Cover Letter — Protocol Registration Submission (PRS) **Dec.09.2025**

Comparative Evaluation of Microneedling with Injectable PRF and Hyaluronic Acid for Papilla Reconstruction: A Randomized Clinical Trial

This prospective, split-mouth, double-blind, randomized controlled clinical trial investigates two minimally invasive interventions—microneedling combined with injectable PRF (i-PRF) versus hyaluronic acid (HA) gel injections—for the management of **Class I interdental papilla deficiency** in adult patients. The primary aim is to assess and compare improvements in papillary height, width, and soft-tissue thickness during the 12-week follow-up period.

The study will be conducted at the Department of Periodontology, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia. Ethical approval was obtained from the Research and Ethics Committee (Reference No. 117-10-24). Written informed consent will be collected from all enrolled participants. The study adheres to institutional regulations and the Declaration of Helsinki (2013) regarding the protection of human subjects.

We are registering this protocol in the PRS to ensure transparency, support the dissemination of scientifically valid results, and contribute to accessible clinical evidence, all while complying with global clinical research standards that prioritize patient safety.

We appreciate your assistance with processing our registration, and we remain ready to provide any further materials or clarifications that may be required.

Sincerely,

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Comparative Evaluation of Microneedling with Injectable PRF and Hyaluronic Acid for Papilla Reconstruction: A Randomized Clinical Trial

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Abstract

Background: Loss of the interdental papilla represents a frequent aesthetic and functional challenge in dentistry. Traditional surgical procedures can partially restore papillary form but are limited by technical sensitivity, donor-site morbidity, and unpredictable long-term results. Consequently, interest has shifted toward minimally invasive, biologically driven strategies for papilla reconstruction. Among non-surgical options, hyaluronic acid (HA) injections and the injectable form of platelet-rich fibrin (iPRF) have shown considerable promise. Combining microneedling (MN) enhances trans-mucosal delivery of the biologic agent and can boost regenerative outcomes. Despite promising results, direct comparisons between HA and MN + iPRF remain scarce. Therefore, this clinical trial aims to compare the clinical effectiveness of MN + iPRF vs. HA gel injection in the reconstruction of Class I interdental papilla loss.

Methods: This study is conducted as a split-mouth, double-blind, randomized, controlled clinical trial to compare the efficacy of MN + iPRF vs. HA injections in the reconstruction of interdental papillae. Adult patients with more than one interdental papillary site that need reconstruction will be enrolled. Each site will be randomly assigned to one of two treatment groups: Group MN + iPRF, and Group HA. Clinical parameters measured at baseline, 6 weeks, and 12 weeks will include papillary height and papillary width at three levels (base, middle, and tip), and papillary thickness.

Keywords: Papilla reconstructions, Hyaluronic acid, PRF, Microneedling, Gingiva, Plasma platelet-rich fibrin

Introduction

Mucogingival deformities, including gingival recession and loss of the interdental papilla, represent frequent aesthetic and functional challenges in periodontology[1, 2]. These conditions are classified under periodontal and peri-implant diseases according to the 2018 World Workshop classification[2]. They often result in root exposure, dentin hypersensitivity, plaque accumulation, and unaesthetic “black triangles” between teeth[1, 3]. The interdental papilla is a key anatomic structure occupying the gingival embrasure between adjacent teeth; its integrity supports periodontal health, prevents phonetic and food impaction problems, and contributes to smile harmony [4, 5]. When the papilla recedes or is lost, the resultant open gingival embrasure compromises both function and appearance. Kokich et al. [6] demonstrated that black triangles larger than 3 mm are perceived as unattractive by both clinicians and patients, reflecting the strong psychosocial and aesthetic impact of these defects.

