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Review Article

The role of stem cells in oral bone regeneration

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Abstract

Oral bone regeneration has revolutionized implant dentistry by enhancing both the quantity and quality of the available bone, ultimately leading to improved treatment outcomes. In recent years, the use of stem cells for regenerative purposes has shown promising results. The aim of this study was to review the current literature concerning the clinical applications of Mesenchymal Stem Cells (MSCs) and/or Adipose Stem Cells (ASCs), for the treatment of oral osseous defects. An electronic search of Ovid Medline database was conducted. All types of human studies that applied MSCs and/or ASCs for implant site development were included. Pre-clinical and in vitro studies were excluded. Out of the initial 282 identified records, 22 were considered for inclusion. Even though most reports showed some benefit after the use of MSCs/ASCs, the review indicated that there is lack of consensus, regarding the methodology and the outcomes assessment. The clinical significance and the feasibility should be further studied with standardized techniques.

Keywords: Bone defects, Bone regeneration, Stem cells

Introduction

Bone defects in the oral cavity differ greatly, ranging from limited alveolar bone loss to large-scale bone atrophy. A "jaw (bone) defect" is defined as the lack of bone volume where it should normally exist[1]. A variety of reasons can cause jaw deformities such as extractions/tooth loss, periodontitis, periapical infections, injuries due to facial trauma, tumors or cyst resections, congenitally missing teeth or developmental conditions (cleft palate defects), osteomyelitis, osteoradionecrosis and drug-induced osteonecrosis[2]. The most common cause is the post-extraction alveolar ridge collapse, which is inevitable and leads to bone loss in both horizontal and vertical dimensions[3]. The above is clinically relevant when implant replacements are to be inserted as their position in the jaws should be driven by the restorative plan[4].

Regardless of the cause of jaw deficiencies, reconstruction of the maxilla and the mandible is imperative for the return to form and function. Reconstructive jaw surgery may involve a multitude of different bone grafting materials such as autografts, allografts, xenografts and alloplasts[5]. Autologous bone grafts are considered to be the gold standard for bone regeneration because of histocompatibility and their osteogenic, osteoconductive and osteoinductive properties[6]. However, autografts show some disadvantages due to their limited supply, donor-site morbidity and potential infections[7]. On the other hand, allografts and xenografts do not have the problem of limited supply and do not require a donor site but they have poor osteoinductive properties due to the absence of cell populations[8]. To overcome these difficulties, new advanced techniques have been employed and one of the most promising is the use of stem cells[9].

By the term "stem cells" we define the cells with the ability to grow into anyone of the human body's more than 200 cell types, responsible for the foundation of each and every organ and tissue[10]. They have two defining characteristics; the ability of unlimited self-renewal and the ability to differentiate into specialized adult cell type with specific functions, when stimulated by both external and internal signals[11].

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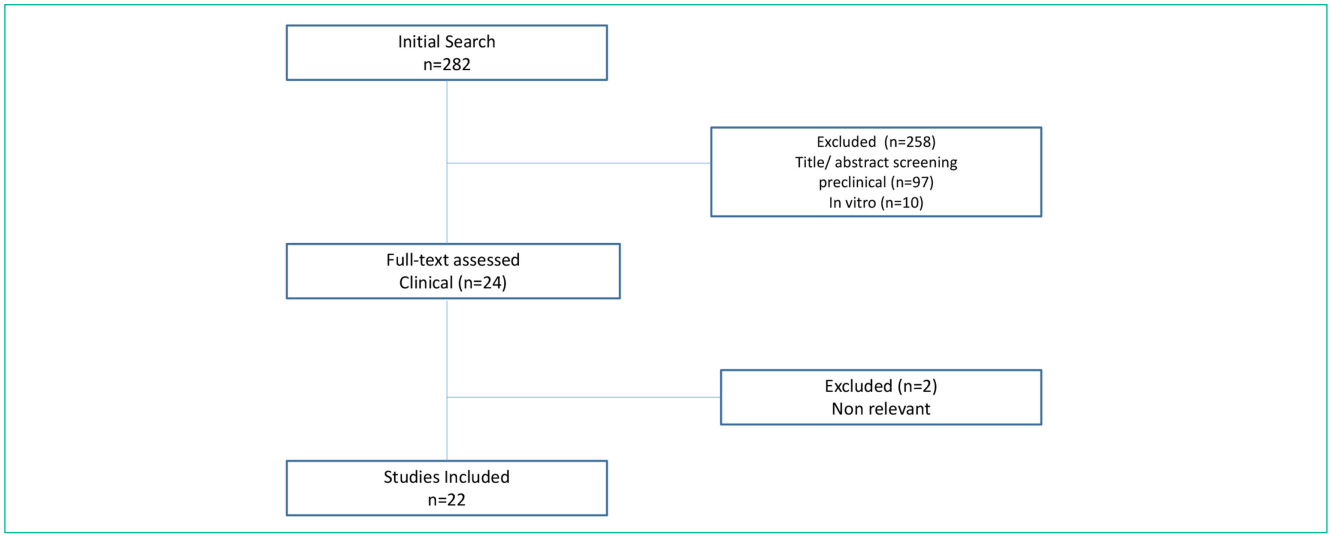

Figure 1. Flow chart of the paper selection process.

