Bone Tissue Engineering: A Literature Review

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Submitted: 2016-03-13; **Accepted:** 2016-04-25

Introduction: Classic bone tissue engineering involves use of osteogenic cells, growth factors, and bone scaffolds to generate a graft material to replace the gold standard which is autogenous bone graft. Several modifications have been applied to the classic approach but none of them can fully regenerate bone defects. The current study reviewes the literatures in applications of bone tissue engineering both *in vivo* and *in vitro*. **Materials and Methods:** An electronic search in MEDLINE was conducted and both *in vivo* and *in vitro* studies were included using bone scaffolds with or without osteogenic growth factors or stem cells. *In vitro* studies which did not investigate cell-scaffold interactions and *in vivo* studies which did not measure new bone formation were excluded. **Results:** Of 86 studies, 38 concerned *in vitro* and 48 *in vivo* studies. These studies were divided into six groups based on scaffold which they used: Synthetic, natural, polymers (non-ceramics), composites (polymer+ceramic), metal-based and nano-scaffolds. The results of the studies were compared in a qualitative manner. *In vitro* studies were mostly conducted on polymers, while relatively more animal and clinical studies were performed on ceramics. The most commonly used scaffolds, stem cells and growth factor were synthetic ceramics, bone marrow stem cells and bone morphgenic protein 2, respectively. **Conclusion:** Determination of the most successful approach was not possible due to the presence of several variable and variances in analyzing methods and data report. However, studies which used all three components of BTE, including scaffolds, growth factors and stem cells, showed good results both *in vitro* and *in vivo*.

Keywords: Tissue engineering; Bone; Stem cell; Growth factor; Scaffold

Introduction

Tissue engineering is a biological cell-based treatment approach and classically involves regenerative cells, inducing factors and scaffolds. It is a relatively new therapy developed to overcome conventional reparative treatments. In tissue engineering, the graft material would be generated outside the body and the limitations of autografts as well as donor site complications would be eliminated (1, 2).

In orthopedics and craniomaxillofacial surgeries, tissue engineering has been applied to reconstruct bone structures and repair bone defects. In bone tissue engineering (BTE) or bone engineering, osteogenesis is initiated by osteoblasts, which are mostly differentiated forms of stem cells provided by graft material (3). After the initiation of osteogenesis, local and distant stem cells and also osteoblasts would be attracted to the field and participate in bone regeneration. The application of different types of cells, such as embryonic stem cells, fetal cells, placenta and amniotic fluid cells, umbilical cord cells, bone marrow haematopoietic stem cells, mesenchymal stem cells, adipose tissue- derived cells and dental-derived tissue stem cells have been investigated for bone tissue engineering (4-7). However, Bone marrow stem cells (BMSCs) have been used more frequently (8).

The presence of growth factors such as bone morphogenetic proteins (BMPs) help to differentiate local or grafted stem cells and they can increase the rate of bone regeneration (9). BTE usually needs growth factors and cytokines to repeat the natural process of bone formation which includes cell colonization, proliferation, differentiation and extracellular matrix (ECM) deposition (10, 11). Several factors exist in natural bone healing and remodeling process. However, providing all these factors for tissue engineering seems neither possible nor necessary. It has been demonstrated that some of the most important factors such as BMP2 could facilitate the bone regeneration process (10). BMPs can regulate many steps in bone morphogenesis (12).

Scaffolds used in bone engineering have an extensive variety. The primary goal of using scaffolds is to transfer stem cells and growth factors to the site of defects (13, 14). This regeneration is influenced by its physical and chemical properties (15, 16). In addition, they assist proliferation, differentiation and biosynthesis of cells similar to the natural extracellular matrix (17). An ideal

Table 1. *In vitro* studies using synthetic ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
Chai *et al.*(18)	CaP-based carriers	hPDSCs	-	-Upregulation of OCN, OPN, BMP2, and Runx2 gene expression of hPDSCs treated with Ca^{2+} or Pi. -At 14 days, mineralization was observed in osteogenic medium -At 21 days, Calcium mineral deposition after cell treatment with Ca^{2+}, Pi, and combined CaP.
Hinze *et al.* (19)	1. Non-sintered, nanocrystalline, phase-pure HA 2. DBX®	Human osteoblast-like cells	-	-Formation of a dense network of multilayered polygonal-shaped cells with processes allowing cell–cell communication on DBX® after 3 weeks. - 10 ng/ml OCN -The ALP staining of these cells showed intensive staining (about 37%). -COLA1 was present in about 3% of the cells. -On HA, the cells showed no tendency to proliferate or migrate.
Zhao *et al.* (20)	CaP constructs (reinforced with chitosan and fibers)	hUCMSCs	-	-Successful synthesis of bone minerals. -The percentage of mineral area: about 3% at day-7, to 12% at day-21. -ALP and OC on the CPC-fiber scaffold was 2-fold those on CPC control without fibers. -This scaffold had mechanical strength matching that of cancellous bone.
Xu *et al.* (21)	CPC scaffolds (consisted of TCP* and dicalcium phosphate-anhydrous with a molar ratio of 1:1)	hBMSCs hUCMSCs	-	-Stem-cell-encapsulating CPC construct with chitosan and fiber reinforcement reached the strength of cancellous bone, which was much stronger than injectable carriers for cell delivery including CaP and Bioglass. - hUCMSCs synthesized nearly three-fold more bone minerals than the hBMSCs in vitro.
Bernhardt *et al.* (22)	Alginate–gelatine–HA scaffolds	hBMSCs	-	-A 10–14-fold increase of cell number after 2 weeks. -The specific ALP activity significant rise from day 1 to day 7.
Cai *et al.* (23)	β-TCP reinforced with 45P2O5–22CaO–25Na2O–8MgO bioglass	Mouse osteoblast cell lines (MC3T3-E1 cells)	-	-Formation of apatite agglomerates -Ca/P molar ratio is 1.42. -Better adhesion and proliferation of cells on porous β-TCP/BG scaffolds than porous β-TCP scaffolds.
Sader *et al* (24)	β-TCMP and β-TCP	Human osteoblast cells (SaOs2)	-	-Osteoblasts adhered and spread on both samples after 7 days -Higher cell proliferation on β-TCMP compared to β-TCP.
Alexander *et al.* (25)	1. OPLA Scaffolds 2.collagen composite scaffolds (mixture of type I and III collagens) 3.CaP scaffolds (5 mm × 4.5 mm)	human JPC	BMP2	-Denser colonization of JPC-seeded OPLA scaffolds and formation of bone nodules after initiation of osteogenesis. - JPCs growing within OPLA scaffolds are able to form CaP particles.
Wagner *et al.* (26)	1. Native and plasma-coated PLGA-scaffolds) 2.agarmatrix with HA (coated with 0.4% hyaluronic acid and native) 3.Tissue Foil E, as reference matrix	Osteoblast (ovine and human)	-	-Proliferation rates: For human cells: TissueFoil E > plasma-coated PLGA-scaffolds > uncoated PLGA-scaffolds. For ovine cells: plasma-coated PLGA-scaffolds> Tissue Foil E > uncoated PLGA-scaffolds. -Average amount of OCN: 9.6 ng/mL for ovine cells and 7.4 ng/mL for human cells.
Hofmann *et al.* (27)	1.Human demineralized cancellous bone 2.Human autoclaved cancellous bone 3.HA ceramic cylinders (control group)	Human osteoblasts	-	-Production of multiple vital and functioning tissue-engineered bone pieces of 1.0 ×0.75 cm size within 10 days in vitro. -Gene expression significantly higher when demineralized bone used as biomatrix.

