ORIGINAL ARTICLE

10.2478/AMB-2025-0073

MINIMALLY INVASIVE REGENERATIVE TREATMENT OF VERTICAL BONE DEFECTS: A CLINICAL TRIAL

K. Kotsilkov, V. Dosseva-Panova, Z. Pashova-Tasseva, H. Maynalovska

Department of Periodontology, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria

Abstract. *The* ***aim*** *of the current study was to analyze and evaluate the outcomes of regenerative therapy using a bone graft and enamel matrix proteins, applied through a minimally invasive surgical approach for the treatment of vertical bone defects.* ***Materials and methods:*** *Ten systemically healthy patients with a total of 30 vertical bone defects were treated using a minimally invasive surgical approach combining enamel matrix derivative (Emdogain) with a bone graft. Probing pocket depth (PPD), clinical attachment level (CAL), and radiographic defect depth were analyzed before and at the 6-month postoperative follow-up. Statistical analysis was performed using descriptive statistics and the paired Student's t-test ($p < 0.05$).* ***Results:*** *At the 6-month follow-up, a statistically significant reduction in probing pocket depth (mean reduction: 4.00 ± 1.44 mm; $p < 0.0001$) and a significant gain in clinical attachment level (mean gain: 4.62 ± 2.13 mm; $p < 0.0001$) were recorded. Radiographic evaluation also revealed a statistically significant reduction in bone defect depth (mean change: 2.95 ± 1.34 mm; $p = 0.0027$), with a residual mean depth of 0.57 ± 0.73 mm.* ***Conclusion:*** *The minimally invasive approach using enamel matrix derivatives (EMD) in combination with bone grafting proved effective in the treatment of vertical bone defects. The favorable clinical and radiographic outcomes suggest periodontal stability and support the long-term preservation of the treated teeth.*

Key words: *vertical bone defects, periodontal regeneration, minimally invasive surgical treatment, enamel matrix derivatives*

Corresponding author: *Hristina Maynalovska, Department of Periodontology, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria, email: h.maynalovska@fdm.mu-sofia.bg*

ORCID: *0000-0001-8331-1514*

Received: *07 April 2025;* ***Accepted:*** *15 April 2025*

INTRODUCTION

Periodontitis is a highly prevalent chronic inflammatory disease affecting a large portion of the human population at all ages [1-5]. It is driven by a dysbiotic plaque biofilm and leads to the gradual destruction of the periodontal supporting tissues. Clinically, it is characterized by the formation of periodontal pockets, gingival bleeding, loss of clinical attachment, and alveolar bone loss detectable on radiographs [6-10].

Vertical (angular) bone defects are anatomical consequences of the apical progression of periodontal infection, where the base of the periodontal pocket lies below the alveolar crest and is surrounded by residual bony walls. Due to limited access for effective removal of subgingival biofilm and the irregularity of the bone architecture, these defects often persist after initial periodontal therapy [11]. As a result, they are associated with ongoing disease progression and an increased risk of tooth loss [12]. Given this elevated risk, surgical intervention is frequently required in addition to cause-related therapy to prevent further breakdown of the periodontal supporting structures [11].

Over the past decade, the diagnostic and surgical approaches to the dental diseases and to periodontal regenerative therapy has evolved significantly, with a growing emphasis on minimally invasive techniques [13-17]. These approaches prioritize gentle handling of soft tissues and aim to optimize clinical outcomes by enhancing the stability of the blood clot, promoting primary intention healing, and reducing postoperative discomfort and tissue contraction. Such refinement in surgical protocols supports better esthetic outcomes and long-term tissue stability. Crucially, successful periodontal regeneration relies on the stable adhesion and maturation of the blood clot on the root surface, combined with tension-free wound closure, to encourage true tissue regeneration rather than mere repair through long junctional epithelium formation [18].

Minimally invasive surgical protocols are often combined with the use of enamel matrix derivatives (EMDs) to enhance regenerative outcomes. EMDs are a group of proteins secreted by Hertwig's epithelial root sheath during the embryonic development of the tooth. When applied to a debrided root surface, they trigger a cascade of biological events that replicate the natural processes involved in periodontal tissue formation, thereby supporting true periodontal regeneration rather than simple repair [19].

