Craniomaxillofacial Bone Engineering by Scaffolds Loaded with Stem Cells: A Systematic Review

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Abstract

Objective: The concept of tissue engineering holds huge promise for thefuture treatment of osseous defects. For bone tissue engineering, stem cells are applied on supporting scaffolds under controlled stimulation with growth factors. Scaffolds are provisional matrices for bone growth providing a specific environment for tissue development and favoring cellular attachment, growth and differentiation. To date, ceramics, polymers, and composite scaffolds have been widely used for bone tissue engineering in various in-vitro and animal studies. The objective of this article was to review the advances in jaw bone engineering from a scaffold material point of view.

Methods: A review of literature was carried out by using Medline database and searching topics like "craniomaxillofacial tissue engineering", "bone regeneration", "scaffold", "oral surgery", "stem cell+ scaffold", "xenograft" and "allograft".Animal and human studies evaluating repair of craniomaxillofacial defects with scaffold and stem cells, were considered in this study.

Results: A total of 64 studies were evaluated.

Conclusion: Based on the results of this literature review, although autogenous bone grafting has remained the preferred strategy for treatment of bone defects, rapid prototyping (RP) techniques do offer great opportunities to generate suitable scaffolds for bone tissue engineering in near future.

Key words: Tissue engineering, Bone regeneration, Scaffold, Stem cells, Craniomaxillofacial, Oral surgery, Allograft

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Introduction:

Craniofacial defects due to trauma, periodontal disease, surgical bone resection and congenital and acquired defects decrease bone volume and do not heal spontaneously. Such defects require bone grafting (1, 2). Autogenous bone grafting is still the gold standard for reconstruction of these defects. However, its disadvantages including its limitation, painful surgery, risk of infection, bleeding, nerve damage and loss of function have made the researchers to look for an alternative (3).

Tissue engineering was first introduced in early nineties to make up for these limitations. It is the science of designing and manufacturing new tissue to resume and restore the function of defected or lost tissues. The principals of tissue engineering are based on biological and molecular cell proliferation, and tissue formation through biologic engineering (4). Correct perception of the function of cells, structure of the extracellular matrix and adequate knowledge about the structure of scaffolds for providing a suitable environment for adhesion and maintenance of cells are the key concepts of tissue engineering (5). Bone tissue engineering necessarily requires 3 components:

preosteoblastic cells, osteogenic growth factor, and a scaffold for cell adhesion and preserving cell function (5-7). The most important superiority of tissue engineering over autogenous bone grafting is that the tissue graft in exogenous tissue engineering can be produced outside the body and therefore has no limitation and does not do any damage to the living cells (7).

Stem cells have a great potential for application in cell-mediated treatments and tissue engineering due to their self-renewal property and ability to differentiate to various cells such as osteoblasts under the induction of host tissue or culture medium (8-10). However, one drawback of tissue engineering is that it requires large amounts of cells (11). In osteogenic tissue engineering, stem cells need growth factors to start their function (5). Growth factors can be provided for cells through different methods. Transmission of gene encoding growth factor to the host cell (12-14), affinity of growth factor to the scaffold and its controlled release during the degradation of scaffold (15, 16), use of scaffolds that contain growth factor in their natural structure (17, 18) and cell culture in presence of growth factors (19) are among these techniques.

A scaffold is required for the migration of cells to the site of defect. It also plays a key role for progenitor cells in tissue engineering (20). Initially, a scaffold should create a suitable extra cellular matrix for growth and differentiation of cells. It should also be able to reconstruct and resume the proper function of tissue (1). Considering the fact that at present numerous commercial scaffolds are available in the market for application in tissue engineering, the cliniciansand researchers should compare their choice of scaffold with theideal one before application for bone reconstruction.

In summary, an ideal scaffold for bone tissue engineering should have the following characteristics:

1- Biocompatibility
2- Easy application and having the ability to be fixed in the defect
3- Having osteoconductive and osteoinductive properties
4- Biodegradation rate similar to the pace of new bone formation in the body
5- Having a continuous porous structure to allow for growth and differentiation of stem cells and import and export of materials and substances
6- Having mechanical properties similar to that of the natural bone
7- Having the potential for rapid angiogenesis (1, 21-23)

To date, no ideal scaffold has been found to meet all the above mentioned criteria for bone tissue engineering.

For three dimensional designing of scaffolds for bone reconstruction, several considerations have to be followed such as having a continuous porous structure (24, 25). A summary of several techniques used for manufacturing of synthetic bone scaffolds is presented below (For further details regarding each technique you can refer to the review articles published in this respect) (22, 26):

1) Solvent-Casting and Particulate Leaching Technique: In this simple method, a water soluble salt is combined with a biodegradable scaffold and an organic solvent and poured in the desired frame. After evaporation of solvent, salt particles are rinsed and a porous scaffold of desired shape is obtained (22).