Based on their biological properties, stem cells can be divided into pluripotent and multipotent. The former, have the ability to differentiate into all cell types in the body. They are only present in the fetus before differentiating into the more specialized multipotent stem cells. In reference to the origin of stem cells, they can be obtained from the inner cell mass of the blastocyst (pre-implantation embryos), fetal tissue and adult tissue[12,13]. Adult stem cells - also called somatic- are found in the mesenchyme of many tissues and organs (Mesenchymal Stem Cells/MSCs) and they are responsible for their maintenance and repair when damaged or lost cells must be replaced. MSCs, apart from the bone marrow where they exist in abundance, have also been isolated from other tissues such as the skin, lung, adipose tissue (ASCs) and teeth (dental pulp, periodontal ligament and exfoliated deciduous teeth)[14,15].

In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed the following minimal criteria to define human MSCs[16]:

- they must be plastic-adherent in standard culture conditions;
- they must express CD105, CD73 and CD90;
- they must not express CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-Dr surface molecules;
- they must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*.

Tissue engineering combines biological components, such as cells and growth factors, with engineering principles and biomaterials[17]. It is based on the ability of stem cells to be artificially guided in the direction of particular cellular phenotypes under the influence of favorable microenvironments. Currently, different technologies and application protocols are being studied on this field.

The aim of this study was to review the recent literature concerning clinical applications of MSCs and/or ASCs, utilized to enhance the regeneration of oral bone tissue prior to implant placement.

Materials and methods

An online research was conducted in September 2020 using the Ovid Medline Database, including articles published in English during the last twenty years. The search strategy used was as follows: ("Bone Regeneration"[MesH] OR "Alveolar Ridge Augmentation"[MesH] OR "Tissue Engineering"[MesH] OR "Guided Bone Regeneration"[MesH] OR "Osseous Repair"[All Fields]) AND ("Jaws" OR "Alveolar Process"[All Fields]) AND ("Wound Healing"[MesH] OR "Biology'[MesH] OR "Physiology"[MesH] OR "Molecular'[MesH]OR "Cellular"[MesH][All Fields]) AND ("Barrier"[MesH] OR "Membrane"[MesH][All Fields]) AND ("Stem Cells"[All Fields]) AND "2000/01/01" AND "English"[Lang].

All levels of evidence were included, except reviews. The following exclusion criteria were applied:

- Pre-clinical studies.
- *In vitro* studies.
-Temporomandibular joint (TMJ) regeneration.
- Regeneration of dental and periodontal defects.
- Studies that involved the use of stem cells for distraction osteogenesis.
- Reconstruction of defects following removal of benign and malignant tumors/cysts.

Table 1. List of the selected clinical trials included in the analysis.

Reference	Stem cell type	Collection	Subculture	Origin	N	Carrier	Defect type	Graft location	Cover	Control	Time for analysis	Analysis	Primary outcomes	Implants	Restoration	Follow-up after restoration	Implant survival rate	Complications
Bajestan et al 2017[35]	MSC + MNC + Monocytes	BMA	Yes (12 days manually, no induction)	IB	18p (8 control + 10 test)	β-TCP	Horizontal	AM, PM,AMn	CM	Autogenous Block+ABG	4-8 months	CT	Gain of crest width: control 3,3±1,4 mm versus test	YES	YES	3-6 months	N/S	1 implant failure
Gimbel et al 2007[11]	N/S	BMA	No (whole aspirate)	IB	21 test + 25 contr + 23 contr	CS	Cleft Palate	AM	NO	IB	1 day - 1 - 3 - 6 weeks - 6 months	Comfort+ complications donor-site	Best results in test group followed by conventional iliac graft	NO	NO	N/A	N/A	test: 2 granulation tissues contr1 + 2: oronasal fistula
Gonshor et al 2011[21]	MSC	CBA	NO	N/S	18/8 bilats + 10 unilats (26 sites)	CBA	Sinus Lift	PM	NO	Allograft	3,7±0,6 months	CT+H+Hm	Vital bone: 32,5±6,8% test vs 18,3±10,6% control	YES	NO	N/A	N/S	2 Patients lost
Kaigler et al 2013[22]	MSC	BMA	Yes (12 days-automated induction)	IB	12+12	CS	Alveolar Reconstruction	M+Mn	CM	CS	6 or 12 weeks	Rx+μCT+H	Linear bone height 6w: 55,3%-78,9% contr vs test/12w: 74,6%-80, 1% contr vs test	YES	YES	1 year	100%	N/S
Khojasteh et al 2016[14]	ASC	SAT	Yes	BFP	4+4	FDBA	Alveolar Reconstruction	M+Mn	CM	IB	5 months	CT+H	Bone width gain: control 3,01±0,89/ test 3,94±1,62	YES	N/S	N/A	N/A	N/S
Katagiri et al 2016[17]	MSC-CM	Commercially available hBM-derived MSC	Yes (48 hours)	N/S	8	TCP or ACS	Sinus Lift/ GBR/ Socket preservation	N/S	PLGA	TCP/ACS	1 - 3 - 6 months	CT+H+ Panor x-Ray	newly formed bone, early mineralization	YES	N/S	N/A	N/A	N/S
Khojasteh et al 2018[16]	ASC	SAT	Yes (1-2 weeks/ no induction)	BFP	7+7	ABBM	Horizontal + Vertical	PMn	TM	Autologous bone	6 months	CT	Area of new bone formation: 169,5±5,9 mm (test) vs 168,05±10,69 mm (contr)	YES	N/S	1 year	100%	N/S
Marx et al 2014[24]	MSC	BMA	No (whole aspirate)	IB	20+20	CS + BMP - 2 + Allogenic bone	Alveolar Reconstruction	Mn	TM or bone struts	CD34+ 54±38 cells/mL	4 weeks - 3 - 6 months	CT+Hm	40% of contr achieved regeneration of implantable bone vs 100% of test/Mean Trabecular Bone Area 36±10% contr vs 67±13% test	YES	N/S	N/A	N/A	N/S
Prins et al 2016[36]	ACS	SAT	NO	AW	10 Pat (5+5)/ 16 sites (6 split mouths)	TCP or BCP	Sinus Lift	PM	NO	TCP or BCP	5 - 6 - 9 months	Panoramic x-Ray+Hm	boned osteoid percentages higher in study biopsies	YES (44)	YES	More than 2,5years	97,70%	1 implant failure
Rickert et al 2011[37]	MNC	BMA	NO	IB	12 split mouths/ 24 sinuses	BBM	Sinus Lift	PM	CM	BBM+ Autogenous graft	14,8±0,7 weeks	Hm	new bone 17,7±7,3% (test) vs 12±6,6% (contr)	YES (66)	YES	N/S	N/S	3 implant failures

