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Intraoral Bone Regeneration Using Stem Cells-What a Clinician Needs to Know: Based on a 15-Year MEDLINE Search

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A B S T R A C T

The choice of an appropriate autogenous source of stem cells has not been adequately addressed especially for intraoral bone regeneration. The current review aims to assess the clinical success of various human stem cells in oral bone regeneration. Articles studying the potential of various stem cells utilized for reconstruction of intraoral bone defects in humans were included in this review. Relevant articles were electronically searched in MEDLINE-PubMed database using keywords with different combinations. Only the articles published in English between 2006 and 2020 were included in this review. It was concluded that intra and extraoral stem cells can be successfully used for bone regeneration of various jaw defects. Depending on the origin, quantity, and quality, each cell type has its own advantages and disadvantages. Also, it brings to the fore the need for more clinical studies to validate and adopt the use of stem cells in regular clinical practice.

Keywords: Adult Stem Cells; Mesenchymal Stem Cells; Review; Bone Regeneration; Cell- and Tissue-Based Therapy

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INTRODUCTION

Alveolar bone defects can range in size from small defects due to periodontal disease to severe bone loss as the result of surgical resection or congenital deformities. While some of these defects, such as extraction socket(s), possess a self-healing ability and exhibit satisfactory healing via secondary intention [1], large osseous defects such as those created as a consequence of cystic defect(s) do not present such healing properties. In contrast, defects at the level of alveolar ridge or clefts do not possess any osteogenic capacity and thus, require additional regenerative interventions for functional rehabilitation.

Autologous grafts, although biocompatible and being a gold-standard treatment modality, present with their own limitations. Unlike intraoral sources, extraoral bone harvesting sites add drawbacks of additional hospitalization time with prolonged recovery course and graft sequestration [1]. Bone tissue engineering is gaining popularity fast as it overcomes these drawbacks, providing favorable results. Stem cells have widespread applications including bone regeneration, which have been vastly utilized in combination with appropriate scaffolds and growth factors. Iliac bone aspirate is the usual source for obtaining the widely used bone marrow derived mesenchymal stem cells (BMMSCs).

Oral stem cells are another potential source which have been proven worthy of consideration in clinical practice. Apart from being readily accessible to oral and maxillofacial surgeons, these stem cells can be obtained with minimal invasiveness in comparison with iliac bone aspirate. Furthermore, these cells can also be collected by practicing dentists from sources like dental pulp of extracted or exfoliated teeth. Despite numerous advantages, limited evidence is available for use of oral stem cells in general practice [2].

This review aims at understanding and discussing the various types of bone defects found in the oral cavity and types of stem cells from intraoral and extra-oral sources used for regenerative treatments based on the studies conducted over the past 15 years (2006-2020). The review was restricted to discussion of human clinical studies, trials and case reports (Table 1).

Extraction site

Non-restorable teeth due to severe caries or infection need to be extracted [2]. Following extraction, the extraction socket undergoes physiologic healing; however, loss in vertical and horizontal dimensions is well evident over time, owing to loss of the pertinent functional stimulus provided by the tooth [3]. With increasing awareness of prosthetic rehabilitation, dental implants are frequently opted by patients. However, as time passes since tooth extraction, gradual decrease in bone height compromises the success of this treatment modality, and would necessitate additional procedures. Thus, bone preservation following extraction is a vital pre-requisite. Extraction of mandibular third molars is quite common owing to a plethora of reasons [4]. Their removal is often accompanied by bone resorption at the extraction site with vertical bone loss leading to probing depth values up to 7 mm distal to the second molar, and thereby affects the treatment prognosis [5].

Table 1: Reconstruction of various intraoral bony defects using intraoral stem cells

