Efficacy of autologous stem cells for bone regeneration during endosseous dental implants insertion - A systematic review of human studies

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ABSTRACT

Availability of adequate quantity and quality of bone is prerequisite for longevity and survival of endosseous dental implants. Most of the clinicians face with the problem of lack of bone due to long-standing edentulism during this treatment modality. Conventional therapies with the use of various types of bone grafts and membranes have provided clinicians with unpredictable and compromised results. Cell-based therapies utilizing undifferentiated cells, that have the potential to differentiate into various cell types including osteoblastic lineages, have demonstrated through various previously conducted in-vitro and animal studies, a successful formation of bone in a predictable manner. Thus the main objective of this review was to evaluate the effectiveness of these therapies when applied on human subjects.

A search was carried out in MEDLINE (via PubMed) and Cochrane CENTRAL databases for completed randomized and non-randomised clinical trials utilizing stem cell-based therapies with histologic and radiographic analysis written in English up to January 2019. This search of the literature yielded 10 studies meeting the inclusion and exclusion criteria. In all these studies, stem cells were primarily used to achieve bone augmentation during insertion of endosseous dental implants. Results of these therapies conducted on human subjects have shown a positive impact on bone regeneration, in particular, therapies utilizing bone marrow and adipose tissue derived stem cells. But the clinicians need to examine the efficacy, safety, feasibility of these therapies while treating large size defects or planning for shorter healing period and early loading of dental implants.

1. Introduction

Replacing lost teeth with endosseous dental implants is a widely-accepted treatment modality among patients, clinicians and academicians.^{1–3} It has been long known that among those who desire to undergo endosseous implant therapy, a substantial number lack adequate amount of bone.⁴ This condition happens as a result of jaw defects, loss of teeth or teeth being congenitally absent. As a result, alveolar bone of the jaw is not subjected to the functional stimulus inherently generated by the teeth and their supporting structures and, thus leading to, further resorption of bone.⁵ This combined effect results in severe horizontal and vertical bone deficiencies and insufficient volume of bone to reconstruct these areas of the jaw with functional and esthetic tooth replacements.⁶

Bone regeneration in the oral and maxillofacial region after its loss, due to various causes as mentioned above, continues to be a challenge and its reconstruction still depends mainly up on employing additive

treatments modalities through application of large autogenous grafts, allografts, xenografts, and synthetic alloplastic materials.⁷

In bone reconstruction procedures, autologous bone is presently considered as the gold standard. In this procedure, autologous bone is harvested from the patient and transplanted to the defect site by surgeons.⁸ However, this procedure has numerous severe drawbacks like procuring of graft requires a second surgical site and generates only meager bone stock, the two-stage procedure prolongs surgery time and patients frequently suffer from pain and damage at the donor's site. Furthermore, autologous bone has an unpredictable resorption rate.^{9–11} All these factors increase patient discomfort and treatment costs.

To overcome the limitations of these conventional therapies, a newer, more targeted, cell and tissue-based therapies are required.^{12,13} Stem cell therapies provides a promising tissue engineering strategy to enhance tissue regeneration and to boost de novo formation of both soft and hard tissues.^{13–16}

In the medical and dental specialities, concepts of tissue-engineering

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therapy, is extensively being used to regenerate the function of lost or damaged tissues. This tissue-engineering therapy relies on a triad, which incorporates cells with regenerative capacity (i.e., stem cells), signalling molecules such as growth factors, and a biocompatible matrix serving as a scaffold.¹⁷ In the field of dentistry, cell-based therapy has been used for rehabilitation of the craniofacial and the temporomandibular complexes,¹⁸ regeneration of the pulpal,¹⁹ and periodontal tissues^{20,21} and bone regeneration.²²

Cell-based therapies utilize undifferentiated cells which are either embryonic stem cells that originates in blastocysts or adult stem cells located in adult tissues like bone marrow.²³ Mesenchymal stem cells (MSCs) are multipotent adult stem cells with distinct biologic characteristics which are most commonly related to their mesodermal lineage (adipogenic, chondrogenic, osteogenic, or myogenic).²⁴ So these MSCs being non-hematopoietic progenitor cells can differentiate into various mesenchymal cell lineages, including osteoblastic lineages. Thus, MSCs provide clinicians with a viable option to various bone graft materials for the regeneration of bone, particularly during placement of dental implants.