Table 2. *In vivo* studies using synthetic ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Study Model	Implantation Site	Result
Wang *et al.* (28)	β-TCP	BMSCs	BM	Dog #8	10 mm diameter Medial orbital wall	New bone formation after 24 weeks: 62.57 ± 7.40%
Agacayak *et al.* (29)	HA/TCP	BMSCs	PRP	Rat #90	7mm diameter Parietal bone	After 2 weeks bone healing was 14.3% and 12 weeks bone healing was 85.7%
Vahabi *et al.* (30)	HA/TCP	BMSCs	-	Dog #5	10 * 10 * 10 mm mandible body	New bone formation after 6 weeks were 46.38%
Dong *et al.* (31)	β-TCP	BMSCs	PRP (control: no factor)	Rabbit #15	Muscle pouches	New bone formation 12th week: 9.33% (control): 7.68%
Mankani M.H. *et al.* (32)	Rigid cubical HA/TCP scaffolds with two size of porosity (200 and 500 μm)	hBMSCs	-	- Harlan-Sprague Dawley female mice: #12	subcutaneous 12*5 mm	-Lamellar spacing within scaffolds regulates the extent of bone formation: 500 μm: the most new bone, 200 μm: the strongest transplants.
Behnia *et al.* (33)	HA/TCP	BMSCs	PDGF	Human #4	Anterior maxillary cleft	After 3 months new bone formation was 51.3%
Zhou *et al.* (34)	β-TCP	A:BMMSCs B:BMMSCs+MSCs Derived ECs		Rabbit #32	Ulnar 1.5 cm defect	-New bone formation at 12th week: A:5.42±0.43% B: 17.12±0.97% -New bone formation at 16th week: A:7.68+-0.84% B: 23.31+-1.41%
Shayesteh *et al.* (35)	HA/TCP	BMSCs	-	Human #7	Maxillary Sinus Floor	After 3 months 41.34% new bone formation
Jafarian *et al.* 2008 (36)	HA/TCP	BMSCs	-	Dog #4	-10mm TAT Mandible -Masseter Muscle	After 6 weeks 29.12% new bone formation
Eslami *et al.* (37)	HA/TCP	BMSCs	-	Dog #4	Masseter Muscle	New bone formation after 2 months was 29.12%
Uchida M. *et al.* (38)	βTCP granules	hBMSCs	rhBMP 2	-Female BALB/cAJcl-nu/nu mice	Subcutaneous pockets	-Lesser cell numbers, greater ALP activity, more osteogenic potential osteocalcin synthesis compared to control (no rhBMP 2)
Khojasteh *et al.* (39)	A:BioOss B: β-TCP	BMSCs	PRP	Rat #15	5mm diameter parietal bone	New bone formation at 6th week: A+PRP:1.27 A+BMMSCs:1.44 B+PRP:1.21 B+BMMSCs:2.53
Marcacci *et al.* (40)	Porous HA ceramic scaffolds	hBMSCs	FGF	Human	Case 1. Proximal tibia: 4 cm × 3 cm Case 2. Proximal ulna: 4 cm ×1 cm Case 3. distal humerus: 7 cm × 2.5 cm Case 4. Distal ulna: 6 cm × 1 cm	-Complete fusion between the implant and the host bone observed 5 to 7 months after surgery. -No late fractures in the implant zone were observed.
Murata *et al.* (41)	Functionally	No cells	rhBMP 2	Male Wistar rats: #10	Subcutaneous pockets	-Giant cells appeared on the fg-HAp at 2 weeks.

	graded apatite (fg-HAp) developed from bovine bone.					-Body fluid permeation was found inside the fg-HAp, and the fluid component was immunopositive for albumin. Albumin was also a main component among proteins collected from the in vivo implanted fg-HAp. After 4 weeks the BMP-2/fg-HAp implant showed 59.0% in the total volume of bone and marrow.
Jansen *et al.* (42)	Ti-fiber mesh and porous CaP cement	No cells	rhBMP 2, S-300 BMP cocktail and rhTGFh1	- Wistar King rats: #56 - Mature male New Zealand White rabbits: #18 - Mature female New Zealand White rabbits: #36	- Subcutaneous - Calvarial defects : 7.3 mm in diameter -Calvarial defects: 8mm in diameter and subcutaneous	-In Ti-BMP2 implant, trabecular bone with hemopoietic bone marrow-like tissue was seen at day 20. Denser lamellar bone at day 40. -In the Ti-TGFβ1 implants, extensive trabecular bone and hemopoietic bone marrow-like tissue was observed and bone fill was 36%. -In porous CaP-rhBMP-2 cranial implants complete closure of the defect was observed after 10 weeks and bone fill was 53%. - In subcutaneous CaP-rhBMP-2 implants bone formation was observed at 10 weeks and bone fill was 18%.
Kroese Deutman *et al.* (12)	Porous Ca-P cement and ACS	No cells	rhBMP 2	- Female New Zealand white rabbits: #54	Subcutaneous	-Bone formation in the rhBMP-2 loaded Ca-P cement discs after 10 weeks. -No signs of bone formation in the ACS (absorbable collagen sponge). -Degradation of the Ca-P cement could not be confirmed after 10 weeks.
Ruhe *et al.* (43)	porous Ca P cement and ACS	No cells	rhBMP 2	- Mature female New Zealand White rabbits: #54	Calvarial defect: 8mm in diameter	-After 2 weeks: complete closure of defects in the ACS group; callus-like bone formation outside the rhBMP 2 loaded CaP cement implants. -After 10 weeks: Complete closure of the defects in all rhBMP 2 loaded specimens and in some unloaded specimens.