The combination of enamel matrix proteins with bone grafts is believed to have a potentially synergistic effect. This is thought to result from two independent and distinct healing processes occurring simultaneously within an intrabony defect. The bone graft acts as an osteoinductive and/or osteoconductive agent and helps maintain space within the defect by stabilizing the blood coagulum. Enamel matrix proteins, on the other hand, act at the root surface level by mimicking the biological processes that occur during root and periodontal tissue development, thereby promoting the formation of new cementum and new attachment structures [20, 21]. From a biological standpoint, the combination of EMD and bone graft can be regarded as a tissue engineering approach, where the bone graft serves as a scaffold and the EMD provides the signaling molecules necessary to promote regeneration [22].

The aim of the current study was to analyze and evaluate the outcomes of regenerative therapy using a bone graft and enamel matrix proteins, applied through a minimally invasive surgical approach for the treatment of vertical bone defects.

MATERIALS AND METHODS

Study design

This study was designed as a prospective single-arm clinical study aimed at evaluating the outcomes of regenerative periodontal treatment applied to 30 vertical bone defects in 10 patients, each presenting with at least one vertical bone defect with a radiographic depth of 3 mm or greater.

Inclusion criteria

The inclusion criteria for the patients were as follows: systemically healthy individuals aged 18 years or older, with no known history of allergies, diagnosed with stage III or IV periodontitis according to current classification guidelines. All participants demonstrated satisfactory oral hygiene, defined as Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS) below 15% following initial periodontal therapy. In addition, participants needed to present with at least one angular bone defect associated with a probing depth (PD) of 6 mm or more and bleeding on probing (BoP) at the time of re-evaluation after non-surgical periodontal treatment.

Exclusion criteria

Patients were excluded from the study if they had any systemic disease that could potentially compromise treatment outcomes or if they required antibiotic prophylaxis due to transient bacteremia during diagnostic or therapeutic procedures. The individuals who smoked, demonstrated inadequate plaque control or were pregnant or breastfeeding were also excluded.

Tooth-level inclusion criteria required the presence of proximal angular bone defects, a radiographically confirmed intrabony component of 3 mm or greater, a probing depth of 6 mm or more, and the absence of periapical lesions.

Teeth were excluded if they had inadequate endodontic treatment, exhibited periapical lesions, presented with defects extending into the furcation area, were third molars, or showed grade III mobility.

At the beginning of the study, the following clinical parameters were recorded in the selected patients to assess their oral hygiene status and to document their periodontal condition: FMPS, FMBS, probing depth (PD), clinical attachment level (CAL), bleeding on probing, and recession (R). The data were recorded in a periodontal chart, with measurements taken at six sites per tooth. A manual graduated periodontal probe (UNC-15, Hu-Friedy) was used. Measurements were documented at baseline (initial diagnosis), at the re-evaluation following non-surgical therapy, and at the 6-month postoperative follow-up.

To assess the bone defect, each patient underwent intraoral periapical radiography at two time points: at the time of initial diagnosis to establish baseline data, and again at 6 months postoperatively to evaluate the treatment outcome. The recorded parameters included the depth of the intraosseous component of the defect, measured as the distance from the base of the defect to the level of the intraosseous septum of the adjacent, healthier tooth, as well as the defect angle, defined as the angle formed between the root surface and the wall of the intraosseous defect.

At the 6-month follow-up, radiographic bone fill and the residual depth of the bone defect were assessed using the same parameters described in the initial evaluation, based on intraoral periapical radiographs.

Ethical approval

Ethical approval for this study was obtained from the Research Ethics Committee at the Medical University of Sofia – KENIMUS (Protocol No. 16/19 December 2023). All the participants received comprehensive information regarding the aims and methods of the study and provided written informed consent.