Many systematic reviews and meta-analysis have analyzed the efficacies of MSCs for the regeneration of bone in intra-oral sites through pre-clinical animal studies^{25–27} and many of them have reported these stem cells to be a promising alternative compared to traditional therapies. But there are almost no reviews conducted till now to demonstrate their efficacies on human subjects. So this systematic review was conducted to find out the effectiveness of stem cell therapies in the regeneration of bone in human subjects during the insertion of dental implants.

2. Material and methods

2.1. Protocol

Guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement²⁸ were followed for conduction of this systematic review.

2.1.1. Focus question

The structured question that was developed for population, intervention, comparison, and outcome (PICO) study design was as follows:

Whether stem cell therapy is effective in the regeneration of bone as compared to conventional therapies for dental implant placement in human subjects?

2.1.2. Types of publications

The review included completed clinical trials done on human subjects written in English language.

2.1.3. Types of studies

Randomised and Non-Randomised Clinical trials were included in this review.

2.1.4. Information sources

Articles were systematically searched in two electronic databases i.e MEDLINE (via PubMed) and Cochrane CENTRAL.

2.1.5. Literature search strategy

A comprehensive search of peer reviewed literature, published up to January 2019, was performed online. The following keywords and Mesh terms were used in combination with Boolean operators 'OR', 'AND' & 'NOT' to search the databases: "Stem cells", "mesenchymal dental stem cells", "mesenchymal stem/precursor cells", "mesenchymal stem/progenitor cells", "mesenchymal stem/stromal cells", "mesenchymal stem cell", "mesenchymal stem cell derived", "mesenchymal stem cell like", "dental pulp stromal", "periodontal ligament like", "periodontal ligament stromal cells", "periodontal mesenchymal

cells", "periodontal ligament progenitor", "periodontal ligament cells", "gingival margin derived cell", "oral mucosa stem cells", "bone marrow cells", "bone marrow derived", "bone marrow stem", "bone marrow stroma", "periosteal cells", "periosteal stem cells", "periosteal progenitor cells", "periosteum derived stem/progenitor cells", "adipose mesenchymal stem", "adipose progenitor cells", "adipose stem cell", "adipose stroma", "adipose stromal cells/ pluripotent cells/ multipotent cells", "embryonic stem cells", "induced pluripotent stem cells", "peri-implant defects", "peri-implantitis", "dental implants", "implant infection", "implant bone defects", "clinical trial", "review".

2.1.6. Inclusion criteria

1. Human studies utilizing stem cells for bone regeneration.
2. Studies using biomaterial/Scaffolds along with/without impregnated stem cells.
3. Studies involving treatment or intervention done for placement of dental implants or periimplantitis.
4. Randomised and Non-randomised clinical trials.
5. Completed clinical trials
6. Studies utilizing histological and radiographic parameters for analysis.
7. Studies documented and reported in English language.

2.1.7. Exclusion criteria

1. Animal trials utilizing stem cells for bone regeneration.
2. Use of stem cell therapy in extraction sockets.
3. Studies using biomaterial/Scaffolds only.
4. Case-reports, Case series & Reviews.

3. Data extraction

Initially, two independent reviewers (AD, VP) identified potentially relevant articles after the screening of titles. On reading the abstracts, articles were further identified depending upon the inclusion and exclusion criteria. The difference of opinion about the study's inclusion was settled through means of discussion with a third independent reviewer.(SV) Repeat articles were excluded at the first stage of screening. Finally, during the second stage of screening, the full text of articles was retrieved to confirm the eligibility of each study.

3.1. Risk of bias assessment

All the included studies in this systematic review were subjected to risk assessment tools for detection of biases in them. Cochrane's risk of bias tool²⁹ was used for the randomized trials and ROBINS-I tool for Non-randomised studies.³⁰

Following criteria were used in the Cochrane's tool for reporting bias: "Random sequence generation", "Allocation concealment", "Blinding of personnel", "Blinding of outcome assessment", "Incomplete outcome data", "Elective reporting" and "Other sources of bias" (Table 2). Based on these criteria, studies were categorized as "Low risk of bias" if all the following criteria were satisfied, "Unclear risk of bias" if one of the latter criteria were missed, and "High risk of bias" if two or more criteria were lacking.

In ROBINS-I tool (Table 3), studies were classified as "Low risk", "Moderate risk" and "High risk" depending on the existence of bias due to confounding, "selection of participants into the study", "classification of interventions", "deviations from intended interventions", "missing data", "measurement of outcomes and selection of the reported result" at Pre and Post-Intervention levels.

3.2. Result

The initial electronic search of databases yielded 43 articles. Articles

Table 1
Studies included in the Systematic Review.