Table 3. *In vitro* studies using natural ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
Hinze *et al.* (19)	1. non-sintered, nanocrystalline, phase-pure HA 2. DBX®	Human osteoblast-like cells	-	DBX® -Formation of a dense network of multilayered polygonal-shaped cells with processes allowing cell–cell communication after 3 weeks. - 10 ng/ml OCN -The alkaline staining of these cells showed intensive staining (about 37%). -Type 1 collagen was present in about 3% of the cells. HA -The cells showed no tendency to proliferate or migrate.
Hofmann *et al.* (27)	1.human demineralized cancellous bone 2.human autoclaved cancellous bone 3.HA ceramic cylinders (control group)	Human osteoblasts	-	-Production of multiple vital and functioning tissue-engineered bone pieces of 1.0 ×0.75 cm size within 10 days in vitro. -Gene expression significantly higher when demineralized bone used as biomatrix.

Table 4. *In vivo* studies using natural ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Study Model	Implantation Site	Result
Khojasteh *et al.* (44)	Allograft	BMSCs	FG	Rabbit #5	Tibia	After 2 months -2.09mm Mean bone volume -New bone formation: 28.5 64.5%
Lee *et al.* (45)	Allograft	BMSCs (control: no cell)	-	Rabbit #30	Femoral 1.5 cm	4th week:2.4±1.1 8th week: 5.4±1.1 12th week:14.4±3.1 (control): 4th week:0.8±0.8, 8th week: 3.2±1.5, 12th week:9.2±1.9 12th week: 3/5 united (control):1/5 united
Reichert *et al.* (46)	autologous cancellous bone graft	No cells	-	sheep #16	Tibial defects: 3cm	-After 12 weeks: healing of all defects according to radiographs. Minor biomechanical properties in new bone (11% of the torsional strength and 19% of the torsional stiffness compared to the collateral tibiae)
Behnia *et al.* (47)	Demineralized bone mineral	BMSCs	-	Human #2	Unilateral Alveolar Cleft	After 4 months -Integrity of nasal floor -35.4% the mean postoperative defect of patient 1 25.6% the mean postoperative defect of patient 2
Lucarelli *et al.* (48)	Allograft	SSCs (control: no cell)	PRP	Sheep #10	3cm wide metatarsal bone	4th month :- :5/6 compelete healing (control) :1/4 distal osteotomy --- line completely healed but the proximal osteotomy line remained detectable - New bone formation: 32.3% to 42.8% (control) : 6.7% to 10.5%

Table 5. *In vitro* studies using polymers and non-ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
Tenorio *et al.* (4)	hyaluronic acid (Mesolis) and collagen foams (Tissue Fleece).	Human fetal bone cells	-	-Cell proliferation in both scaffolds at 70% capacity compared to monolayer culture. -Collagen foam: better structure for cell delivery if cavity filling is necessary. -Hydrogels: injectable technique for difficult to treat areas.
Naito *et al.* (49)	1. 3D collagen hydrogels 2. 2Dculture 3. Collagen hydrogel-based ring-shaped bone-like tissues conditioned with osteoinductive supplements	Rat MSCs*	-	-Better osteoblastic differentiation in the hydrogel cultures than 2D cultures. -Enhanced biomechanical properties in ring-shaped bone-like tissues with osteoinductive supplements
Idris *et al.* (50)	Poly (LLA-co-CL)*, Poly (LLA-co-DXO)* and PLLA * scaffolds. (12.5 mm ×1.5 mm)	Human osteoblast-like cells	-	-After 14 days extensive ECM formation and cell growth were seen on test scaffolds. -Increased mRNA expression of the ALP, COL 1, BSP, OCN, OPN, and Runx2 genes.
Sailon *et al.* (51)	Polyurethane scaffolds	Murine preosteoblasts (MC3T3-E1)	-	By day 8: -static scaffolds cell density was 67% ± 5.0% in periphery and 0.3% ± 0.3% in core. -Flow-perfused scaffolds cell density was 94% ± 8.3% in periphery and 76% ± 3.1% in core. -Thick (>6 mm) 3D constructs are sustainable using a flow-perfusion bioreactor.
Ekaputra *et al.* (52)	PCL and PCL/Col fabricated by	pBMMCs	-	-PCL+Col: better attachment, spreading, and proliferation of cells.

	electrospinning technique			-Culturing under dynamic conditions enhanced bone-like tissue formation and mechanical strength.
Alexander *et al.* (25)	1. OPLA* scaffolds, 2.collagen composite scaffolds (mixture of type I and III collagens) 3.CaP scaffolds (5 mm × 4.5 mm)	human JPC	BMP 2	-Denser colonization of JPC-seeded OPLA scaffolds and formation of bone nodules after initiation of osteogenesis. - JPCs growing within OPLA scaffolds are able to form CaP particles.
Lode *et al.* (17)	3D porous scaffolds of biomimetically mineralized collagen type I (10 mm × 4 mm)	hBMSCs	-	-Higher cell numbers: better colonization. -Lowest cell density: the highest proliferation rate and specific ALP activity -A saturation phenomenon was observed; On average 1.7×10^3 cells were attached to the polystyrene surface of the seeding wells.
Santos-Ruiz *et al.* (53)	bioglass/ PLGA80 composite, PLGA80	Osteoblastic cell lines	-	-Mutated cells adhered more to each other than wild-type. They tended to grow in patches which formed bone nodules. -Poorer culture growth in syndromic patients.
Wagner *et al.* (26)	1. native and plasma-coated PLGA-scaffolds) 2.agarmatrix with HA (coated with 0.4% hyaluronic acid and native) 3.Tissue Foil E, as reference matrix	Osteoblast	-	-Proliferation rates: For human cells: TissueFoil E > plasma-coated PLGA-scaffolds > uncoated PLGA-scaffolds. For ovine cells: plasma-coated PLGA-scaffolds> TissueFoil E > uncoated PLGA-scaffolds. -Average amount of osteocalcin: 9.6 ng/mL for ovine cells and 7.4 ng/mL for human cells. - 59% collagen and 46% alkaline phopsphatase in human cells.
Chen *et al.* (54)	Collagen scaffolds prepared with or without glutaraldehyde crosslinking at different freezing temperatures (_20°C or 80°C)	Osteoblastic Cells (MC3T3-E1)	-	-Different freezing temperatures affected the architecture of the collagen sponges, but did not affect osteoblastic responses to them. -Glutaraldehyde crosslinking process resulted in higher cell number, might compromise the osteoblastic differentiation and mineralization.
Ciapetti *et al.* (55)	PCL	hBMSCs	-	-The use of unfractionated marrow tissue with blood can provide a high number of osteogenic precursors.
Eyckmans *et al.* (56)	in vivo: Collagraft carrier	RPDCs HPDCs	BMP6	-In vitro, RPDCs expanded than HPDCs. -BMP6 stimulated ALP activity but osteogenic medium didn't. -In vivo, HPDCs: extensive bone formation; RPDCs: no bone formation.
Weinzlerl *et al.* (57)	3D osteogenic constructs, based on collagenous micro-particles	Human ATSCs	-	-Calcium deposition in the cell matrix of ATSCs with no correlation with the age of the donor. -In Two out of three 3D construcs clear zones of mineralization (v. Kossa) detected in the area of osteocalcin-producing cells.
Wang *et al.* (58)	PhosPEG	gMSCs	-	- PhosPEG–PEG cogels: the greatest accumulation of calcium after 3 weeks. Mineralization primarily in the core of the construct. (for cogels in the perimeter of the construct and in PEODA gels, homogeneous mineralization).

