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Role of mesenchymal stem cells in the periodontal tissue regeneration of class II & class III furcation defects: A systematic review

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Abstract

Background: To investigate the role of stem cell therapy in enhancing periodontal tissue regeneration in class II and class III furcation defects.

Methodology: The systematic review was performed according to the PRISMA statement. PubMed, Embase, MEDLINE and Google Scholar were searched using online search strategies like ("Stem cells" OR "Stem cell transplantation" OR "Mesenchymal Stem cell transplantation") AND ("Furcation" OR "Furcation defects" OR "Periodontal defect" OR "Periodontitis" OR "Periodontal attachment loss") AND ("Regeneration" OR "Periodontal" OR "Periodontal Healing" OR "Periodontal regeneration). Then 260 articles were hand searched for quantitative studies examining the outcome of stem cells transplantation from the following origin sites- periodontal ligament, alveolar bone, bone marrow, adipose tissue, embryonic and gingiva into class II and class III furcation involvement. After the literature search, 12 studies fulfilled the inclusion criteria. A wide variety of surgical defects were created in experimental animals like dogs,

minipigs and rats. Outcome measures included new cementum, alveolar bone and connective tissue formation.

Results: Various studies have shown that stem cell regenerative therapy had a statistically significant improvement in periodontal tissue regeneration.

Conclusion: Stem cell implantation irrespective of the defect type and animal model can be expected to result in a beneficial outcome for periodontal regeneration.

Keywords: animal models, class II and class III furcation defects, periodontal regeneration, stem cells

Introduction Periodontitis is an infectious oral disease resulting in clinical attachment loss, alveolar bone resorption, periodontal pocket formation and gingival inflammation eventually leading to tooth loss.[1] The impact of periodontal disease on general health and quality of life increases the need to manage this condition more effectively. Periodontal therapy is done to control the infection and regenerate the tissues that have been previously lost due to destructive periodontal disease.[2]

Traditional treatment strategies surgical or non- surgical, fail to restore the periodontal supporting structures, which are damaged by periodontal disease due to the limited capacity of the tissues for regeneration.[3] Regeneration of destroyed periodontium includes various processes - migration, proliferation of periodontal cells and their differentiation.[4] Periodontal tissue regenerative therapies like guided tissue regeneration, bone grafting, platelet rich plasma have been utilized in clinical practice which have resulted in overall improvement of periodontal tissue health.[5,6]

Periodontal regenerative treatment in furcation defects pose major problem in the treatment of periodontal disease due to their complex and irregular anatomy which increases the risk for tooth loss.[7] Hirschfeld and Wasserman (1978) reported that percentage of tooth loss in furcation involved molars was 31.4% when compared to 4.9% in single-rooted teeth after a period of 22 years.[8] Various bone grafts and alloplastic materials used in class II and class III furcation defects have shown promising results but advances in tissue engineering has enhanced the periodontal tissue treatment with superior efficacy and predictability.[9] Tissue engineering in combination with mesenchymal stromal cells and progenitor cells, growth factors, cytokines and biomaterials has been observed as a future approach.[10] Mesenchymal stem cells (MSCs) are self-renewable and highly proliferative progenitor cells. When delivered in-situ, growth and differentiation factors in MSCs stimulate neo-vascularization and lead to faster tissue regeneration in periodontal defects.[11,12] They have the ability to differentiate into distinct mesenchymal cell types, including osteoblastic and cementoblastic lineages.[13] These newer approaches have shown predictable results in treating various periodontal defects such as fenestration, dehiscence, intrabony defects, furcation involvement and so on.[14] However, current ethical codes preclude a determination that these clinical improvements represent true periodontal regeneration and the histological proof of evidence for regeneration must be demonstrated in animal models.[15]

Methodology - This systematic review follows the guidelines of the PRISMA (Preferred Reporting items for Systematic Review and Meta-analysis) statement.

a. **Focused Question-** A Systematic review of literature was conducted to address the following question: “Does Stem cell therapy help in improving the quantitative result of periodontal tissue regeneration in furcation defects (class II and class III) and which type of stem cell, material and duration of the treatment influence the periodontal tissue regeneration” in these type of defects.

b. **Study population-** The population of study for this review included animals (Minipigs, Beagle dogs, Mongrel dog, Rats) with no systemic complications.

c. **Type of intervention and comparison-** Treatment of furcation defects (class II and class III) with various type of stem cells. The protocol of treatment varied in each study.