STUDY	STUDY DESIGN	STEM CELL SOURCE	CARRIER / SCAFFOLDS	TREATMENT DONE	STUDY GROUPS	HEALING PERIOD	NEW FORMED TISSUE QUATIFICATION	FOLLOW-UP PERIOD	RESULTS
1 YAMADA ET AL;2008 ³³	Non-Randomised	Bone Marrow Derived Stromal Cells (BMDSCs) from posterior iliac crest	PRP	MSFE (Lateral window technique)	Nil	5–7 months	Clinical,OPG,CT Scan & Histology	2–6 years	Use of injectable tissue-engineered bone statistically significantly regenerated bone compared to pre-operative levels.Also no remarkable bone absorption was seen during 2–6 year follow-up.
2 SCHIMMING et al;2002 ³⁷	Non-Randomised	Tissue engineered autologous bone	Polymer fleeces	MSFE (Lateral window technique)	Nil	3 months	Clinical,OPG,CT Scan & Histology	NA	Periosteum-derived osteoblasts on a suitable matrix can form lamellar bone within 3 months after transplantation and provide a reliable basis for simultaneous or secondary insertion of dental implants
3 P. VOSS ET AL;2010 ³⁸	Non-Randomised	Tissue engineered autologous bone	Polymer fleeces	MSFE (Lateral window technique)	Test: Tissue engineered autologous bone with Polymer fleeces Control: Autologous bone Test: Autologous stem cells with Bio-Oss Control: Bio-Oss mixed with autogenous bone	15 weeks	Clinical, Radiological, and Histological	Up to 24 months	Both implants and augmentation were significantly more successful in the control group Lifting the sinus floor with autologous bone is more reliable than with tissue-engineered transplants Significantly more bone formation was observed in the test group when compared with the control group.
4 RICKERT ET AL;2011 ³¹	Randomised	Bone marrow derived MSC from Posterior Iliac Crest	Bio-Oss®	MSFE (Lateral window technique)	Test: Autologous stem cells with Bio-Oss Control: Autologous bone	13–16 weeks	Clinical,Radiographs, Histologic & Histomorphometric	NA	
5 RICKERT ET AL;2014 ³²	Randomised	Bone marrow derived MSC from Posterior Iliac Crest	Bio-Oss®	MSFE (Lateral window technique)	Test: Autologous stem cells with Bio-Oss Control: Autogenous bone with Bio-Oss	3–4 months	Clinical and Radiographic	12 months follow-up	No differences in soft tissue parameters or peri-implant bone loss were observed between the control and test sides
6 WILDBURGER ET AL;2014 ³⁶	Randomised	Bone marrow derived MSC from Posterior Iliac Crest	Bio-Oss®	MSFE [Lateral window technique (modified)]	Test: Autologous stem cells with Bio-Oss Control: Autogenous bone with Bio-Oss	3–6 months	Clinical, Radiographic,Histologic & Histomorphometric	10 months	There was no significant difference in new bone formation between the test and control group
7 KAIGLER ET AL;2015 ³⁵	Randomised	Bone marrow derived MSC from Posterior Iliac Crest	β-TCP	MSFE (Lateral window technique)	Bio-Oss Control:β-TCP Stem cell therapy: β-TCP with Stem cells	4 months	Clinical, Radiographic (micro CT) & Histomorphometric	18 months	No difference was seen in total bone volume between the 2 groups but stem cell therapy group demonstrated more bone density compared to control group.
8 PRINS, SCHULTEN, TEN BRUGGENKATE ET AL;2016 ³⁴	Non-Randomised	Autologous adipose derived MSC	β-TCP and BCP	MSFE (Lateral window technique)	Control: β-TCP or BCP Test: β-TCP or BCP with Stem cells	6 months	Clinical, Radiographic (micro CT),Histology & Histomorphometric	≥ 3 years	More Bone & Osteoid formation occurred in Test than control group.Similarly β-TCP + Stem cells formed more bone compared to Stem cells + BCP
9 BAJESTAN ET AL;2017 ⁴⁰	Randomised	Bone marrow derived MSC from Posterior Iliac Crest	Imxycelocel-t with β-TCP	Alveolar ridge deficiency due to trauma and Cleft-palate	Control 1:Autogenous Bone Graft(Block Grafting) Stem cell therapy group: Imxycelocel-t with β-TCP	4 Months	Clinical, Radiographic (CBCT)	16 months	The ability of stem cells to treat large alveolar defects is safe, yet, their ability to completely reconstitute large alveolar defects is limited.
10 GJERDE ET AL;2018 ³⁹	Non-Randomised		BCP granules		Nil	4–6 months		18 months	The regenerated bone volume was adequate for dental implant