Table 6. *In vivo* studies using polymers and non-ceramic scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Study Model	Implantation Site	Result
Lin *et al.* (59)	Porous disc shaped PLGA scaffolds (8mm × 2 mm)	Modified rabbit BMSCs	Human BMP-2 and VEGF165 genes	Female NZW rabbits	Parietal defects: 8 mm in diameter	-The short-term BMP2/VEGF expression by BMSCs: 21% bone regeneration at week 12. -Bone ingrowth from the edge at week 4. 83% bone regeneration at week 12.
Xia *et al.* (60)	An injectable sodium alginate/	Rabbit BMSCs	-	New Zealand white rabbits: #10/#15	- Subcutaneous -Calvarial defects: 15 mm in diameter	-After 12 days formation of multilayered and mineralized nodules was observed -2 weeks later: high proliferative capacity of cells and cluster formation.

						-After 8 weeks: Closure of the calvarial defects. -After 12 weeks: complete filling of the defect with mature bone tissue (with the Haversian system)
Wadagaki *et al.* (61)	SRBFS*	Mouse BMSCs	-	Mice	Subcutaneous pockets	-SRBFS promoted osteoblastic differentiation of BMSCs in vitro and in vivo. - Better mineralization in the SRBFS group after 12 weeks.
Zhang *et al.* (62)	Fibronectin	Mouse preosteoblast MC3T3-E1 cells, mouse embryonic mesenchymal cells, C3H10T1/2 cells and human cervical cancer HeLa cells	BMP-2	Male Wistar rats: #9	Cranial defects: 5 mm in diameter	-The tissue concentrations of BMP-2 in animal models: Over 100 pg/mg in the HAP-BMP-FB group and 50 pg/mg in the control groups. -Enhanced bone formation in the HAP-BMP-FB group after 8 weeks.
Graziano *et al.* (63)	absorbable PLGA	Human $CD34^+$ stem cells capable of differentiating into pre-osteoblasts	-	Rats: #12	Subcutaneous pockets	-Bone formation occured in two steps: fibrous bone at day 30 and adult-type bone by day 60. -Neo-angiogenesis within bone nodules. -No bone formation in CD34– cells.
Kim *et al.* (64)	collagen matrix scaffolds	hADSC	-	Balb/C nude mice	Calvarial defects: 4 mm in diameter	- flavonols increase osteogenic differentiation. Expression of the genes were as follows: quercetin>kaempferol>chrysin
Eyckmans *et al.* (56)	Collagraft carrier	RPDCs HPDCs	BMP6	NMRI-nu mice	Subcutaneous (on the back in the cervical region)	HPDCs: extensive bone formation; RPDCs: no bone formation.
Ruhe *et al.* (43)	1.porous CaP cement and ACS	No cells	rhBMP 2	Mature female New Zealand White rabbits: #54	Calvarial defect: 8mm in diameter	-After 2 weeks: complete closure of defects in the ACS group; callus-like bone formation outside the rhBMP-2 loaded Ca-P cement implants. -After 10 weeks: Complete closure of the defects in all rhBMP-2 loaded specimens and in some unloaded specimens.
Fialkov *et al.* (65)	PLGA foam with similar porosity to human trabecular bone (1 cm ×1.2 cm)	Autologous rabbit bone marrow cell	-	New Zealand White rabbits: #27	Femoral defects: 1.2 cm long	-Significant bone formation happened. -The mean bone formation index (BFI) for the cell-loaded group was greater than for the control group at all periods especially at 2- and 8-week radiographs. -A trend of resorption adjacent to the fixation plate and bony deposition on the opposing side was detected. This bone started to form in the distant area from the osteotomy site.
Redlich *et al.* (66)	PGLA fleeces	Rabbit periosteal cells	-	Rabbits: #5	Calvaria defects: 15 mm in diameter	-In group with cell/fleece constructs: intense formation of uncalcified bone from both the margin and center of the defect. Mean defect closure =65% -Control groups: marginal bone formation. Defect closure=31%

Table 7. *In vitro* studies using composite scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
Sicchieri *et al.* (11)	PLGA–CaP scaffolds (10mm × 2mm)	Rat BMSCs	-	Statistically significant positive correlation between pore size and gene expression (only osterix showed a weak correlation)
Roberts *et al.* (67)	5 orthopedic 3D matrices composed of CaP* particles in an open collagen network (3mm × 3mm)	hPDCs	-	hPDCs were differentiated to osteoblasts in vitro.
Reichert *et al.* (68)	Type I collagen-coated circular mPCL–TCP scaffolds (5 mm×3 mm)	Ovine MPCs and OBs	-	-Proliferation rate in vitro: MPCs>OBs (after 1, 3 and 5 days) -A plateau phase in both cells (After 6–7 days). -Mineralization potential in 3D culture: MPCs>OBs (after 4 weeks) - cell viability on the scaffolds >90%.
Akkouch *et al.* (69)	3D porous Coll/HA/PLCL	Saos2 osteoblast-like cells(a human osteosarcoma cell line with osteoblastic properties)	Human epidermal growth factor	-Mineralized Col and HA particles detected on the surface of the scaffold. -The infrared spectra of the Col/HA/PLCL scaffold presented significant similarities with the FTIR spectra of healthy human bone.
Meretoja *et al.* (70)	Poly(e-caprolactone/D,L lactide)- based scaffolds with and without 30 wt % BAG S53P4	rat BMSCs, bone marrow stromal cells	-	-Static culture (no change in medium): a thick collagen rich matrix formation after 3 weeks with cells not penetrating deeper than 500 μm into the scaffolds. Bioactive filler enhanced proliferation, early osteogenic differentiation, and mineralization. -Dynamic cultures: cells present throughout the scaffold interior, even in the center. Bioactive filler decreased cell numbers and inhibited differentiation.
Santos-Ruiz *et al.* (53)	Composite, and PLGA80	Osteoblastic cell lines (mutated from craniosynostotic patients and normal)	-	-Mutated cells adhered more to each other than normal type. They tended to grow in patches which formed bone nodules. -Poorer culture growth in mutated cells.
Lechner *et al.* (71)	Composite grafts (a fibrin–thrombin shell and fibrin, thrombin, Collagen, HA and β-TCP)	Bone marrow and peripheral blood-derived Murine progenitor cells	BMP 2	-After 17 days: modification of appearance and growth behavior in cells. -In vitro pre differentiated osteoblasts of the same clones maintained their osteogenic phenotype for more than 28 days in a tissue collagen matrix.
Arnold *et al.* (72)	Fibrin– alginate–hydroxyapatite composite.	Porcine periosteal cells	F XIII, TGF-β1, and b-FGF	-Control group: fivefold cell count increase up to the 26th day. -Addition of 10.0 ng/mL b-FGF: over seven fold increase in 33 days. -The strongest proliferation in the group with 0.5 ng/mL TGF-β1
Karp *et al.* (13)	Fibrin-CaP-PLGA scaffolds2. precise cylindrically shaped scaffolds	rat BMSCs	-	Spindle like cells migrating above the underlying cell layer after 9 days.