MSC=Mesenchymal Stem Cells, MNC=MonoNuclearCells, BMA=Bone Marrow Aspirate, IB=Iliac Bone, TCP=TriCalciumPhosphate, AM=Anterior Maxilla, PM=Posterior Maxilla, AMn=Anterior Mandible, CM=Collagen Membrane, ABG=Allogenic Bone Graft, CT=Computed Tomography, N/S=Not Specified, CS=Collagen Sponge, N/A=Not Applicable, CBA=Cellular Bone Allograft, H=Histology, Hm=Histomophometry, M=Maxilla, Mn=Mandible, Rx=Radiography, ACS=Adipose Stem Cells, SAT=Subcutaneous Adipose Tissue, BFP=Buccal Fat Pad, FDBA=Freeze Dried Bone Allograft ,MSC-CM=Mesenchymal Stem Cells-Cultured Medium, ACS=AteloCollagenSponge, GBR=Guided Bone Regeneration, PLGA=PolyGalacticAcid, ABBM=Anorganic Bovine Bone Mineral, PMn=Posterior Mandible,BMP-2=Bone Morphogenetic Protein-2, TM=Titanium Mesh, AW=Abdominal Wall ,BCP=Biphasic Calcium Phosphate, BBM=Bovine Bone Marrow

Table 2. List of the selected case series/case reports included in the analysis.