	Type of Study	Stem Cells	Outcome Measure	Follow up (years)	Result
			Extraction site		
Monti et al [6]	Case control	DPSCs	Histologic	0.3	More lamellar and organized bone at the test site than the control site
Graziano et al [10]	Split mouth case report	PDLSCs	Clinical Radiographic	0.5	More mineralization at the test side
d'Aquino et al [5]	Case series	PDSCs	Clinical Radiographic	0.3	Lower horizontal and vertical bone resorption by 38.3% and 36.5% compared with the control site Successful bone formation
Aquino et al [8]	Case control	DPSCs	Radiographic Histologic Immunologic	1	Successful lamellar bone formation distal to second molar tooth
Giuliani et al [7]	Case control	DPSCs	Radiographic Histologic Histomorphometric Holotomographic	3	Successful cortical bone formation distal to second molar tooth
Barbier et al [9]	RCT	DPSCs	CT	0.5	No difference in bone fill in the test and control sites
Kaigler et al [12]	RCT	BMMSCs	Radiographic µCT AP activity	1	Successful bone formation and implant placement
			Sinus lift		
Carinci et al [14]	Case report	DPSCs	CBCT	0.25	Bone density double of native bone
Graziano et al [15]	Case report	DPSCs	Histologic Immunofluorescence	0.5	Successful bone formation

Pradel et al [16]	Case report	SCAB	Clinical Radiographic	0.3	Successful bone formation
Nagata et al [17]	Case series	PDSCs	Radiographic Histomorphometric	1	Successful bone formation Reduction in autogenous bone graft by 40%
Prins et al [18]	Clinical trial	Adipose stem cells from SVF	Clinical Radiographic Histologic µCT Histomorphometric	3	Successful bone formation
Gonshor et al [19]	RCT	Mesenchymal stem cells	Histomorphometric	0.3	Vital bone content of 32.5%±6.8% in the test group compared with 18.3%±10.6% in the control group
Sauerbier et al [20]	RCT	BMMSCs	Radiographic Histologic Histomorphometric	0.3	Successful bone formation
Rickert et al [21]	RCT	BMMSCs	Clinical Histologic Histomorphometric	0.3	Successful bone formation
Shayesteh et al [22]	Case series	BMMSCs	Clinical Histologic	1.1	Successful bone formation
Wildburger et al [23]	RCT	BMMSCs	Histologic Histomorphometric	0.5	No significant difference between the test and control groups
Bertolai et al [24]	RCT	BMMSCs	Clinical Histologic	0.25	Successful bone formation
			Periodontal defect		
Li et al [26]	Case report	DPSCs	Clinical Radiographic	0.75	Successful bone formation
Aimetti et al [27]	Case report	DPSCs	Clinical Radiographic	1	Successful bone formation
Aimetti et al [28]	Case series	DPSCs	Clinical Radiographic	1	Successful bone formation
Aimetti et al [29]	Case series	DPSCs	Clinical Radiographic	1	Successful bone formation
Ferrarotti et al [30]	RCT	DPSCs	Clinical Radiographic	1	Successful bone formation
Feng et al [31]	Case report	PDLSCs	Clinical	6	Decreased probing depth with increased clinical attachment
Shalini et al [32]	RCT	PDLSCs	Clinical RVG	1	Bone fill – 51% in PDLSCs vs. 13% in the control group
Chen et al [33]	RCT	PDLSCs	Clinical Radiographic	1	Successful bone formation
Cystic cavity					
Bertolai et al [36]	Clinical trial	BMMSCs	Clinical Radiographic	1	Bone formation by 85%-90%
Meshram et al [38]	Case report	BFPDSCs	Histologic Histomorphometric (CBCT)	1	Bone density- 76% voxels Mature lamellar bone formation
Pradel et al [39]	Case control	SCAB	Radiographic	1	No significant difference
Redondo et al [40]	Clinical trial	ABMSCs	CT	-	Increased bone density
Colangeli et al [41]	Clinical trial	BMMSCs	CT	0.2	Successful bone formation
			Alveolar cleft		
Hibi et al [45]	Case report	BMMSCs	CT	0.75	Successful bone formation

