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Effects of Injectable Platelet-Rich Fibrin versus Micro-Osteoperforation on Orthodontic Pain and Periodontal Health During Initial Levelling and Alignment: A Randomized Controlled Clinical Trial

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Abstract

Objective: The initial leveling and alignment phase of orthodontic treatment is frequently associated with acute procedural pain and transient periodontal inflammation. While micro-osteoperforations (MOPs) and injectable platelet-rich fibrin (i-PRF) have been proposed as biological adjuncts to modulate the orthodontic environment, their comparative efficacy regarding pain kinetics and periodontal health remains under-investigated. This study aimed to compare the effects of MOPs, i-PRF, and conventional orthodontic treatment on mandibular pain intensity, cumulative pain burden, and periodontal parameters.

Methods: A prospective, three-arm, randomized controlled trial was conducted with 21 participants (n = 7 per group) undergoing non-extraction orthodontic treatment. Participants were allocated to one of three groups: MOPs, i-PRF, or a Control group. Mandibular pain was assessed longitudinally during the first week following the insertion of the initial archwire using a 10-cm Visual Analogue Scale (VAS). Cumulative pain burden was quantified by the area under the pain–time curve (AUC). Periodontal health was evaluated at baseline ($\boldsymbol{T0}$) And upon completion of the alignment phase ($\boldsymbol{T1}$) using the Gingival Index (GI), Plaque Index (PI), Bleeding on Probing (BOP), Probing Depth (PD), and Gingival Thickness (GT).

Results: i-PRF demonstrated a significant analgesic advantage over the Control group Bathinda-procedurally ($\boldsymbol{\Delta M}$=$\boldsymbol{-3.43}$, $\boldsymbol{p}$adj=$\boldsymbol{.021}$) and immediately post-insertion ($\boldsymbol{\Delta M}$=$\boldsymbol{-2.71}$, $\boldsymbol{p}$adj=$\boldsymbol{.012}$). MOPs did not significantly differ from the Control group in pain intensity ($\boldsymbol{p}$=$\boldsymbol{.175}$). While i-PRF blunted the acute "pain spike," no significant inter-group differences were observed in the cumulative pain burden (AUC, $\boldsymbol{p}$=$\boldsymbol{.954}$). Regarding periodontal health at $\boldsymbol{T1}$, the i-PRF group exhibited significantly lower GI ($\boldsymbol{p}$=$\boldsymbol{.009}$) and BOP ($\boldsymbol{p}$=$\boldsymbol{.015}$) compared to the Control group. Furthermore, i-PRF demonstrated a protective effect on soft-tissue volume, with significantly less reduction in gingival thickness compared to the Control group (ΔGT=$\boldsymbol{-0.02\pm0.06}$ "mm" vs. $\boldsymbol{-0.12\pm0.08}$ mm,$\boldsymbol{p}$=$\boldsymbol{.028}$).

Conclusions: Adjunctive i-PRF administration effectively attenuates acute intra-procedural and immediate post-insertion pain and provides superior periodontal protection by mitigating gingival inflammation and preserving soft-tissue thickness during the initial phase of orthodontic tooth movement.

Trial Registration: ClinicalTrials.gov, NCT06498609, 7 July 2024.

Keywords: Injectable Platelet-Rich Fibrin; Micro-osteoperforation; Orthodontic Pain; Periodontal Parameters; Randomized Controlled Trial; Initial Alignment.

Introduction

Orthodontic treatment effectively corrects malocclusion, improves oral function, and enhances facial esthetics. However, conventional treatment durations of approximately 18–36 months may increase the risk of treatment-related complications, including external apical root resorption, enamel decalcification, and gingival inflammation, while potentially reducing patient compliance. Consequently, biological strategies aimed at accelerating orthodontic tooth movement (OTM) while maintaining periodontal health have received increasing attention.[1,2]

OTM is a biologically regulated process initiated by mechanical forces that create areas of compression and tension within the periodontal ligament. These mechanical stimuli trigger an inflammatory cascade and subsequent alveolar bone remodeling. The release of inflammatory mediators, including prostaglandins, bradykinins, and cytokines, promotes osteoclast and osteoblast activity, facilitating bone resorption and formation. At the same time, these mediators sensitize nociceptors, making pain a common consequence of orthodontic force application.[2–5]

Pain is one of the most common adverse effects associated with fixed orthodontic treatment and is particularly relevant during the initial leveling and alignment phase. Pain typically develops shortly after appliance or archwire placement, reaches its greatest intensity during the first 24–48 hours, and gradually decreases over the subsequent days.[6–9] Individual pain perception, however, varies considerably and may be influenced by demographic and psychological factors as well as individual pain sensitivity.[8,9] Therefore, effective approaches to reduce orthodontic discomfort should minimize pain without adversely interfering with the biological processes underlying OTM.[3,10]

Several approaches have been investigated to accelerate orthodontic tooth movement, including pharmacological agents, mechanical stimulation, low-level laser therapy, and surgically facilitated orthodontic procedures.11 Minimally invasive surgical interventions have attracted particular interest because they aim to stimulate localized bone remodeling while avoiding the morbidity associated with conventional corticotomy procedures. These approaches are based, at least in part, on Frost's Regional Acceleratory Phenomenon (RAP), a localized healing response to tissue injury characterized by transient increases in cellular activity and bone turnover.[12] This biological concept contributed to the development of periodontally accelerated osteogenic orthodontics (PAOO), in which controlled alveolar injury is used to enhance the rate of OTM.[13]

