

Healing effect of hyaluronic acid gel in adjunct with periodontal surgery. A randomized controlled clinical and biomarkers study.

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The study was approved by the Ethics Committee of the School of Dentistry (Protocol No. 31/21-03-2023). All participants were informed about the study procedures and provided written informed consent, including consent for the use of their clinical data and biological samples for research purposes.

Abstract:

Aim: This study aimed to evaluate the effectiveness of hyaluronic acid (HA) gel in promoting soft tissue healing following periodontal surgery for residual periodontal pockets.

Materials and Methods: A prospective, randomized controlled clinical trial was conducted over three months, including 26 patients from the Postgraduate Periodontology Clinic at the Aristotle University of Thessaloniki. Participants had completed initial cause-related therapy and presented with at least one residual periodontal pocket of 5-8. Patients were randomly assigned to: (A) the test group receiving surgical therapy with 0.6 ml of HyaDENT® BG HA gel applied, or (B) the control group receiving periodontal surgery with 0.6 ml of saline solution (placebo). Gingival crevicular fluid (GCF) was collected from the target site preoperatively, at 14 days for VEGF, and at 3 months for IL-1 β analysis using ELISA. Clinical parameters, including probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), and plaque index (PI), were recorded at baseline and at three months. Soft tissue healing was assessed using the Landry Wound Healing Index (WHI), and patient-reported outcomes, including pain, swelling, function, and aesthetics, were evaluated using a Visual Analog Scale (VAS).

Results: Baseline demographics and clinical parameters were comparable between groups ($p > 0.05$). Both groups showed significant improvements in PPD and CAL at three months ($p < 0.001$), with no intergroup differences. Sites treated with HA demonstrated a significant reduction in BOP ($p < 0.05$) and lower postoperative swelling ($p = 0.039$), while smoking status and tooth type had no impact on outcomes. Biomarker analysis showed no statistically significant differences between groups; however, VEGF levels were higher at 14 days and IL-1 β showed a greater reduction at three months in the HA group, with a significant overall within-group decrease in IL-1 β ($p = 0.046$). Soft tissue healing and patient-reported outcomes showed an improvement in both groups ($p < 0.001$).

Conclusions: Both surgical approaches resulted in significant clinical improvements. Adjunctive HA application enhanced site-specific inflammation control and postoperative comfort, with trends toward angiogenic and anti-inflammatory effects. These findings support HA's potential as a biologically active adjunct in periodontal surgery.

Key- words: hyalouronan acid, surgical periodontal therapy, healing , soft tissues, biomarkers, IL-1 β , VEGF, probing depth, attachment level

1. Introduction

Periodontitis is a chronic inflammatory disease of microbial aetiology characterized by progressive loss of the supporting tissues of the teeth, including alveolar bone and periodontal ligament, leading to pocket formation and tooth loss (Papapanou et al., 2018, AAP 1998). Affecting 20-50% of the global population, it primarily manifests in adults, with prevalence increasing with age (Kassebaum et al., 2014, 2017); (Sanz et al., 2015), (Billings et al., 2018). Periodontitis is characterized by a sequence of inflammatory and microbial events that result in dysbiotic subgingival biofilms and a dysregulated host immune response, resulting in tissue destruction (Hajishengallis C Korostoff, 2017). Risk factors such as smoking, uncontrolled diabetes, systemic conditions, genetic predisposition, and local anatomical features modulate disease progression and therapeutic outcomes (Tonetti et al., 2018), (Kinane et al., 2017), (Larsson et al., 2015), (Jeffcoat C Howell, 1980), (Jansson et al., 1994).

Diagnosis relies on clinical examination, radiographic assessment, and careful exclusion of non - periodontally derived causes of attachment loss (Papapanou et al., 2018). Clinical attachment loss (CAL), probing pocket depth (PPD), and bleeding on probing (BOP) are key parameters, while emerging biomarkers, including interleukin-1 beta (IL-1 β) and vascular endothelial growth factor (VEGF), provide insight into current disease activity and tissue regeneration potential (Khiste et al., 2011); (Herrera et al., 2025).

Treatment of periodontitis involves non-surgical therapy, including scaling and root planing (SRP), and surgical interventions such as access flap surgery, resective surgery, and regenerative approaches. Non-surgical therapy has been shown to lead to an inflammation resolution by reducing microbial load, while surgical therapy addresses residual deep pockets and may promote periodontal regeneration (Sanz et al., 2020), (Graziani C Tsakos, 2020), (Smiley et al., 2015) Supportive periodontal therapy (SPT) is essential to maintain long-term stability and prevent disease recurrence (Chapple et al., 2018), (Becker et al., 1984), (Costa et al., 2014).