Table 8. *In vivo* studies using composite scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Study Model	Implantation Site	Result
Liao *et al.* (73)	PCL/HA	BMSCs	-	Pig	2*2cm Temporal bone	After 6 months new bone formation was 64%
Liao *et al.* (74)	PCL/TCP + collagen I	pASCs	-	Mice	Gluteal Muscle Pocket	More Woven Bone Than Control Group
Khojasteh *et al.* (44)	PCL/TCP	BMSCs	-	Dog #4	20*10*10 mm3 Posterior Mandibular	After 2 months 48.63% of the defect was filled with lamellar bone and 24.1% of scaffold was remained.
Sicchieri *et al.* (11)	PLGA–CaP scaffolds (5mm × 2mm) with various pore size	Rat BMSCs	-	Rat # 24	Calvarial defects: 5 mm in diameter	-After 2 weeks: similar amount of bone and blood vessels formation in all scaffolds; At 4 and 8 weeks much more bone formation in scaffolds with pores of 470–590 μm. -Pore sizes around 1000 μm: better osteoblast phenotype expression, -Pore sizes around 500 μm: more bone formation. -In vivo experiment: woven bone overlaid by lamellar bone lined by bone-lining cells after 8 weeks.
Roberts *et al.* (67)	3D matrices composed of CaP particles in an open collagen network	hPDCs	-	Mice	Subcutaneous	-Highest bone and bone marrow formation in NuOss/hPDC implant at 13.03%. - The bone formed in this implant: 65% originating from implanted cells. -Formation of a cartilage intermediate.
Reichert *et al.* (46)	Type I collagen-coated mPCL–TCP	ovine MPCs and OBs	-	Mice #4	Subcutaneous pockets	-Osteogenic potential in vivo: OBs>MPCs.
Davies *et al.* (75)	Composite scaffold from PLGA and two CaP phases	HUCPVCs	-	nude rats	Femoral osteotomies	- scaffold contributed to bone healing in osteotomies - Also the application of scaffold for socket preservation and sinus lift was demonstrated.
Kim *et al.* (76)	PCL/TCP	BMSCs	rhBMP-2	Dog #2	5.0×5.0×8.0 mm scapular region	Bone ingrowth occurred in PCL-TCP scaffold which was transplanted with rhBMP-2, and MSCs did not affect bone growth
Lin *et al.* (14)	Poly(propylene fumarate)/ β-TCP composite scaffolds	Transduced/ untransduced modified human gingival fibroblasts	Murine BMP 7	mice	Subcutaneous pockets	-New bone localized on the scaffold surface, following its contours. -The total stiffness of the constructs was retained for up to 12 weeks.
Karp *et al.* (13)	Filled/-PLGA-CaP cylindrical scaffolds (2.4 mm×2.5 mm) 2. precise cylindrically shaped scaffolds	-	-	rat	Femoral defects: 2.5 mm in diameter	New bone begun to form after 4 days, and after 7 days bone completely invaded the pores of scaffolds.

Table 9. *In vitro* studies using metal- based scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
De peppo *et al.* (77)	EBM-fabricated cp-Ti and Ti6Al4V porous Scaffolds	hES-MPs	bFGF	-These scaffolds supported cell attachment and growth -They did not change the expression of osteogenic genes and affect the alkaline phosphatase activity.
St-Pierre *et al.* (78)	Titanium	Newborn mouse calvaria-derived MC3T3-E1 pre-osteoblastsMouse MC3T3-E1 pre-osteoblasts	-	-Cells proliferate on all materials with a plateau phase at day 9. -Proliferation rates higher on foams (123 to 163 percent per day) than on the reference material (80% per day). -On polished titanium: greater osteocalcin release, earlier mineralization of the extracellular matrix. -Similar calcium content on all materials at the end.

Table 10. *In vivo* studies using metal- based scaffolds

Study	Scaffold	Type Of Cells	Type Of Growth Factor	Study Model	Implantation Site	Result
Ye *et al.* (79)	Silver	iPSCs modified	SATB2 gene	Mice	4mm calvaria	After 5 weeks, histometric analysis showed %59.58±7.00 new bone formation
Duan *et al.* (80)	Silver	iPSCs	Enamel Matrix Derivatives	Mice #24	1.5*2mm periodontal	After 24 days, histometric analysis revealed %58.53±2.67 new bone formation
Meretoja *et al.* (81)	titania (TiO2)-coated, titania-silica (TiSi 30:70 mol %)-coated, and uncoated (cp-Ti) titanium fiber meshes	Rat BMSCs	-	Rats: #18	subcutaneous	-After 1 week: detection of multiple patches of unorganized mineralizing tissue in all implants. -After 12 weeks: new bone maturation, mesh fibers embedded in lamellar bone. -Enhanced distribution of bone in the sol-gel coatings -bone formation mainly in the center of the uncoated scaffolds.

Table 11. *In vitro* studies using nano-scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Result
Hinze *et al.* (19)	1. non-sintered, nanocrystalline, phase-pure HA 2. DBX®	Human osteoblast-like cells	-	-Formation of a dense network of multilayered polygonal-shaped cells with processes allowing cell–cell communication on DBX® after 3 weeks. - 10 ng/ml osteocalcin -The alkaline staining of these cells showed intensive staining (about 37%). -Type 1 collagen was present in about 3% of the cells. -On hydroxyapatite, the cells showed no tendency to proliferate or migrate.

Table 12. *In vivo* studies using nano-scaffolds

Study	Scaffold	Type of Cells	Type of Growth Factor	Study Model	Implantation Site	Result
Behnia *et al.* (82)	nanocrystal line HA and silica gel matrix)	BMSCs	-	Rabbit #8	8mm in diameter Parietal bone	Bone formation 29.45% after 6 weeks and 44.55% after 12 weeks
Liu et al. (83)	nHAC/ PLA	DPSCs	rhBMP 2 (control: no growth factor)	Rabbit #36	10*4*3 mm Alveolar	After 12 weeks 2.52 increase in bone height compared to 1.77 in control group
Liu *et al.* (84)	nHACP/ CF	BMSCs	-	Goat #32	25-mm tibia	After 4 weeks new bone formation was 16.56% with no bone union. However after 8 weeks all samples showed bone union
El-Ghannam *et al.* (85)	SCPC	No cells	rhBMP 2	rabbits	Segmental ulnar defects: 10mm	- After 4 weeks: defect replacement by new bone with mature bone characteristics. -The SCPC-rhBMP-2 hybrid enhanced bone regeneration in defect to form a mature bone

scaffold should be biocompatible and reproducible and also easily be sterilized (11, 70); rate of scaffold degradation should be the same as the rate of regeneration (54); they should have suitable porosity to maintain cell attachment and proliferation (54) and mechanical stability to resist external forces, cell contraction forces in healing process (23, 32, 54). Scaffolds' mechanical properties are really important in load-bearing sites. Their compressive strength should be great, but not exceed that of the surrounding bone, because it may cause bone resorption (16).