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Table 1 (continued)

STUDY	STUDY DESIGN	STEM CELL SOURCE	CARRIER / SCAFFOLDS	TREATMENT DONE	STUDY GROUPS	HEALING PERIOD	NEW FORMED TISSUE QUANTIFICATION	FOLLOW-UP PERIOD	RESULTS
		Bone marrow derived MSC from Posterior Iliac Crest		Mandibular posterior ridge augmentation			Clinical, Radiographic (CBCT), Histologic & Histomorphometric		installation. t MSCs can successfully induce significant formation of new bone, with no untoward sequelae.

selection approach is outlined in the flow diagram (Fig. 1). Among all the obtained articles, 21 of them were excluded during the screening of their titles and thus only 22 clinical studies estimating the effects of stem cells on bone regeneration were recognized. No other study was added by any other search methodology. On reading their abstracts, 7 studies were excluded due to reasons mentioned in Fig. 1. Out of the 15 studies selected, 2 articles were found to be repeat articles and on examination of their full texts, 3 were excluded as they did not satisfy the inclusion and the exclusion criteria. Thus overall, 10 studies were incorporated for this systematic review.

3.3. Characteristics of included studies

All the 10^{31–40} included studies were human clinical trials in which autogenous stem cells were used to regenerate bone for implant placement (Table 1). Out of these, five^{31,32,35,36,40} were randomized and five were non-randomised^{33,34,37–39} clinical trials. In these clinical trials stem cells were procured from posterior iliac spine^{31–33,35,36,39,40} through aspiration and adipose tissue through lipoaspiration.³⁴ In two studies, periosteal cells^{37,38} were used to develop tissue-engineered bone.

Various types of carrier or scaffolds were used alone or in combination with these stem cells. They included biphasic calcium phosphate (BCP),^{34,39} β -tricalcium phosphate (β -TCP),^{34,35,40} bovine bone (Bio-Oss®),^{31,32,36} Polymer fleeces^{37,38} and Platelet rich plasma (PRP).³³ These combinations of stem cells and scaffolds were utilized to regenerate bone in maxillary sinus lifts,^{31–38} mandibular ridge augmentations,³⁹ cleft palate and traumatic bone deficiencies.⁴⁰ Bone regeneration was evaluated after a healing interval of 4–6 months. These studies were followed for a period of 1–2 years after implant placement except one which had upto, 6 years of follow-up.³³

3.4. Maxillary sinus floor elevation (MSFE)

Out of the 10 included clinical trials, 8 studies^{31–38} utilized stem cells for Maxillary sinus floor elevation (MSFE). Among those 8 studies, 5 studies^{31–33,35,36} isolated stem cells from the posterior iliac spine through aspiration and 1 from adipose tissue³⁴ and 2 utilized tissue-engineered bone containing periosteal cells.^{37,38} Stem cells derived from posterior iliac spine were mixed with scaffolds like β -TCP, Bovine bone, PRP & BCP whereas stem cells from adipose tissue were combined with β -TCP and tissue-engineered bone was mixed with Polymer fleeces. Stem cells mixed with PRP was utilized in an injectable form whereas others were in putty form.

Operators in most of the studies had utilized Lateral window technique for sinus elevation. In most of the studies, implants were placed in second stage i.e after 4–6 months of healing (2- Stage procedure) whereas in few they were inserted simultaneously (1-Stage procedure) during the sinus elevation procedure. None of the studies utilized osteotomy technique for sinus elevation.

Study done by Rickert et al.³¹ was a randomized controlled split-mouth clinical trial in which combination of mesenchymal stem cells and Bio-Oss® (Bovine bone) were used and it significantly regenerated more bone compared to the control group where Bio-Oss® was combined with autogenous bone harvested from the retromolar area. Although, out of 66 implants placed, 3 implants failed in the test subjects and none in the control subjects before the prosthetic placement. These subjects were further followed for another 12 months and Rickert et al.³² reported in 2014 that there was no further loss of either implants or peri-implant soft tissue & bone in both these groups. Thus, concluded that both these procedures were equally reliable.