2) Emulsion Freeze Drying: This method has been used for the fabrication of PLGA or poly (lactic-co-glycolic acid) scaffold with high porosity (>90%)(27). In this method, an emulsion of organic solvent, scaffold and water is cooled down quickly. Water and organic solvent are then extracted using Freeze Drying and a high porosity scaffold is obtained (22).

3) Gas-Foaming Process: In this method a highly porous scaffold is produced without using a solvent by increasing and decreasing the pressure of gas like CO2 (22)

4) Electro-spinning: In this method an electrical charge is used to control the deposition of polymer fibers on a substrate. Eventually a polymer fiber scaffold of desired size is formed (22).

5) Thermally Induced Phase Separation: In this method, the scaffold is dissolved in the solvent at high temperatures. By reducing the temperature, separation of 2 phases of solid-liquid or liquid-liquid is done and a porous scaffold is obtained (22, 26).
6) Rapid-Prototyping Technique: In this method, designing and fabrication of the scaffold are done by computer software. Shape of defects determined using CT scan or MRI image and the computer commands the fabrication of scaffold with desired shape to the bioreactor (22,28). Bioreactor can load the cells and growth factor onto the scaffold simultaneous with its formation (29). Thus, a scaffold including cells and growth factor is obtained in the form of defect. The obtained scaffold has relatively low porosity and its mechanical properties need to be improved (30). However, this method can cause great advancements in the field of tissue engineering.
The present study aimed to discuss different types of scaffolds and their fabrication technique and review the scaffolds used for craniomaxillofacial bone reconstruction through the application of stem cells.

Methods:

A Pub-Med search was done using the key words "tissue engineering", "bone regeneration", "stem cell+ scaffold", "oral surgery", "scaffold", "allograft" and "xenograft". Manual search was done through selected journals published by February 2012. All articles evaluating reconstruction and repair of craniomaxillofacial bone defects in humans or animal models using a scaffold and stem cells were entered the study. Those assessing the reconstruction of bone defects due to infection, malignancy, systemic disease and osteonecrosis were excluded from the study.

Results:

Of a total of 1,144 articles found through searching the databases using key words and manual search in selected journals, 113 were chosen after reviewing their title and abstract. The full texts of these articles were reviewed and in the next phase, 64 articles that matched our inclusion and exclusion criteria were selected and evaluated qualitatively. The process of article selection is demonstrated in Figure 1.

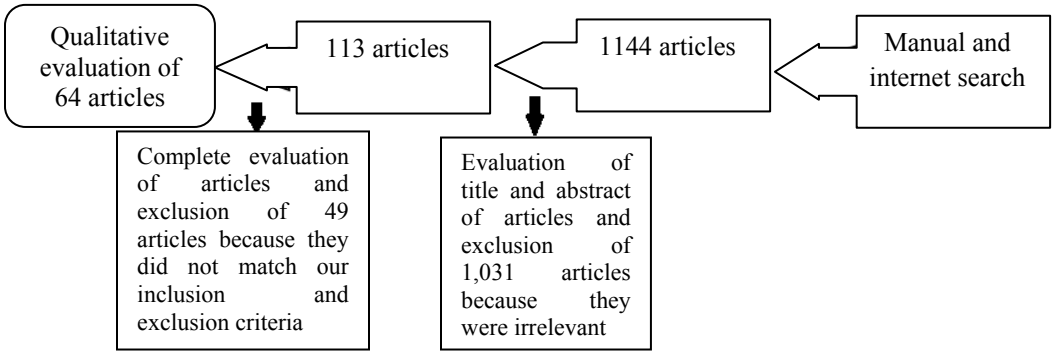

Figure 1- The process of article selection in this review study

Findings obtained from qualitative evaluation of articles:

Scaffolds for bone tissue engineering:

Based on the obtained results, scaffolds used for bone tissue engineering are divided into 3 main groups of natural, synthetic, and composite (31-34)(Table 1). Hydrogels are a group of scaffolds that are presently used and can have natural (collagen, hyaluronic acid, alginate, etc.) or synthetic (poly ethylene glycol, self-assembling peptides, etc.) origin (35).

Table 1- Different scaffolds used for craniomaxillofacial bone tissue engineering

Natural scaffolds		Synthetic scaffolds		Composite scaffolds
Natural organic	**Natural inorganic**	**Synthetic Polymer**	**Synthetic Ceramic**	
Collagen Sponge	Silver	PLGA	Calcium Magnesium Phosphate Cement (CMPC)	nono-hydroxyapatite/collagen/PLLA
PRP	Coral	PLG	βTCP	Octacalcium phosphate/collagen
Gelatin Sponge	Silk fibroin protein	PLLA	HA/TCP	Nano-hydroxyapatite/polyamide 6
Gelatin Hydrogel	Premineralized silk fibroin protein	PGA	Flurohyhroxyapatite	nono-hydroxyapatite/polyamide66
PuraMatrix	ABB	PLA	Ca-deficient hydroxyapatite (CDHA)	hydroxyapatite-coated PLGA
Alginate	Deer antler	PLA-PEG		HA/PLGA
Partially demineralized bone matrix		fibronectin-coated PLA		βTCP/Collagen
Bio-Oss		PEG-DA		DBM/PLA
Allograft		PEG-MMP		nano-hydroxyapatite/polyamide
Fibrin Sealant		PVDC		OsteoSet
Gelatin foam		polycaprolactone		octacalcium phosphate precipitated (OCP) alginate
Collagen gel				demineralized bone powders/PLA
hyaluronic acid-based hydrogel				apatite-coated PLGA

