EFFICACY OF PERIODONTAL LIGAMENT CELLS FOR THE TREATMENT OF INTRA-BONY DEFECTS OF PERIODONTIUM AMONG THE POPULATION OF KARACHI: RANDOMIZED CLINICAL TRIAL

[1]Dr Aeliya Nabeel, [2]Dr Ujala Mushtaque, [3]Dr Hina Khan, [4]Dr Hina Nafees, [5]Dr Muhammad Zohaib Younus, [6]Dr Fariha Irfan

[1]Dr Aeliya Nabeel
BDS, Graduate of Liaquat College of Medicine and Dentistry, Karachi, Pakistan

[2]Dr Ujala Mushtaque (Corresponding Author)
Assistant Professor, Head, Department of Periodontology, Jinnah Medical and Dental College, Karachi, Pakistan

[3]Dr Hina Khan
Assistant Professor, Operative dentistry, Hamdard University, Karachi, Pakistan

[4] Dr Hina Nafees
Lecturer, Operative Dentistry, Hamdard University, Karachi, Pakistan

[5]Dr Muhammad Zohaib Younus
Assistant Professor, Endodontic Department, Hamdard University Dental Hospital, Karachi, Pakistan

[6]Dr Fariha Irfan
Assistant Professor Operative Dentistry, Hamdard University, Karachi, Pakistan

Author's Contribution:

A.N. and U.M. designed the model and the computational framework and analyzed the data. H.K. and H.N. carried out the implementation. M.Z.Y. performed the calculations. F.I. and A.N. wrote the manuscript with input from all authors. U.M. and H.K. conceived the study and were in charge of overall direction and planning.

Corresponding Author:

Dr Ujala Mushtaque
Assistant Professor, Head, Department of Periodontology, Jinnah Medical and Dental College, Karachi, Pakistan

ABSTRACT:

Aims and objectives:

Periodontitis is the most common disease of periodontium leading to the loss of teeth in adults. Various studies have shown that periodontal ligament cells help in the regeneration of damaged periodontium.

Methodology:

A randomized control trial from a multiple-center study was conducted among the population of Karachi. To repair the periodontal intra-bony abnormalities, autologous periodontal ligament cells were combined with bony minerals. Patients were grouped into Group A (Control group) and Group B (Treatment group). After 12 months of follow up the extent of the disease was analyzed among the population of Karachi, Pakistan. To assess the treatment's efficacy, the study's outcome was based on the regeneration of alveolar bone followed by surgery.

Results:

Ninety-two patients with the disease of periodontium with intra-bony defects were enrolled belonging to the age group 20 to 55 years. Out of these patients, 48 were enrolled in the control group and 44 were enrolled in the treatment group. These patients were clinically evaluated along with the surgical procedure. Clinically no adverse signs were observed concerning periodontal ligament cells. The study's results revealed a significant rise in the height of the bone along with a decrease in the depth of bone defect ($p < 0.001$). There was no statistically significant difference between the two groups ($p > 0.05$).

Conclusions:

This study concluded that periodontal intra-bony defects can be treated effectively with the help of autologous periodontal ligament cells.

Keywords: Periodontium, intra-bony defects, autologous, periodontal ligament cells

INTRODUCTION:

Globally, periodontitis is one of the most common localized infectious diseases of periodontium caused by opportunistic bacteria and sub-gingival plaque accumulation in the oral cavity (1). It is also the main cause of tooth loss in middle age adults with which time destroys the supporting structures of teeth (2). Hence, if it is left untreated it may result in severe pathological inflammatory consequences due to the changes which occur in the supporting structure of teeth (3). The Clinical symptoms of periodontitis include loss of clinical attachment level and probing pocket depth on the contrary radiographically loss of alveolar bone is observed (4). Various studies have shown that the most common type of periodontitis among adults is moderate periodontitis, and only 15% of people at some point in their lives experience severe generalized periodontitis (5). Periodontal disease is one of the most significant issues which is being faced by dentists, patients, and public healthcare professionals. It should be noted that in traditional treatment methods, whether through non-surgical or surgical therapy, the periodontal supporting structures are destroyed by periodontal disease to some extent (6). There is a dire need to have efficient treatment options for intra-bony defects so that a quality and healthy lifestyle can be adopted (7). Usually, the conventional method of treating the disease of periodontium arrests the disease but unfortunately, it does not regain the support of bone or connective tissue which is lost during the disease process (8, 9). In recent years, dentists have been using regenerative procedures to restore bone, cementum, and the periodontal ligament (10). Numerous pieces of evidence have shown that treating single or numerous intra-bony abnormalities with periodontal regeneration is effective and predictable (11). According to recent developments in stem cell biology and regenerative medicine, cell-based therapy is possible now for the treatment of periodontium. Various studies have also shown that auto-grafts, xeno-grafts, allografts, and alloplastic materials can also be used for periodontal ligaments to re-grow (12). Numerous cells such as osteoblastic, and cementoblasts can be used to regenerate a variety of periodontal tissues. Several in vitro and in vivo models have produced positive results in various pre-clinical studies (13). There is evidence that the periodontal ligament tissue cells can develop full periodontal attachment apparatus. Few progenitor cells of periodontal ligament cells are thought to be responsible for the regeneration of periodontal ligament cells; as a result, the involvement of these mesenchymal stem/stromal cells (MSCs) is necessary for the regeneration of periodontium (14). The long-term objective of periodontal therapy is to restore the functional attachment apparatus of the periodontium, which involves the distinct tissues, of periodontium which is eventually damaged due to periodontitis. Hence, deep intraosseous defects even persisted after the therapy of periodontium (15). Various methods have been used for periodontal regeneration to enhance the immediate and long-term clinical results of periodontally damaged teeth presenting with decreased periodontal support and deep pockets. Patients who participate in supportive periodontal-care programs have an increased risk of tooth loss if their deep pockets continue after active periodontal therapy (16). Deep intra-bony abnormalities and teeth with deep pockets are regarded as clinically challenging cases (17). To treat periodontal intra-bony defects, periodontal ligament stem cells will be utilized to evaluate their efficacy. This study will help to

provide the effectiveness of periodontal stem cells in regenerative dentistry among the population of Karachi, Pakistan.

MATERIALS AND METHODS:

A randomized control trial was conducted among the four dental hospitals situated in Karachi, Pakistan. Ninety-two patients from these hospitals were recruited. From each hospital, 23 patients were randomly assigned to the study. Only those patients were recruited in the study that had the disease of periodontium with intra-bony defects belonging to the age group 20 to 55 years. Out of these 48 patients were enrolled in the control group and 44 in the treatment group. This study was conducted from 1 June 2019 to 31 December 2019 and by the end of December 2022 the study was completed with a follow-up of twelve months. The study was approved by the ethical review board of Jinnah Medical and Dental College and Hospital. All the patients were informed about the study subject. Only those patients were included in the study that fulfilled the criteria of selection while the rest of the patients was excluded from the study. Once the oral and clinical diagnosis of all the patients was done they were asked to fill out the informed consent. For the assessment, initially, the patient was asked to maintain oral hygiene, scaling and root planning were also recommended to avoid any bacterial infection in the mouth. To analyze the osteogenic activity, the third molar of the patients was used to provide a periodontal ligament sheet. For this purpose, the patient was recommended to extract the third molar because of its non-functional ability. Post-operative complications were recorded and were also examined. At different stages, patients were asked to get their blood and immunoglobulins tests done. In this study, all the teeth which have received the treatment were randomly included in the study. The data were analyzed and subjected to SPSS software version 21. The primary outcome was based on protocol. Independent group tests were performed for the baseline comparison between age and clinical indices. The comparison of gender between the groups was analyzed by using Fisher's exact probability test. Hence, repeated measure analysis of variance was used to assess the changes in clinical indices. A P-value of < 0.05 was considered significant.