Clinical trial conducted by Yamada et al.³³ utilized injectable tissue-engineered bone, which was a formed by combining stem cells derived from bone marrow (BMDSCs) and PRP. In this clinical trial, 16 sinus augmentations were performed on 12 patients. Overall 41 dental implants were inserted simultaneously. After 6 months of healing, bone

Table 2

Risk assessment FOR randomised trials- Cochrane COLLABORATION'S TOOL.

Study	Random sequence generation	Allocation concealment	Blinding of personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias	Overall bias
Bajestan et al;2017 ⁴⁰	+	–	–	–	+	?	?	–
KAIGLE RET A;2015 ³⁵	+	?	–	–	+	+	?	?
Rickert et al;2011 ³¹	+	?	+	–	+	+	?	?
Rickert et al;2015 ³²	+	?	+	?	–	+	?	?
Wildburger et al;2014 ³⁶	+	?	–	–	–	+	?	–

Low risk Bias

Unclear Risk Bias

High risk bias

biopsies were taken for histologic and histomorphometric analysis. Result demonstrated a significant difference ($p < 0.01$) in the height of regenerated bone compared to pre-operative levels at 3,6,12 and 24 months (3.8 ± 1.8 , 7.4 ± 1.8 , 8.9 ± 1.6 , and 8.8 ± 1.6 mm, respectively). Also, no significant bone resorption was seen even after 2–6 years of follow up.

Prins, Schulten, ten Bruggenkate et al.³⁴ did a clinical trial using autologous stem cells derived from stromal vascular fraction [SVF] of adipose tissue. This was the first human study in which freshly isolated, autologous adipose stem cell preparation was used. In this trial, out of 10 patients, 6 were bilaterally treated with calcium phosphate carrier with SVF (Study side) or calcium phosphate carrier alone (Control side). Calcium phosphate carrier was either β -TCP or BCP. Rest 4 cases were made to undergo unilateral sinus lift procedure with either combination of SVF and β -TCP ($n = 2$) or SVF and BCP ($n = 2$). All unilaterally treated patients belonged to the study group. After a healing period of 6 months, dental implants were placed and biopsies were taken for histologic and histomorphometric analysis. Result demonstrated that more bone and osteoid formation had occurred in the study subjects compared to the control subjects and in particular, among β -TCP treated patients. No negative outcomes were reported either during the surgery or during the follow-up period (> 3 years). The author reported only 1 implant failure in the study group among all the 44 implants placed.

Kaigler et al.³⁵ did a randomized clinical trial in which MSFE was performed in Stem cell therapy (Ixmyelocel-t + β -TCP) and the Control group (β -TCP). A total of 30 patients with 15 in each group, were included for this study. After a healing period of 4 months, implants were inserted and biopsies were taken. Results demonstrated no difference in total bone volume, but increased bone density in Stem cell therapy group compared to the Control group. These patients were followed for one year and during that period author reported 1 graft failure in stem cell therapy group and 1 implant failure in the control group.

Wildburger et al.³⁶ had done MSFE procedure using MSCs in a split-mouth randomized control trial, among seven subjects having a highly atrophic maxilla on both right and left sides of the jaw. Test side was treated with Stem cells and Bio-Oss® whereas the control side was treated with Bio-Oss® alone. Biopsies were obtained from the planned implant sites at 3 and 6 months after sinus elevation procedure for

histologic and histomorphometric evaluation. Examination of biopsies between the control and test sides, revealed no significant change in the formation of new bone. Implants were placed at 6 months along with simultaneous harvesting of the second biopsy. The prosthesis was fabricated after 4 months of implant insertion. In all, 52 implants were placed and the author reported no loss of implants.

Schimming and Schmelzeisen³⁷ in their clinical trial used tissue-engineered bone containing periosteal cells derived from periosteum from the lateral cortex of the mandibular angle for sinus augmentation in 27 patients either by performing one stage or two-stage procedure. These periosteal cells were incorporated in polymer fleeces to form a cell-polymer construct. These patients were evaluated clinically, radiographically and histologically after 3 months of completion of the procedure. In 18 patients, bone biopsies showed the formation of calcified trabecular bone with a trace amount of the biomaterial indicating successful augmentation procedure. Whereas unsuccessful results were seen in 8 cases.

P. Voss et al.³⁸ did a non-randomized clinical trial in which tissue-engineered bone containing periosteal cells derived from peri-ostium, was used in combination with Polymer fleeces in the study subjects and whereas, in the control subjects, autogenous bone procured from the iliac crest, was utilized. In both the groups, implants were inserted either as one-stage i.e simultaneously along with sinus elevation or as a two-stage procedure i.e with implant being inserted 3 months after bony augmentation. These patients were followed clinically and radiographically for 24 months. During this follow-up period, the author reported failure of an only single implant in the control subjects compared to 11 eleven implants in the study subjects. Thus authors concluded that both augmentation procedure and implant placements were significantly more successful in the control subjects.

