PLGA/β-TCP/COL I	rASCs	–	12	15 mm Radial	8, 16, and 24 weeks	HLE	NBF: COL/PLGA/β-TCP + rASCs: 8th week, 44.2 ± 3.9% 16th week, 75.3 ± 2.6% 24th week, 96.4 ± 2.3%

aFG auto fibrin glue, BF bone formation, β-TCP tricalcium phosphate, BPSA bone percentage surface area, BV bone volume, COL I collagen I, CPC calcium phosphate cement, dBMMSCs dog bone marrow mesenchymal stem cells, HA hydroxyapatite, hASCs human adipose-derived stem cells, hBMMSCs human bone marrow mesenchymal stem cells, HLE histological evaluation, HMMA histomorphometrical analysis, HU Hounsfield unit, NBF new bone formation, μ-CT micro-computerized tomography, PRP platelet-rich plasma, PCL poly ε-caprolactone, pBMMSCs porcine bone marrow mesenchymal stem cells, PEG poly(ethylene glycol), PLGA poly(lactic-co-glycolic acid), rBMMSCs rabbit bone marrow mesenchymal stem cells, rASCs rat adipose-derived stem cells, rhBMP-2 recombinant human bone morphogenetic protein-2, TRS thrombocyte-rich solution

^a Hu is the numeric information in a CT image. It is related to the composition and nature of the tissue imaged used to represent the density of tissue. Air has a value of -1000, water is 0, and compact bone is considered +1000

compared. μ -CT results indicated the highest NBF in the fresh ASCs group (80%) [62].

Discussion

Bone tissue engineering mainly includes the triad of an artificial extracellular matrix/scaffold, stem cells, and growth factors [1, 2]. The efficacy of this combination has been studied in various animal and human studies. A perfect scaffold for bone tissue engineering should have the following features: (1) biocompatibility; (2) convenience for application in defect site, osteoconductivity, and osteoinductivity; (3) biodegradation rate close to natural bone formation rate; (4) structure with proper porosities providing a dynamic extracellular matrix for cell proliferation and differentiation; (5) mechanical properties near to natural bone; and (6) proper structure for vascularization [13]. However, an ideal scaffold having all of these features has not been found for bone tissue engineering. Several studies have attempted to select the most qualified source of stem cells for bone tissue engineering [39, 42, 46, 57]. Expression of special surface markers and capability of differentiation into different cell lineages are the main criteria for detecting stem cells [67, 68]. However, these criteria might not guarantee the efficacy of cells for tissue engineering [47]. Finally, application of exogenous growth factors and osteoinductive cytokines, as the third side of bone tissue engineering, have been shown as important factors in promoting cell adhesion, proliferation, and differentiation in many studies [18, 34, 40, 49].

Combinations of various scaffolds, stem cells, and exogenous factors have been used in tissue engineering, causing much confusion. The current systematic review aimed to help reconstructive surgeons to find proper combination which are more efficient for bone regeneration. In this study, the most commonly used scaffolds including allograft, NBBM (Bio-Oss), β -TCP, nano-HA, HA/TCP, and composite scaffolds were assessed. Scaffolds were categorized according to the types of scaffolds and animal models. In each study, the application of growth factor, types of stem cells, sample size, follow-up periods, and assessment test also were reported. Although not all the criteria assessed in these studies were the same, they all indicated higher bone formation at follow-up sessions. Several major technical advances have been achieved in the field of bone tissue engineering during the past decade. Particularly, the increased understanding of bone healing at the cellular and molecular levels allowed the conduction of numerous animal and pilot clinical studies using tissue-engineered constructs for local regeneration.

Scaffolds In general, most of the papers included in this study, according to inclusion criteria, were categorized as composite scaffolds.

Bone allografts are commonly used for reconstruction and repair of skeletal defects due to their appropriate mechanical strength and osteoconductive properties [69]. Although allografts contain protein factors that enhance bone formation, failures due to fracture and infection have been reported in many cases [70–72]. Allograft has been extensively used in combination with BMMSCs [16, 28, 32–34]. Several studies attempted to enhance bone regeneration capability of allograft-BMMSC constructs by addition of various factors. Studies by Khojasteh et al. and Lucarelli et al. demonstrated that the addition of PRP [34] and fibrin glue [28] to allograft bone can increase the amount of bone formation in animal models. Similarly, a study by Lee et al. showed that the amounts of NBF were greater when Gelfoam was added to cell-scaffold constructs [33].

NBBM (Bio-Oss) has been well-established in reconstructive surgeries due to its biocompatibility and osteoconductive properties [73]. Various MSC types have been reported to be used in combination with Bio-Oss. In rat models, the combination of MSCs and NBBM indicated more NBF in alveolar defects compared to calvarial defects [38, 39]. However, in those studies, different types of MSCs were used. Also, different methods were used for assessment of NBF. Khojasteh et al. demonstrated that application of PRP instead of rat BMMSCs can decrease the amount of bone formation [18].