Figure-1 Flow diagram for patient selection, randomization, and follow-up

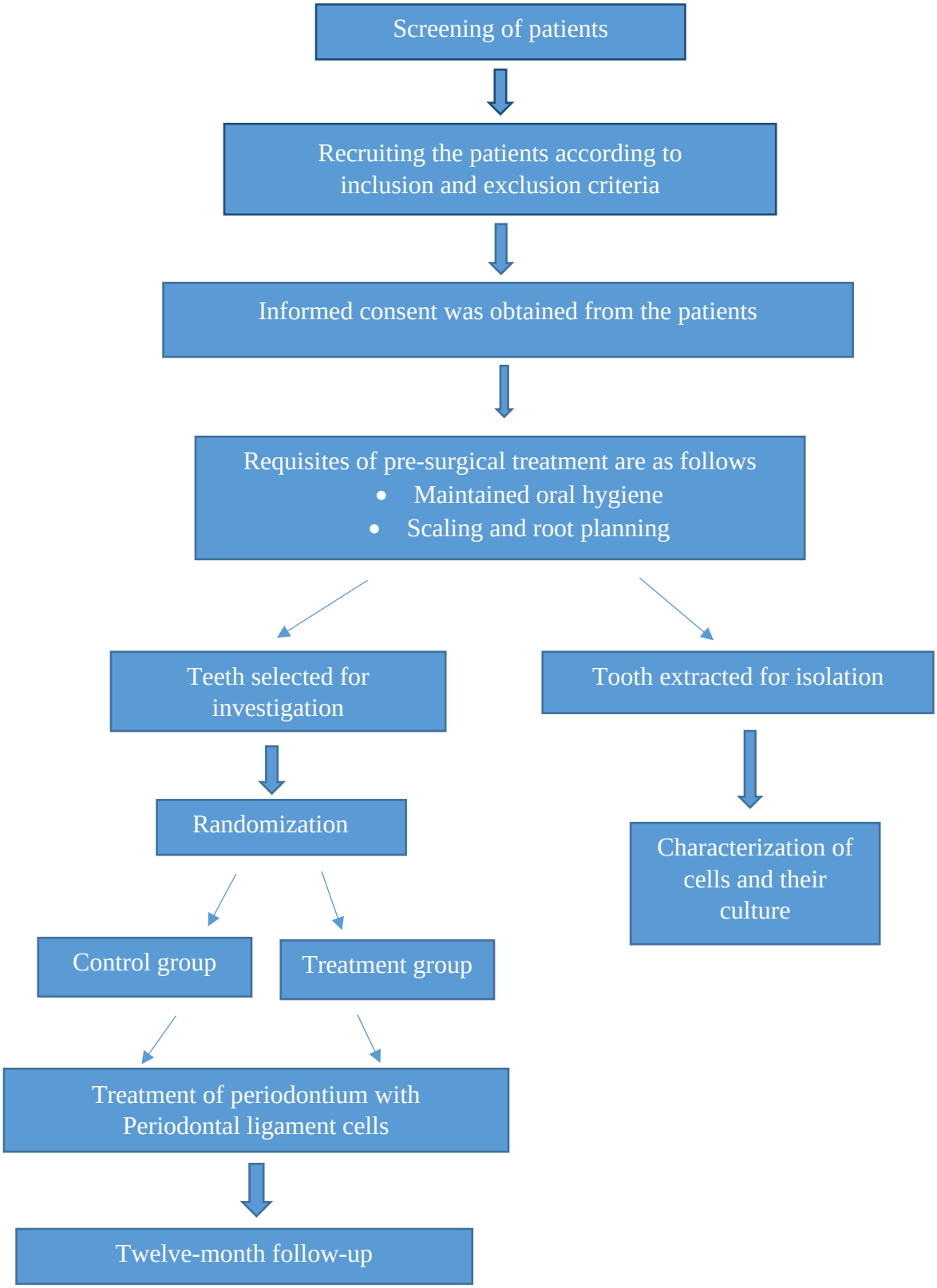
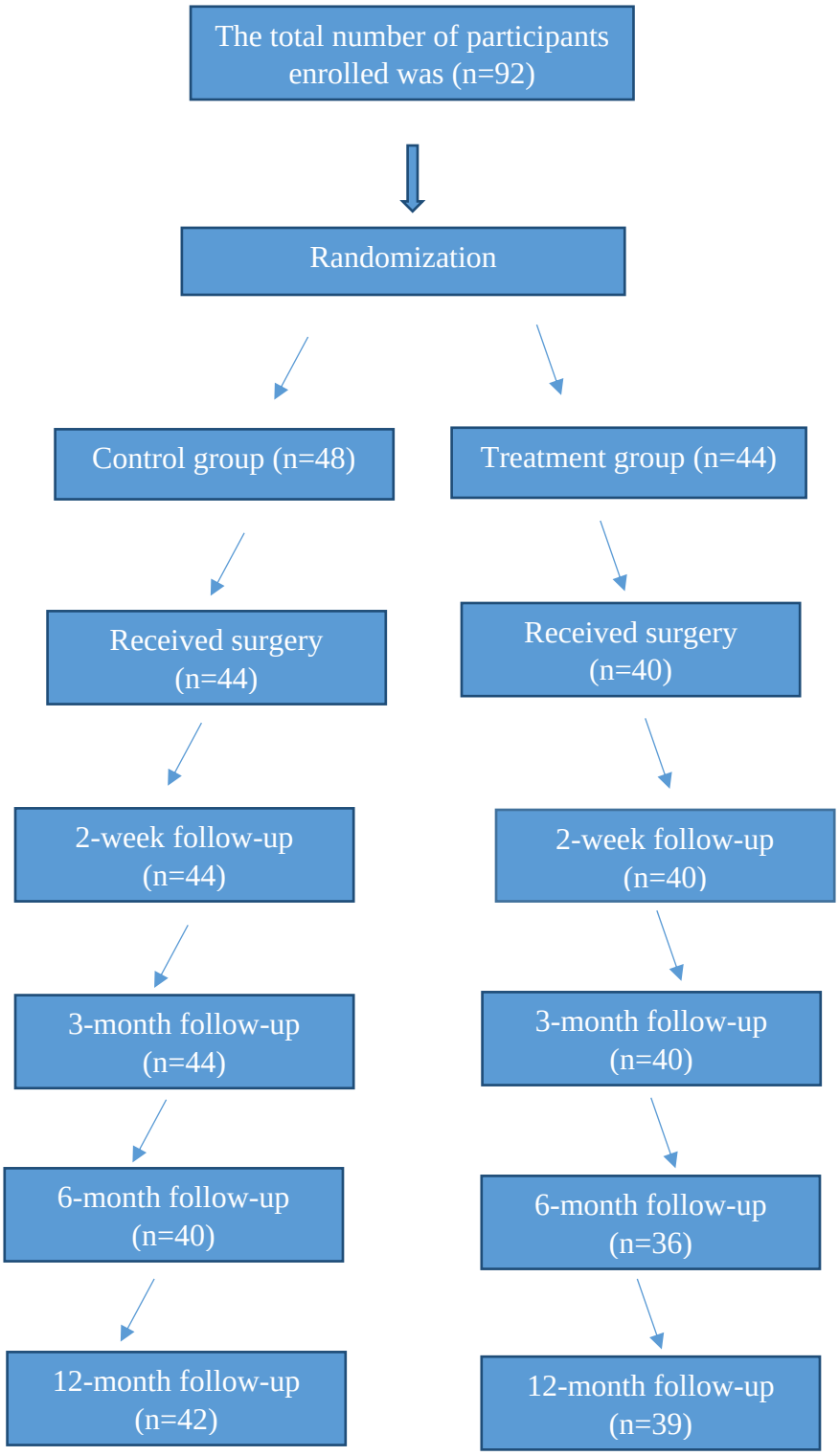