Khojasteh et al [46]	Case control	BFPDSCs	Histomorphometric(CBCT)	0.5	New bone formation – 82% in the test group vs. 75% and 70% in the 2 control groups
Pradel et al [47]	Case report	SCAB	Clinical Radiographic	1.5	Successful bone formation and tooth eruption
Pradel et al [48]	Case control	SCAB	Clinical Radiographic	0.5	Osteogenesis at the defect site 40% in the test group vs. 36% in the control group
Bajestan et al [49]	RCT	BMMSCs	Clinical CBCT	0.8	Successful bone formation with implant placement
Behnia et al [50]	Case report	BMMSCs	Clinical Radiographic CT	0.3	Successful bone formation
Gimbel et al [51]	Case control	BMMSCs	Clinical	0.5	Reduced donor site morbidity and decreased donor site pain with the use of tissue engineered bone
Behnia et al [52]	Case report	BMMSCs	Clinical Histomorphometric (CBCT)	0.25	Bone fill by 51.3%
Behnia et al [59]	Case series				
			Alveolar width deficiency		
Khojasteh et al [54]	Case control	BFPDSCs	Histologic Histomorphometric (CBCT)	0.3	Bone width gain – 3.9 vs. 3.0 mm Newly formed bone – 65% in the test group vs. 49% in the control group
Gjerde et al [55]	Clinical trial	BMMSCs	CBCT µCT Histologic	1	Successful bone formation and implant placement
			Large alveolar defects		
Khojasteh et al [58]	Case report	BFPDSCs	Histologic Histomorphometry (CBCT)	4	Successful bone formation and implant placement

RCT: Randomized controlled trial; **DPSCs:** Dental pulp stem cells; **BMMSCs:** Bone marrow derived mesenchymal stem cells; **PDLSCs:** Periodontal ligament derived stem cells; **PDSCs:** Periosteum derived stem cells; **SCAB:** Stem cells from alveolar bone; **ABMSCs:** Alveolar bone derived mesenchymal stem cells; **BFPDSCs:** Buccal fat pad derived stem cells; **CT:** Computed tomography; **µCT:** Micro-computed tomography; **AP:** Alkaline phosphatase; **RVG:** Radiovisiography; **CBCT:** Cone-beam computed tomography; **SVF:** Stromal vascular fraction

Measures aiding to bone regeneration in the denuded area distal to the second molar will potentially benefit the patients by preventing postoperative complications. Numerous studies and case reports have been documented in the literature, utilizing various sources of stem cells for their osseous healing efficacy in this particular and other similar defects [2].

Monti et al. [6] evaluated the healing potential of dental pulp stem cells (DPSCs) in a split-mouth trial. They first performed an in-vitro assessment, characterizing these cells and then used them in-vivo in allogenic hosts. Next, the cells obtained from extracted third molars, combined with a collagen scaffold (test side), were placed in extraction sockets of six patients undergoing tooth extraction. Collagen sponge without cells was packed at the control sites to enhance physiologic healing of the socket. In all patients, dental implants were placed within a span of 45 to 70 days after extraction. Test sites showed denser radiopacity as compared to the control sites. Hematoxylin-eosin staining of bone samples obtained during implant placement displayed well organized bone with the Haversian system at the test sites compared with poor tissue formation at the control side. Similar results were reported by Giuliani et al, [7] and also by d'Aquino et al, [5,8] in their first publication which was later followed by a second publication concerning the follow-up of

the same patients. In contrast, Barbier et al. [9] did not find any significant difference in clinical, radiographic, and surgical characteristics of the control and experimental groups. A similar case report with a split-mouth design was presented by Graziano et al, [10] who utilized human periodontal ligament derived stem cells (hPDLSCs) from the periodontal tissue obtained from extraction of impacted mandibular third molars. Clinical assessment of the test and control sites after six months revealed lower probing depth at the test site compared with the control site. Radiographic examination also showed higher mineralization at the test site.

Periosteum is a highly vascularized layer, the inner cambium layer of which is a rich source of osteoblasts and other osteogenic cells [11]. However, the true bone forming capacity of periosteum has not been fully utilized in the field of dentistry. D'Aquino et al. [5] showed successful utilization of periosteum-derived stem cells for bone regeneration in extraction sockets, which were later rehabilitated by dental implants. During the second surgery performed for the purpose of implant placement, clinical re-assessment revealed decreased resorption of alveolar bone by 38.3% and 36.5% in horizontal and vertical dimensions, respectively at the test sites compared with the control sites. Histological examination of bone obtained from both sites revealed faster bone formation at the test site with more organic matrix than the control site.

Kaigler et al. [12] utilized BMMSCs for bone regeneration in the extraction sockets. In a randomized controlled trial (RCT), a total of 24 patients were enrolled of which, 12 were treated with a stem cell suspension with gelatin sponge while 12 were treated with a saline-soaked sponge. At 6 weeks postoperatively, significantly greater (P=0.01) radiographic bone height was achieved in the stem cell group. The same was noted at 12 weeks, but it was not statistically significant (P=0.28).