d. **Outcome measures-** The outcome was measured as the amount of periodontal regeneration seen histologically as formation of new bone, cementum and PDL in the furcation defects.

e. **Search Strategy-** Online search strategies were ("Stem cells" OR "Stem cell transplantation" OR "Mesenchymal Stem cell transplantation") AND ("Furcation" OR "Furcation defects" OR "Periodontal defect" OR "Periodontitis" OR "Periodontal attachment loss") AND ("Regeneration" OR "Periodontal" OR "Periodontal Healing" OR "Periodontal regeneration).

f. **Study Selection Criteria -** Literature search was performed by screening various articles and the abstracts of the relevant articles. Full texts fulfilling inclusion criteria were included in the systematic review. Literature search was limited to studies published in English language from 2002-2020.

g. **Inclusion and exclusion criteria -** Inclusion criteria for the selection were preclinical trials conducted in animals whose main aim was regeneration of class II and class III furcation defects using stem cell transplantation from the following origin sites- Periodontal ligament, alveolar bone, bone marrow, adipose tissue, embryonic and gingiva. Exclusion criteria was preclinical studies conducted on human in-vitro and animal ex-vivo whose main aim was not the regeneration of class II and class III furcation defects.

h. **Data Collection-** The data of the included articles was collected in data extraction files. The eligibility of the articles for the inclusion in the systematic review was decided by two reviewers independently based on the reported parameters. The data collected from the included studies were summarized according to the clinical and radiographical outcome in the follow up period. Any dis-agreement among the reviewers was re-examined and the decisions were made unanimously.

Results

1. **Characteristics of included studies :-** Online library search through databases like Pubmed and online internet resource of Google scholarTM yielded 1120 results. After screening of title for relevancy 260 abstract were found relevant: out of which only 12 abstracts fulfilled the inclusion criteria of the systematic review. The 12 abstracts included were published between the year 2002-2020, out of which each article was published in the year 2002, 2006, 2010, 2011, 2014, 2015 & 2019; while two studies in 2012 and three studies in 2013 respectively.Studies involving 5 different animal species: minipig (1); dog(1); Beagle dog(6); Mongrel dog(3); Rats (1)- met the inclusion criteria of the review.
2. **Exclusion of studies:-** The reasons for excluding the studies were that they did not meet inclusion criteria i.e. regeneration of furcation class II and III quantitatively. Human studies were also excluded due to lack of clinical evidence.
3. **Risk of Bias in studies:-** The assessment items includes blinding of personnel, participants and examiners and other sources of bias. None of the studies reported that allocation concealment had been carried out. Out of 12 studies only 2 had reported blinding and 3 studies had reported to be carried out

by randomization. The judgment of other risk indicated low risk of bias, unclear risk of bias and high risk which indicates either lack of information or uncertainity over potential for bias.

4. **Summary of outcome measure:-** All defects were created surgically on premolars & molars. Lang et al included both premolars & molars and Suiad et al & Iwasaka et al included only molars while only premolars were included in other studies. A variety of periodontal defects were studied which included class II furcation (n=7) and class III furcation (n=5). Majority of the studies showed positive regeneration of periodontal tissues. The animals included in this systematic review are dogs, mini-pigs and rats.[26,27]

Dogs: Number of experimental studies on periodontal diseases have been conducted in dogs among which beagle dogs are most commonly used due to its size, temperament and similarities with human periodontal tissues & teeth due to reproducible critical-sized defects and susceptibility to periodontal disease.

Mini-pigs: In terms of anatomy, physiology and disease development, the miniature pig has oral & maxillofacial structures and periodontium similarities to that of humans. They have been frequently used for testing the regenerative potential of various compounds and technologies.

Rats: They are frequently used in models of experimental periodontitis because periodontal anatomy in the molar region shows some resemblance with that in humans. They are easy to handle and can be obtained with different genotypes and immunological status and most of the histological structure of the epithelium as well as connective tissue are comparable to those of human tissue.