Fig 2- Flow diagram of a procedure



RESULTS:

In the study, Ninety-two patients were recruited. Forty-eight patients were randomly taken for the control and forty-four patients were taken in the treatment group. Although, only 61 teeth received surgery. Hence, Fisher's exact probability test revealed no significant group differences. In this study, those patients were enrolled who had an extraction of wisdom teeth due to impaction and non-functionality. Before surgical extraction, two assessors of teeth concluded that teeth need extraction. For cell isolation, the sample of the extracted tooth was used. The colony-forming abilities of all the cells were positive for the mesenchymal stem cells (MSC markers), and on the contrary, it was negative for the hematopoietic markers. Hence no adverse effects were observed among the patients after post-operative healing. Patients were asked to get their blood and urine test post-operatively within the following timeframe (two weeks, three months, and twelve months postoperatively). Hence the results of these tests revealed that none of the values exceeded its clinical reference value. The results of the study revealed that no tooth was lost during this procedure. Hence the bone height was increased during the 3, 6, and 12 months with a decrease in bony defects. With time, bone height increased significantly ($p < 0.001$). However, no statistically significant differences were found between the treatment group and the Control group ($p > 0.05$) as shown in Table 2. At 3 months, post-surgery, the results revealed that no significant difference was observed in the clinical periodontal parameters ($p > 0.05$) as shown in Table 3.

Table 1- Demographic characteristics of patients with baseline measurements of the teeth.

Characteristics	Control Group (n=48)	Treatment Group (n=44)	P-value
Gender			0.130
Male	22	19	
Female	26	25	
Age (mean ± SE)	27.04 ± 5.90	29.08 ± 2.22	0.052
Clinical Attachment Level (mean ± SE)	4.28 ± 2.40	4.14 ± 1.32	0.0792
Bone Defect (mean ± SE)	7.10 ± 1.23	7.01 ± 1.62	0.891
Pocket Depth (mean ± SE) **Facial** **Palatal**	 4.88 ± 1.39 4.78 ± 1.21	 5.42 ± 1.12 5.34 ± 1.10	 0.176 0.272

Table 2- The depth of intra-bony defects observed between the control and treatment group within a time frame of (3,6 and 12 months)

	Number of Teeth	Baseline survey	Three months	Six months	Twelve months	F value	P value
Control Group (n=48)	48	6.19 ± 1.77	3.81 ± 1.82	4.11 ± 1.43	4.79 ± 1.31	0.10	0.741
Treatment Group (n=44)	44	6.20 ± 2 55	3.89 ± 163	4.51 ± 1.77	4.39 ± 2.01		

Table 3- Clinical periodontal parameters observed between control and treatment group

	Number of teeth	Surfaces of teeth	Baseline survey	Three months	F value	P value
Clinical attachment loss						
• **Control Group**	48		5.18 ± 1.50	5.01 ± 1.40	0.810	0.361
• **Treatment Group**	44		5.10 ± 1.42	4.40 ± 1.10		
Pocket depths						
• **Control Group**	48	Buccal	5.60 ± 1.50	3.80 ± 0.71	0.890	0.323
• **Treatment Group**	44	Buccal	6.40 ± 1.90	3.79 ± 1.02		
• **Control Group**	48	Palatal	5.80 ± 1.40	3.70 ± 0.50	2.109	0.140
• **Treatment Group**	44	Palatal	6.20 ± 1.30	4.19 ± 0.80		

DISCUSSION:

Various procedures are available for the treatment of periodontal intra-bony defects clinically, but dentists in Karachi, Pakistan are still looking for more reliable regenerative treatments that are less technically sensitive, promote the regeneration of tissues rapidly, and apply to a wide range of periodontal conditions that are seen regularly in dental hospitals and OPD. Various animal studies have revealed that periodontal ligament cells have played a vital role in the therapy of periodontium (18). Several pieces of evidence have been revealed from various studies that periodontal regeneration is an effective way to treat the diseases of the periodontium as a result of which regenerative procedure of periodontium has become common (19-21). Before this therapy progresses, critical challenges such as the immunogenicity of cells must be resolved and it should be economical (22-25). Cell-based therapies are more appropriate for incurable and life-threatening illnesses such as diabetes, Parkinson's, muscular dystrophy,

Alzheimer's, neurological and cardiac diseases, and refractory systemic lupus erythematosus (26).