Reference	Study design	Stem cell type	Collection	Subculture	Origin	N	Carrier	Defect type	Graft location	Cover	Time for analysis	Analysis	Primary outcomes	Implants	Restoration	Follow-up after restoration	Implant survival rate	Complications
Ahn et al 2018[23]	CR	MSC	BMA	No (whole aspirate)	IB	1	PCL	Cleft Palate	AM	NO	6 months	CT	oronasal fistula closure+BV of newly formed 45% of total defect volume	NO	NO	N/A	N/A	4months: exposure of PCL and removal of plate +screws
Behnia et al 2009[38]	CS	MSC	BMA	Yes (2 wks manually/ no induction)	IB	2	DBM + Calcium Sulfate	Cleft Palate	AM	NO	4 months	CT	oronasal fistula closure+25,6-34,5%bone defect fill	NO	NO	N/A	N/A	N/S
Behnia et al 2012[39]	CS	MSC	BMA	Yes (2 wks manually/ no induction)	IB	4	HA / TCP + PDGF	Cleft Palate	AM	Fibrin clot	3 months	CT	oronasal fistula closure+51,3% bone defect fill	NO	NO	N/A	N/A	N/S
Cerruti et al 2007[10]	CS	MNC	BMA	No (whole aspirate)	IB + SB	32	AB + PPP + PRP	Vertical, Horizontal, Sinus lift	AM + PM	N/S	4 months	H+CT	Width 6-14mm Height= 10mm (AM) and 6-15mm (PM)	YES	YES	4 years	100%	1 graft not intergrated+1 sinus infection
Hibi et al 2006[34]	CR	MSC	BMA	Yes (2 wks manually/ osteogenic induction)	IB	1	PRP	Cleft Palate	AM	TM	3-6-9 months	CT	79,1% bone coverage	NO	NO	N/A	N/A	N/S
Kaigler et al 2010[40]	CR	MSC	BMA	Yes (12 days manually/ no induction)	IB	1	GS	Extraction Socket	N/S	CM	6 weeks	μCT+H	Bone defect fill	NO	NO	N/A	N/A	N/S
Khojasteh et al 2019[15]	CS	ASC	SAT	Yes (manually/ no induction)	BFP	2	BBM	Horizontal + Vertical	M + Mn	CM	6 months	CT+H	Newly formed bone	YES	NO	48 months	100%	N/S
Rajan et al 2014[41]	CR	MSC	BMA	Yes (12 days manually/ no induction)	IB	1	TCP	Horizontal + Vertical	AM	CM	4-6-12 months	CT+Hm	Width 5-6 mm gain +80% regeneration Height	YES	YES	6 months	100%	N/S
Shayesteh et al 2008[31]	CS	MSC	BMA	Yes (4 wks manually/ no induction)	IB	7	HA / TCP	Sinus Lift	PM	CM	3-12 months	Rx+Hm	New bone: 41.34% / radiographic bone height: 2.25–12.08–10.83 (baseline-postgraft-1 y)	YES[30]	YES	6 months	93%	2 implants lost
Smiler et al 2007[32]	CS	N/S	BMA	No (whole aspirate)	IB	5 (7 sites)	Xenograft, Allograft or Alloplast	Sinus Lift or Horizontal	PM	CM + TM	4-7 months	H+Hm	23-45% new bone formation / No difference between carriers are statically reported	NO	NO	N/A	N/A	N/S
Valdivia et al 2017[42]	CR	MSC + Monocytes	BMA	NO	IB	1	Xenograft	Horizontal + Vertical	AM	CM	7 months	CT+Hm	New bone formation / presence of osteocytes	YES	NO	N/A	N/A	N/S
Yamada et al 2013[33]	CR	MSC	BMA	Yes (4wks manually / osteogenic induction)	IB	1	PRP	Horizontal +Vertical	PMn	CM +TM	7 months	CT+H	4,2mm height bone gain /new mature bone	Yes (3)	YES	2 years	100%	N/S

CR=Case Report, CS=Case Series, MSC=Mesenchymal Stem Cells, MNC=MonoNuclearCells, ACS=Adipose Stem Cells, N/S=Not Specified, BMA=Bone Marrow Aspirate, SAT=Subcutaneous Adipose Tissue, IB=Iliac Bone, SB=Sternum Bone, PCL=PolyCaproLactone, DBM=Demineralized Bone Marrow, HA/TCP=Hidroxyapatite/tricalcium phosphate, PDGF=Platelet Derived Growth Factor, AB=Allograft Block, PPP=Platelet Poor Plasma, PRP=Platelet Rich Plasma, GS=Gelatine Sponge, BBM=Bovine Bone Marrow, AM=Anterior Maxilla, PM=Posterior Maxilla, M=Maxiila, Mn=Mandible, PMn=Posterior Mandible, CM=Collagen Membrane, TM=Titanium Mesh, CT=Computed Tomography, H=Histology, Hm=Histomorphometry, Rx=Radiography, N/A=Not Applicable

Results

The author (GK) carried out the search in duplicate and in case of uncertainty about the inclusion of an article the decision was taken after consulting an experienced reviewer (KS). A total of 282 articles were identified by the database search. Applying the exclusion criteria at the title and abstract level, 24 articles were finally selected for full-text screening. Of those, 22 met the inclusion criteria and were considered for analysis and discussion (Figure 1). Out of the 22 articles, 10 corresponded to clinical trials (Table 1), 7 to case reports and 5 to case series (Table 2). In total, they assessed the application of MSCs in 289 patients.

Cell Type

All studies reviewed used adult stem cells from various tissues. Specifically, 16 studies reported the use of Bone Marrow Aspirate (BMA)[18-33], 4 the use of Subcutaneous Adipose Tissue (SAT)[34-37] and 2 used cellular bone allograft (CBA)[38,39].

Stem cells were generally isolated from bone marrow aspirates from the iliac bone. Only one reported as a source the sternum bone along with the iliac crest[26]. This highlights that this specific harvesting technique is widely adopted. BMSCs (Bone Marrow-derived Stem Cells) from the iliac bone have been thoroughly studied and documented, as they are easily collected and expanded and retain great potential for differentiation. Gimbel et al. confirmed that BMA from the iliac crest resulted in reduced morbidity and increased patient comfort compared to the traditional harvesting technique for autologous graft, from same location[19].

ASCs (Adipose-derived Stem Cells) are easily accessible via liposuction (less invasive harvesting procedure) from areas such as the abdominal wall or the buccal fat pad and they are much more abundant (0,5-2 x 10^6 ASCs/g adipose tissue)[40,41]. Applications in the human body[34-37] evaluated the efficacy of ASCs for bone regeneration. In all cases, newly formed bone was seen and there was no evidence of immune response.

There are also commercially available human MSCs. Katagiri et al.[39] applied hMSCs from Lonza Inc. (Walkersville, MD, USA) and cultured them in a mesenchymal stem cell basal medium. Following incubation, the cell culture-conditioned medium was collected, and this was defined as MSC-CM. The results in all patients showed early bone formation with increase trabecular density. This fact justified the potential of MSCs to differentiate and promote osteogenesis responding to the environment, while there is no need for autograft harvesting, protecting the patients from discomfort and possible complications in the donor-site.