Surgical Procedure

The surgical procedure was performed under local anesthesia by a single experienced operator (K.K). A horizontal incision was made at the base of the papilla associated with the treated defect, followed by a vertical incision extending to the marginal bone crest to ensure access to the bony defect. A full-thickness flap was elevated, extending 2 mm beyond the crestal bone margin to adequately expose the defect. An incision was made at the level of the intraosseous septum to separate the supracrestal soft tissues from those occupying the intrabony component of the defect. The surgical technique followed the principles of the single flap approach as proposed by Trombelli et al. [23] and was performed in accordance with the modified minimally invasive surgical technique (MIST) described by Cortellini and Tonetti [24-26], aiming to preserve soft tissue integrity and optimize healing. Thorough instrumentation of the root surface and the bony defect was performed using both mechanical and manual instruments, including scaling and root planing.

The root surface was conditioned with 24% ethylenediaminetetraacetic acid (EDTA) gel (pH 6.7, PrefGel, Straumann) for 2 minutes, followed by copious irrigation of the surgical site with sterile saline solution. Emdogain was then applied, beginning from the base of the bone defect and covering the entire exposed root surface. The bone graft was mixed with Emdogain and placed into the intrabony component of the defect. The flap was repositioned and sutured using a combination of horizontal mattress and O-shaped sutures.

Systemic antibiotic therapy with Augmentin 1000 mg every 12 hours for 7 days was prescribed, beginning one day prior to surgery. The patients were instructed to rinse with a 0.2% chlorhexidine gluconate solution three times daily for one week before and for two weeks after the surgical procedure, followed by twice-daily rinsing for an additional two weeks.

Sutures were removed after two weeks. Patients received instructions on maintaining appropriate home oral hygiene practices.

Statistical Methods

Statistical analysis was performed on raw data compiled in an Excel spreadsheet. The applied methods included descriptive statistics and the paired Student's t-test. A p-value of less than 0.05, with a 95% confidence interval, was considered statistically significant for rejecting the null hypothesis. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

RESULTS

The study included 10 patients (3 men and 7 women) at a mean age of 42 ± 6 years (range: 36-53). Each patient presented with at least one vertical bone defect and met the inclusion criteria for the clinical trial. A total of 30 defects were surgically treated.

The distribution of participants by sex and of the defects according to the type of affected tooth (non-molar/molar) is presented in Table 1.

All the patients underwent non-surgical periodontal therapy, which included motivation and instruction for optimal personal oral hygiene, supra- and subgingival instrumentation, and the elimination of plaque-retentive factors where present. Substantial improvement was observed in the recorded parameters: FMPS decreased from 87.77% to 17.63%, and FMBS from 61.10% to 6.01%. These results clearly indicate that effective plaque control was achieved by the participants in the clinical study. A significant reduction was also noted in bleeding on probing (BoP), which decreased from 47.16% to 11.90%. The favorable outcomes of the initial therapy permitted progression to the next phase of periodontal treatment – surgical intervention.

Table 2 presents the baseline data on the characteristics of the treated defects.

Table 1. Distribution of participants by sex and of treated teeth by tooth type

Category	Number (N)	Percentage (%)
Participants (N = 10)		
Male	3	30%
Female	7	70%
Treated Teeth (N = 30)		
Non-molars	15	50%
Molars	15	50%

Table 2. Baseline characteristics of the defects

	Mean ± SD	95% CI
PPD (mm)	6.80 ± 1.49	6.24 - 7.36
CAL (mm)	7.77 ± 2.13	6.97 - 8.56
Infrabony defect (mm)*	5.17 ± 2.12	4.38 - 5.96

Acronyms: CAL = clinical attachment loss; CI = confidence interval; PPD = probing pocket depth; SD = standard deviation; *intra-operatively measured depth

None of the patients experienced complications postoperatively or throughout the follow-up period.

Table 3 presents the clinical outcomes measured six months following the surgical procedure.