The aim of the current study was to review the results of studies that used the terms tissue engineering or bone engineering for describing the nature of their work.

Materials and Methods

Study selection

In vitro and *in vivo* experiments in the field of BTE were reviewed. Studies which used bone scaffolds with or without cells and growth factors were included. Only studies which proposed their method as BTE or tissue engineering were included. *In vivo* studies including animal, human experiments and case reports were excluded. *In vitro* experiments which did not investigate the behavior of cells on the scaffold, including cell attachment, proliferation or osteoblastic differentiation, were excluded. *In vivo* studies which did not measure new bone formation were also excluded.

Search Strategy

Published papers on BTE were found using the following keywords alone or ensemble: bone tissue engineering, tissue engineering, bone, stem cells, scaffold, growth factor.

The initial paper selection was done by examining titles and abstracts of all selected papers. The full texts of potentially suitable articles were obtained for final assessment according to the exclusion and inclusion criteria. The reports consist of the most relevant data were included and analyzed in a qualitative manner.

Results

After data base search 2148 articles were retrieved, finally, 157 of them were selected after screening the titles and abstracts. Then the full text of these 157 articles was reviewed and finally 86 of them were selected based on our data selection criteria (Figure 1). These 86 articles include 38 *in vitro* studies and 48 *in vivo* studies. Included studies were categorized according to the scaffold that they used: ceramic, polymer (non-ceramic), composite (ceramic + polymer), nano-scaffolds and metal-based scaffolds. Ceramic scaffolds were either natural or synthetic (Figure 2).

1. Synthetic ceramic scaffolds

Ceramic scaffolds including hydroxyapatite (HA), tricalcium phosphate (TCP), HA-TCP, beta-tricalcium phosphate / bioactive glass (β-TCP/BG), β-TCP with Mg (β-TCMP), Calcium phosphate cement (CPC) have been used. From the total number of ten *in vitro* studies (Table 1), two of them used BMSCs, and the rest of them used cells, such as osteoblast cells, periosteum-derived cells and umbilical cord mesenchymal stem cells. The results were observed one to four weeks after the surgical procedure.

From total number of 17 *in vivo* studies (Table 2), 13 studies used BMSCs, four used no cells, and the rest used multipotent adult progenitor cells and pre-osteoblasts. Animal models were rats, rabbits and mic. Also, two human studies were reported.

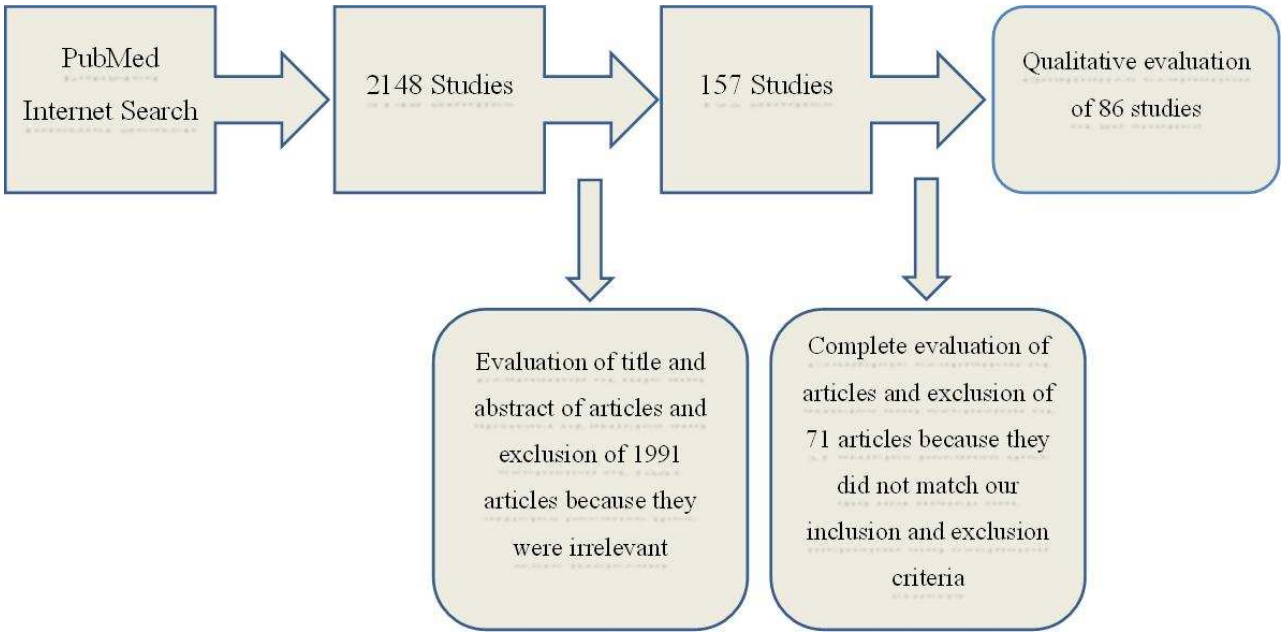

Figure1. Study design

Defects were either cranial, calvarial, or subcutaneous. Their sizes were at a range of 5 mm to 12 cm. The defect closure was observed one to 10 weeks after the surgery.

2. Natural ceramic scaffolds

Scaffolds such as human demineralized cancellous bone, human autoclaved cancellous bone, demineralized bone matrix (DBX®) have been used in seven studies. As demonstrated in Table 3, two *in vitro* studies used human osteoblast cells.

In five *in vivo* studies (Table 4), three studies used BMSCs, one of them used skeletal stem cells (SSCs), and two others did not use any cells. Animal models included rabbit and sheep and one study was performed on human. Defect sizes were between 1.5 to 3 cm and the results were analyzed after one to four months.

3. Polymers and non-ceramic scaffolds

Scaffolds including polycaprolactone (PCL), PCL/collagen (PCL/Col), polyurethane, phosphoester-poly (ethylene glycol) (PhosPEG), poly (lactic-co-glycolic) acid (PLGA) and open-cell poly-L-lactic acid (OPLA) have been used in 24 studies. As shown in Table 5, from the total of 14 *in vitro* studies, 6 of them used BMSCs, and the rest of them used cells such as osteoblast cells, periosteum-derived cells, human primary osteoprogenitors from craniosynostotic patients, human adipose tissue- derived stem cells (AdSCs). The results were observed 1 to 3 weeks after.