Culture and Preparation methods

There are several different approaches in terms of handling the population of the collected cells. Some researchers used the whole aspirate without expansion or differentiation, loaded into scaffolds[19,21-23,26,31,32,36,38]. In other studies, stem cells were isolated and expanded, under certain conditions (in a basal medium with autologous serum/no osteogenic induction)[18,24,25,28-30,34,35,37]. Finally, other studies used an expansion protocol with osteogenic factors such as dexamethasone (100 μM), beta-glucophosphate (10 μM) and L-ascorbic acid (25-50 μg/ml) known to induce osteogenic differentiation[20,27,33]. This automated culture system expands the quantity of bone reconstruction cells (hematopoietic and mesenchymal stem cells)[42]. Although no clinical comparison has been performed between processed BMA and non-processed BMA, the outcomes from the clinical trials showed similar efficacy in terms of bone regeneration whether osteogenic induction was performed or not[20,38].

Carriers and Membranes

Several types of materials have been used as carriers(scaffolds) to deliver the cells into the defect being treated. These vary from alloplastic (β-TCP with or without HA)[18,25,29,30,36,39] to xenografts (mainly bovine bone)[22,31,32,35,37], allograft (freeze-dried bone allograft/ FDBA)[34] or autografts (PRP concentrate)[27,33].

The ideal scaffold must be biocompatible (without immune response), biodegradable (preferably the time of resorption to follow the time of bone reconstruction), porous so as to allow the cell to adhere, and with the appropriate shape for filling the bony defect[43]. One study compared the use of β-TCP to atelocollagen sponge (ACS) as carriers for MSCs[39]. Newly bone formed was seen in both cases with mild presence(β-TCP) or absence of inflammatory cells (ACS). ACS was more easily resorbed than β-TCP and its use resulted into denser bone formation.

3-D printing technology offers the possibility of patients-specific custom-made scaffolds. Ahn et al. reported in 2018[23] the first case of alveolar cleft palate repair using a 3D printed bioresorbable polycaprolactone (PCL) scaffold loaded with MSCs from iliac bone marrow aspirate. After 6 months 45% of the total defect volume was full of new bone.

Some studies used these carriers combined and mixed with or without additional factors such as Bone Morphogenetic Protein-2 (BMP-2)[21] or Platelet-Derived Growth Factor (PDGF)[25].

Cerruti et al.[26] used bone marrow whole aspirate combined with bone allograft and platelet growth factors (PPP: platelet-poor plasma and PRP: platelet rich plasma). PPP was used to produce fibronectin and laminin, known to attract MSCs[44]. PRP was mixed with the bone allograft and the stem cells and were all placed into the grafted area. Even though some studies have shown contradictory results regarding the regenerative capacity of PRP[45,46,47,48], it can be concluded that it presents with multiple advantages: it offers bleeding reduction[49], rapid healing of the soft tissues[50] and due to its contents, especially PDGF and TGFs (Transforming Growth Factors -α, -β) influences bone regeneration[51]. These factors along with VEGF (Vascular Endothelial Growth

Factor) and IGF (Insulin-like Growth Factor) play significant role in the angiogenesis, the proliferation and differentiation of MSCs, enhancing the regenerative procedure[52].

Another important difference was identified on the use and the type of membrane/barrier. Some studies used collagen membranes[30,37] or titanium mesh[31,33] to cover the grafted area while others did not use any membrane at all[19,27].

Defect Type

Regarding the type of defect being treated, this ranged from small sized deficiencies (post-extraction sockets)[28,39] to extensive bone deformities (cleft palate)[19,23-25,27]. Specifically, 77 cleft palates were repaired, 115 sinus elevations were performed, 109 horizontal and/or vertical augmentations and 4 post-extraction sockets were preserved. Bone regeneration varied greatly from site to site.

Clinical Results

Overall, the methodologies applied were not comparable to extrapolate robust conclusions. However, several studies indicated the benefit of the use of stem cells. Gonshor et al.[38] reported a histological advantage of using stem cells carried in a cellular bone allograft after sinus augmentation (32.5%±6.8% vital bone for the test group versus 18.3%±10.6% of the control group). Another study demonstrated that cell therapy accelerated the bone regeneration process[20]. The treated sites were analyzed clinically, radiographically and histologically and at 6 weeks, the linear bone height was 55.3% in the control group versus 78.9% in the test group (p=0.01). After 12 weeks, it was 74.6% (control) versus 80.1% (test) (p=0.28). Katagiri et al.[39] reported newly formed bone, early mineralization and dense trabecular bone when MSC-CM were used. Without cell loading, β-TCP was scarcely replaced, and inflammatory cells were found around the alloplastic β-TCP. A study worth mentioning was conducted from Marx and coworkers[21]. The aim of this trial was to investigate the role of the bone marrow derived CD34+cells in bone regeneration. Both groups received the same grafting materials (BMA+rhBMP-2+ allogenic bone). The difference was that in control group, BMA contained 54±38 cells/mL CD34+ whereas in test group the CD34+ cell concentration was 1012±752 cells/mL. All patients in test group achieved regeneration of implantable bone but only 40% of the control group did so. This difference was statically significant (p=0.006). These findings showed that CD34+ cell (hematopoietic stem cell) is a master signaling cell and a cell count of 200/mL CFU or greater was correlated to a successful clinical outcome, probably driving regeneration via angiogenesis and vasculogenesis.