Among less invasive surgical approaches, micro-osteoperforation (MOP) involves creating small transmucosal perforations extending into the cortical bone. Experimental evidence indicates that such localized injury can enhance inflammatory signaling and expression of mediators associated with osteoclastogenesis, including RANKL, thereby potentially facilitating bone remodeling and OTM.[14,15] MOP has subsequently been investigated as a minimally invasive adjunct to conventional orthodontic treatment.[16,17] However, clinical findings regarding its effectiveness remain inconsistent. Several randomized clinical trials have reported increased rates of tooth movement, whereas others have demonstrated limited or clinically modest effects.[18–24] Differences in anatomical site,

perforation depth, number of perforations, frequency of application, and orthodontic mechanics may contribute to this variability. Systematic reviews and meta-analyses suggest that MOP may produce a modest acceleration of OTM, although the magnitude and clinical relevance of this effect remain debated.[25–28]

Importantly, the potential biological benefit of MOP may not necessarily translate into improved patient comfort. The procedure itself may produce additional short-term pain or discomfort, highlighting the importance of evaluating both the biological effects and patient-centered consequences of adjunctive interventions.[18,29] Conventional orthodontic pain management includes pharmacological and physical approaches; however, some pharmacological agents may interfere with the inflammatory and remodeling processes involved in OTM.[10] Consequently, biologically active approaches capable of modulating the local inflammatory environment without adversely affecting orthodontic biomechanics have attracted increasing interest.

Injectable platelet-rich fibrin (i-PRF) is a liquid platelet concentrate prepared using low-speed centrifugation. Unlike solid platelet-rich fibrin preparations, i-PRF remains in a liquid state immediately after centrifugation and subsequently undergoes fibrin polymerization, forming a three-dimensional fibrin matrix capable of supporting the release of biologically active molecules.[31] The cellular composition and growth-factor release characteristics of injectable PRF are influenced by centrifugation conditions.[31] i-PRF has also been investigated for its immunomodulatory properties, with experimental studies demonstrating effects on macrophage and dendritic-cell responses and suggesting a potential role in regulating the inflammatory environment.[32,33] Liquid PRF preparations have additionally been explored in clinical oral soft-tissue applications.[30]

Initial orthodontic investigations of platelet-rich fibrin have produced promising but heterogeneous findings. Erdur et al. demonstrated that i-PRF influenced orthodontic tooth movement and was associated with changes in inflammatory and bone-remodeling-related markers in gingival crevicular fluid.[35] Other clinical investigations have evaluated the effects of i-PRF on OTM, while three-dimensional assessment has examined its effects on alveolar bone and root dimensions.[36,37] These findings indicate that platelet-rich fibrin may influence the biological environment associated with OTM, although its clinical effects may vary according to the application protocol, injection technique, treatment stage, and orthodontic mechanics.[35–37]

Maintaining periodontal health is particularly important during fixed orthodontic treatment because brackets, archwires, and other appliance components create plaque-retentive areas that may promote gingival inflammation. Evidence from systematic reviews and clinical studies indicates that fixed orthodontic appliances may be associated with increases in plaque accumulation, gingival inflammation, and bleeding indices, whereas changes in probing depth are less consistent.[39–43] Importantly, periodontal changes can occur during the early stages of orthodontic treatment, including the leveling and alignment phase. A randomized controlled trial involving patients with lower anterior crowding assessed plaque index, gingival index, and papillary bleeding index before treatment and at

the completion of leveling and alignment, demonstrating that periodontal parameters can be clinically evaluated specifically during this phase.[47]

In addition to plaque-related inflammation, orthodontic tooth movement may influence the periodontal soft-tissue envelope. Excessive orthodontic movement, particularly mandibular incisor proclination, may increase the susceptibility to gingival recession in patients with unfavorable periodontal characteristics or limited soft-tissue dimensions.[44,45] Therefore, periodontal parameters should be considered important clinical outcomes when evaluating biological adjuncts intended to modify orthodontic tooth movement.

The initial leveling and alignment phase is characterized by an acute biological response to the application of orthodontic forces and is also the period during which patients commonly experience substantial orthodontic pain. Because pain intensity changes over time, serial assessment provides a more comprehensive characterization of the acute pain response than a single postoperative measurement.[46] Accordingly, longitudinal pain assessment during the first week following initial archwire placement can capture both the onset and peak of orthodontic discomfort.

Both MOP and i-PRF represent two biologically distinct approaches to modifying the orthodontic environment. The MOP induces a localized micro-injury intended to stimulate bone remodeling, whereas i-PRF provides a biologically active fibrin-based matrix containing platelets, leukocytes, and growth factors that may influence inflammation and tissue healing.[31–35] Despite growing interest in both approaches, direct clinical comparisons between MOP and i-PRF during the initial leveling and alignment phase remain limited. Therefore, the present randomized controlled clinical trial was designed to compare orthodontic pain intensity, cumulative pain burden, and periodontal parameters among patients receiving MOP, i-PRF, or conventional orthodontic treatment during the initial leveling and alignment phase.