Although extraction sockets completely heal physiologically, there is often a bony defect left behind especially in cases of impacted mandibular third molars. The above-mentioned studies show the promising results of potential use of stem cells like DPSCs, hPDLSCs, BMMSCs and periosteum-derived cells in management of post-extraction bony defects. This can aid in preservation of the alveolar ridge and decrease the physiological residual ridge resorption which can contribute to better treatment planning for implant-based treatments.

Sinus lift

Present in a bone of the same name, the maxillary antrum is the largest, pyramidal-shaped paranasal sinus, located bilaterally in the facial skeleton. The sinus floor is formed by the alveolar and palatine processes which lie in close approximation to the root apices of posterior teeth. With age, the sinus pneumatization increases, which leads to decrease in distance between the sinus floor and the alveolar crest. Loss of posterior teeth, followed by a prolonged period of edentulism, lead to a further decrease in this distance, compromising the height of the available bone. Sinus lift procedures, also known as subantral augmentation, were introduced in the mid-1970s to increase this compromised height, and enhance dental implant placement [13]. Since then, many researchers have used various kinds of materials for alveolar bone regeneration in sinus lift procedures.

Carinci et al. [14] presented a case report wherein DPSCs obtained from an extracted third molar, combined with collagen were autologously placed in the sinus cavity to receive a dental implant at a later stage. Postoperative evaluation after 4 months showed complete resolution of the defect and bone density twice that of the native bone. Graziano et al, [15] also presented a case report wherein DPSCs were successfully used for bone augmentation in a sinus lift procedure.

The choice of scaffold is an important aspect of tissue regeneration. Pradel et al. [16] showed successful bone regeneration and implant placement after using a combination of osteoblast-like cells with solvent dehydrated mineralized bovine bone scaffold while use of demineralized bovine bone matrix scaffold resulted in fibrous formation which was considered as failure. This study proved that

the choice of biomaterial affects osteogenesis in bone tissue engineering.

Periosteal-derived stem cells have also been used in glue-based formulations. Cultured autogenous periosteal cells obtained predominantly from the anterior region of the mandibular ramus, were used by Nagata et al [17]. They mixed these cells with platelet-rich plasma (PRP), 2% calcium chloride ($CaCl_2$) and particulate autogenous bone to obtain a glue-like material. The glue was transplanted at 33 intraoral bone defect sites; of which, 18 were for maxillary sinus augmentation. Histological evaluation was done at 4 months post-grafting, which revealed evidence of active bone formation around the grafted particulate autogenous bone. Cone beam computed tomography (CBCT) scans done 3 months post-operatively presented evidence of cortical bone formation and a smooth surface area with high density.

In one of the first in-human phase I trials, Prins et al. [18] showed enhanced bone formation and subsequent dental implant placement by using adipose derived stem cells (ADSCs) isolated from stromal vascular fraction. In another clinical trial, Gonshor et al. [19] compared bone formation by a conventional allograft with a cellular bone matrix allograft containing native mesenchymal stem cells wherein vital bone content of 32.5%±6.8% was seen in the latter group compared with 18.3%±10.6% in the former group.

Sauerbier et al. [20] utilized BMMSCs for bone formation in sinus augmentation procedures. In the conducted RCT, bovine bone mineral was placed in conjunction with bone marrow aspirate at the test sites and with milled autologous bone at the control sites. In addition, thrombin was added to the test sites along with the mixture to enable the aspirate solution to form a clot around the bovine bone mineral. The authors found that new bone formation was not significantly (P=0.333) higher at the test sites than the control sites. The test sites reported a 3.3% decrease in non-mineralized tissue and the volumetric analysis showed significantly higher (P=0.02) radiographic gain and increased bone height. In another RCT by Rickert et al, [21] BioOss® with either the same stem cell source or with autogenous bone was used for sinus lift followed by dental implant placement. Superior bone formation was seen with the former. A case series of 6 patients was presented by Shayesteh et al, [22] wherein, BMMSCs were used in conjunction with either biphasic hydroxyl apatite or beta tricalcium phosphate followed by implant placement. They reported 93% clinical success rate of implants with a mean bone regeneration of 41.34% at the grafted site. However, in a split-mouth RCT, Wildburger et al. [23] found no significant difference in bone formation between the control and test groups by the use of bovine bone with or without BMMSCs.