Table 3. Clinical outcome at six months

	Mean ± SD	95% CI
PPD (mm)	2.80 ± 0.81	2.50 - 3.10
CAL (mm)	3.13 ± 1.70	2.50 - 3.77
CAL gain (mm)	4.63 ± 2.13	3.84 -5.43
PPD reduction (mm)	4.00 ± 1.44	3.46 - 4.54

Acronyms: CAL = clinical attachment loss; CI = confidence interval; PPD = probing pocket depth; SD = standard deviation

To evaluate the outcome of the surgical treatment, changes in pocket probing depth (PD) and clinical attachment level (CAL) were carefully analyzed. The reduction in probing depth was statistically highly significant ($p < 0.0001$; 95% CI: 3.46-4.54). The mean reduction was 4.00 ± 1.44 mm, with a mean post-operative probing depth of 2.80 ± 0.81 mm. The observed gain in clinical attachment was also statistically highly significant ($p < 0.0001$; 95% CI: 3.84-5.43), with a mean attachment gain of 4.62 ± 2.13 mm.

Another important aspect in evaluating the outcome of regenerative therapy is the bone fill within the treated defect. This analysis was based on changes observed in intraoral segmental radiographs taken before and six months after the surgical procedure. The paired t-test revealed a statistically significant reduction in the depth of the bone defect ($p = 0.0027$; 95% CI: 1.364-6.014) (Fig. 1). The mean depth of the residual bone defect was 0.57 ± 0.73 mm, while the mean change in defect depth was 2.95 ± 1.34 mm.

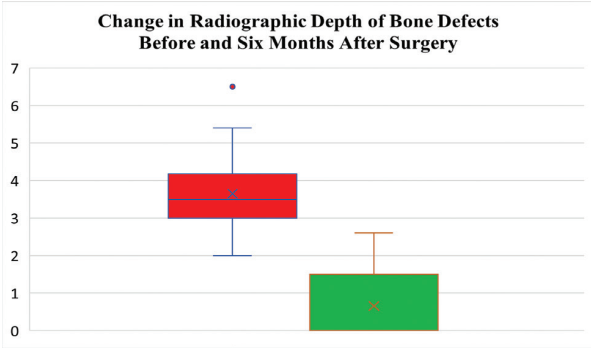

Figure 1. Reduction in radiographic depth of vertical bone defects – 6 months after surgery

DISCUSSION

The regenerative approach to the treatment of intraosseous bone defects has been proven to be an effective therapeutic model, leading to improvement in key periodontal parameters and contributing to a more favorable long-term prognosis of the treated teeth. Contemporary regenerative techniques consistently demonstrate reliable clinical outcomes, and their indications continue to expand as evidence supporting their efficacy grows [24-26].

In the present study, 30 vertical bone defects were treated using a minimally invasive surgical approach combined with a regenerative therapy involving a bone graft and Emdogain. The postoperative results at six months demonstrated reduced probing depth, gain in clinical attachment and radiographic bone fill.

The mean probing depth decreased from 6.80 ± 1.49 mm at baseline to 2.80 ± 0.81 mm postoperatively. The achieved reduction of 4.00 ± 1.44 mm was statistically significant. These findings are consistent with data reported in a review by Trombelli et al. [27], who observed a mean pocket depth reduction of 4.53 mm with single flap approaches, 4.58 mm with minimally invasive techniques, and 3.95 mm with papilla preservation flaps.

In 25 of the treated sites (83%), the probing depth was reduced to 3 mm or less – the values typically associated with a healthy gingival sulcus and considered by some authors as a meaningful clinical endpoint for regenerative periodontal therapy [28]. In the remaining 5 defects, the residual probing depth was 4 mm, a depth that has been shown to be associated with a very favorable long-term prognosis for the affected tooth [12]. These findings confirm the effectiveness of the applied therapeutic modality in reducing pocket depth in sites associated with angular bone defects.

Gain in clinical attachment is widely regarded as a qualitative indicator reflecting the extent of regenerated attachment tissues [29]. In the present study, a statistically significant mean attachment gain of 4.62 ± 2.13 mm was recorded. This outcome exceeds the mean values reported in the review by Trombelli et al. [27], which summarized data from 13 studies on the combined use of Emdogain and bone grafts. The authors reported a mean attachment gain of 3.65 mm for papilla preservation flaps and 4.10 mm for minimally invasive techniques.