TCP: Tricalcium phosphate – HA: hydroxyapatite – DBM:Demineralized bone matrix – PLGA: poly (lactic-co-glycolic acid) – PLA: poly (D, L-lactic acid) – PGA: poly (glycolic acid) – PLLA: poly (L-lactic acid) – PVDC: Polyvinylidene chloride – PEG:polyethylene glycol – DA:diacrylate – MMP:matrix metalloproteinases – ABB: anorganic bovine bone – Puramatrix: a self-assembling peptide nanomaterial

Natural scaffolds:

Natural scaffolds are derived from the materials present in the nature, human body, plants, insects, or animals. These scaffolds are either ceramic having a structure similar to natural bone because of having hydroxyapatite or a similar substance, or have a structure similar to bone organic matrix proteins such as collagen, osteopontin and osteonectin.

Natural organic scaffolds:

Organic scaffolds include platelet-rich plasma (PRP), collagen, gelatin, fibrin, alginate, Chitosan and demineralized natural bone (allograft). These materials usually provide a natural substrate for adhesion, differentiation and proliferation of cells. However, this group of scaffolds usually has uncontrollable poor mechanical properties. Table 2 demonstrates natural organic scaffolds used in various studies conducted within the time period of the present review.

natural Inorganic scaffolds:

natural Inorganic scaffolds possess suitable biocompatibility and osteoconductive properties because they are structurally similar to the mineralized tissues in the body. Table 3 summarizes the inorganic natural scaffolds used in various studies conducted during the present study period.

Table 2- Natural organic scaffolds

Authors	Year of Publication	Site & form of lesion	Type of stem cell	Type of scaffold	Growth factor	Result
Zou et al. (36)	2012	5mm calvaria	BMSCs modified	Gelatin Sponge	HIF-1α gene	After 8 weeks, histological evaluation revealed 57.43%±0.21 bone formation and 9.6%±0.27angiogenesis
Yamada et al. (37)	2011	Posterior maxilla	BMSCs	PRP		After 3 months, radiography demonstrated 8.2±1.6 mm increase in bone height
Yamada et al. (38)	2011	10*10mm mandible	BMSCs DPSCs DTSCs	PRP		After 8 weeks, histometric analysis demonstrated 54.7%±2.2, 16.6%±1.3% and 52.8±3.5% new bone formation in pDTSCs/PRP, cDPSCs/PRP and cMSCs/PRP, respectively
Kohgo et al. (39)	2011	5mm mandible	BMSCs	PuraMatrix	PRP	After 8 weeks, histometric analysis demonstrated 55.64% bone formation around the implant
Ben-David et al. (40)	2011	5mm calvaria	BMSCs	Gelatin Hydrogel		After 8 weeks, µCT showed 65% bone formation
Chang et al. (41)	2010	5*2mm Calvaria	BMSCs modified	Alginate و Collagen type 1	BMP2 gene	After 3 months, histological evaluation and CT scan showed almost complete repair of defect with spongy bone and MPa 81/112 in the collagen group and almost no new bone formation in the alginate group.
Cheung et al. (42)	2010	3.5 mm calvaria	ADSCs	Collagen Sponge		After 3 weeks, histological evaluation revealed a mean number of 34.7 vessels per mm^3 and osteogenesis at the defect margins
Liu et al. (43)	2010	5mm calvaria	UCBMSCs	partially demineralized bone matrix		After 12 weeks, µCT showed almost complete repair of defect and formation of bone with a density approximately similar to that of natural bone
Kim et al. (44)	2010	5*5 mm calvaria	BMSCs	small intestine submucosa (SIS) sponge		After 4 weeks, histological evaluation demonstrated 46% new bone formation
d'Aquino et al. (45)	2009	15mm mandible	DPSCs	Collagen Sponge		After 3 months, clinical and radiographic assessments revealed more than 70% bone reconstruction
Arpornmaeklong et al. (46)	2009	5mm calvaria	ESCMSCs	Bio-Oss		After 6 weeks, histological examination revealed bone formation at the defect margins and formation of fibrous and granulation tissue at the center of defect.
Usas et al. (14)	2009	5mm calvaria	MDSCs modified	CG , FS & GS	BMP4 gene	After 6 weeks, µCT showed the volume of newly formed bone to be 102.85±51.4 mm^3 in repair with GS, 11.57±0.6 mm^3 in repair with CG and 12.02±6.2 mm^3 in repair with FS
Bohnenblust et al. (47)	2009	8mm calvaria	ADSCs	Allograft		After 6 weeks, mineral density of the newly formed bone was 1365±160.4
Kim et al. (48)	2007	8mm calvaria	BMSCs	hyaluronic acid-based hydrogel	BMP2	After 4 weeks, histological evaluation showed mature bone formation and vascular factors were also traced.
Smiler et al. (49)	2007	Maxillary sinus floor & maxillary ridge	BMSCs	βTCP, xenograft & resorbable algae		After 4 to 7 months, histomorphogenic analysis demonstrated 43% and 45% new bone formation in resorbable algae, 40%, 23% and 16% in βTCP and 14% and 32% in xenograft, respectively.
Dudas et al. (19)	2006	8mm calvaria	ADSCs	gelatin foam	BMP2	After 6 weeks, quantitative radiographic assessment revealed repair of 65% of the defect.
Ito et al. (50)	2005	10mm mandible	BMSCs	PRP		Histologic evaluation demonstrated mature bone formation. After 2 weeks, Vickers hardness test yielded a value of 17
Lee et al. (51)	2001	5mm	MDSCs modified	Collagen sponge	BMP2 gene	Repair of 95-100% of defects after 4 weeks