Sinus lift procedures have shown great success with the use of synthetic grafts or modified allografts alone. It was necessary to compare the results of grafting with and without stem cells to justify the use of these cells in sinus lift procedures.

Bertolai et al. [24] compared bone formation with corticocancellous freeze-dried bone allograft (FDBA) (control group) and BMMSCs treated corticocancellous FDBA (test group) for maxillary ridge augmentation by sinus floor elevation. Although FDBA proved to be a successful graft material, the BMMSCs treated engineered material presented greater histological integration potential.

Given the increased demand for implant-supported prosthesis following long periods of edentulism in the maxillary molar region, sinus lift procedures are routinely performed. However, insufficient bone makes implant placement a clinical challenge. Sinus lift procedures aid in providing sufficient bone width in such cases. Apart from regular bone grafts, use of stem cells from dental pulp, periosteum, and bone marrow in conjunction with a variety of scaffolds further assist in enhancement of bone quality and reduce the time interval between the two procedures.

Periodontal defects

Chronic periodontitis is a common oral condition, presenting with characteristic features of varied extent and intensity of bone loss, often leading to tooth mobility and

eventual tooth loss in advanced cases. The treatment in such cases is aimed at regeneration of the lost periodontium and restoring the tooth supporting tissues. Growth factors, guided tissue regeneration, and bone grafts have been found to serve the purpose to satisfactory levels; however, stem cell use has gained much popularity in the past few years [25].

In an observational study by Li et al, [26] DPSCs caused successful bone formation in periodontal defects, showing an increased amount of bone formation over a period of 9 months. This was confirmed both radiographically and clinically, with decreased furcation grade, reduced mobility, and increased gingival attachment [24]. Utilizing the same stem cell source, Aimetti et al. [27] looked forward to rectifying infrabony periodontal defects. In their attempt, they found completely filled defects with bonelike material at the end of one year. Similar successful clinical results in healing of periodontal defects by DPSCs were reported by Aimetti et al, [28,29] in two case series and by Ferrarotti et al in a RCT [30].

Feng et al. [31] achieved successful bone formation in periodontal defects utilizing hPDLSCs after 32-72 months. In a RCT conducted by Shalini et al, [32] on similar cell lines, a reduction in periodontal defects by 5.8% to 9.2% was reported in the control group which was much lower than 23.9% to 28.4% reduction reported in the test group. Also, the test side showed bone fill by up to 51% in comparison with 13% bone fill at the control sites. Combined use of hPDLSCs, guided tissue regeneration membrane, and Bio-Oss® has also shown good clinical results [33].

Periodontal bone defects predispose the patients to early tooth loss, and the bone loss makes rehabilitation a challenge. The abovementioned case reports highlight the great potential of DPSCs and hPDLSCs for regeneration of periodontal defects, thereby helping in establishment of a healthy periodontium. Clinical parameters such as reduction in the probing depth, improvement in gingival recession, and attachment gain indicate their great periodontal regenerating capability.

Cystic cavity

Arising from the odontogenic epithelium in tooth-bearing areas, odontogenic cysts develop due to either proliferation or degeneration of this epithelium [34]. These cysts are usually painless, and are mostly diagnosed incidentally on radiographs. Over time, they can increase in size, encroaching upon the neighboring structures. Cystic lesions with a diameter ≤3 cm are easier to be eradicated with a potential of satisfactory bone formation [35]. However, decompression following marsupialization is opted for lesions bigger than 3 cm. The literature indicates that only up to 50% of large lesions are healed by the end of one year with healing reaching up to 91% by 2 years [36]. Also, the quality of bone formed in large defects is subpar than that formed in smaller defects, necessitating additional bone grafts [36]. Autogenous, allogenic and xenogeneic bone graft materials have been used in such defects with few attempts of use of stem cells of different origins [36,37].

Bertolai et al. [36] used a combination of BMMSCs from iliac crest aspirate and PRP in mandibular and maxillary cystic defects to assess the bone regeneration potential of BMMSCs. This was compared to control cases where patients were treated without stem cells. The authors reported bone formation by 85% to 90% in the residual bone cavity in a span of 12 months which was half the time documented in the literature for the same amount of bone formation in a similar defect.