Another commonly reported outcome in studies evaluating regenerative therapeutic approaches is the change in the radiographic depth of the intrabony component of the defect. In the aforementioned review, the mean reduction in bone defect depth was 3.29 mm for single flap approaches and 2.89 mm for minimally invasive techniques [15]. In the present study, the radiographic depth of the bone defect decreased from 3.55 ± 0.89 mm to 0.57 ± 0.73 mm. The resulting mean reduction of 2.95 ± 1.34 mm is consistent with the findings reported in the literature. The substantial reduction in radiographic bone defect depth – from 3.55 ± 0.89 mm to 0.57 ± 0.73 mm – reflects a nearly complete resolution of the intrabony defects. This outcome indicates not only the success of the regenerative approach but also its potential to restore periodontal structures to a clinically stable condition. When the residual defect depth is minimal or almost eliminated, as seen in the present study, the long-term prognosis of the affected teeth is considered highly favorable [30]. These results contribute to the overall goal of maintaining long-term periodontal stability.

CONCLUSION

The findings of this clinical study confirm the effectiveness of a minimally invasive surgical approach combined with enamel matrix derivatives and bone grafting in the treatment of vertical bone defects. The applied therapeutic modality resulted in significant improvement in probing depth reduction, clinical attachment gain, and radiographic bone fill. These favorable outcomes indicate clinical resolution of the defects and suggest a stable periodontal condition for the treated sites. The results support the use of minimally invasive techniques with biologically active agents as a viable strategy for treatment and improving the long-term prognosis of affected teeth.

Funding: *This research was funded by the Medical University – Sofia under Agreement No. D 305/18 December 2023, as part of a research project financed through the 2023 competition "Stimulation of Scientific Research in Fields with High Scientific Achievements."*

Ethics Approval Statement: *Ethical approval for this study was obtained from the Research Ethics Committee at the Medical University – Sofia – KENIMUS (Protocol No. 16/19 December 2023). This study has been performed in accordance with the ethical standards as laid down in the Declaration of Helsinki.*

Informed Consent Statement: *All the participants received comprehensive information regarding the aims and methods of the study and provided written informed consent.*

REFERENCES

1. Kotsilkov K, Popova C, Boyanova L et al. Comparison of culture method and real-time PCR for detection of putative periodontopathogenic bacteria in deep periodontal pockets. Biotechnology & Biotechnological Equipment. 2015;29(5):996–1002.
2. Kotsilkov K. Modified coronally advanced flap approach with isthmus deepithelialization for the treatment of multiple recession defects in cases with interdental tissue loss. J of IMAB. 2021;27(2):3784- 3792.
3. Pilloni A, Zeza B, Kuis D, et al. Treatment of residual periodontal pockets using a hyaluronic Acid-Based gel: a 12 month multicenter randomized Triple-Blinded clinical trial. Antibiotics. 2021;30;10(8):924.
4. Dimova-Gabrovska MI, Maksimovskaya LN, Dimitrova DG. Changes in the oral mucosa in patients with bruxism. Stomatology. 2021;100(6):48.
5. Dimova-Gabrovska M, Dimitrova D. Study of the dietary minerals intake of children with early childhood caries. Archives of the Balkan Medical Union, 2019;54(3):540-548.
6. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018;45 Suppl 20:S162–70.
7. Dimova-Gabrovska M, Shopova D, Stamenova M. Oral Clinical Manifestations in Patients with COVID-19: A Review. Acta Medica Bulgarica. 2024;51(s2):188–94.
8. Kotsilkov K, Popova C. Effectiveness of target antimicrobial therapy of severe chronic periodontitis. Part III: clinical atachment gain. J IMAB. 2010;16(4):24–26.
9. Kotsilkov K, Popova C. Real time PCR identification for target adjunctive antibiotic therapy of severe chronic periodontitis. Part II – microbiological effetivenes. J IMAB. 2014;20(5):610–616.
10. Maynalovska H, Mlachkova A, Kotsilkov K.Comparative analysis of microorganisms around teeth and implants in health and disease. J of IMAB. 2025;31(1):6004-6008.
11. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47(Suppl 22):4–60.
12. Matuliene G, Pjetursson BE, Salvi GE, et al. Influence of residual pockets on progression of periodontitis and tooth loss: Results after 11 years of maintenance. J Clinic Periodontology. 2008;35(8):685–95.
13. Kotsilkov K, Popova C, Dosseva V. Effectivness of target antimicrobial therapy of severe chronic periodontitis part I: Reduction of gingival inflamation and active periodontal disease sites. J of IMAB, 2010;16:18-20.