Fig 1: Flow chart of study selection using PRISMA guidelines

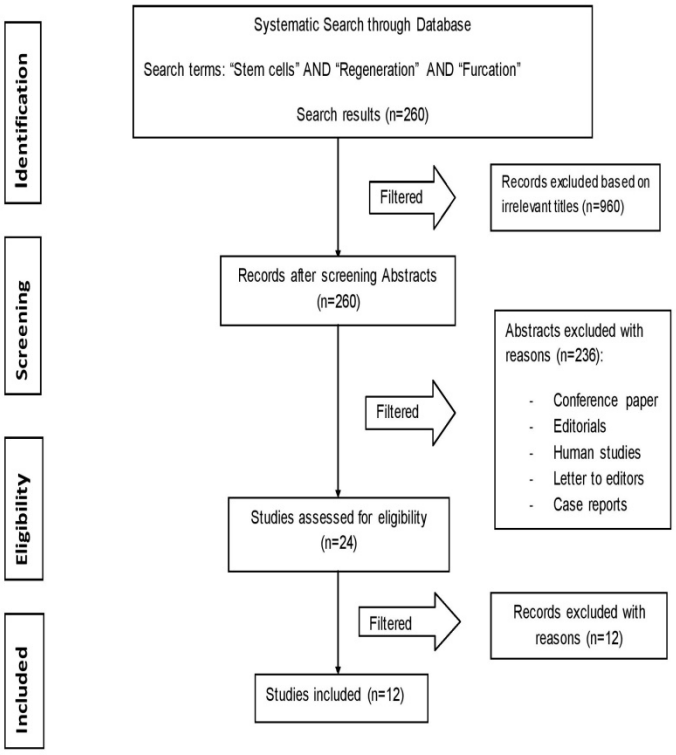

Table 1: Summary of risk bias in individual

References	Animal Model	Randomization (Selection Bias)	Allocation Concealment (Selection Bias)	Blinding (Performance/Detection Bias)	Other source of bias
Dogan et al (2002)[16]	Dogs	No	No	No	Unclear
Murano et al (2006)[17]	Mongrel dogs	No	No	No	Unclear
Jiang et al (2010)[18]	Beagle dogs	No	No	No	Unclear
Suiad et al (2011)[19]	Beagle dogs	Yes (defects were randomly assigned to one of the treatment i.e. control group or test group)	No	Yes (Blinded split mouth study)	Unclear
Suiad et al (2012)[20]	Beagle dogs	Yes (defects were randomly assigned to one of the treatment i.e. control group, GTR group, Sponge group, cell group.	No	Yes (Blinded split mouth study)	Unclear
Simek et al (2012)[2]	Mongrel dogs	No	No	No	Unclear
Tobita et al (2013)[10]	Beagle dogs	No	No	No	Unclear
Yang et al (2013)[21]	Minipigs	Yes (furcation defects were randomly assigned to control or test treatment group)	No	Yes	Unclear
Yu et al (2013)[22]	Beagle dogs	No	No	No	High (no details about location of the notch)
Iwasaki et al (2014)[23]	Rats	No	No	No	Unclear
Nagahara et al (2015)[24]	Beagle dogs	No	No	No	Unclear
Rezaei et al (2019)[25]	Mongrel dogs	No	No	No	Unclear

In this systematic review various stem cells included are PDL stem cells, alveolar bone stem cells, periosteal stem cells, bone marrow mesenchymal stem cells, adipose

tissue derived cells, embryonic stem cells and gingival mesenchymal stem cells.

Periodontal ligament stem cells (PDSCs): These cells have the ability to form cementum and periodontal ligament structures when transplanted into surgically created periodontal defects in animals.[28]

Periosteal stem cells: Periosteal cells are clonogenic, display long telomers and have expressed markers of MSCs.[29] They are capable of differentiating into chondrocytes, osteoblasts, adipocytes and have also proved to form cartilage or bone in vivo.[30]

Bone marrow mesenchymal stem cells (BM-MSCs): They are self-renewing multipotent cells that have the potential to differentiate into chondrocytes, tenocytes, adipocytes, muscle cells, and nerve cells in vitro and in vivo and also into cementoblasts, osteoblasts, and periodontal fibroblasts.[31-33]

Adipose tissue derived stem cells (A-MSCs): They are isolated from fat tissue and can differentiate into cells & tissues of mesodermal origin.[34,35] They not only regenerate mesodermal tissue but may also contribute to both ectodermal and endodermal tissues. They are easily accessible and present in abundance.[36,37]

Gingiva derived mesenchymal stem cells (G-MSC): They possess stem cell properties and have the capacity of multilineage differentiation in-vitro and have the ability to form new bone in vivo.[38-40] They show stable morphology, uniform homogeneity and fast proliferation, while still maintaining a normal karyotype in long term culture.[39] As GMSCs are readily accessable and are present in abundance, they can be used in regenerative medicine and they also promote periodontal regeneration.[41]