BMSCs: Bone marrow mesenchymal stem cells – ADSCs: Adipose derived mesenchymal stem cells – MDSCs: Muscle derived mesenchymal stem cells – DPSCs: Dental pulp stem cells – DTSCs: Deciduous tooth stem cells – UCBMSCs: Umbilical cord blood mesenchymal stem cells – ESCMSCs: Embryonic stem cell derived mesenchymal stem cells – PRP: Platelet rich plasma – BMP: Bone morphogenetic protein – TCP: Tricalcium phosphate – Puramatrix: a self-assembling peptide nanomaterial – CG: Collagen gel, – FS: Fibrin Sealant – GS: Gelatin Sponge

Table 3- Natural inorganic scaffolds

Authors	Year of Publication	Site & form of lesion	Type of stem cell	Type of scaffold	Growth factor	Result
Ye et al. (52)	2011	4mm calvaria	iPSCs modified	silver	SATB2 gene	After 5 weeks, histometric analysis showed %59.58±7.00 new bone formation
Duan et al. (53)	2011	1.5*2mm periodontal	iPSCs	silver	Enamel Matrix Derivatives	After 24 days, histometric analysis revealed %58.53±2.67 new bone formation
Xiao et al. (54)	2010	12mm orbital wall	BMSCs modified	coral	BMP2 gene	After 24 weeks, histometric analysis demonstrated 74.63%±7.94 formation of a bony bridge
Zhang et al. (55)	2010	3mm calvaria	BMSCs modified	silk fibroin protein	BMP7 gene	After 4 weeks, histological evaluation demonstrated new bone formation at the margins and bony islands in the center of defect
Pieri et al. (56)	2010	8mm vertical calvaria	ADSCs	ABB		After 1 month, μCT demonstrated 33.18%±3.92 new bone formation and 3.11±0.56 mm increase in height. Histometric analysis showed 47.96%±8.53 new bone formation
Lucaciu et al. (17)	2010	3mm Parietal	BMSCs	Deer antler		After 2 and 4 months, histological evaluation demonstrated bone formation of a good quality
Jiang et al. (13)	2009	5mm Ramus	BMSCs modified	Pre-mineralized silk fibroin protein	BMP2 gene	After 8 weeks, histometric analysis revealed 57.79±7.96% new bone formation
Cui et al. (57)	2007	20*20mm Parietal	ADSCs	coral		After 24 weeks, radiographic assessment revealed repair of 84.19%±6.45% of the defect
Hou et al. (58)	2007	15mm calvaria	BMSCs	coral	BMP2	After 16 weeks, radiographic assessment demonstrated formation of bone with 77.45% opacity
Al-Salihi (59)	2004	mandible	BMSCs	coral		After 3 months, histological assessment revealed mature bone formation with abundant blood vessels

BMSCs: Bone marrow mesenchymal stem cells – ADSCs: Adipose derived mesenchymal stem cells – iPSCs: induced pluripotent stem cells –BMP: Bone morphogenetic protein – ABB: anorganic bovine bone

Synthetic scaffolds:

Synthetic scaffolds are divided into 2 groups of polymer and ceramic.

Synthetic polymer scaffolds:

Synthetic polymer scaffolds used in different studies are derived from polylactic acids or poly glycolic acids. The main advantage of these scaffolds is the ability to fabricate several similar scaffolds and determining their mechanical and chemical properties. Also, growth factors can be incorporated into the biodegradable scaffolds in this group like PLA or poly (D, L-lactic acid), PGA or poly (glycolic acid) and PLGA or poly (lactic-co-glycolic acid). Therefore, during the degradation of scaffold, these factors are gradually released. Table 4 presents the studies conducted on synthetic polymer scaffolds with the aim of repairing maxillofacial bone defects.