β -TCP is a bioactive bone substitute ceramic which has high biocompatibility and better stability than polymeric bone substitutes [74]. Most of the studies used β -TCP scaffolds alone or in combination with other scaffolds. Since the measurement techniques and follow-up periods to assess NBF were different, it is hard to decide which combinations would be the most suitable. In studies by Hao et al. and Pang et al., β -TCP was used in combination with PLGA (PLGA 70%/ β -TCP 30%). The amounts of NBF according to HLE, after 24-month follow-ups, were nearly the same, although different types of stem cells had been used in these studies [27, 61]. Similar amounts of NBF were observed in the studies by Vahabi et al., Jafarian et al., and Kim et al. after 6, 8, and 16 weeks [17, 56, 57]. In these studies, dogs were used as their animal model and HA/TCP loaded with autogenous BMMSCs was used for bone regeneration. Kim et al. also compared BMMSCs with PDLSCs, and they observed higher bone regeneration with BMMSCs. In the studies by Vahabi et al. and Jafarian et al., the bone regeneration capability of HA/TCP were compared with Bio-Oss; they reported better results in HA/TCP loaded with cells. The study by Vahabi et al. was the only study that included negative control in their study design. Although the NBF was highest in HA/TCP + BMMSCs, they observed significantly higher NBF compared to HA/TCP or Bio-Oss-alone groups. In addition, they showed that mean woven bone was significantly higher in the “empty cavity” group (60.80%) than other groups (40.60–46.38%) [56]. Moreover, Eftekhari et al. compared

the bone regeneration potential of TCP and HA. The result indicated that combination of hBMMCs and β -TCP for treatment of rat femoral defects showed increased quantity of newly formed lamellar bone compared to HA with hBMMCs in 45 days [51].

The structure of scaffold materials in nanoscale to micro-scale can resemble the natural structure of cancellous bone [75]. Nanocrystalline HAs were incorporated with silica gel matrix, collagen, chitin fibers, and poly (L-lactide) [9, 52–54]. Behnia et al. performed a study in a rabbit model using a combination of nano-HA, silica gel matrix, autologous PRGF, and BMMSCs. They showed that nano-HA and autologous PRGF group had more bone formation than nano-HA and autogenous BMMSCs. Moreover, application of both autogenous PRGF and BMMSCs showed the best results among the experimental groups. However, Ling et al. indicated significantly higher percentage of mature bone formation area in rat DPSCs loaded on β -TCP compared with nHAC + PLA [55]. Liu et al. indicated that application of hBMP-2 could significantly increase protein content, alkaline phosphatase activity and NBF in a rabbit model [53].

Collagen was the most common natural polymer used in combination with composite and nano-HA scaffolds [53–55]. Composition of a biodegradable material, integrating bioactive particles, might improve structural and biological properties of the scaffolds [76–79]. However, there are controversies within the literature regarding advantages and disadvantages of incorporation of collagen in composite scaffolds. Many studies showed better compressive modulus, strength, proliferation, and expression of osteoconductive factors [27, 60, 80]. Others demonstrated that its incorporation in the ceramic phase might cause inadequate mechanical properties [81, 82].

Osteoprogenitor/stem cells Osteoprogenitor/stem cells are one of the most important parts in bone tissue engineering, and their capabilities in proliferation and osteogenic differentiation have been proven in many studies. In this review, it is also confirmed that the addition of MSCs to different types of scaffolds enhances NBF [16, 28, 34, 45, 52], although in some studies, no statistically significant difference was seen in the amount of NBF while using MSCs [9, 49, 52]. Using enzymatic digestion, MSCs can be isolated from buccal fat pad [83], muscles [84], embryonic tissues [85], and dental tissues [14, 68, 85, 86]. Although in this review the majority of papers used BMMSCs, much evidence indicated other source of MSCs with the same or greater results in NBF. BMMSCs are derived from bone marrow aspirates and are defined as clonogenic cells with the capability of easily culturing and multilineage differentiation [35].

In two different studies on rat, the results demonstrated improvement of bone formation after 28 days and also 12 weeks with different analysis (histological and radiographic evaluation) due to addition of stem cells [43, 50]. In one of

them, adipose-derived stem cells were used and in another one human urine stem cells. As a matter of fact, Guan et al. also present a novel idea and suggested that human urine stem cells can be act as a reliable cell source for bone tissue engineering [50].

In the study carried out by Thesleft et al., the combination of β -TCP and ASCs were used in patients following cranioplasty. One-year follow-ups, using CT analysis, revealed successful bone regeneration. Also, satisfactory results were reached in cranium contour regeneration and healing of the wound [45]. Likewise, in three other studies, the application of human BMMSCs in treatment of maxillary sinus floors and clefts indicated acceptable results in bone regeneration [19, 20, 37]. Lucarelli et al. reported higher rates of NBF and new vascularization using the combination of SSCs and PRP with allograft [34]. In the study by Kim et al., two types of stem cells, dog BMMSCs and dog PDLSCs, were used separately with HA/TCP scaffolds. Eight- and 16-week follow-ups showed better outcomes in the dog BMMSCs group although the differences between the two groups were not outstanding [57]. Unlike the previous study, Yu et al. indicates higher bone formation in treatment with Bio-Oss and autogenous PDLSCs compared to Bio-Oss and autogenous BMMSCs in rat and dog models [39, 42]. In studies by Arinze et al. and Bruder et al., dog femur defects were treated with HA/TCP loaded with dog allogenic BMMSCs. Although they used allogenic BMMSCs, without any immunosuppressive therapy, no adverse immune reaction was seen [35, 58].