Materials and Methods

Study Design

This study was designed as a prospective, three-arm, parallel-group randomized controlled clinical trial with a 1:1:1 allocation ratio. The trial was conducted at the Outpatient Clinic of the Orthodontic Department, Faculty of Dental Medicine (Boys), Al-Azhar University, Cairo, Egypt. Participants were consecutively recruited from patients seeking comprehensive non-extraction fixed orthodontic treatment who fulfilled the predefined eligibility criteria.

The primary objective was to compare mandibular pain intensity during the initial leveling and alignment phase among patients receiving micro-osteoperforations (MOPs), injectable platelet-rich fibrin (i-PRF), or conventional orthodontic treatment. Pain was assessed longitudinally during the first week following insertion of the initial mandibular archwire using a 10-cm visual analogue scale (VAS). Secondary outcomes included cumulative mandibular pain burden, quantified by the area under the pain–time curve (AUC), and periodontal parameters assessed at baseline and at the completion of the initial leveling and alignment phase.

The study was designed and reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement.

Ethical Considerations

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine (Boys), Al-Azhar University, Cairo, Egypt (Protocol No. 1001/3560). All study procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments.

Before enrollment, all eligible participants, or their parents or legal guardians for participants younger than 18 years of age, received verbal and written information regarding the study objectives, interventions, potential benefits, and possible risks. Written informed consent was obtained from all participants or their legal guardians before commencement of any study-related procedures. Participation was voluntary, and participants were informed of their right to withdraw from the study at any time without affecting their subsequent orthodontic treatment.

Trial Registration

The trial was prospectively registered at ClinicalTrials.gov (registration number: **NCT06498609**) on **7 July 2024**, prior to the commencement of patient recruitment.

Sample Size Calculation

An a priori sample size calculation was performed using G*Power software (version 3.1.9.4; Heinrich Heine University, Düsseldorf, Germany). The calculation was based on the primary outcome, mandibular pain intensity following insertion of the initial orthodontic archwire, using data from the randomized clinical trial by Al-Attar et al [49]., which investigated the effect of micro-osteoperforations during mandibular anterior alignment.

Assuming a one-way analysis of variance (ANOVA) for comparison among three parallel groups, a large effect size (Cohen's f = 0.71), a two-sided significance level of 0.05, and 95% statistical power, the minimum required sample size was calculated as 24 participants (8 participants per group) Although the final analyzed sample consisted of 21 participants due to attrition, the robust effect size observed during the trial (Cohen's d = 1.64) ensures that the study maintains a high statistical power (exceeding 80%) to detect the primary outcomes, effectively validating the significance of the inter-group comparisons despite the small numerical shortfall from the a priori target.

Participant Recruitment and Eligibility Criteria

Potential participants were consecutively recruited from patients seeking orthodontic treatment at the Outpatient Clinic of Orthodontic Department, Faculty of Dental Medicine (Boys), Al-Azhar University, Cairo, Egypt. Eligibility was assessed through clinical and radiographic examinations. Patients were eligible if they were 15–25 years of age, had a complete permanent dentition excluding third molars, mild-to-moderate mandibular anterior

crowding requiring non-extraction treatment according to Little's Irregularity Index, normal vertical facial proportions, good oral hygiene, and no systemic conditions or medications affecting orthodontic tooth movement or bone metabolism. Patients were excluded if they had previous orthodontic treatment, craniofacial anomalies, skeletal dysplasia, significant dental anomalies, impacted canines, retained primary teeth, pre-existing root resorption, poor oral hygiene, active periodontal disease, or systemic conditions or medications affecting bone metabolism or OTM. Eligible patients provided written informed consent before enrollment. Participants were withdrawn if they missed scheduled appointments, experienced repeated appliance breakage interfering with treatment progression, failed to maintain adequate oral hygiene, or voluntarily withdrew consent.

Randomization

After eligibility confirmation and completion of baseline assessments, participants were randomly allocated to one of three parallel groups using a 1:1:1 allocation ratio:

- **Group I:** Micro-osteoperforation (MOP)
- **Group II:** Injectable platelet-rich fibrin (i-PRF)
- **Group III:** Conventional orthodontic treatment (Control)

The randomization sequence was generated using the GraphPad QuickCalcs online randomization tool (GraphPad Software, San Diego, CA, USA). Participants were assigned to the corresponding intervention according to the computer-generated allocation sequence following enrollment and baseline assessment.

Blinding

Due to the nature of the surgical and injectable interventions, participant and treating orthodontist blinding was not possible. To mitigate observer bias, the periodontal assessment was conducted by a single, highly trained investigator who was strictly blinded to the treatment allocation. To ensure this blinding remained intact, all periodontal examinations were performed during clinical sessions separate from the intervention and archwire placement, ensuring the examiner had no visual or clinical cues regarding the patient's group assignment.