Embryonic stem cells (ES Cells): They are pluripotent cells isolated from the inner cell mass of blastocysts and have unlimited life span.[42,43] The major drawbacks of these cells are ethical consideration and the risk of teratoma formation.[44]

Discussion- Periodontal regeneration with stem cells has shown predictable outcomes in animal models. This systematic review aims to compare the various types of stem cells in different periodontal defects in furcation class II and class III in relation to the periodontal tissue regeneration. Also, an attempt has been made to provide evidence of the efficacy of stem cells for the periodontal regeneration of animal furcation defects. Various types of stem cells like periodontal ligament, gingiva, adipose tissue, alveolar periosteal, bone marrow and embryonic stem cells have been used as treatment options for periodontal tissue regeneration.

Almost 30 years ago, Melcher proposed the concept of stem cells residing in the periodontal tissue stating that fibroblast, cementoblast and osteoblasts were responsible for maintenance of homeostasis in periodontium.[45]

Transplantation of BM-MSCs have led to new treatment modalities for many diseases. They are self renewing multipotent cells capable of differentiating into several phenotypes including osteogenic, adipogenic, cardiogenic and neurogenic cells.[46-48] They have been observed to have significant clinical potentials in cell therapy and for periodontal tissue regeneration. Several studies have shown that transplantation of BM-MSCs into class III furcation defects in animals promote periodontal regeneration but cannot be used for large volume alveolar bone defects.[32,33] Transplantation of BM-MSCs with biocompatible material like β-tricalcium phosphates has been shown to accelerate alveolar bone regeneration but statistically they yielded conflicting results.[49]

In 2004, periodontal ligament stem cells (PDSCs) were first isolated from bovine dental follicles of progenitor cells.[50] These were confirmed to have mesenchymal stem cell like feature along with plastic adherence,

expression of verified mesenchymal stem cells markers, formation of fibroblast resembling clonogenic clusters and the capacity of differentiating into adipocytes, osteoblasts and cementoblast like cells in vitro.[51] Studies showed that the use of PDL-MSCs resulted in increased formation of cementum and PDL. These findings demonstrated that PDL-MSC express high level of several PDL specific proteins which are capable of forming cementum, PDL structure in-vivo when compared to other MSCs.[50,52] For these reasons PDL-MSC has been observed to show more favorable clinical outcome for periodontal defect regeneration.[49]

Another stem cell i.e. adipose tissue derived stem cells can differentiate into cells and tissues of mesodermal origin. Various studies reveal that ASCs not only regenerate mesodermal tissues but also contributes to ectodermal and endodermal tissue growth.[15] Many growth factors like vascular endothelial growth factor, hepatocyte growth factor and angiogenic factors like MMP-1 & MMP-9 has been secreted by adipose stem cells which are main factors for periodontal tissue defect repair by angiogenesis.[53,54] ASCs along with PRP have reported to regenerate periodontal tissue including alveolar bone, cementum like structure and PDL structure.[55]

GMSCs also possess stem cell property, including formation of clonogenic colonies, expression of a typical MSC surface marker profile & capacity of mutilineage differentiation in-vitro. Gingival MSCs are more superior to bone marrow MSCs because they are homogenous, easy to isolate and proliferate faster.[39] They have been found to promote significant improvement in periodontal regeneration but more studies are required to explore the efficacy of GM-MSC in periodontal regeneration.[22]

This systematic review clearly demonstrated that in a variety of animal models the implantation of stem cells had positive outcome in terms of enhanced periodontal regeneration but fewer studies have shown that MSCs with PRP have no better efficacy than autogenous bone.[2] For the occurrence of periodontal regeneration, the potential of the stem cells to recapitulate periodontal development needs to be studied.

The relevant assessment needs to be done before this becomes a routine clinical procedure such as designing appropriate cell delivery matrices, understanding the immunogenic and immune-regulatory properties of these cells and defining which cells may have the greatest effect on regeneration of tissue.[56] Regeneration of periodontal defects in humans have already been attempted by researchers using non invasive method to measure tissue regeneration level: radiologic evaluation, probing depth, clinical attachment level and evaluation of gingival recession.[57,58,59]

Various studies carried out in animals till date provide an excellent body of evidence to support that stem cells based periodontal regeneration is a viable treatment for periodontitis and clearly indicating that now it is time to move from animal studies to human clinical trials. Recently, one of the human RCT has shown significant gain in alveolar bone and no immunogenic reaction in infrabony defects treated by GTR, Bio-oss® and PDLSC sheets.[59]