Nordland and Tarnow [5] proposed a simple classification system for the degree of papillary loss based on identifiable anatomical landmarks—the contact point, facial CEJ, and interproximal CEJ—facilitating clinical assessment and standardized outcome measurement. Traditional surgical procedures, including coronally advanced flaps, subepithelial connective tissue grafts, and tunneling techniques, can partially restore papillary form but are limited by technical sensitivity, donor-site morbidity, and unpredictable long-term results [7, 8]. Consequently, interest has shifted toward minimally invasive, biologically driven strategies for papilla reconstruction.

Among non-surgical options, hyaluronic acid (HA) injections have shown considerable promise. HA is a natural glycosaminoglycan abundant in connective tissue and the extracellular matrix, with strong hygroscopic and viscoelastic properties that allow volumization and hydration of soft tissue.

It also promotes fibroblast proliferation, angiogenesis, and collagen synthesis, contributing to wound healing and tissue regeneration[9-11]. Several clinical studies have reported significant increases in papilla height and fill, and reductions in the black-triangle area after HA injection[12-15]. Bal et al. [12] observed that combining HA with plasma rich in growth factors (PRGF) enhanced papillary regeneration more effectively than HA alone, emphasizing the potential of biologically active adjuncts to prolong HA's regenerative effects.

Platelet-rich fibrin (PRF), a second-generation autologous platelet concentrate, provides a reservoir of growth factors, including PDGF, VEGF, and TGF-β, that accelerate neovascularization and extracellular matrix formation. The injectable form (i-PRF) serves as a bioactive scaffold and gradually releases these factors, enhancing soft-tissue healing while minimizing immune reactions[16, 17].

Microneedling (MN), first introduced in dermatology as percutaneous collagen induction therapy, involves controlled micro-perforations that stimulate fibroblast activation, collagen deposition, and the release of endogenous growth factors[18, 19]. The technique enhances transmucosal delivery of biologic agents and can synergize with materials such as HA or PRF to boost regenerative outcomes. In a recent in-vivo study, Fan et al. [20]demonstrated that MN combined with HA gel significantly improved periodontal soft-tissue regeneration compared with HA alone. Similarly, clinical trials have shown that MN combined with platelet concentrates increases papillary height and gingival thickness, outperforming single-agent therapies[21-23].

Despite these promising results, direct comparisons between HA and MN + iPRF remain scarce. The biological synergy between mechanical collagen induction and autologous growth-factor delivery may provide a more durable and natural restoration of interdental papilla than passive

fillers alone. This approach could overcome HA's transient effects by stimulating intrinsic tissue remodeling and angiogenesis.

Therefore, this randomized split-mouth clinical trial aims to compare the clinical effectiveness of MN combined with iPRF versus HA gel injection in the reconstruction of Class I interdental papilla loss. The study hypothesizes that MN + iPRF will yield greater papillary regeneration, offering a minimally invasive, biologically active alternative to traditional surgical approaches for soft-tissue esthetic rehabilitation.

Materials and Methods

This study will be a split-mouth, double-blind, randomized, controlled clinical trial comparing the efficacy of microneedling combined with injectable platelet-rich fibrin (i-PRF) versus hyaluronic acid (HA) injections in the reconstruction of interdental papillae. The study will be carried out at the Department of Periodontology, King Abdulaziz University Faculty of Dentistry (KAUFD), Jeddah, Saudi Arabia, between November 2025 *and* March 2026. Ethical approval was obtained from the Research and Ethics Committee of the Faculty of Dentistry, King Abdulaziz University (Approval #117-10-24). Written informed consent will be obtained from all participants before enrollment. The trial adhered to CONSORT (2010) guidelines (Figure 1) and the Declaration of Helsinki (2013)[24].

The inclusion criteria are age between 18 and 45 years, Plaque index = 0[25], Gingival index = 0[26], Class I papillary loss (Nordland and Tarnow classification)[5], probing depth ≤4 mm at the test site, and interproximal bone crest distance ≤7 mm from the contact point. Exclusion criteria include pregnancy,

lactation, uncontrolled systemic diseases (e.g., diabetes), anticoagulant therapy, recent radiotherapy, orthodontic treatment, midline diastema, high frenum attachment, and Class II/III papillary loss.