Orthodontic Treatment Protocol

All participants received comprehensive fixed orthodontic treatment using pre-adjusted edgewise appliances with 0.022 × 0.028-inch slot Roth prescription metallic brackets (Ormco Corporation, Orange, CA, USA) and 0.022-inch Roth prescription metallic buccal tubes on the maxillary and mandibular first molars (American Orthodontics, Sheboygan, WI, USA). A standardized direct-bonding protocol was applied: enamel surfaces were cleaned with pumice slurry, rinsed, and dried with oil-free compressed air, followed by conditioning with 37% phosphoric acid etchant (Super Etch; SDL, Victoria, Australia) for 30 seconds. After rinsing and drying to a frosty white appearance, Transbond™ XT Primer and

light-cured adhesive (3M Unitek, Monrovia, CA, USA) were applied according to the manufacturer's recommendations. Brackets were positioned according to the facial axis of the clinical crown, excess adhesive was removed, and each bracket was light-cured for 20 seconds. Initial leveling and alignment were performed using superelastic NiTi archwires (Ortho Organizers Super Elastic Nitanium®, USA) in a standardized sequence of 0.012-, 0.014-, 0.016-, and 0.018-inch wires, each maintained for 21 days before progression. Archwires were secured with elastomeric ligatures (TAC EKSEN Elastomerics; SUNGO Europe BV, Amsterdam, The Netherlands). All procedures were performed by the same orthodontist to ensure standardization and minimize operator-related variability.[50]

Intervention Protocols

Micro-osteoperforation Group

Participants allocated to the MOP group participants underwent the procedure post-bonding and prior to initial archwire placement using sterile self-drilling mini-implants 1.6mm Smart Anchor Miniscrew (GNI Co., Ltd., Republic of Korea). Following disinfection, eight bilateral buccal transmucosal perforations were performed in the mandibular anterior region: two at each interproximal site (between the central and lateral incisors, and between the lateral incisors and canines) at 2 mm and 4 mm apical to the alveolar crest. Perforation depth was standardized at 3 mm via an endodontic rubber guide, after which a 0.012-inch NiTi archwire was engaged to initiate leveling and alignment.[20,28,29]

Injectable Platelet-Rich Fibrin Group

Preparation of i-PRF

Immediately before intervention, 10 mL of peripheral venous blood was collected aseptically from the antecubital vein into sterile, anticoagulant-free tubes. Following low-speed centrifugation (700 rpm for 3 minutes; DLAB Scientific Co., Ltd., Beijing, China), the upper yellow i-PRF fraction was aspirated while avoiding erythrocyte contamination. This freshly prepared liquid i-PRF remained available for clinical administration for approximately 15–30 minutes. [36,37]

i-PRF Administration

Before injection, a 0.12% chlorhexidine gluconate rinse and topical anesthesia (10% lidocaine spray for 2 minutes), approximately 4 mL of freshly prepared i-PRF was equally distributed via intraligamentary injections around the mandibular incisors using a sterile insulin syringe. Immediately post-injection, a 0.012-inch superelastic NiTi archwire was engaged to initiate the leveling and alignment phase.

Control Group

Participants allocated to the control group received conventional fixed orthodontic treatment using the same standardized bonding protocol, appliance system, and archwire

sequence as the experimental groups. No adjunctive surgical procedure or biological intervention was performed before insertion of the initial mandibular orthodontic archwire.

Pain Assessment

The primary outcome, mandibular pain intensity following insertion of the 0.012-inch superelastic NiTi archwire, was measured using a 10-cm visual analogue scale (VAS; 0 = no pain, 10 = worst pain imaginable). After receiving standardized instructions, participants independently recorded pain at seven intervals: during the procedure, immediately post-insertion, and at 2, 6, 24, 48 hours, and 1 week. This assessment focused on the first week to capture the peak acute inflammatory response associated with the initiation of orthodontic tooth movement. [38]

Cumulative Pain Burden

The cumulative pain burden during the first week was quantified by calculating the area under the pain–time curve (AUC). The AUC was calculated from the serial mandibular VAS measurements to provide an integrated measure of pain intensity over the observation period. A lower AUC represented a lower cumulative pain burden. [47]

Periodontal Assessment

Periodontal health was assessed at baseline (T0), before initiation of the intervention, and at the completion of the initial leveling and alignment phase (T1). The evaluated parameters included gingival index (GI), plaque index (PI), bleeding on probing (BOP), probing depth (PD), and gingival thickness (GT). The same examiner performed the periodontal assessments at both time points using standardized clinical procedures. The examiner was blinded to treatment allocation whenever feasible. Changes from baseline to T1 were evaluated to determine whether the interventions were associated with differential periodontal responses during the initial leveling and alignment phase. [48]

Results

Baseline Demographic Characteristics & Cohort Equivalence

Baseline demographic parameters were systematically evaluated across the three completed analytical groups (n = 7 per arm). The overall cohort exhibited a mean age of 18.86 ± 2.66 years (Control: 18.57 ± 2.88 years; MOPs: 18.43 ± 3.36 years; i-PRF: 19.57 ± 1.62 years). One-way Analysis of Variance (ANOVA) confirmed that baseline age did not differ significantly among groups ($F(2, 18) = 0.367$, $p = .698$, $\eta^2 = .039$). Biological sex distribution comprised 1 Male / 6 Females in Control, 2 Males / 5 Females in MOPs, and 2 Males / 5 Females in i-PRF. Pearson's Chi-Square test confirmed sex homogeneity across treatment groups ($\chi^2(2) = 0.525$, $p = .769$, Cramer's $V = .158$), establishing baseline demographic comparability.