Synthetic ceramic scaffolds:

These scaffolds include synthetic hydroxyapatite (HA), Nano HA, Bioglass, Beta Tricalcium Phosphate (βTCP), and Calcium Phosphate (CaP). Studies conducted with these scaffolds are summarized in Table 5.

Table 4- Synthetic polymer scaffolds

Authors	Year of Publication	Site & form of lesion	Type of stem cell	Type of scaffold	Growth factor	Result
Songsong et al. (60)	2011	3*5mm Condyle	BMSCs modified	PLGA	NELL-1	After 24 weeks, μCT demonstrated 60.7%±9.4% bone formation in the defect site
Hamajima et al. (61)	2011	5mm calvaria	BMSCs	PVDC		After 6 weeks, CT scan showed almost complete repair of defect
Wang et al. (62)	2010	7mm calvaria	BMSCs	PLGA	alendronate	After 12 weeks, radiographic assessment demonstrated 65% radio-opacity
Zong et al. (63)	2010	5mm calvaria	BMSCs	PLGA		After 20 weeks, histometric analysis revealed 53.9%±6.2% new bone formation
Terella et al. (64)	2010	8mm calvaria	BMSCs	PEG-DA, PEG-MMP	BMP2	After 8 weeks, μCT showed 42% decrease in size of defect in the PEG-DA group and 77% decrease in the PEG-MMP group
Di Bella et al. (65)	2008	15mm calvaria	ADSCs	fibronectin-coated PLA		After 6 weeks, histometric analysis revealed 12.09% new bone formation and radiographic assessment revealed 30.60% radiodensity.
Liu et al. (66)	2007	6.5mm calvaria	BMSCs	PLG	BMP2	After 12 weeks, histometric analysis showed 78.8% new bone formation.
Ren et al. (67)	2007	5*12mm mandible	BMSCs	PLGA, PLLA & PLA-PEG		After 12 weeks, histometric analysis revealed 81.9% new bone formation in PLGA group and 72.7% in PLA-PEG group
Ren et al. (68)	2005	5*12mm mandible	BMSCs	PLGA		After 3 months, histological examination showed complete repair of the bone defect
Marei et al. (69)	2005	Mandibular central incisor socket	BMSCs	PLA/PGA		After 4 weeks, histological evaluation revealed bone formation with a density of 74.94% in the site
Schantz et al. (70)	2003	15mm calvaria	BMSCs	polycaprolactone		After 3 months, histological evaluation revealed newly formed bony islands and abundant blood vessels but the defect was not repaired completely.

BMSCs: Bone marrow mesenchymal stem cells – ADSCs: Adipose derived mesenchymal stem cells –PRP: Platelet rich plasma – BMP: Bone morphogenetic protein – PLGA: poly (lactic-co-glycolic acid) – PLA: poly (D, L-lactic acid) – PGA: poly (glycolic acid) – PLLA: poly (L-lactic acid) – PVDC: Polyvinylidene chloride – PEG:polyethylene glycol – DA:diacrylate – MMP:matrix metalloproteinases

Table 5- Synthetic ceramic scaffolds

Authors	Year of Publication	Site & form of lesion	Type of stem cell	Type of scaffold	Growth factor	Result
Zou et al. (71)	2011	5mm Calvaria	BMSCs modified	Calcium Magnesium Phosphate Cement (CMPC)	HIF-1α	After 8 weeks, histometric analysis showed 25.31% ±5.16% new bone formation
Zhao et al. (12)	2010	5mm Mandible	BMSCs modified	βTCP	BMP2 gene	After 8 weeks, histometric analysis showed 31.83±5.35% new bone formation
Kim et al. (72)	2009	10*5mm Mandible	BMSCs &PLSCs	HA/TCP		After 16 weeks, histometric analysis showed 40.17% new bone formation in the BMSCs group and 36.51% new bone formation in PLSCs group around the implant
Zheng et al. (73)	2009	1.5*2.5*2.5mm Mandibular Symphysis	DTSCs	βTCP		After 6 months, histometric analysis showed 83.1% new bone formation
Pieri et al. (74)	2009	3.5*8mm edentulous ridge	BMSCs	FAP		After 3 months, histometric analysis showed 45.28% new bone formation
Guo et al. (75)	2009	3*6*10mm mandible	BMSCs	Ca-deficient hydroxyapatite (CDHA)		After 8 weeks, histologicalevaluation showed complete repair of the defect
Jafarian et al. (76)	2008	10mm Mandibular alveolar ridge	BMSCs	HA/TCP & Bio-Oss		After 6 weeks, histometric analysis showed 65.78% and 36.84% bone formation in HA/TCP and Bio-Oss groups, respectively
Shayesteh et al. (77)	2008	Maxillary sinus floor	BMSCs	HA/TCP		After 3 months, histometric analysis revealed a mean of 41.34% bone formation
Khojasteh et al. (78)	2008	5mm Parietal	BMSCs	Bio-Oss & βTCP		After 6 weeks, histometric analysis revealed 1.44 mm and 2.53 mm bone repair in the Bio-Oss and βTCP groups, respectively
Pieri et al. (79)	2008	Maxillary sinus augmentation	BMSCs	FAP		After 3 months, histometric analysis showed 42.51% new bone formation