Bajestan et al.[18] treated 18 patients with horizontal alveolar ridge atrophy-10 secondary to trauma and 8 with cleft palate. The patients received either conventional autogenous block graft or stem cell therapy. After 4 months, the mean gain in bone width was 1.5±1.5 mm in the stem cell therapy group and 3.3±1.4 mm in the control group. The mean bone gain was higher in trauma patients as compared to cleft palate patients for both the control and the study groups, showing that stem cells may have the ability to treat large alveolar defects but their ability to completely reconstruct a large craniofacial defect (cleft palate) was limited.

In 2016, Khojasteh et al.[34] assessed the efficacy of buccal fat pad derived stem cells (BFPDSCs) with iliac bone block grafting for the regeneration of extensive jaw atrophy. New bone formation was 65.32% in the test group versus 49.21% in the control group. It appears that the application of BFPDSCs may increase the amount of newly formed bone and also may decrease secondary bone resorption. Another study[35] where BFPDSCs were used, revealed new bone formation of 169.5±5.9 mm^2 in the test group versus 166.75±10.05 mm^2 in the control group. In both studies, there were no statistically significant differences between the two groups (p>0.05).

Prins et al.[36] used freshly isolated autologous adipose stem cells (ASCs), seeded on either β-tricalcium phosphate or biphasic calcium phosphate for maxillary sinus floor elevation in one-step surgical procedure. Bilaterally treated patients received CaP plus ASCs on one side and CaP only on the opposite side. This intra-patient evaluation clearly demonstrated the efficacy of ASCs for bone regeneration in the grafted areas which were supplemented with ASCs as higher volume of osteoid and higher bone contents were found in the study group. Micro-CT analysis showed higher BV (Bone Volume)/ TV (Tissue Volume) percentages at test sides than at control sides (19.5%±3.8% versus 13.7%±4.4%). The difference was statically significant (p=0.03).

Similar results were also concluded from other study, using mononuclear stem cells harvested from the iliac crest. Twelve patients were treated, needing reconstruction of their atrophic maxilla. A bilateral sinus floor augmentation was performed to each patient (split-mouth design). The test side was augmented with bovine bone mineral (BioOss®) mixed with autogenous stem cells. In the control side, BioOss® mixed with autogenous bone harvested from the retromolar area was applied. Significantly more bone formation was observed in the test group: 17.7±7.3% compared to the control group, 12.0±6.6% (p=0.026).

In all case reports included in this review, the results of stem cell therapy were encouraging. When MSCs were used for cleft palate reconstruction[23-25,27], oronasal fistula closure was observed and a total of 34.5% to 79.1% bone defect filling was occurred depending on the follow-up period (3 to 9 months). Where horizontal and vertical augmentation were performed[29,32,33,37] newly formed bone was present and there was a gain in both width and height ridge's dimensions.

Discussion

Bone regeneration is often needed prior or during dental implant placement as it has been suggested that up to 40% of implants require some kind of GBR (Guided Bone Regeneration)[53]. A retrospective analysis showed that especially in the anterior maxilla, which is an esthetically demanding region, 74.7% of implants inserted, required additional local bone augmentation procedure[54]. Implant therapy has become an integral part of restorative dentistry and there is a growing demand for reduced healing times and application in medically compromised patients. Following the principles of personalized medicine and tissue engineering, stem cells have been used to enhance the regenerative procedures with promising results.

This review was performed to evaluate the clinical applications of stem cells in the treatment of oral bone defects using clinical, radiographic and histological analyses. Most reports demonstrated a greater amount of newly formed bone in the short term, when stem cells were applied, but the majority of the studies were case reports and case series. The absence of standardized clinical protocols and the fact that until now the existent clinical trials have failed to demonstrate statistically significant differences, clearly highlights the need for further research in the field. The feasibility of the use of different cell lines and scaffolds has been established but there is limited evidence regarding the selection of optimal combination for superior results in specific defect types. In addition, financial and patient reported outcome measures have not been consistently discussed and these should be incorporated in the design of future trials in order to evaluate the long-term benefits of stem cell-based therapies both at implant- and patient- level.

Our review had some inherent limitations, the most obvious of which was the small number of included reports for analysis. We restricted our search into human studies published during the last 20 years as we attempted to report on the most updated techniques and materials. The inclusion of preclinical investigations would have offered a greater perspective on the biological mechanisms but the results cannot be directly translated into clinical practice.

Conclusion

The review of the published literature has shown the advantages of mesenchymal stem cells in the field of oral bone regeneration. However, most studies failed to show a significant difference between control and test groups. Better knowledge of MSCs potential and the use of new advanced treatment strategies being developed now could establish cell therapy as a future clinical tool and reliable solution for use on patients.