3.4.1. Mandibular posterior ridge augmentation

Among the 10 included studies in this analysis, only in one clinical trial, authors had used stem cells for the augmentation of posterior mandibular ridge. This was a non-randomized clinical trial³⁹ consisting of 11 subjects, where bone marrow derived MSCs from posterior iliac spine were combined with BCP (20%HA + 80% β -TCP) for augmentation procedure. After 4–6 months of healing interval, biopsies were taken and implants were placed. Formation of new bone was evaluated

Table 3
Risk assessment for NON-RANDOMISED studies-robins-I tool.

STUDY	Pre-intervention		At intervention	Post-intervention		Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Over all risk of Bias Judgement
	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions					
YAMADA ET AL,2008 ³³	Low risk of Bias	Moderate Risk of Bias	Low risk of Bias	Low risk of Bias	No Information	Low risk of bias	Low risk of Bias	Moderate risk of Bias	
P. VOSS ET AL,2010 ³⁸	Moderate risk of bias	Moderate Risk of Bias	Low risk of bias	Low risk of bias	No Information	Low risk of Bias	Low risk of bias	Moderate Risk of Bias	
PRINS, SCHULTEN, TEN BRUGGENKATE ET AL,2016 ³⁴	Low risk of Bias	Low risk of bias	Moderate Risk of Bias	Low risk of Bias	No Information	Low risk of bias	Low risk of Bias	Moderate risk of bias	
GJERDE ET AL,2018 ³⁹	Low risk bias	Low risk of bias	Moderate Risk of Bias	Low risk of bias	No Information	Low risk of Bias	Low Risk of Bias	Moderate risk of Bias	
SCHIMMING AND SCHMELZEISEN,2002 ³⁷	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	No information	Low risk of bias	Moderate risk of bias	Moderate risk of bias	

through clinical and radiographic examination. These patients were followed for a period of another 12 months. The author reported that use of MSCs with BCP regenerated an adequate volume of bone for implant insertion and no implants were lost during the follow-up period.

3.4.2. Alveolar ridge deficiency due to trauma and cleft-palate

Bajestan et al.⁴⁰ did a randomized controlled clinical trial using bone marrow derived mononuclear cells (BMMNC) for the regeneration of craniofacial bone deficiencies as a result of trauma or cleft palate. Total of 18 patients were divided into stem cell therapy (n = 10) and control group (n = 8). In stem cell therapy group, patients were treated with BMDSC (Ixmylelocel-t) with β -TCP whereas in the control group patients had undergone treatment with a traditional cortico-cancellous block bone grafting. Both groups consisted of patients having bone deficiencies due to cleft palate and trauma. After 4 months of healing, re-entry was made and dental implants were placed. Bone gain was assessed clinically and radiographically. Overall, for both the control and the stem cell therapy groups, the patients with trauma demonstrated greater amount of bone regeneration as compared to patients with the cleft palate. The author also reported that implants could be successfully placed only among 5 out of 10 patients and in all 8 patients, in the stem cell therapy and the control group respectively. While most of the implants were successfully loaded and remained stable for 6 months, only one implant failed before loading in the control/cleft palate group. Thus author inferred that it was safe to use stem cell therapy to treat large size alveolar defects but their ability to entirely reconstruct these types of defects was limited.

3.5. Risk of bias within studies

For randomised trials, Cochrane collaboration's tool,²⁹ revealed (Table 2) that most of the selected studies were having an “Unclear” to “High risk of bias”. “High risk of bias” was seen in “Blinding of personnel” and “Blinding of outcome assessment” whereas “unclear risk of bias” was seen in “allocation concealment” and “other sources of bias”. “Random sequence generation” and “Selective reporting” demonstrated a “low risk of bias”.

Using Robins-I tool³⁰ (Table 3) for investigating non-randomised trials, showed the presence of “moderate risk of bias” in almost all the included studies. Within the domains, subgroup analysis revealed “low risk of bias” to “moderate risk of bias” in controlling confounding factors and “selection of participants into the study”. Post-intervention analysis demonstrated a “low risk of bias” in “deviations from intended interventions”, “measurement of outcomes” and “selection of the reported result”. None of the studies provided any information on missing data.