BMSCs: Bone marrow mesenchymal stem cells – PLSCs: Periodontal ligament stem cells – DTSCs: Deciduous tooth stem cells – PRP: Platelet rich plasma – BMP: Bone morphogenetic protein – TCP: Tricalcium phosphate – HA: hydroxyapatite – FAP: Fluorohydroxyapatite

Composite scaffolds:

Composite scaffolds are a combination of polymer and ceramic scaffolds aiming at reducing each one's disadvantages. For example, when a biodegradable polymer like PLGA is combined with a bioactive material like CaP, the alkaline products derived from degradation of CaP can neutralize the acidic products derived from the degradation of PLGA. Also, these scaffolds imitate the complex properties of natural bone. However, some composite scaffolds are made by the combination of natural and synthetic materials.

Various combinations like hydroxyapatite/polyamide, hydroxyapatite/PLGA, Octacalcium Phosphate/Alginate, TCP/collagen and demineralized bone/PLA have been used in different studies (Table 6).

Table 6- Composite scaffolds

Authors	Year of Publication	Site & form of lesion	Type of stem cell	Type of scaffold	Growth factor	Result
Liu et al. (80)	2011	10mm Alveolus	DPSCs	nono-hydroxyapatite/ collagen/PLLA	BMP2	After 12 weeks, histometric analysis showed 61.16%±2.18% new bone formation
Kawai et al. (81)	2011	9mm Calvaria	BMSCs	Octacalcium phosphate/collagen		After 8 weeks, histometric analysis demonstrated 44.1%±1.7% new bone formation
Khadka et al. (82)	2011	8mm Calvaria	BMSCs	Nano-hydroxyapatite/ polyamide 6		After 8 weeks, histometric analysis showed more than 70% new bone formation
Qu et al. (83)	2011	8mm Calvaria	BMSCs modified	nono-hydroxyapatite/ polyamide6	bFGF gene	After 4 weeks, density of microvessels was about 70% and after 12 weeks they had more than 80% new bone formation
Kwan et al. (84)	2011	4mm Parietal	ADSCs modified	hydroxyapatite-coated PLGA	FGF2 gene	After 20 weeks, µCT showed almost complete repair of defect and formation of bone with more than 180 mg/cc mineral content
James et al. (85)	2011	4mm Calvaria	ADSCs	HA/PLGA		After 4 weeks, µCT demonstrated 80% new bone formation
Tsumanuma et al. (86)	2011	5*5mm Periodontal	BMSCs PLSCs APSCs	βTCP/Collagen		After 8 weeks, in all groups 70% new bone was formed but a higher amount of cement was formed when using PLSCs
Rhee et al. (87)	2011	8mm Calvaria	ADSCs	DBM and/or PLA		After 8 weeks, radiodensitometric analysis demonstrated 42.75% new bone formation in DBM/PLA+ADSCs group and 57.69% in DBM+ADSCs group
Li et al. (88)	2010	8*12mm Mandible	BMSCs modified	nano- HA /polyamide	BMP7 gene	After 8 weeks, histometric analysis showed 85.54%±2.07 new bone formation
Behnia et al. (18)	2009	Alveolar cleft	BMSCs	OsteoSet		After 4 months, CT scan showed 34.5% and 25.6% new bone formation
Fuji et al. (24)	2009	4.2mm Calvaria	BMSCs	octacalcium phosphate precipitated (OCP) alginate		Results demonstrated that OCP can enhance the adhesion of cells to the scaffold
Ko et al. (89)	2008		hMSCs	demineralized bone powders/PLA		After 12 weeks, histological assessment and µCT showed almost complete repair oflesion and formation of bone with a density of 0.30 g/cm^2
Wang et al. (90)	2007	8*12mm Mandible	BMSCs	nano- HA /polyamide		After12 weeks, histological assessment showed more than 80% new bone formation
Cowan et al. (91)	2005	4mm Calvaria	BMSCs, ADSCs	apatite-coated PLGA	BMP2 & Retinoic acid	µCT demonstrated new bone formation with 60-70% density after 2-4 weeks

BMSCs: Bone marrow mesenchymal stem cells – ADSCs: Adipose derived mesenchymal stem cells – DPSCs: Dental pulp stem cells – APSCs: alveolar periosteal stem cells – PLSCs: Periodontal ligament stem cells – hMSCs: human mandibular mesenchymal stem cells – FGF:Fibroblast growth factor – BMP: Bone morphogenetic protein – TCP: Tricalcium phosphate – HA: hydroxyapatite – DBM:Demineralized bone matrix – PLGA: poly (lactic-co-glycolic acid) – PLA: poly (D, L-lactic acid)

Discussion:

Although the importance of cell-and growth factor-driven bone reconstruction strategies has been well documented, this study focused on scaffold-driven approaches.