From the total number of ten *in vivo* studies (Table 6), four of them used BMSCs, and the rest of them used human AdSCs, periosteal- derived cells, pre-osteoblasts. One study did not use any types of cells. Animal models were rats, rabbits and mice. Defects were cranial, calvarial, femoral, or subcutaneous and their sizes were at a range of 4 mm to 1.2 cm. The defect closure was observed 12 days to 12 weeks after the surgery.

4. Composite scaffolds (polymer+ceramic)

Scaffolds including PCL/TCP, fibrin-alginate-HA, PLGA-CaP, PLGA-bioactive glass have also been used. As shown in Table 7, from nine *in vitro* studies, 3 of them used BMSCs, and the rest of them used cells such as osteoblast cells or periosteum-derived cells. The results were observed 4 days to 8 weeks after.

Review of *in vivo* experiments in this category shows that from ten studies, four of them used BMSCs (Table 8). The other cell types were osteoblasts, gingival fibroblasts, and periosteal cells. Animal models were dog, pig, rat and mice. Defects were cranial, femoral, or subcutaneous and their sizes were at a range of 2.5 mm to 5 mm. The defect closure was observed 4 days to 6 months after the surgery.

5. Metal-based scaffolds

Scaffolds such as titanium and titanium alloys (Ti_6 AL_4 V and Ti_6 AL_4 V with a CaP coating), titania-silica coated Ti fibers and silver have been used in five studies. In two *in vitro* studies, pre-osteoblasts, periosteal cells, or human embryonic stem cell-derived mesodermal Progenitors were mixed with metal scaffolds (Table 9).

In three *in vivo* study (Table 10), two of them used induced pluripotent stem cells (iPSCs) and the other one used BMSCs. Animals were mice and rats. Defects were 1.5 to 4 mm and histologic evaluations were performed 1 to 12 weeks after surgery.

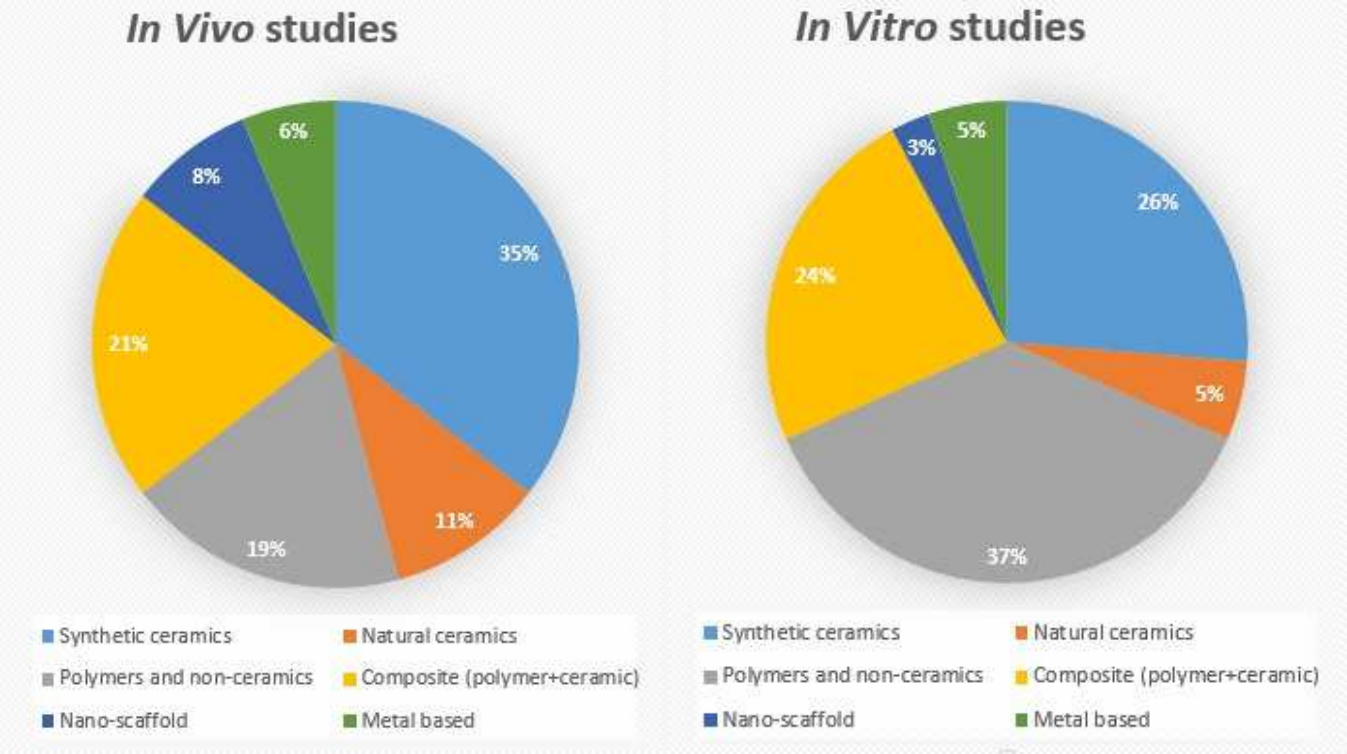

Figure 2. Distribution of studies based on the types of scaffolds

6. Nano-scaffolds

Nano-scaffolds have been used in recent studies. Nono-sintered, nanocrystalline, phase-pure HA and silica -CaP nanocomposite are used in the included studies. Only one *in vitro* study has been reported (Table 11). Of the four *in vivo* studies (Table 12), two of them used BMSCs and others either used dental pulp stem cells (DPSCs) or no cells. Animals were rabbits and goats with 8 mm to 25 mm defects. Animals were sacrificed 4 to 12 weeks post-surgery.

Discussion

The current review was performed to investigate the current trends in the field of BTE. Due to the presence of several variables including type of scaffolds, stem cells, growth factors, study models and defect sizes (in *in vivo* studies) as well as variability in analyzing methods and data report, determination of the most successful approach was not possible. However, the results of the studies could be compared in a qualitative manner. Most studies in the primary electronic search were *in vitro* studies, only a few of them introduced their experiment *in vivo*. *In vitro* studies were more focused on polymers, while relatively more animal and clinical studies were performed on ceramics (Figure 2).