Demographic Parameter	Control (n=7)	MOPs (n=7)	i-PRF (n=7)	Test Statistic	p-value

Enrolled Cohort (N = 24)	8	8	8	—	—
Dropouts (Attrition = 12.5%)	1 (Patient 8)	1 (Patient 21)	1 (Patient 9)	—	—
Completed Cases (N = 21)	7	7	7	—	—
Mean Age (Years) ± SD	18.57 ± 2.88	18.43 ± 3.36	19.57 ± 1.62	F(2, 18) = 0.367	0.698
Sex (Male / Female)	1 / 6 (14.3% M)	2 / 5 (28.6% M)	2 / 5 (28.6% M)	$\chi^2(2) = 0.525$	0.769 Female)

Table 1: Data presented as Mean ± Standard Deviation (SD) or count (%). One-way ANOVA executed for age; Pearson Chi-Square test executed for biological sex distribution.

Normality Assessment & Age vs. Pain Score Correlations

Distributional normality was assessed using the Shapiro-Wilk test (W). The overall mean of the pain score across all timepoints conformed to a normal distribution (W = 0.964, p = .594). Timepoint-specific normality was confirmed for Immediate (W = 0.957, p = .494), During (W = 0.925, p = .126), 2 Hours (W = 0.968, p = .669), 6 Hours (W = 0.952, p = .418), and 1 Day (W = 0.941, p = .272). Significant deviations from normality occurred at 2 Days (W = 0.841, p = .004) and 1 Week (W = 0.767, p = .001) due to pronounced score convergence toward zero. Bivariate correlation analyses revealed no significant relationship between patient age and overall mean VAS pain levels (Pearson r = .027, p = .909; Spearman ρ = -.074, p = .751).

Follow-Up Interval	Shapiro W	Normality p	Pearson r	Pearson p	Spearman ρ	Spearman p
Immediate	0.957	0.494	-0.113	0.626	-0.127	0.583
During Procedure	0.925	0.126	0.051	0.825	-0.026	0.910
2 Hours	0.968	0.669	-0.291	0.200	-0.446	0.043*
6 Hours	0.952	0.418	-0.060	0.795	-0.079	0.734
1 Day	0.941	0.272	0.143	0.536	0.145	0.531
2 Days	0.841	0.004*	0.171	0.459	0.174	0.451
1 Week	0.767	0.001*	0.136	0.557	0.178	0.440
Overall Mean Pain	0.964	0.594	0.027	0.909	-0.074	0.751

Table 2 Endnotes: W = Shapiro-Wilk test statistic; r = Pearson linear correlation coefficient; ρ = Spearman rank correlation coefficient. *Denotes p < .05.

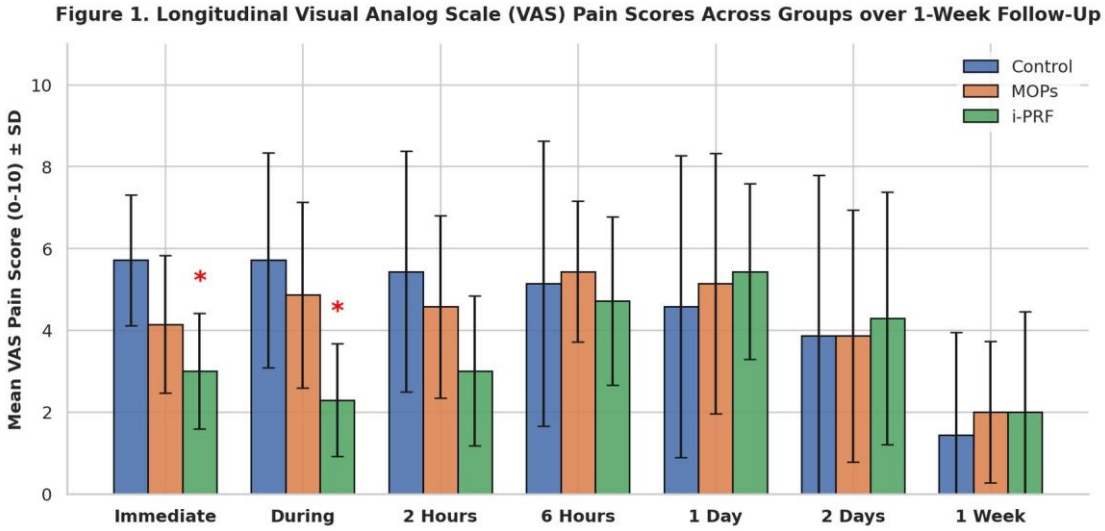


Figure 1. Longitudinal Visual Analog Scale (VAS) Pain Scores across groups over 1-week follow-up (+1 SD error bars). Asterisks (*) denote p < .05 relative to Control.