Meshram et al. [38] conducted a study on 5 patients with pathologic lesions in either of the jaw bones. The patients' buccal fat pad was used to isolate ADSCs prior to final enucleation of the pathology. The obtained tissue was centrifuged and made into pellets which were delivered drop by drop at the defect site post-enucleation. Radiographic examination showed peripheral blending of bone margins as early as 1 month with dense compact bone formation at the end of 6 months. CBCT analysis showed a mean bone density gain of 73.8% voxels at 6 months with significant differences ($P<0.05$) among all three analyses conducted at

different durations in the study.

In 2006, Pradel et al. [39] published a study involving bone regeneration of defects following enucleation of odontogenic cysts. Eleven of the 22 sites received alveolar bone derived stem cell (ABDSC) engineered bone grafts (test group) while the other half received iliac bone grafts. ABDSCs were obtained from bone biopsies done at the time of enucleation. Radiographic evaluation was done at 3, 6, and 12 months which showed little variation in bone density in the two groups at 6 and 12 months. However, considerably stronger ossification was seen at the end of one year in the test group.

Redondo et al, [40] in their clinical trial assessed the bone forming potential of autologous alveolar bone derived stem cells in maxillary cystic defects of 2-4 cm. Using these cells, a BioMax serum scaffold was prepared over a duration of 4 weeks which was then implanted at the defect site. Computed tomography analysis of the intervened defect site showed increased amounts of radiodensity over a period of 7 months with no signs of inflammation or any other adverse effects.

Colangeli et al. [41] used BMMSCs for bone regeneration following removal of dentigerous cysts in 5 patients. The authors evaluated the volume as well as density in Hounsfield units of the newly formed bone. At the end of 6 months, a mean bone volume of 2.44 cm^3 was seen with a mean density of 1137 Hounsfield units.

Removal of large cystic lesions often renders the bone prone to pathologic fractures. In severe cases, it becomes even more important to place a bone graft in the operated area in order to rehabilitate the jawbone and support various surrounding structures. Bone regeneration procedures also add to the esthetics and help the patient to return to normalcy. Stem cells along with conventional bone grafts have been seen to aid bone regeneration in patients with such defects. The studies listed herewith also prove the great defect healing capacity of stem cells such as BMMSCs, ADSCs and ABDSCs which help in formation of a higher quality bone as compared to the conventional bone grafts.

Alveolar cleft

Orofacial cleft is the most common head and neck congenital malformation with a worldwide and Indian prevalence of approximately 1 in 1000 live births [42,43]. This anomaly comprises a plethora of defects wherein more than 60% of the cases have some levels of defect in the alveolus region [44]. Lacking physiologic bone formation ability, the defect site is subjected to grafting procedures with the use of autogenous grafts in most cases to facilitate unhindered growth, eruption of permanent lateral incisors and canines, and enhance facial esthetics. However, autogenous bone grafts as the gold standard treatment procedure have limitations such as donor site morbidity and limited tissue availability. These shortcomings call for techniques that can aid in bone regeneration in areas at which tissue engineering provides a viable alternative.

Probably in the first clinical attempt, Hibi et al. [45] formulated an injectable tissue-engineered bone material comprising of BMMSCs, PRP, $CaCl_2$ and human thrombin which was applied for a 9-year-old female patient who presented with a left-sided unilateral cleft lip and alveolar defect. The authors reported evidence of extension of bone from the cleft walls 3 months post-operatively with bridging of the cleft defect after 6 months and bone formation of 79.1% at the end of 9 months when the canine of the affected site erupted into the oral cavity.

Khojasteh et al. [46] presented a clinical trial on 10 unilateral cleft patients. They studied the bone regeneration potential of ADSCs in combination with autogenous bone grafts. Patients in the first group (AIC group) were treated with anterior iliac crest bone along with a collagen membrane. The second group [lateral ramus cortical bone plate (LRCP) + buccal fat pad derived mesenchymal stem cells (BFSC)] received lateral ramus cortical bone plate in conjunction with stem cells derived from the buccal fat pad, on a bone mineral of bovine origin and collagen membrane. The last group (AIC+BFSC group) received similar grafts as the second group with AIC in place of LRCP. Computed tomography evaluation done

6 months post-surgically showed maximum bone formation in the AIC + BFSC group followed by LRCP + BFSC and AIC groups respectively, thus validating the extensive bone regenerative power of the BFSCs.