The procedure of cell therapy to regenerate periodontal tissue is not an economical option or competitive as compared to dental implants or root canal treatments. Unfortunately, due to the harmless nature of periodontitis, periodontal tissues have not been considered appropriate for stem cell regenerative therapy. Hence, patients with impacted teeth are perfect cases for experimenting with new treatments as teeth are easily accessible (27).

The results of the study revealed that periodontal ligament cells have a therapeutic effect and the patients had no side effects during the whole follow-up period (27). In our study, no clinical safety issues related to the experimental periodontal ligament cells were found. Further the results of the study revealed that with time, the height of the alveolar bone was significantly increased in each group ($p < 0.001$) as shown in Table 2. At 3 months, post-surgery, the results revealed that no significant difference was observed in the clinical periodontal parameters ($p > 0.05$). Although no statistically significant differences were found in bone and clinical parameters between the treatment and Control groups ($p > 0.05$) as shown in Tables 2 and 3. Hence, similar results were revealed in a study conducted by Feng et al., which showed a significant increase in the height of the alveolar bone ($p < 0.001$). The efficacy of periodontal ligament cells has been utilized in previous studies as well for the treatment of periodontium (28). Numerous studies conducted globally have proven that local cell injection had no adverse side effects (29). In periodontal regenerative dentistry, periodontal stem cell therapies should be further investigated by utilizing appropriate study designs and clinical trials in more challenging clinical circumstances.

CONCLUSION:

Regenerative therapies are leading the way towards evolutionary fundamental scientific research in the field of clinical stem cell procedures. As it is a potential new therapeutic approach that may make it possible to regenerate destroyed periodontium. Clinicians should continue to follow the development of regenerative therapies even though several challenges need to be resolved before regenerative therapies are widely used and accepted in the world. The results of this study revealed that periodontal ligament cells are effective for the treatment of periodontal intra-bony defects.

REFERENCES:

1- Shaikh MS, Zafar MS, Alnazzawi A. Comparing Nanohydroxyapatite Graft and Other Bone Grafts in the Repair of Periodontal Infrabony Lesions: A Systematic Review and Meta-Analysis. International Journal of Molecular Sciences. 2021 Nov 6;22(21):12021. https://doi:10.3390/ijms222112021. PMID: 34769451; PMCID: PMC8584357.

2- Kao RT, Nares S, Reynolds MA. Periodontal regeneration–intrabony defects: a systematic review from the AAP regeneration workshop. Journal of Periodontology. 2015 Feb;86:S77-104. http://doi:10.1902/jop.2015.130685. Epub 2014 Sep 12. PMID: 25216204

3- Miron RJ, Moraschini V, Fujioka-Kobayashi M, Zhang Y, Kawase T, Cosgarea R, Jepsen S, Bishara M, Canullo L, Shirakata Y, Gruber R. Use of platelet-rich fibrin for the treatment of periodontal intra bony defects: a systematic review and meta-analysis. Clinical oral investigations. 2021 May;25:2461-78. http://doi:10.1007/s00784-021-03825-8. Epub 2021 Feb 20. PMID: 33609186; PMCID: PMC8060184

4- Vera J, Ochoa J, Romero M, Vazquez-Carcano M, Ramos-Gregorio CO, Aguilar RR, Cruz A, Sleiman P, Arias A. Intracanal cryotherapy reduces postoperative pain in teeth with symptomatic apical periodontitis: a randomized multicenter clinical trial. Journal of Endodontics. 2018 Jan 1;44(1):4-8. http://doi:10.1016/j.joen.2017.08.038. Epub 2017 Nov 1. PMID: 29079057.