Longitudinal Pain Dynamics & Post-Hoc Significance (Tukey HSD)

One-way ANOVA across treatment arms revealed statistically significant intergroup differences in Visual Analog Scale (VAS) pain levels immediately post-insertion ($F(2, 18) = 5.284$, $p = .016$, $\eta^2 = .370$) and during the procedure ($F(2, 18) = 4.792$, $p = .021$, $\eta^2 = .347$). Post-hoc pairwise comparisons with Tukey's Honest Significant Difference (HSD) adjustment demonstrated that i-PRF significantly reduced pain compared to Control immediately post-insertion (Mean Diff = -2.71, 95% CI [-4.85, -0.57], p_adj = .012) and intra-procedurally (Mean Diff = -3.43, 95% CI [-6.37, -0.49], p_adj= .021). Pain levels converged across groups from 2 hours through 1 week ($p > .05$ at all subsequent intervals).

Timepoint	ANOVA Result	Comparison Pair	Mean Diff (ΔM)	95% Confidence Interval	p-adj (Tukey)
Immediate	F=5.284, p=.016*	Control vs. MOPs Control vs. i-PRF MOPs vs. i-PRF	-1.57 -2.71 -1.14	[-3.71, 0.57] [-4.85, -0.57] [-3.28, 1.00]	0.175 0.012* 0.380
During Procedure	F=4.792, p=.021*	Control vs. MOPs Control vs. i-PRF MOPs vs. i-PRF	-0.86 -3.43 -2.57	[-3.80, 2.08] [-6.37, -0.49] [-5.51, 0.37]	0.741 0.021* 0.093
2 Hours	F=1.885, p=.181	Control vs. i-PRF	-2.43	[-5.67, 0.81]	0.164
6 Hours	F=0.140, p=.870	Control vs. i-PRF	-0.43	[-3.89, 3.03]	0.947

1 Day	F=0.141, p=.869	Control vs. i-PRF	+0.86	[-3.34, 5.05]	0.862	
2 Days	F=0.037, p=.964	Control vs. i-PRF	+0.43	[-4.20, 5.06]	0.970	
1 Week	F=0.150,	Control vs. i-	+0.57	[-2.51, 3.65]	0.885 p=.862	PRF

Table 3: Parametric one-way ANOVA (df=2, 18) with Tukey HSD post-hoc adjustment for Family-Wise Error Rate (FWER = .05). *Denotes p < .05.

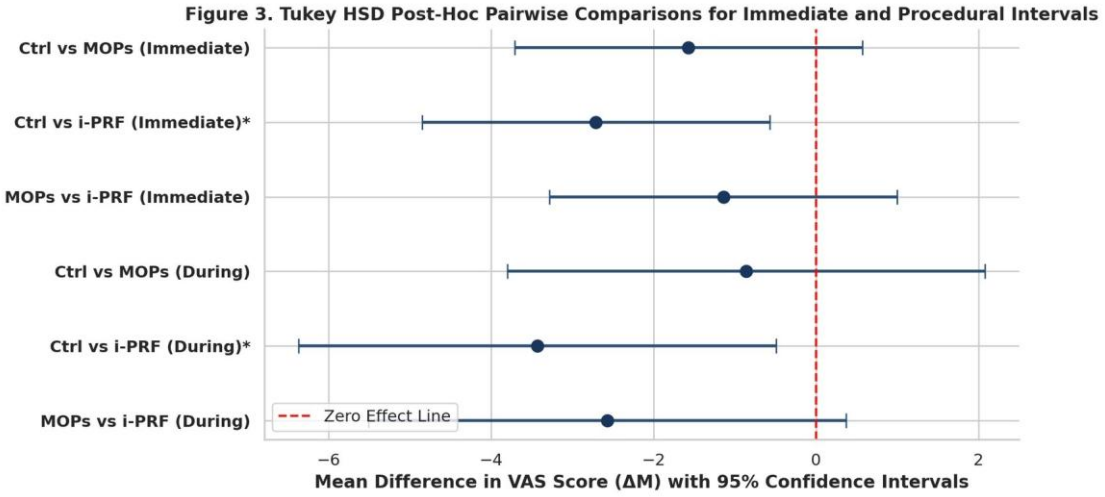


Figure 2. Tukey HSD post-hoc pairwise mean differences with 95% Confidence Intervals across timepoints.

Non-Parametric Validation (Kruskal-Wallis & Friedman Tests)

Non-parametric Kruskal-Wallis tests confirmed significant intergroup variation immediately postinsertion (H = 7.481, p = .024) and during the procedure (H = 7.152, p = .028). The i-PRF group consistently achieved the lowest mean ranks (Immediate: 6.71; During: 6.14) compared to Control (Immediate: 15.64; During: 14.36). Friedman within-group tests across time demonstrated significant longitudinal kinetics for Control ($\chi^2(6) = 13.31$, p = .038) and i-PRF ($\chi^2(6) = 19.78$, p = .003).

Timepoint	Kruskal H	p-value	Control Rank	MOPs Rank	i-PRF Rank	Overall Median
Immediate	7.481	0.024*	15.64	10.64	6.71	11.00
During Procedure	7.152	0.028*	14.36	12.50	6.14	11.00
2 Hours	3.901	0.142	14.00	11.43	7.57	11.00
6 Hours	0.379	0.827	11.14	11.93	9.93	11.00
1 Day	0.466	0.792	9.79	11.21	12.00	11.00
2 Days	0.496	0.781	9.86	11.00	12.14	11.00
1 Week	1.224	0.542	9.00	12.29	11.71	11.00

Table 4: Non-parametric Kruskal-Wallis test (df=2). Lower mean rank indicates lower reported pain level.