Hyaluronic acid (HA), a high-molecular-weight glycosaminoglycan, is widely distributed in connective tissues and plays a key structural and functional role in the periodontium, contributing to tissue hydration, elasticity, and regeneration (Necas et al., 2008); (Bukhari et al., 2018). HA exerts anti-inflammatory effects by modulating cytokines (IL-1 β , TNF- α) and matrix metalloproteinases (MMPs), it may also enhance cell migration

and proliferation, stimulate collagen synthesis, and promote angiogenesis through VEGF upregulation (Misra et al., 2015), (Wulandari et al., 2022); .It may also accelerate epithelialization, lead to a reduction of scar formation, and exhibit bacteriostatic activity, making it a valuable adjunct in both non-surgical and surgical periodontal therapy (Bhati et al., 2022).

Recent evidence highlights HA's therapeutic potential in both non-surgical and surgical periodontal interventions. Clinically, HA adjunctive use with SRP has demonstrated reductions in PPD, BOP, and plaque indices, although improvements in CAL and bone height are less consistent (Bertl et al., 2024), (Eliezer et al., 2019), (Karakostas et al., 2022). In surgical interventions, HA has been associated with improved CAL, reduced PPD, and enhanced postoperative comfort and healing, particularly when combined with regenerative approaches, although standardized protocols and biomarker data remain limited (Fawzy El-Sayed et al., 2012), (Briguglio et al., 2013), (Mamajiwala et al., 2021). Aslı Şener et al. (2025) demonstrated that adjunctive 0.8 % HA gel with open flap debridement significantly modulated inflammatory biomarkers, increasing IL 10 and decreasing IL 17A in gingival crevicular fluid, suggesting an enhanced host response and improved soft tissue healing. Pilloni et al. (2025) further showed that HA promotes periodontal ligament cell proliferation, growth factor expression (PDGFB, FGF 2, EGF), and blood clot stability, collectively supporting accelerated wound healing and improved clinical outcomes in both non-surgical and surgical contexts. These findings reinforce HA's mechanistic and clinical value as an adjunctive biomaterial in periodontal therapy.

Biomarkers such as IL-1B and VEGF in gingival crevicular fluid (GCF) provide valuable insights into periodontal inflammation, tissue destruction, and regenerative activity. IL-1B is a pro-inflammatory cytokine central to disease progression (Armitage et al, 2004), while VEGF promotes angiogenesis and tissue repair following periodontal surgery (Mansour et al., 2024). Despite promising evidence of HA's anti-inflammatory and pro-angiogenic properties, few studies have evaluated its adjunctive use in surgical therapy with corresponding biomarker analyses, highlighting the need for further research in understanding its mechanistic effects.

Aim of the Study

This study aims to evaluate the clinical efficacy of HA gel as an adjunct to periodontal surgery in treating residual pockets with PPD of 5-8 mm, focusing on reductions in probing depth and BOP, as well as potential regenerative effects reflected by changes in biomarkers (IL-1B and VEGF). The findings seek to provide evidence for HA's role as a biocompatible adjunct in biologically enhanced periodontal therapy.

The primary outcome was the reduction or elimination of periodontal pocket depth (PPD \leq 4 mm and absence of bleeding on probing) from baseline to 3 months. Secondary outcomes included clinical parameters—clinical attachment level (CAL), bleeding on probing (BoP), and total plaque index (PI) at baseline and 3 months; immunological markers—IL-1 β at baseline and 3 months and VEGF at 14 days; and soft tissue healing at 14 days, assessed using the Landry, Turnbull, and Howley Wound Healing Index (0-5) and patient-reported outcomes via a Visual Analog Scale (VAS, 1-10). Given that PPD was the primary outcome used for sample size calculation, the study was underpowered for secondary outcomes, limiting the ability to draw definitive conclusions.

2. Materials & Methods

2.1. Study Design and Participant Recruitment

This single-center, parallel-group, prospective, randomized controlled clinical trial (RCT) was undertaken over a three-month study period at the Department of Preventive Dentistry, Periodontology and Implant Biology, Aristotle University of Thessaloniki.

- Inclusion Criteria

Participants were eligible if they were:

1. \geq 18 years old,
2. systemically healthy or medically stable for periodontal surgery, and
3. presented at least one residual periodontal pocket with probing depth (PPD) of 5-8 mm after Step 1 (initial cause-related therapy) and Step 2 (re-evaluation) according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.

- Exclusion Criteria

Participants were excluded if they had:

1. severe systemic disease contraindicating surgery (e.g., uncontrolled diabetes, chemotherapy, immunosuppressive therapy, bisphosphonate use),
2. antibiotic intake within 30 days prior to surgery, prior periodontal surgery at the target site within six months,
3. smoked $>$ 10 cigarettes/day,
4. had alcohol or drug abuse,

5. or were pregnant/breastfeeding.
6. Intraosseous defects requiring regenerative therapy were excluded and managed separately.