Growth factors A wide range of exogenous growth factors are currently being used in bone tissue engineering: transforming growth factor beta (TGF- β 1), fibroblast growth factor (FGF), insulin growth factor (IGF), vascular endothelial growth factor (VEGF), PDGF, and bone morphogenic proteins (BMPs) [19, 54]. Moreover, rhBMP-2 (INFUSE®, Medtronic, Minneapolis, MN) for alveolar ridge augmentation and sinus lift surgeries and rhBMP-7 (OP-1™, Stryker Biotech, Hopkinton, MA) have received Food and Drug Administration (FDA) approval for long bone nonunion and spinal fusion [87]. BMP-2 is one of the most frequently used growth factor within the studies. PRP and PDGF are two of the most commonly used growth factors in this review. However, due to their differences, a reasonable comparison was not possible. An autologous source of growth factors would be ideal due to low risk of infection transmission and immunological responses. In fact, PRP act as this autologous source and contains not only various growth factors such as TGF- β 1, FGF, VEGF, and PDGF, but also adhesion molecules like fibrin, fibronectin, and vitronectin [88–90]. Application of PRP is well established in the treatment of skeletal defects [89, 90]. In this review, three articles also confirmed the beneficial effect of PRP in bone regeneration [34, 44]. PDGF has different types of activity that seem to be beneficial in bone

regeneration. PDGF has been shown to have a chemotaxis effect for MSCs. Also, it promotes IGF-1 signaling and VEGF and BMP antagonist expressions [91]. The application of PDGF in the combination of HA/TCP and human BMMSCs in the anterior maxillary cleft among humans showed increased bone formation [20]. In 2013, Behnia et al. showed more NBF (up to 18%) when PRGF was added to nanocrystalline hydroxyapatite silica gel matrix (nHASGM) loaded with rabbit BMMSC [9].

Another biologic adhesive molecule, widely used in reconstructive surgeries, is fibrin glue. Fibrin glues activate fibrinogen and imitate the last part of coagulation, resulting in formation of a fibrin clot with adhesive properties. This element enhances initial stability of scaffolds [16, 17]; it promotes angiogenesis, cell attachment, and proliferation [18].

Almost all studies indicated higher bone formation when growth factor was added, except the study carried out by Liu et al., in which the addition of rhBMP2 to nHA/PLA scaffold had adverse effects on bone regeneration in alveolar defects [53].

Animal models Animal models within in vivo studies make transitional research between in vitro and clinical trials [92]. Animal models including dogs, rabbits, mice, sheep, pigs, and rats have been frequently used to assess bone regeneration. Each animal model has its own advantages over others, and selection of the best animal model for a specific research study depends on several factors. These factors include cost, availability, life span, animal size, and similarities in physiological and pathological conditions to human beings [93–96]. Dogs, sheep, and pigs have more similarities to human [97]. In this review study, the most common animal model used was the dog. Rabbits, rats, pigs, sheep, and goats were also used. Studies using the same animal model with the same assessed criteria were in agreement and accordance in results. Other studies, differing in their animal models, had no significant differences in NBF. However, factors such as type and size of the defects have great impact on bone regeneration. In this review, defects were placed in various anatomical sites: temporal, parietal, mandibular, maxillary, radial, ulnar, metatarsal, scapula, femoral, and tibial bone. Among them, femoral and mandibular defects were most frequently seen as defect sites.

Human In general, six studies were done on humans. All the investigations were conducted in the oral cavity except one study that was performed on cranial defects [45]. All studies used BMMSCs, except the study by Thesleft et al., which applied ASCs for the treatment of cranial defects [45].

Limitations The following variations were observed between included studies which resulted in heterogeneity in reported data and made a reasonable comparison between studies difficult: difference in cell source, scaffold type, animal model,

and evaluation method and sacrifice time. Also, several studies did not have the control groups which made in feasible to draw a conclusion. Moreover, not all the studies included growth factors, and some of them which utilized growth factors did not determine dose-response curve.

Conclusion

Tissue engineering is a fast-growing area. Various treatment strategies are already being used clinically. The current review aimed to inform reconstructive surgeons of how combinations of various MSCs, scaffolds, and growth factors enhance bone regeneration. The great success has been achieved using HA/TCP, β -TCP, NBBM, DBBM, scaffolds, nano-HA, and allografts scaffolds in combination with various MSCs and growth factors. The highest bone regeneration has been achieved when combination of all three elements, given scaffolds, MSCs, and growth factors, were used. Among studies being reported in this review, BMMSCs are the most studied MSCs, B-TCP is the most frequently used scaffold, and PRP is the most commonly used growth factor.

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