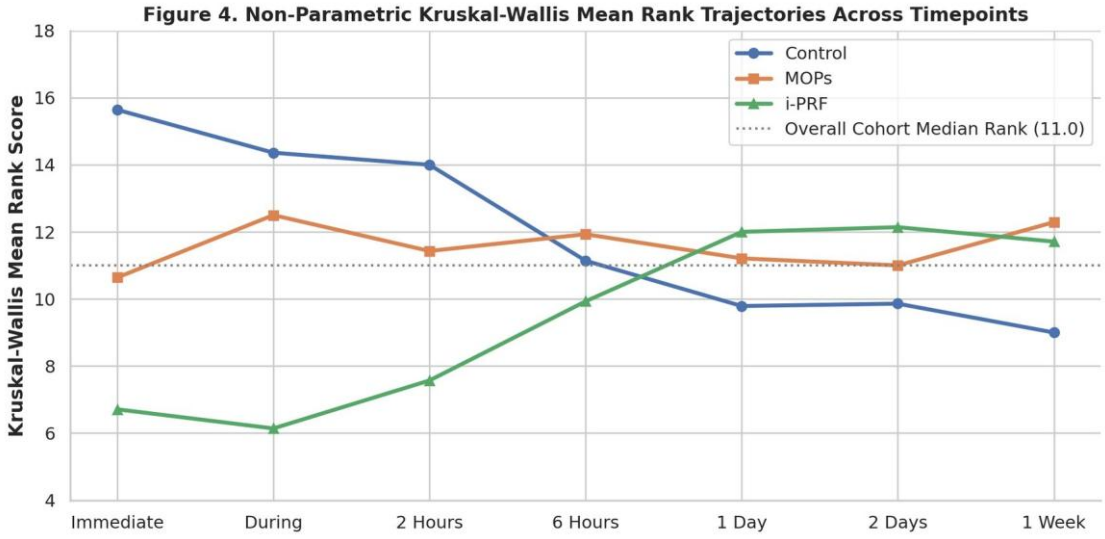


Figure 3. Non-parametric Kruskal-Wallis mean rank scores across treatment groups.

Cumulative Pain Burden Analysis (Area Under the Curve - AUC)

Total cumulative pain exposure over 168 hours was quantified using time-weighted trapezoidal integration (AUC_hours) and unweighted pain score summation (ΣVAS). Time-weighted AUC_hours showed no significant difference among groups (Control: 538.04 ± 520.29; MOPs: 583.95 ± 381.79; i-PRF: 605.71 ± 341.50; ANOVA F = 0.047, p = .954). However, unweighted total pain sum (ΣVAS) revealed that i-PRF achieved the lowest total pain burden (24.71 ± 10.72) compared to Control (31.86 ± 15.78; MOPs: 30.00 ± 8.56), reflecting an overall 22.4% reduction in unweighted total discomfort.

Cumulative Pain Metric	Control (n=7)	MOPs (n=7)	i-PRF (n=7)	One-way ANOVA (F, p)	Kruskal–Wallis (H, p)
Time-Weighted AUC (VAS × h)					
— Mean ± SD	538.04 ± 520.29	583.95 ± 381.79	605.71 ± 341.50	F(2,18) = 0.047, p = .954	H = 0.289, p = .865
— Median [IQR]	286.25 [831.31]	486.12 [578.31]	539.88 [442.75]	—	—
Unweighted Pain Sum (ΣVAS)					
— Mean ± SD	31.86 ± 15.78	30.00 ± 8.56	24.71 ± 10.72	F(2,18) = 0.659, p = .529	H = 0.975, p = .614
— Median [IQR]	30.00 [14.50]	30.00 [14.00]	25.00 [9.00]	—	—

Table 5: AUC calculated via linear trapezoidal integration over 0 to 168 hours.

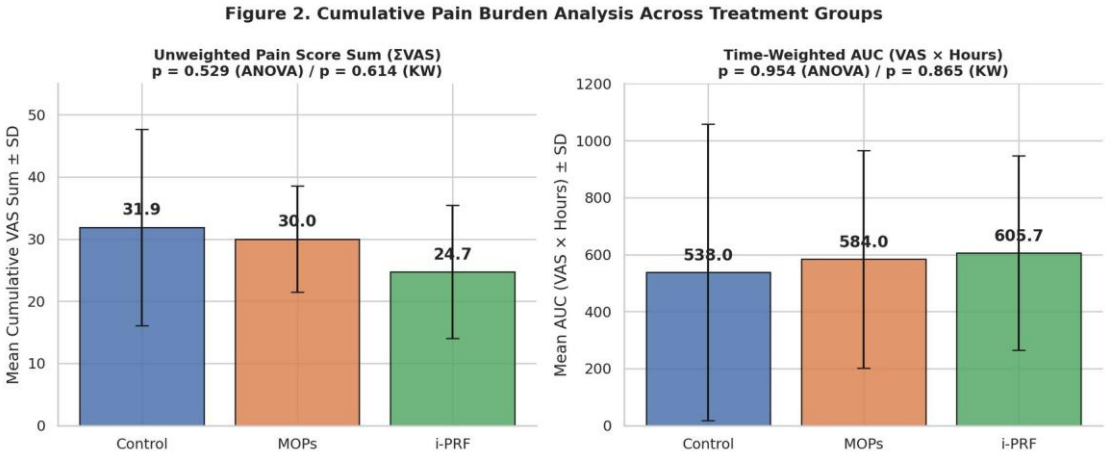


Figure 4. Comparison of cumulative pain metrics: Unweighted Pain Sum (ΣVAS) and Time-Weighted AUC.