Table 2: Details of the included studies

Study	Cell	Defect	Animal model	Observation	Results	Conclusion

	source	type(Dimension)	(n)/Groups	period		
1. Dogan et al (2002)[16]	PDL Stem cells	Class II furcation (5×2mm)	Dog (n=1) a) Cell-seeding group b) Control group	42 days	Cell seeding defects showed new connective tissue attachment with cementum formation 75.5% and bone fill 51%.Control sites showed new connective tissue attachment with cementum formation 72% and bone fill 35%.	Cell seeding provided periodontal regeneration in furcation defects by fibroblast – like cells.
2. Murano et al (2006)[17]	PDL stem cells	Class III furcation (4×4)	Mongrel Dogs (n=15) a) Experimental group b) Control group	8 weeks	New cementum formation in experimental group (98.1±2.3%) and control group (22.3±1.6%) New bone formation in experimental group (84.8±3.9%) and in control group (12.2±1.3%)	VEGF levels were increased in PDL group which enhanced bone formation and healing process by angiogenesis
3. Jiang et al (2010)[18]	Autologous periosteal stem cells	Class III furcation (4×3mm)	Adult Beagle Dogs (n=4) a) e-PTFE plus β-TCP and periosteal cells b) e-PTFE plus β-TCP c) e-PTFE	12 weeks	NP (new periodontal ligament formation) was 3.71 ± 1.82 in group A, 2.34 ± 0.47 in group B and 1.33 ± 0.31 in group C. NC (new cementum formation) was 5.53 ± 1.34 in group A, 4.71 ± 1.47 in group	In class III furcation defects periosteal cell stimulated more new bone formation (NBA) and new periodontal ligament (NP) than other group.

					B and 3.37 ± 1.21 in group C.NBA (new bone formation) was 6.21 ± 2.18 in group A, 3.71 ± 2.00 in group B and 2.35 ± 1.26 in group C.	
4. Suiad et al (2011)[19]	Autologous PDL-MSCs	Bilateral class II furcation (5×2mm)	Beagle dogs (n=14 defects) a) Control group: a collagen sponge scaffold, without cells, was applied with an absorbable membrane. The sponge was soaked with DMEM. b) Test group: a cell-seeded collagen scaffold with an absorbable membrane	3 months	Cell-treated group and control group presented new cementum (6.00 ± 1.50 and 8.08 ± 1.08 mm), periodontal regeneration (3.94 ± 1.20 and 7.28 ± 1.00mm) and new bone (7.01 ± 0.61 and 9.02 ± 2.30mm).	PDL Cells and GTR provided significant formation of bone, cementum and periodontal ligament cells than control group
5. Suiad et al (2012)[20]	Autologous PDL-MSCs	Class III furcation (5mm from CEJ)	Beagle dogs (28 defects) A) Control group B) GTR group: C) Sponge group D) Cell group	3 months	New cementum (4.82 ± 0.61 mm; 3.66 0.95 mm; 2.87 ± 0.74 mm and 1.70 ± 0.60 mm), new periodontal regeneration (3.43 ±	PDL cells with GTR lead to more periodontal tissue regeneration by stimulating progenitor cells by cytokines from

					1.44 mm; 2.33 ± 0.95 mm;1.52 ± 0.39 mm and 0.69 ± 0.59 mm) and and new bone (5.45 ± 1.58 mm; 3.94 ± 1.52 mm; 2.91 ± 0.56 mm and 1.89 ± 0.95 mm) for Cell, Sponge, GTR and Control group respectively.	seeding cells.
6. Simsek et al (2012)[2]	BM-MSCs	Bilateral class II furcation	Mongrel dogs (30 defects) Group 1- (Control) Group 2 - (PRP) Group 3 - (ACB) Group 4 - (ACB + PRP) Group 5 - (MSCs + PRP)	8 weeks	% of new cementum- Group 1 :3.33±3.33 Group 2 :36.6±20.17 Group 3 :93.62±4.09 Group4 :66.83±10.78 Group5 :70.47±11.75 % of new alveolar bone-Group 1 : 31.98±6.67Group 2 :33.95±15.39Group 3:84.6±4.85Group 4 :68.80±14.20Group 5 :80.47±8.23	Cementum, alveolar bone, and periodontal ligament formed after 8 weeks in all 3 groups but none of them showed higher efficacy.
7. Tobita et al (2013)[10]	Adipose tissue derived (A-MSCs)	Bilateral class III furcation(5mm)	Beagle dogs (48 defects) Group 1 - non-implanted Group 2- PRP Group 3- ASC+PRP	8 weeks	% of new cementum Non-implanted: 38.7, PRP:37.7,PRP+ASC: 36.4-8 weeks Non-implanted: 61.7, PRP: 62.5 PRP+ASC:84.7 % of new bone area-4 weeks Non-implanted: 37.0, PRP:33.6,PRP+AMS	Periodontal regeneration due to ASC and PRP possessed the ideal periodontal tissue morphology.