In a case report presented by Pradel et al, [47] a 10-year old boy with unilateral cleft lip, alveolus and palate was subjected to secondary osteoplasty before the eruption of permanent canines. They used osteoblasts from the cancellous bone of the maxilla, cultured on Osteovit®, a demineralized bone matrix to produce tissue engineered bone grafts which were used to treat the alveolar cleft defect. Radiographic evaluation done 8 months post-operatively showed ossified cleft area with successful migration of permanent canine and supernumerary teeth into the cleft space. Complete radiographic bone closure was seen 18 months post-operatively with spontaneous eruption of canine. In a successive clinical trial by Pradel and Lauer, [48] eight children were equally divided into two groups; control group treated with iliac bone graft and test group treated with tissue-engineered bone (autogenous osteoblasts on demineralized bone matrix Osteovit®). Comparison of pre- and post-operative CBCT analysis revealed ossification of 40.9% of the cleft defects in the test group compared with 36.6% ossification seen in the control group, thus proving higher bone regeneration capacity of the tissue engineered graft.

Bajestan et al. [49] conducted a RCT wherein bone formation efficacy of autologous BMMSCs was evaluated in patients with large alveolar defects due to congenital clefts or trauma. The control group was treated with conventional autogenous bone graft while the test group was treated by stem cell therapy (ixmyelocel-t). Stem cells were transplanted with β-tricalcium phosphate scaffold as cell carrier. Re-entry into the surgical sites was performed 4 months after initial surgery to compare pre- and post-surgical results. Although the authors reported no adverse effects with the use of autologous stem cells, the ex-vivo expanded cells had limited role in repairing large alveolar defects, recommending larger multicenter clinical trials.

Behnia et al. [50] presented a case report of two cases wherein unilateral cleft patients were treated with a composite scaffold comprising of BMMSCs, demineralized bone mineral, and calcium sulfate. Computed tomography scans showed 34.5% regenerated bone after a span of 4 months in one case while it was 25.6% in the second case. These percentages were not revealed on the scans, but the authors advocated the use of these stem cells citing the features of lack of donor site morbidity, decreased patient hospitalization time, and good soft tissue healing. BMMSCs have also been utilized in a larger patient group (n=21) with alveolar cleft for bone formation; however, the study was performed aiming to assess and compare donor site morbidity, pain intensity, and frequency of pain experience among three procedures namely tissue engineering, traditional iliac crest bone grafting, and minimally invasive iliac bone grafting [51]. Thus, the study did not comment on bone forming capacity of the three procedures. Taking their attempts further, as a primary report, Behnia et al. [52] used the same stem cell source in combination with scaffold and platelet derived growth factors for secondary alveolar cleft repair. They reported a 51.3% bone defect fill after 3 months of surgery, which was assessed by CBCT.

Alveolar width deficiency/atrophied jaw bone

Jaw bone atrophy and alveolar width deficiency are manifested as sequels of trauma, tooth extraction, periodontal disease, tumors, or congenital defects. They pose a challenge for dental clinicians to rehabilitate the defect site especially when dental implants are the proposed treatment. Alveolar ridge resorption also leads to esthetic and functional defects, debilitating patient's regular activities [1]. Ridge augmentation techniques using hard and soft tissue grafts have been widely employed to achieve adequate bone but they have drawbacks of limited bone availability, donor site morbidity, uncertain bone quality, and postoperative discomfort [1,53].