References

1. Gordon L, Chiu EJ. Treatment of infected non-unions and segmental defects of the tibia with staged microvascular muscle transplantation and bone-grafting. J Bone Joint Surg Am 1988;70(3):377-86.
2. Giannobile WV. Commentary: treatment of periodontitis. Destroyed periodontal tissues can be regenerated under certain conditions. J Periodontol 2014;85(9):1151-1154.
3. Tan WL, Wong TLT, Wong MCM, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res 2012;23(suppl. 5):1-21.
4. Jivraj S, Reshad M. Esthetic implant dentistry: diagnosis and treatment planning. In: Frost RJ, editor. Oral and Maxillofacial Surgery. Elsevier; 2018. pp. 391-409
5. Dimitriou R, Jones E, McGonagle D, and Giannoudis PV. Bone regeneration: current concepts and future directions. BMC Med 2011;9:66.
6. Cypher TJ, Grossman JP. Biological principles of bone graft healing. J Foot Ankle Surg 1996;35(5):413-417.
7. Caminiti MF, Sandor GK, Carmichael RP: Quantification of bone harvested from the iliac crest using a power-driven trephine. J Oral Maxillofac Surg 1999;57:801-805.
8. Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Glogauer M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. Biomater Res 2017;21:9.
9. Padial-Molina M and Rios HF. Stem cells, scaffolds and gene therapy for periodontal engineering. Cur Oral Health Rep 2014; 1: 16-25.
10. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. Feeder Layer- and Serum-Free Culture of Human Embryonic Stem Cells, Biol Reprod2004;70(3):837-845.
11. Biehl JK, Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs 2009;24(2):98-105.
12. Pera MF, Cooper S, Mills J, Parrington JM. Isolation and characterization of a multipotent clone of human embryonal carcinoma-cells. Differentiation 1989;42:10-23.
13. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282:1145-1147.
14. Phinney DG and Prockop DJ. Concise review: mesenchymal stem/ multipotent stromal cells: the state of transdifferentiation and modes of tissue repair - current views. Stem Cells 2007;25(11):2896-2902.
15. ShiS, Bartold PM, Miura M, Seo BM, Robey PG, and Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8(3):191-199.
16. Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315-317.
17. Howard D, Buttery LD, Shakesheff KM, Roberts SJ. Tissue engineering: Strategies, stem cells and scaffolds. J Anat 2008; 213:66-72.
18. Bajestan MN, Rajan A, Edwards SP, Aronovich S, Cevidanes LHS, Polymeri A, Travan S, Kaigler D. Stem cell therapy for reconstruction of alveolar cleft and trauma defects in adults: A randomized controlled, clinical trial. Clin Implant Dent Relat Res 2017;19(5):793-801.
19. Gimbel M, Ashley RK, Sisodia M, Gabbay JS, Wasson KL, Heller J, Wilson L, Kawamoto HK, Bradley JP. Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cells in a resorbable matrix. J Craniofac Surg 2007;18(4):895-901.
20. Kaigler D, Pagni G, Park CH et al. Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial. Cell Transplant 2013;22(5):767-777.
21. Marx RE, Harrell DB. Translational research: The CD34+ cell is crucial for large-volume bone regeneration from the milieu of bone