					C:35.1-8 weeksnon-implanted: 40.3, PRP: 53.7, PRP+ASC:63.9	
8. Yang et al (2013)[21]	Embryonic stem cells	Furcation (4×5×3)	Minipigs (12 defects) a) SRP b) SRP + ES Cells	3 months	Test group after 12 weeks had significantly better clinical results for probing depth with p=0.043 and attachment level with p=0.043	Feasibility of using ES cells to improve the regeneration of periodontal furcation defects.
9. Yu et al(2013)[22]	G-MSC	Class III furcation defects	Beagle dogs (16 defects) a) SRP b) SRP+G-MSC	8 weeks	% of new cementum SRP:24, SRP+G-MSC sheet:68% Area of new bone SRP: 10.37±9.53, SRP+G-MSCsheet:47.11±7.91	Periodontal fibers formed by GMSCs are oriented perpendicularly which play an important role for masticatory function and GMSCs regenerated bone, cementum and periodontal ligament effectively.
10. Iwasaki et al(2014)[23]	Human PDL-MSCs	Bilateral class II furcation defects (1.5×2mm)	Rats (12 defects) a) amniotic membrane b) PDL-MSC	4 weeks	Newly formed cementum in the PDSLC-amnion site was 3.441 ± 0.485 mm and not	Periodontal regenerationoccurred by differentiation of PDL-SC into cementoblast and

			+ amniotic membrane		measurable in amniotic transplanted sites	later on cementogenesis
11. Nagahara et al (2015)[24]	BM-MSC	Bilateral class III furcation defects (4mm from CEJ)	Beagle dogs (72 defects) a) col b) TCP/col c) MSC/col d)MSC/col/TCP	8 weeks	% of new cementum groups a, b, c and d were 36.8 ± 7.1%, 38.5 ± 12.7%, 69.9 ± 30.3% and 79.4 ± 15.7%, respectively. % of new bone area in groups were 17.5 ± 8.3%, 38.5 ± 15.1%, 23.5 ± 17.4% and 65.3 ± 13.1%, respectively	Alloplast act as scaffold for bone regeneration with MSC but at 8 weeks statistically non-significant regeneration was found in other groups.
12. Rezaei et al (2019)[25]	BM-MSC	Class II furcation	Mongrel dogs (n=5) a) PRP+Fibrin glue b) PRP+ cBM-MSCs+ fibrin glue c) Fibrin glue d) Fibrin glue+ cBM-MSCs e) Control	8 weeks	% of new cementum: PRP+Fibrin glue:2.95 PRP+ cBM-MSCs+ fibrin glue:2.53Fibrin glue: 1.24Fibrin glue+ cBM-MSCs: 3.33 Control :5.30% of new one: PRP + Fibringlue:74.81PRP + BM-MSCs+ fibrin glue:66.11Fibrin glue: 67.83Fibrin glue+ cBM-MSCs: 76.76 Control: 56.61	Statistically non-significant results were observed between the groups though, higher rate of bone and PDL formation was observed in MSC group as compared to other.

Limitations: However despite the positive results, a number of important issues need to be looked upon before human trials become a reality such as designing appropriate delivery devices, understanding the immunogenicity and immune-regulatory properties of various stem cells, defining which tissues can provide the most appropriate donor cells, devising ways to control the overall regenerative process and making it cost effective.

Conclusion- Current scientific data is unanimous on the fact that stem cell therapy has a positive impact on periodontal tissue regeneration. Stem cell based periodontal regeneration is considered to be biologically

possible but clinically unpredictable. Randomized control trial on humans with more sample size and follow-ups are required to provide the evidence for periodontal regeneration using stem cells. Establishment of large scale preparation facility incorporating the stringent protocols of good manufacturing practice (GMP), as dictated by bodies such as the Food and Drug Administration will be an absolute necessity.

Conflict of interest and source of funding- The authors have reported no conflicts of interest related to this study.

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