5- Chen, FM., Gao, LN., Tian, BM. *et al.* Treatment of periodontal intra-bony defects using autologous periodontal ligament stem cells: a randomized clinical trial. *Stem Cell Res Ther* **7**, 33 (2016). https://doi.org/10.1186/s13287-016-0288-1

6- Nuñez J, Vignoletti F, Caffesse RG, Sanz M. Cellular therapy in periodontal regeneration. Periodontology 2000. 2019 Feb;79(1):107-16. http://doi:10.1111/prd.12250.PMID: 30892768

7- Graziani F, Karapetsa D, Alonso B, Herrera D. Nonsurgical and surgical treatment of periodontitis: how many options for one disease? Periodontology 2000. 2017 Oct;75(1):152-88. http://doi:10.1111/prd.12201. PMID: 28758300

8- Giannobile WV. Commentary: treatment of periodontitis: destroyed periodontal tissues can be regenerated under certain conditions. http://doi:10.1902/jop.2014.140254. PMID: 25168257

9- Queiroz A, Albuquerque-Souza E, Gasparoni LM, de França BN, Pelissari C, Trierveiler M, Holzhausen M. Therapeutic potential of periodontal ligament stem cells. World Journal of Stem Cells. 2021 Jun 6;13(6):605. http://doi:10.4252/wjsc.v13.i6.605 PMID: 34249230; PMCID: PMC8246246

10- Kao DW, Fiorellini JP. Regenerative periodontal therapy. Front Oral Biol. 2012;15:149-59. http://doi:10.1159/000329677. Epub 2011 Nov 11. PMID: 22142962.)

11- Cortellini P, Tonetti MS. Clinical concepts for regenerative therapy in intra-bony defects. Periodontol 2000. 2015 Jun;68(1):282-307. http://doi:10.1111/prd.12048.PMID: 25867990

12- Tatullo M, Codispoti B, Sied J, Makeeva I, Paduano F, Marrelli M, Spagnuolo G. Stem cells-based and molecular-based approaches in regenerative dentistry: A topical review.

Current Stem Cell Research & Therapy. 2019 Oct 1;14(7):607-16. http://doi:10.2174/1574888X14666190626111154. PMID: 31271121.

13- Bright R, Hynes K, Gronthos S, Bartold PM. Periodontal ligament-derived cells for periodontal regeneration in animal models: a systematic review. Journal of periodontal research. 2015 Apr;50(2):160-72. http://doi:10.1111/jre.12205. Epub 2014 Jun 26. PMID: 24965968.

14- Liu J, Ruan J, Weir MD, Ren K, Schneider A, Wang P, Oates TW, Chang X, Xu HH. Periodontal bone-ligament-cementum regeneration via scaffolds and stem cells. Cells. 2019 Jun 4;8(6):537. http://doi:10.3390/cells8060537. PMID: 31167434; PMCID: PMC6628570.

15- Lu H, Xie C, Zhao YM, Chen FM. Translational research and therapeutic applications of stem cell transplantation in periodontal regenerative medicine. Cell Transplant. 2013;22:205–29. http://doi:10.3727/096368912X656171.Epub 2012 Oct 1. PMID: 23031442

16- Kao RT, Nares S, Reynolds MA. Periodontal regeneration–intra-bony defects: a systematic review from the AAP regeneration workshop. Journal of Periodontology. 2015 Feb;86:S77-104. http://doi:10.1902/jop.2015.130685. Epub 2014 Sep 12. PMID: 25216204

17- Xu XY, Li X, Wang J, He XT, Sun HH, Chen FM. Concise review: periodontal tissue regeneration using stem cells: strategies and translational considerations. Stem cells translational medicine. 2019 Apr;8(4):392-403. http://doi:10.1002/sctm.18-0181. Epub 2018 Dec 26. PMID: 30585445; PMCID: PMC6431686

18- Tomokiyo A, Wada N, Maeda H. Periodontal ligament stem cells: regenerative potency in the periodontium. Stem cells and development. 2019 Jul 1;28(15):974-85. http://doi:10.1089/scd.2019.0031. PMID: 31215350.