Eligible participants were randomized 1:1 into control (C) and test (HA) groups using a computer-generated sequence. For patients with multiple eligible lesions, a second randomization was performed. Allocation was concealed in sealed opaque envelopes opened by the treating periodontist immediately prior to surgery. Outcome assessors were blinded to group allocation.

2.2. Intervention Procedures

Control Group (C): Open-flap periodontal surgery with mechanical debridement followed by 0.6 mL sterile saline application.

Test Group (HA): Open-flap surgery with identical debridement, followed by 0.6 mL hyaDENT® BG hyaluronic acid gel applied within the defect and over the sutured site.

All surgeries were performed under local anaesthesia by the same experienced periodontist (E.V.). Standardized intraoral periapical radiographs were obtained using the long-cone paralleling technique with individualized positioning stents for baseline assessment and future evaluation of bone healing.

2.3. Treatment Timeline

Patients were screened post-Step 1 and Step 2 therapy. Baseline clinical measurements and gingival crevicular fluid (GCF) samples were collected. Additional oral hygiene instructions and non-surgical therapy (scaling and root planing) were provided if necessary. Surgical intervention followed, with follow-up visits at 2 weeks and 3 months. Clinical measurements were recorded at baseline and 3 months, while biomarker assessments (IL-1 β , VEGF) were performed at baseline, 2 weeks, and 3 months. Soft tissue healing was evaluated at 2 weeks using the Landry Wound Healing Index (Wound Healing Index (by Landry, Turnbull and Howley), 1988), and patient-reported outcomes were assessed via Visual Analog Scale (VAS) questionnaires.

Figure 1: Treatment Timeline

2.4. Clinical Periodontal Measurements

The following parameters were recorded at six sites per tooth using a 15-mm periodontal probe:

- PPD: Gingival margin to base of sulcus
- CAL: Cementoenamel junction to base of sulcus
- FMPS: Full-mouth plaque score
- BOP: Presence of bleeding 30 s after probing

Deepest sites per tooth were used for statistical analysis. Intra-examiner reliability was high (ICC = 0.93, 95% CI 0.89-0.96).

2.5. Surgical Procedures

Full-thickness (mucoperiosteal) flaps were elevated to gain access to the underlying periodontal lesions. Intrasulcular incisions were made at the affected sites, following flap elevation, thorough debridement of granulation tissue and meticulous scaling and root planing of the exposed root surfaces were performed. Where indicated, hyaluronic acid or saline solution were applied.

- Control Group: 0.6 mL saline applied after suturing.
- Test Group: 0,6mL hyaDENT® BG HA gel, approximately half an ampoule, applied using a 27G needle within the defect and over the sutured flap.

Flaps were closed with 5/0 PGA single interrupted sutures. Postoperative care included analgesics (ibuprofen 400 mg TID if needed), chlorhexidine 0.12% rinse BID for 2 weeks, and oral hygiene reinforcement. Sutures were removed at 7-10 days.

2.6. Biomarker Collection and Evaluation

GCF samples were collected from the deepest pocket using two sterile paper strips for 30 s, avoiding blood contamination, and stored at -80°C. IL-1 β and VEGF levels were quantified using ELISA kits (Abbkine, EliKine™) per manufacturer instructions, with detection limits of 194 pg/mL (IL-1 β) and 460 pg/mL (VEGF). Optical densities were read at 450 nm and concentrations expressed as pg/mL.

2.7. Soft Tissue Healing Assessment, PROMS

Landry Wound Healing Index (WHI): Gingival tissue healing scored 1-5 based on redness, granulation tissue, bleeding, swelling, and epithelialization (Wound Healing Index (by Landry, Turnbull and Howley). | Download Scientific Diagram, n.d.)

Visual Analog Scale (VAS): Patients assessed pain, swelling, functional ability, and aesthetics at 24 h and 14 days post-surgery. Analgesic consumption and overall treatment satisfaction were recorded.

2.8. Statistical Analysis - Sample Size Calculation

A patient-based analysis was performed to detect a clinically significant PPD difference of 1.5 ± 1 mm between groups and over time, with $\alpha = 0.05$ and power = 0.80, requiring 12 patients per group. Considering a 30% dropout rate, 26 participants (13 per group) were recruited ((Bhowmik C Rao, 2021)).

Continuous variables were expressed as mean \pm SD, categorical as n (%). Normality was checked with Shapiro-Wilk test. Between-group comparisons used independent t-test or Mann-Whitney U test; within-group comparisons used paired t-test or Wilcoxon signed-rank test. Categorical variables were analysed with Chi-square or Fisher's exact test. Statistical significance was set at $p \leq 0.05$. Analyses were performed using STATA 13 (StataCorp LP, Texas, USA) and IBM SPSS Statistics 29.