4. Discussion

Regeneration of bone in the oral and craniofacial region is a major challenge and stem cell therapy provides substantial advantages compared to traditional approaches which have resulted in the emergence of an enormous volume of work describing diverse stem cell populations and their regenerative capabilities.

Thus regenerative medicine is regarded as a suitable treatment modality for future therapy. In this respect, the current systematic review was designed to analyse the noteworthy findings reported in the various published data, on the efficacy of the bone regeneration strategies, using a combination of stem cells and scaffolds in human subjects.

Our systematic search yielded 10 studies done on human subjects utilizing stem cells with scaffolds for bone regeneration. In most of the studies, bone regeneration was performed for Posterior maxillary sinus floor elevation, Mandibular ridge augmentation and Craniofacial & traumatic defects. No studies were retrieved in which stem cells were

Fig. 1. PRISMA flow diagram.

used to regenerate bone in peri-implant bone defects.

In most of the studies clinicians, in our systematic review had employed stem cells obtained from bone marrow and in few, adipose tissue and periosteal cells. It is interesting to note that none of the authors employed embryonic, dental pulp or periodontal ligament derived stem cells.

Though the use of BMDSC has few limitations like, being a time-consuming procedure, incurring high establishing costs and the requirement for a good manufacturing practice laboratory, which makes it poorly suited for routine practice, but it turned out to be the choice of material for bone augmentation during clinical trials. Possible reasons for this include, the results obtained in an animal study conducted by Gutwald et al in 2010.⁴¹ This study demonstrated that combining of natural bone mineral with concentrated mononuclear bone marrow cells as well as mesenchymal stem cells (MSCs) obtained from the iliac crest results in bone-forming kinetic identical to the regions reconstructed with autogenous bone alone. Also, multipotential MSCs have the unique potential to differentiate into a variety of cell types depending on the type of inducing signals perceived from the recipient tissues.⁴²

Out of all the studies included, eight studies combined BMDSC with BBM, β -TCP or BCP granules. Among these 8 studies, 6 studies reported a greater amount of bone formation in biopsies taken at a healing interval of three to six months through histologic and histomorphometric analysis.

Rickert et al.^{31,32} reported that mesenchymal stem cells obtained from aspirate of the posterior iliac crest and seeded on Bio-Oss® particles can lead to regeneration of an adequate quantity of new bone,

enabling a reliable insertion of dental implants within a time limits that was similar to applying either purely autogenous bone or a mixture of autogenous bone and Bio-Oss®. However, the survival rates of implants after 12 months of loading were 91% in the test group and 100% in the control group. Other authors^{41–43} have reported survival rates ranging from 94% to 100% in MSFE during the first year of functional loading. Also, much more favourable implant survival rates have been reported^{44,45} in edentulous maxillae which were less resorbed. In the present study, the maxillae were moderate to severely resorbed. Histomorphometrical examination did not show a difference in histomorphology between bone biopsies derived from sites of implants success and sites where implants had failed.³¹

Key findings of Kaigler et al. study³⁵ where Enriched CD90⁺ Stem Cell therapy used in MSFE was the formation of a better quality of bone and also the percentage of autologous CD90⁺ cells transplanted significantly correlated with the quality of new bone formed.

Gjerde et al.³⁹ reported successful augmentation of the posterior alveolar ridge in his study participants following a novel protocol using bone marrow derived mesenchymal stem cells. Though reconstruction of this region was challenging, because of the presence of comparatively poor blood supply,⁴⁶ nonsterile environment,⁴⁷ and oral functions such as mastication, speaking, and swallowing, which interfered with the survival of the graft, but the key determining factor as underlined by the author was the use of critical number of cells or a critical cell-to-biomaterial ratio used for regeneration. The critical number of cells and the cell-to-biomaterial BCP ratio, employed in this clinical trial were taken from the findings of a pre-clinical study, where 20×10^6 mesenchymal stem cells were combined with 1 cm³ biphasic

calcium phosphate.⁴⁸

Bajestan et al.⁴⁰ used Ixmyelocel-t seeded on β -TCP for the reconstruction of alveolar clefts and trauma deformities in adult individuals. Ixmyelocel-t therapy employs reproducible cell isolation and expansion protocols that can generate cell populations, characterized by the presence of CD90⁺ MSCs, CD14⁺ monocytes/macrophages, and mononuclear cells⁴⁹ in a consistent and predictable manner. Use of Ixmyelocel-t therapy in the present clinical trial was based on the successful regeneration of bone obtained in alveolar sockets and bone augmentation of maxillary antrum in the previously conducted randomized clinical trials.^{35,50} However, in the current trial, the ability of these cells to entirely reconstruct large craniofacial defects was compromised, especially in individuals having cleft palate defects. Compromised results obtained could be due to availability of insufficient amount of mucosa to cover the cleft palate deformities, presence of scar tissue from prior surgical procedures and poor blood supply with low oxygen tension due to the presence of scar tissue.^{51,52}