Adult patients with at least two interdental papillary sites will be enrolled. Each site will be randomly assigned to one of two treatment groups: Group MN + iPRF, which will receive microneedling with i-PRF, and Group HA, which will receive hyaluronic acid injection (Juvéderm Ultra, Allergan). Randomization will be achieved using computer-generated random numbers and a sealed opaque envelope. The surgeon will draw one envelope to determine which treatment (MN + i-PRF or HA) will be applied to each site. The split-mouth design effectively controls for intrasubject variability, improving internal validity, while double-blinding minimizes experimenter and response biases.

Patients will receive standardized oral hygiene instruction and supragingival prophylaxis 1 week before baseline measurement. The i-PRF will be prepared by drawing one tube of venous blood from the antecubital vein, placing it into anticoagulant-free Choukroun i-PRF 13 ml tubes, and centrifuging at 700 rpm for 3 minutes using a Process for PRF centrifuge system (Choukroun PRF Duo Quattro Centrifuge). The freshly obtained i-PRF (0.2 mL) will be immediately injected into the papilla using a 0.25 mm x 6 mm sterile insulin syringe (BD Microfine Plus), followed by microneedling with a sterile blood lancet to create microchannels (ACCU-CHEK®). This will be performed at baseline and 3 weeks, with a standalone microneedling session (no i-PRF) at 1 week. The control site will receive 0.2 mL of HA gel, split into 0.1 mL at baseline and 0.1 mL at 3 weeks. All procedures will be performed under local anesthesia (2% Mepivacaine with 1:100,000 epinephrine). After the injection, patients will be advised to avoid mechanical plaque control in the treated area for the first 24 hours and to use a 0.2% chlorhexidine digluconate mouthwash twice daily. After the initial 24 hours, they will be instructed to resume gentle brushing with a soft toothbrush, combined with the mouthwash.

A blinded, calibrated examiner will perform all clinical assessments. All instruments will be calibrated, and measurement procedures will be standardized across all patients to ensure validity and reliability. At baseline, pocket depth, bone-to-contact distance, and gingival recession type will be recorded. Clinical parameters will be measured at baseline, 6 weeks, and 12 weeks included papillary height (from the mucogingival junction to the papilla tip), papillary width at three levels (base, middle, and tip) using a University of North Carolina (UNC-15) periodontal probe, and papillary thickness, which will be measured using an endodontic K-file size 10 with a silicone stop inserted perpendicularly into the soft tissue 2 mm apical to the midbuccal region[21]. Standardized intraoral photographs will be taken with a camera, using cheek retractors and occlusal mirrors under consistent lighting conditions.

Statistical Analysis

Descriptive statistics (mean ± standard deviation) summarize baseline and follow-up values (6 weeks and 12 weeks) within each group. Longitudinal and between-groups comparisons will be analyzed using linear mixed-effects models. For each outcome, fixed effects will be group (MN + iPRF vs HA) and time (baseline, 6 weeks, 12 weeks), with their interaction; random effects will be specified as sites nested within subjects. Pairwise contrasts (inter-group differences at each time point and intra-group change over time from baseline) will be estimated from marginal means with false discovery rate (FDR) adjustment. Analyses will be performed in R software version (4.5.1) within RStudio version (2025.05.1) [27]using the packages lme4, lmerTest, and emmeans. The percent change from baseline to 6 and 12 weeks will be calculated for each clinical parameter, with values expressed as percentages relative to baseline, where positive values indicate increases and negative values indicate decreases. In addition, correlations among clinical parameters for each group will be assessed using the repeated-measures correlation (rmcorr) package, with results

reported as the repeated-measures correlation coefficient (r_{rm}),[28]. P-values from (rmcorr) will be corrected with FDR. A p-value < 0.05 will be considered statistically significant.

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