In a preliminary study by Khojasteh and Sadeghi [54], BFPSCs were used for correction of maxillomandibular atrophy. Eight patients

with extensive jaw atrophy received non-vascularized blocks of anterior iliac crest bone with cyrodesiccated bone granules covered with a collagen membrane, filling the gaps between the blocks. The patients in the test group received BFPSCs along with bone granules while stem cells were not used for the control group. In all cases, implant placement was done 5 months postoperatively during which trephine bone biopsies of 2 mm were taken for histological analysis. The test group showed 65.32% new bone formation as compared to 49.21% in the control group. The observed mean bone width change was higher in the test group as compared to the control group. Gjerde and coworkers [55] assessed the feasibility, safety, and efficacy of BMMSCs combined with synthetic biphasic calcium phosphate in augmenting the alveolar bone of a severely atrophic mandible. The combination was placed subperiosteally in the resorbed ridge and was clinically and radiographically assessed after 4 to 6 months of healing. The authors reported new bone formation to be adequate for dental implant installation with the bone regeneration method to be safe and free of any adverse effects.

Large alveolar defects

Orofacial trauma, resection of large tumors, or simultaneous extraction of multiple teeth may lead to formation of tridimensional defects in the alveolar bone, compromising physiologic functioning and patient appearance [56].

In a published case report, Rajan et al. [57] highlighted the successful use of ixmyelocel-T based cells seeded on β-tricalcium phosphate for reconstruction of jaw with loss of 75% bone support and subsequently rehabilitated with implants for prosthesis support. The authors reported 80% regeneration of the original defect with sufficiently mineralized and vascularized bone.

Khojasteh et al. [58] reported utilizing BFPSCs in adjunct with guided bone regeneration. Their case report comprised of 2 cases where simultaneous extraction of multiple teeth left behind a large osseous defect in the alveolar bone. In the first case, a 19-year-old female was treated with BFPSCs loaded natural bovine bone mineral, and implants were placed 6 months postoperatively which were intact after 10 months. In the second case, a 22-year-old male patient treated similarly received implants 6 months after guided bone regeneration. Radiographic evaluation done at 48 months, postoperatively revealed complete survival of implants. The given case report showcases the promising regenerative capabilities of BFPSCs in rehabilitation of large alveolar defects, and re-establishment of patient's normal form and function.

CONCLUSION

The evidence available in the literature justifies the use of stem cells in various bone defects of the jaws. Each cell type has various advantages and disadvantages based on the origin, and quality and quantity of cells. While bone marrow derived MSCs are the gold standard for bone regeneration, the studies mentioned in this review bring to light the versatility of oral origin stem cells in intraoral bone regeneration. Intraoral defect sites like extraction sockets can be used as test sites to assess the regenerative potential of any stem cell source without significantly affecting the functional rehabilitation of the site. The studies in this review have shown that concomitant use of osteogenic stem cells and osteoinductive synthetic bone grafts demonstrates higher mineralization in sinus lift procedures. The same is also true at sites like the alveolar cleft. There is sufficient evidence in the literature in the form of RCTs to prove the effectiveness of stem cell-based bone regeneration over physiologic healing in intraoral cysts. Sites like periodontal bone defects are treated adequately by stem cells with appropriate membranes and without the need for additional synthetic bone grafts.

This review provides a comprehensive update on the various stem cells used in the past 15 years for intraoral bone regeneration. Although BMMSCs are the most preferred autogenous source of stem cells, the intraoral sources have also been widely studied. Periosteum, adipose tissue, dental pulp, cancellous bone, and periodontal ligament have proven clinical success in providing cells with good osteogenic

differentiation ability and consequently good bone regeneration capacity in intraoral bone defects. It would be difficult to comment on the most suitable or successful source of stem cells based on the human clinical trials of the past 15 years. The authors suggest further studies to analyze various stem cells with osteogenic potential. The assessment criteria could include quantitative analyses, cell viability tests, mineralization potential, protein markers, and gene markers.

Another area that needs further research is the scaffold suitability for each cell type specific for the site. There is also a need to standardize the techniques for cell isolation and differentiation for various stem cells used for intraoral bone regeneration.

The authors found great difficulty in comparing the results of various studies assessed in this review. The main reason for this was the lack of uniformity in assessment of bone regeneration. Researchers have used various methods to assess the efficacy of stem cells in bone regeneration, complications, failure rates, and clinical success, such as radiographic analysis (either simple or computed radiographs), volumetric radiographic analysis, and histological analysis. The authors suggest further research to develop baseline criteria to measure the success of stem cell-based bone regeneration techniques. This will not only help clinical scientists to guide their procedures towards favorable results but also researchers to frame appropriate study designs.

CONFLICT OF INTEREST STATEMENT

None declared.

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