Another major reason cited by the author for obtaining inadequate results was the combining of Ixmyelocel-t with β -TCP. The use of this combination was based on findings of pre-clinical studies in which human bone marrow mononuclear cells or human bone marrow derived mesenchymal stem cells were seeded on β -TCP cells and this attachment of β -TCP with stem cells persisted for a period of 3 weeks.^{53,54} It was the first preclinical trial that evaluated the adherence of Ixmyelocel-t cells on β -TCP and thus in the current human study, this attachment may not have persisted long enough and therefore these cells might have moved out of the defect, thereby must have not contributed to the regenerative process.

Injectable tissue-engineered bone was used by Yamada et al.³³ in which BMDSC were mixed with PRP to form a coagulated mass. Use of PRP with BMDSC was based on the assumption that PRP contained fibrinogen, which on activation transforms itself into a fibrin network forming a matrix and cytokinetic elements like transforming growth factor, platelet-derived growth factor, vascular endothelial growth factor and insulin like growth factor, which could induce MSCs to convert into osteoblasts.⁵⁵ Also, the formed bone graft matrix could easily be fitted into the complicated shaped defects around implants which resulted in the successful regeneration of bone and simultaneous insertion of implants. In this study, the mean residual bone height was 5.5 ± 1.6 mm (range: 2–10 mm) before surgery and this increased to 8.8 ± 1.6 mm, at 2 years after the operation. However, no further resorption of the regenerated bone was observed radiographically and also this regenerated bone persisted in the sinus floor that had been elevated by tissue-engineered bone. Also, all the 41 endosseous implants placed in conjunction with this material, remained stable even after the second-stage surgery.

Another noticeable study was conducted by Prins, Schulten, ten Bruggenkate et al.³⁴ where MSFE procedure was performed by combining Adipose tissue-derived mesenchymal stem cells (ASCs) with calcium phosphate (CaP) ceramics scaffolds in a one-step surgical procedure. Interestingly, it was observed that, in the grafted area, the percentage of uncalcified osteoid formed was more at the study sides when compared to the control sides. Also, another peculiar phenomenon observed by the author was that out of 10 selected bone biopsies in the study group, 7 demonstrated, active bone formation at the cranial side whereas only one biopsy demonstrated this phenomenon in the control side. This observed phenomenon was attributed to either transdifferentiation of the ASCs within SVF toward bone-forming cells or to increased ASC paracrine recruitment of progenitor cells from the lateral bony window capping the bone reconstruction compartment.

Tissue-engineered bone containing periosteal cells were used by P. Voss et al.³⁸ to augment maxillary sinus floor. Isolation of periosteal cells was done using biopsy specimens taken from periosteum which were then resuspended and cultured. This cell suspension was loaded in polymer fleeces. The cell-polymer constructs were then transplanted into the sinus lift in the test group, after 8 weeks of harvesting. But,

both implant insertion and sinus augmentation were significantly more successful in the control group. According to the author, this unaccepted result may have been due to the low pH produced by the resorption of polymer construct which must have prevented the survival of osteoblasts.

A similar study was conducted by Schimming and Schmelzeisen³⁷ using tissue-engineered bone containing periosteal cells and Polymer fleeces. There was no control group formed in this study. Though augmentation failed in 8 cases out of 27 patients but still author described periosteum-derived stem cells loaded on a suitable matrix, to be a reliable technique for sinus augmentation and simultaneous or secondary placement of dental implants.

5. Conclusion

Current scientific literature is unanimous on the fact that stem cell therapy has a positive impact on regeneration of bone. Most of the studies analyzed in this systematic review reported positive results when they used BMDSC for bone tissue engineering. Although bone tissue engineering has proven its value in animal studies particularly in bone regeneration in long-bone critical size defects^{56,57} as well as in their oral application in the extraction socket model⁵⁸ but it becomes a slightly unpredictable method in humans while treating large size defects or during early insertion or loading of implants. This unpredictable nature may be due to variation in source or expansion of these stem cells. Thus, these limitations remain a challenge for the implementation of stem cell therapies in clinical practice in the future.

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