14. Kotsilkov K, Dimitrov R. Complex treatment in a patient with severe chronic periodontitis (Case Report). J of IMAB. 2015;21(1):687-689.
15. Dimova-Gabrovska M, Stamenova M, Shopova D, Yankova M. The modern digital intraoral scanning systems: a review. Acta Medica Bulgarica. 2024; 51(2):58-64.
16. Dimova M, Svechtarov V. Quantitative Assessment of Pterygomasseteric Hypertrophy. Folia medica. 2003, 45(4):56-58.
17. Dimitrova D, Dimova-Gabrovska M, Maksimovskaya L. Aesthetic crowns in primary teeth – a new methodology for evaluating the preventive value. Comptes rendus de l'Académie bulgare des Sciences, 2021;74(2),300-308.
18. Polimeni G, Xiropaidis AV, Wikesjö UME. Biology and principles of periodontal wound healing/regeneration. Periodontology 2000. 2006;41(1):30–47.
19. Bosshardt DD. Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels. J Clin Periodontol. 2008;35(8 Suppl):87–105.
20. Esposito M, Grusovin MG, Papanikolaou N, et al. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. A Cochrane systematic review. Eur J Oral Implantol. 2009;2(4):247–66.
21. Sculean A, Windisch P, Szendröi-Kiss D, et al. Clinical and Histologic Evaluation of an Enamel Matrix Derivative Combined With a Biphasic Calcium Phosphate for the Treatment of Human Intrabony Periodontal Defects. Journal of Periodontology. 2008;79(10):1991–9.
22. Tavelli L, Barootchi S, Rasperini G, et al. Clinical and patient-reported outcomes of tissue engineering strategies for periodontal and peri-implant reconstruction. Periodontology 2000. 2023;91(1):217–69.
23. Trombelli L, Farina R, Franceschetti G, et al. Single-Flap Approach with Buccal Access in Periodontal Reconstructive Procedures. Journal of Periodontology. 2009;80(2):353–60.
24. Cortellini P, Tonetti MS. A minimally invasive surgical technique with an enamel matrix derivative in the regenerative treatment of intra-bony defects: a novel approach to limit morbidity. J Clinic Periodontology. 2007;34(1):87–93.
25. Cortellini P, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intra-bony defects. I: clinical outcomes and morbidity. J Clinic Periodontology. 2007;34(12):1082–8.
26. Cortellini P, Pini-Prato G, Nieri M, et al. Minimally invasive surgical technique and enamel matrix derivative in intrabony defects: 2. Factors associated with healing outcomes. Int J Periodontics Restorative Dent. 2009;29(3):257–65.
27. Trombelli L, Simonelli A, Quaranta A, et al. Effect of Flap Design for Enamel Matrix Derivative Application in Intraosseous Defects. JDR Clinical & Translational Research. 2021;6(2):184–94.
28. Aimetti M, Fratini A, Manavella V, et al. Pocket resolution in regenerative treatment of intrabony defects with papilla preservation techniques: A systematic review and meta-analysis of randomized clinical trials. J Clinic Periodontology. 2021;48(6):843–58.
29. Simonelli A, Severi M, Trombelli L, et al. Minimal invasiveness in the surgical treatment of intraosseous defects: A systematic review. Periodontology 2000. 2023;91(1):20–44.
30. Saleh MHA, Mallala D, Alrmali A, et al. Residual vertical defects: risk of disease progression, retreatment rates, and cost: a retrospective analysis. Clin Oral Invest. 2024 25;28(8):446.