The term bone engineering classically refers to application of stem cells, growth factors and a scaffold for bone regeneration. However, the results showed presentation of the tissue engineering triangle only in 7 out of 38 in vitro experiments (18.4%) and in 16 out of 48 *in vivo* studies (33.3%). Although, due to variability in methods, comparison of the results of these studies with those which did not use cells or growth factor was not possible, it seems that classic BTE triad would result in better stem cells differentiation, proliferation and attachment *in vitro* and proper bone healing *in vivo*. Combination of HA with fibroblast growth factors (FGF) and BMSCs in human with 4-7 cm tibial and ulnar bone defects resulted in complete fusion between the implant and the host bone (40). More than 50% of bone formation in maxillary alveolar cleft was reported using HA/TCP scaffold loaded with BMSCs and platelet-derived growth factor (PDGF) (33). In animal studies application of HA/TCP with platelet-rich plasma (PRP) in rats after 2 and 12 weeks resulted in 14.3% and 85.7% bone healing, respectively. Short term bone formation with PLGA discs with BMSCs expressing BMP-2 and vascular endothelial growth factors (VEGF) was 21% in rabbit (59).

Ceramic materials, including natural ceramics like allografts and synthetic ceramic like β-TCP and HA/TCP are the most used scaffold in the reviewed literature. Allografts, as an alternative to autogenous bone grafts, primarily show *in*

vivo osteoconductive properties and less commonly osteoinductive properties because of the variable attendance of growth factors. A combination of allogenic BMSCs in the reconstruction of the tibia in sheep showed nearly as much bone formation as autogenic BMSCs (86). Human demineralized cancellous bone as an allogenic source of bone substitute induced more osteoblastic differentiation *in vitro* compared with synthetic bone materials (19). β-TCP is a synthetic calcium phosphate, which have good biocompatibility and osteoconductivity in both animal experiments and clinical settings (87-93). Biphasic ceramics of HA/TCP are increasingly being used as a bone substitute in orthopedic and maxillofacial regenerative surgeries (94-96). According to some investigations, these scaffolds may possess osteoinductive property in addition to their osteoconductive effects, but these properties are not enough for complete regeneration of bone defects, particularly in cases of extensive tissue loss (97, 98). One approach used to overcome this problem, is to enrich them by osteogenic factors such as stem cells, which has been shown to increase the amount of bone formation in dogs (36).

Apart from the variability of scaffolds, other factors may also influence treatment outcome in BTE. These factors, mainly include the type of cell, the presence and type of growth factor, and animal model (for *in vivo* experiments). Adult stem cells (ASCs), the subject of most investigations in bone regeneration research, show great promise for using in the oral and maxillofacial regions. Undifferentiated cells found in almost all specialized tissues. ASCs are able to self-replicate and differentiate into various cell types (99, 100). ASCs with their potential to bone generating include BMSCs, AdSCs, DPSCs, stem cells from human exfoliated deciduous teeth (SHED), and periodontal ligament stem cells (PDLSCs) (101). In the current review, BMSCs were the most applied stem cell in BTE. Successful repair of bone defects with autologous BMSCs has been achieved in various animal models (102). The application of BMSCs with bone allografts increased bone union in rabbits after three months (45). In addition, optimal outcome has been achieved using autologous BMSCs to repair human bone defects, particularly mandible defects (103-105). These studies have shown that BMSCs possessed favorable potential for bone regeneration in the oral and maxillofacial regions. On the other hand, hUCMSCs synthesized nearly three-fold more bone minerals than the hBMSCs *in vitro* (21). Yu et al. (106) demonstrated that bone regeneration with DPSCs is comparable with BMSCs.

Cell proliferation, chemotaxis, differentiation, and matrix synthesis could be the results of local delivery of growth factors, hence demonstrating possible bone regeneration (107). Bone morphogenetic protein-2 (BMP 2) was the most widely used growth factor in animal studies. Even if recombinant human bone morphogenetic protein 2 (rhBMP2) be used by itself, can amplify bone formation, but, its quick spread in the implanted spot can reduce its osteoinductive power (76, 108). Previous studies have proved that BMP2 can promote osteogenic differentiation of ASCs and enhance matrix secretion in the process of bone repairs both in vitro and in vivo using a scaffold releasing BMP2 (83, 109). The use of skeletal stems cells with sheep allograft and PRP compared with control group (with no cell and factor), significantly increased new bone formation (48). Although addition of PRP to β-TCP scaffold seems to increase in vivo bone formation (31), BMSCs can induce more bone formation compared with PRP (39).

Different animal models including dog, sheep, goat, pig, rabbit, rat and mouse have been used in tissue engineering processes. Larger animals like dog, sheep, and pig are known as the models with the highest similarity to human (110). However, our results show that most of animal models were not large. Given the considerable dissimilarities with human bone and the small size of the animal, mice and rats are not counted as desirable models for bone studies (110). Moreover, the type of the defect might also be considered as an influencing factor on bone regeneration. There are different defect sites with different sizes according to our different animal models in studies which were reviewed.

Future prospects

Most of the studies in TBE were *in vitro* trials, but in the last 15 years, little progress has been made *in vivo*. Bone engineering has not yet achieved a major progress in the clinic. In order to provide accurate data in this field, some standards need to be defined and used in clinical studies. There are some challenges in this field. BMSCs are the most common cells used in bone engineering, however, there are some questions of whether they lead to the ultimate results or not. Common scaffold materials also have some problems, for example, there is a risk of inflammation *in vivo* due to the acidic pH change caused by the degradation of biodegradable polymers. The rapid prototyping technique has been used to produce specific scaffolds with characterized architecture. Ceramics seem to be more successful due to osteoconductivity, easy access, and the absence of immunological reaction. One method in designing scaffolds is the creation of cell environments that resemble natural human tissues. New technologies in scaffold fabrication such as bioreactors and rapid prototyping may help to produce these scaffolds.

Ex vivo gene therapy is a novel approach in BTE. In this method, progenitor cells are transduced by gene therapy to express bone forming factors, therefore there is no need for the expensive and difficult production of recombinant cells or growth factors. This approach has been successful in healing of bone defects in animal models. Combination of genetically- modified cells with scaffolds has also increased bone formation *in vivo*.

Conclusion

Among the reviewed studies, the most commonly used scaffold, stem cells and growth factor were synthetic ceramics, BMSCs and BMP2, respectively. Studies, that used these components showed high proliferation, attachment and differentiation of cells *in vitro* and new bone formation *in vivo*. The presence of stem cells and growth factors increased the amount of bone formation *in vivo*. However, some of other studies that did not use one of the components of BTE triad also reported relatively acceptable results. This shows that modification of scaffolds or methodology may facilitate the clinical application of BTE. Nano-scaffolds and composite polymer-ceramics have been developed more recently and may be alternative to conventional scaffolds. Although, bone regeneration using BTE seems to face several challenges, new technology in this field may help reach a final solution.

Conflict of Interest: 'None declared'.

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Please cite this paper as: Motamedian SR, Iranparvar P, Nahvi G, Khojasteh A. Bone Tissue Engineering: A Literature Review. Regen Reconstr Restor. 2016; 1(3):103-120.