marrow progenitor cells in craniomandibular reconstruction. Int J Oral Maxillofac Implants 2014;29(2):e201-9.
22. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A and Raghoebar GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. Clin Oral Implants Res 2011;22(3):251-258.
23. Ahn G, Lee JS, Yun WS, Shim JH, Lee UL. Cleft Alveolus Reconstruction Using a Three-Dimensional Printed Bioresorbable Scaffold with Human Bone Marrow Cells. J Craniofac Surg 2018; 29(7):1880-1883.
24. Behnia H, Khojasteh A, Soleimani M, et al. Secondary repair of alveolar clefts using human mesenchymal stem cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108(2):e1-e6.
25. Behnia H, Khojasteh A, Soleimani M, Tehranchi A and Atashi A. Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report. J Cranio-Maxillofac Surg 2012;40(1):2-7.
26. Filho Cerruti H, Kerkis I, Kerkis A, Tatsui NH, da Costa Neves A, Bueno DF, da Silva MC. Allogenous bone grafts improved by bone marrow stem cells and platelet growth factors: clinical case reports. Artif Organs 2007;31(4):268-73.
27. Hibi H, Yamada Y, Ueda M and Endo Y. Alveolar cleft osteoplasty using tissue-engineered osteogenic material. Int J Oral Maxillofac Surg 2006;35(6):551-555.
28. Kaigler D, Pagni G, Park CH, Tarle SA, Bartel RL, Giannobile WV. Angiogenic and osteogenic potential of bone repair cells for craniofacial regeneration. Tissue Eng Part A 2010;16(9):2809-2820.
29. Rajan A, Eubanks E, Edwards S, Aronovich S, Travan S, Rudek I, Wang F, Lanis A, Kaigler D. Optimized cell survival and seeding efficiency for craniofacial tissue engineering using clinical stem cell therapy. Stem Cells Transl Med 2014;3(12):1495-503.
30. Shayesteh YS, Khojasteh A, Soleimani M, Alikhasi M, Khoshzaban A, and Ahmadbeigi N. Sinus augmentation using human mesenchymal stem cells loaded into a β-tricalcium phosphate/hydroxyapatite scaffold. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106(2):203-209.
31. Smiler D, Soltan M and Lee JW. A histomorphogenic analysis of bone grafts augmented with adult stem cells. Implant Dent 2007;16(1):42-53.
32. Valdivia E, Mendoza-Azpur G, Pando J, Cornejo H, Carrasco A, Nevins M, Kim D. Tissue Engineering Therapy for Atrophic Maxilla Using Minimally Manipulated CD90 and CD105 Bone Marrow Stem Cells: A Case Report. Int J Periodontics Restorative Dent 2017;37(3):355-361.
33. Yamada Y, Hara K, Nakamura S, Ueda M, Ito K and Nagasaka T. Minimally invasive approach with tissue engineering for severe alveolar bone atrophy case. Int J Oral Maxillofac Surg 2013;42(2):260-263.
34. Khojasteh A, Sadeghi N. Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study. Int J Oral Maxillofac Surg 2016;45(7):864-871.
35. Khojasteh A, Hosseinpour S, Rezai Rad M, Alikhasi M, Zadeh HH. Buccal fat pad-derived stem cells with anorganic bovine bone mineral scaffold for augmentation of atrophic posterior mandible: An exploratory prospective clinical study. Clin Implant Dent Relat Res 2019;21(2):292-300.
36. Prins HJ, Schulten EA, Ten Bruggenkate CM, Klein-Nulend J, Helder MN. Bone Regeneration Using the Freshly Isolated Autologous Stromal Vascular Fraction of Adipose Tissue in Combination with Calcium Phosphate Ceramics. Stem Cells Transl Med 2016;5(10):1362-1374.
37. Khojasteh A, Hosseinpour S, Rad MR, Alikhasi M. Buccal Fat Pad-Derived Stem Cells in Three-Dimensional Rehabilitation of Large Alveolar Defects: A Report of Two Cases. J Oral Implantol 2019;45(1):45-54.
38. Gonshor A, McAllister BS, Wallace SS, and Prasad H. Histologic and histomorphometric evaluation of an allograft stem cell-based matrix sinus augmentation procedure. IntJ Oral Maxillofac Implants 2011;26(1):123-131.
39. Katagiri W, Osugi M, Kawai T, Hibi H. First-in-human study and clinical case reports of the alveolar bone regeneration with the secretome from human mesenchymal stem cells. Head Face Med 2016; 12:5.
40. Baer PC. Adipose-derived mesenchymal stromal/stem cells: an update on their phenotype in vivo and in vitro. World J Stem Cells 2014;6(3):256-265.
41. Baer PC and Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. Stem Cells Int 2012; 2012:812693.
42. Temenoff JS, Park H, Jabbari E et al. In vitro osteogenic differentiation of marrow stromal cells encapsulated in biodegradable hydrogels. J Biomed Mater Res A 2004;70:235-244.
43. Kearney CJ, Mooney DJ. Macroscale delivery systems for molecular and cellular payloads. Nat Mater 2013;12(11):1004-1007.
44. Zangi L, Rivkin R, Kassis I, Levdansky L, Marx G, Gorodetsky R. High-yield isolation, expansion, and differentiation of rat bone marrow-derived mesenchymal stem cells with fibrin microbeads. Tissue Eng 2006;12:2343-54.
45. Luboshitz J, Berger U, Yaari O, Wiessman A, Moshonov J. Platelet rich plasma (PRP) as adjuvant tool for bone augmentation. Refuat Hapeh Vehashinayim 2004;21:63-70.
46. Oyama T, Nishimoto S, Tsugawa T, Shimizu F. Efficacy of platelet-rich plasma in alveolar bone grafting. J Oral Maxillofac Surg 2004; 62:555-558.
47. Sanchez AR, Sheridan PJ, Eckert SE, Weaver AL. Regenerative potential of platelet-rich plasma added to xenogenic bone grafts in peri-implant defects: a histomorphometric analysis in dogs. J Periodontol 2005;76:1637-44.
48. Jakse N, Tangl S, Gilli R et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res 2003; 14:578-83.
49. Anitua E, Andia I, Ardanza B, Nurden P, Nurten AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004;91:4-15.
50. Nikolidakis D, Jansen JA. The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng Part B Rev 2008;14(3):249-58.
51. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(6):638-646.
52. Tozum TF, Demiralp B. Platelet-rich plasma: a promising innovation in dentistry. J Can Dent Assoc 2003;69:664.
53. Elgali I, Omar O, Dahlin C, Thomsen P. Guided bone regeneration: materials and biological mechanisms revisited. Eur J Oral Sci 2017;125(5):315-337.
54. Bornstein MM, Halbritter S, Harnisch H, Weber HP, Buser D. A retrospective analysis of patients referred for implant placement to a specialty clinic: indications, surgical procedures, and early failures. Int J Oral Maxillofac Implants 2008;23:1109-1116.