The exact characteristics of scaffolds have been evaluated in several review articles (31-33). The scaffold of choice can be selected through evaluation of these scaffolds and comparing them with an ideal model. However, it should be noted that to date, no scaffold has been found to meet all the required criteria for an ideal scaffold. In order to ensure successful treatment of a bone defect, a scaffold should have at least 3 characteristics. Firstly, it should have correct anatomical geometry. Secondly, the scaffold should be able to endure mechanical loads present in the area especially if it has not undergone incubation in bioreactor and has been implanted directly in its respective location in the tissue. Last but not least, the scaffold should enhance the regeneration ability of growth factors.

Natural scaffolds:

Natural organic scaffolds:

Review of literature reveals that out of 18 studies using natural scaffolds, 11 had used bone marrow stem cells (BMSCs). Other cells used in these studies with a lower frequency included dental pulp stem cells (cDPSCs) or puppy deciduous teeth stem cells (pDTSCs), adipose-derived stem cells (ADSCs), umbilical cord blood mesenchymal stem cells (UCB-MSCs), human embryonic stem cell-derived mesenchymal stromal cells (ESC-MSCs) or muscle-derived stem cells (MDSCs).

The animal models in these studies were mice, rats, dogs, Guinea pigs or rabbits. The highest number of studies has been conducted on rats. Three studies had been conducted on humans.

Size of bone defects created in understudy animal models was usually between 5 to 15mm and these defects were usually created in calvaria and skull and occasionally in alveolar ridge, maxilla or mandible.

Of these 18 studies, in 4 a growth factor was used along with a scaffold. In 4 studies, cells were transfected with the growth factor. In other studies no growth factor was used and sometimes the type of scaffold used contained growth factors (like PRP).

Evaluation of the repair and filling of bone defects was usually done after 1 to 3 months using different methods. The more common procedures included histology, histomorphometry, and μCT tomography. Bone densitometry, spectroscopy, biomechanical tests and Vickers hardness test were also used. Some studies have used Immunohistochemistry and gene expression analysis in the laboratory phase.

Evaluation of outcome in these studies reveals that in the majority of them, concomitant use of scaffold, cells and growth factor or scaffold and cells yields better results compared to using cells or scaffold alone. In 2 studies however, no significant difference was detected between repair of defect after using scaffold and cells and repair without the graft material. The results of human studies have been satisfactory.

In some studies, a comparison was done between different natural scaffolds and results demonstrated that type I collagen yields better results compared to alginate. Also, comparison of repair of 5 mm bone defects in mice calvaria by using muscle-derived stem cells (MDSCs) transfected with BMP4 gene along with 3 types of scaffolds of collagen gel (CG), Fibrin sealant (FS) and Gelatin sponge (GS) demonstrated that defect repair with these 3 scaffolds is different in terms of volume, shape and morphology. Defects repaired with GS scaffold produced hypertrophic bone while CG and FS scaffolds resulted in formation of bone very similar to calvaria natural bone (14).

Natural inorganic scaffolds

Of a total of 10 studies using inorganic natural scaffolds, 6 used bone marrow stem cells (BMSCs). The remaining studies used adipose-

derived stem cells (ADSCs) and induced pluripotent stem cells (iPSCs).

Animal models used in these studies were mice, dogs, cows and rabbits. The highest number of studies was conducted on mice. To date, no human study has been performed using these scaffolds. Size of bone defects created in animal models was usually between 1.5 to 30 mm and these defects were usually created in calvaria, and sometimes in parietal bone, orbit, ramus, skull or mandible.

In 4 of these 10 studies, no growth factor was used. In another 4 studies, cells were transfected with the respective growth factor. In the remaining 2 studies, growth factors were used.

Evaluation of the repair of defects within 1 to 6months after the intervention was done using histology, histomorphometry, micro CT and sometimes radiography. These studies also confirmed the synergistic effect of simultaneous use of scaffold, cells and growth factor or scaffold and cells.

Synthetic scaffolds:

Synthetic polymer scaffolds:

In these 11 studies bone marrow stem cells (BMSCs) were mainly used and only in one study adipose-derived stem cells (ADSCs) were employed.

The animal models used in these studies were goats, mice, and rabbits. The majority of studies were conducted on mice and rabbits. No human study has been conducted by using these scaffolds. Size of created defects was usually within 5 to 15 mm and these defects were often created in calvaria and sometimes in the skull, condyle, mandible or extracted tooth socket. In 7 of these 11 studies no growth factor was used.

Evaluation of the repair of bone defects was usually done within 1 to 6 months after intervention using methods similar to those used in previous studies.

In these studies chemical modifications of scaffolds were also noted. For example, comparison of the protease sensitive PEG matrix metalloproteinases (PEG-MMP) scaffold with Poly (Ethylene Glycol)-Diacrylate(PEG-DA) scaffold revealed that PEG-DA even in presence of stem cells and growth factors inhibits bone formation (64). Additionally, use of fibronectin-coated polylactic acid (PLA) with adipose-derived mesenchymal cells (ADCs) for repair of rabbit skull defect demonstrated that fibronectin-coated PLA scaffold can efficiently enhance its effect on repair of such defects. Also it was shown that when stem cells are placed in an osteogenic environment before implantation, a higher percentage of new bone formation is achieved (65). Gradual release of growth factors by using these scaffolds has also been noted.