19- Iwata T, Yamato M, Washio K, Yoshida T, Tsumanuma Y, Yamada A, Onizuka S, Izumi Y, Ando T, Okano T, Ishikawa I. Periodontal regeneration with autologous periodontal ligament-derived cell sheets–a safety and efficacy study in ten patients. Regenerative therapy. 2018 Dec 1;9:38-44. http://doi:10.1016/j.reth.2018.07.002. PMID: 30525074; PMCID: PMC6222282.

20- Bartold M, Ivanovski S. Stem cell applications in periodontal regeneration. Dental Clinics. 2022 Jan 1;66(1):53-74. http://doi:10.1016/j.cden.2021.06.002.Epub 2021 Aug 13. PMID: 34794554.

21- Tassi SA, Sergio NZ, Misawa MY, Villar CC. Efficacy of stem cells on periodontal regeneration: a systematic review of pre-clinical studies. Journal of periodontal research. 2017 Oct;52(5):793-812. http://doi:10.1111/jre.12455. Epub 2017 Apr 10. PMID: 28394043

22- Gaubys A, Papeckys V, Pranskunas M. Use of autologous stem cells for the regeneration of periodontal defects in animal studies: a systematic review and meta-analysis. Journal of oral & maxillofacial research. 2018 Apr;9(2). http://doi:10.5037/jomr.2018.9203. PMID: 30116515; PMCID: PMC6090251.

23- Trubiani O, Pizzicannella J, Caputi S, Marchisio M, Mazzon E, Paganelli R, Paganelli A, Diomede F. Periodontal ligament stem cells: current knowledge and future

perspectives. Stem cells and development. 2019 Aug 1;28(15):995-1003. http://doi.10.1089/scd.2019.0025. Epub 2019 May 20. PMID: 31017047.

24- Fawzy El-Sayed KM, Elahmady M, Adawi Z, Aboushadi N, Elnaggar A, Eid M, Hamdy N, Sanaa D, Dörfer CE. The periodontal stem/progenitor cell inflammatory-regenerative cross-talk: A new perspective. Journal of Periodontal Research. 2019 Apr;54(2):81-94. http://doi:10.1111/jre.12616. Epub 2018 Oct 8. PMID: 30295324.

25- Nagata M, Iwasaki K, Akazawa K, Komaki M, Yokoyama N, Izumi Y, Morita I. Conditioned medium from periodontal ligament stem cells enhances periodontal regeneration. Tissue Engineering Part A. 2017 May 1;23(9-10):367-http://doi:10.1089/ten.TEA.2016.0274.Epub 2017 Jan 27. PMID: 28027709; PMCID: PMC5444511

26- Mitsiadis TA, Orsini G, Jimenez-Rojo L. Stem cell-based approaches in dentistry. European Cells and Materials (ECM). 2015;30:248-57. http://doi:10.22203/ecm.v030a17. PMID: 26562631.

27- Feng F, Akiyama K, Liu Y, Yamaza T, Wang TM, Chen JH, Wang BB, Huang GJ, Wang S, Shi S. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. Oral diseases. 2010 Jan;16(1):20-8. http://doi:10.1111/j.1601-0825.2009.01593.x PMID: 20355278; PMCID: PMC2848819

28- Ouchi T, Nakagawa T. Mesenchymal stem cell-based tissue regeneration therapies for periodontitis. Regenerative Therapy. 2020 Jun 1;14:72-8. http://doi:10.1016/j.reth.2019.12.011. PMID: 31970269; PMCID: PMC6962327.

29- Sheth N, Parmar B, Patel H, Makwana K. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells—a pilot study. International Journal of Oral and Maxillofacial Surgery. 2017 Mar1;46:215.https://doi.org/10.1016/j.ijom.2017.02.730