Comparison of 3 scaffolds of PLGA, PLLA and PLA-PEG revealed that PLGA had the greatest ability to enhance adhesion, proliferation and differentiation of cells and subsequent repair of bone defect (67).

Since 2003, use of rapid prototyping technique for fabrication of these scaffolds has been the focus of attention aiming at obtaining scaffolds with controlled geometry and optimal microscopic structure (70).

Synthetic ceramic scaffolds:

These studies have been conducted on mice, rabbits, pigs, Guinea pigs, and dogs. One clinical study on human using these scaffolds has also been reported. In these studies, bone marrow stem cells (BMSCs) has been mainly used and only in one study deciduous teeth stem cells were employed.

The important point that needs to be considered when using this type of scaffolds is their porosity. Comparison of these scaffolds with Bio-Oss demonstrated their superiority. However, when compared with Bioglass no difference was detected.

Composite scaffolds:

Studies performed on composite scaffolds used along with stem cells and growth factors have shown that compared to all groups even autogenous bone grafts, quicker and greater osteogenesis was observed inside the defect

when using these scaffolds.

Comparison of the efficacy of bone marrow stem cells (BMSCs), periodontal ligament stem cells (PLSCs) and alveolar periosteal stem cells (APSCs)used along with βTCP/collagen scaffold for repair of a 5 mm defect on a periodontal wall in a dog revealed that greater amounts of new cement, new periodontal fibers and alveolar bone were formed in the group where PLSCs along with βTCP/collagen scaffold had been used. Also, only in this group nerve filaments were observed in reconstructed PDL (86).

Conclusion:

Based on the present study results, in the majority of reviewed articles BMSCs were used for bone reconstruction and they are still considered as the gold standard for bone tissue regeneration. Growth factor is one of the three elements of tissue engineering. However, it has been less frequently used in the mentioned studies and occasionally scaffolds that are a reservoir for growth factors have been used. Among growth factors, BMPs especially BMP2 have had the highest application. Additionally, PRP which has also been used as a scaffold contains osteoinductive proteins and can be used as a growth factor as well.

Among organic natural scaffolds, PRP and collagen sponge have been more frequently used compared to others and it seems that collagen sponge has had more satisfactory results. Coral and silver are the most frequently used organic natural scaffolds and the outcome of using corals has been better than that of silver.

Among the synthetic polymer scaffolds, PLGA has been the most commonly used yielding favorable results. Also, HA/TCP has been more successful than other synthetic ceramic scaffolds. Composite scaffolds are usually fabricated by combining hydroxyapatite and synthetic polymer scaffolds. Application of composite scaffolds for reconstruction of craniomaxillofacial defects has been less common than other scaffolds but has increased during the recent years.

In general, the best results were obtained when using coral scaffolds followed by composite scaffolds with more than 80% osteogenesis.

In order to achieve optimal results in bone tissue engineering, there are some factors that have to be considered by researchers including:

1- Clinical studies: To date, limited number of clinical studies has been conducted on humans and in each study sample size has been very small.
2- Animal model studies: These studies have mostly focused on reconstruction of small defects in small animals. In order to be able to generalize the results to human beings, larger animals with biological structures and immune systems similar to those of humans have to be studied.
3- Control groups: Appropriate selection of control groups can help in better interpretation of results and more clearly demonstrating the effect of each variable.
4- Time: Most studies have evaluated the outcome after a short time period. Follow up of samples for longer periods of time can better demonstrate the results and the possible side effects of materials used.
5- The tissue engineering triangle: In a small number of studies all 3 elements of tissue engineering have been used. It is important to know what factors are required to imitate natural healing and repair and be aware of the fact that these factors have to be gradually released into the environment
6- Evaluation of results: There are various methods to present the study results; but, it should be noted that angiogenesis and immunologic reactions are among the most important factors in bone reconstruction and therefore have to be precisely evaluated. Also, presenting the qualitative results of osteogenesis can help for comparison with similar studies.

Although tissue engineering can theoretically have an application in dentistry, its clinical application has not gained popularity since it is not cost effective and has some considerations related to cellular manipulation. However, its perspective attracts dentists and researchers.

In this review study on the scaffolds used for reconstruction of craniomaxillofacial bone defects, it was revealed that there is still a long way till achieving an ideal treatment. Although scaffold has been proposed as the key factor in success of tissue engineering, after 20 years of tissue engineering introduction, an ideal scaffold has yet to be designed. Studies have been mostly performed on reconstruction of small defects and characteristics like angiogenesis and bone physiology have been less considered.

The future perspective of bone tissue engineering seems to include application of rapid prototyping technique for the fabrication of a custom-made composite scaffold using patient's CT and MRI images along with the use of genetically modified stem cells.

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