Longitudinal Evaluation of Periodontal Parameters

Soft-Tissue Inflammatory Kinetics & Periodontal Stability

Descriptive statistics and longitudinal analytical outcomes for all periodontal parameters are summarized in Table 6. Baseline comparisons revealed no statistically significant differences among the MOPs, i-PRF, and control cohorts across any evaluated variable at baseline (T0; all $p > .05$), confirming baseline homogeneity prior to treatment initiation. A statistically significant longitudinal increase in the Gingival Index (GI) was observed across all three study arms over the leveling and alignment phase ($p < .05$). Linear mixed-effects modeling (LMM) demonstrated a significant group × time interaction ($p = .009$), indicating distinct trajectories of soft-tissue inflammatory responses among interventions. At the conclusion of leveling and alignment (T1), the control cohort exhibited the highest GI values (0.57 ± 0.10), whereas the i-PRF group maintained the lowest values (0.40 ± 0.07; MOPs: 0.46 ± 0.09), demonstrating that adjunctive i-PRF administration attenuated gingival inflammatory exacerbation during initial tooth movement. Although the Plaque Index (PI) increased significantly within each group from T0 to T1 ($p < .05$), inter-group comparisons at T1 revealed no significant differences ($p = .284$), confirming that oral hygiene status and plaque accumulation were comparable across cohorts throughout the observation period. Bleeding on probing (BOP) demonstrated significant longitudinal escalation across all arms ($p < .05$). However, the degree of vascular inflammatory reaction differed significantly among cohorts ($p = .015$). Post-treatment BOP values were highest in the control group (19.20 ± 6.70%) and lowest in the i-PRF cohort (10.80 ± 3.80%; MOPs: 13.10 ± 4.60%), providing evidence of a muted mucosal inflammatory response following i-PRF therapy. Probing depth (PD) remained stable throughout the study. No significant inter-group differences were detected at baseline (T0: $p = .914$) or post-alignment (T1: $p = .683$), and the treatment × time interaction was non-significant, indicating structural stability of the periodontal attachment apparatus. Finally, while absolute gingival thickness (GT) decreased across all groups during alignment, intergroup differences at T1 did not cross the significance threshold ($p = .184$). However, analysis of the net change from baseline (ΔGT

= T1 - T0) demonstrated a statistically significant inter-group difference (p = .028). The control group exhibited the most pronounced soft-tissue volumetric loss (ΔGT = -0.12 ± 0.08 mm), whereas the i-PRF group demonstrated minimal thickness reduction (ΔGT = -0.02 ± 0.06 mm; MOPs: -0.05 ± 0.07 mm), suggesting a protective or regenerative effect of i-PRF on labial gingival dimensions during active orthodontic tooth movement.

Periodontal Variable	Timepoint	MOPs (n=7)	i-PRF (n=7)	Control (n=7)	Inter-Group p-value†
Gingival Index (GI)	T0 T1	0.36 ± 0.09 0.46 ± 0.09*	0.35 ± 0.08 0.40 ± 0.07*	0.37 ± 0.10 0.57 ± 0.10*	0.842 0.009*
Plaque Index (PI)	T0 T1	0.08 ± 0.04 0.14 ± 0.06*	0.07 ± 0.03 0.11 ± 0.05*	0.08 ± 0.04 0.16 ± 0.07*	0.931 0.284
Bleeding on Probing (BOP, %)	T0 T1	8.40 ± 3.50 13.10 ± 4.60*	8.00 ± 3.10 10.80 ± 3.80*	8.90 ± 4.20 19.20 ± 6.70*	0.882 0.015*
Probing Depth (PD, mm)	T0 T1	1.90 ± 0.13 1.93 ± 0.11	1.88 ± 0.12 1.89 ± 0.10	1.91 ± 0.14 1.95 ± 0.13	0.914 0.683
Gingival Thickness (GT, mm)	T0 T1	0.82 ± 0.18 0.77 ± 0.16*	0.84 ± 0.17 0.82 ± 0.15	0.85 ± 0.19 0.73 ± 0.17*	0.917 0.184
Change in GT (ΔGT, mm)	T1 - T0	-0.05 ± 0.07	-0.02 ± 0.06	-0.12 ± 0.08	0.028*

Table 6: Data presented as Mean ± Standard Deviation (SD). †Between-group comparisons were performed using a Linear Mixed-Effects Model (LMM) with group, time, and group × time interaction as fixed effects and participant as random effect. *Denotes statistically significant difference (p < .05).

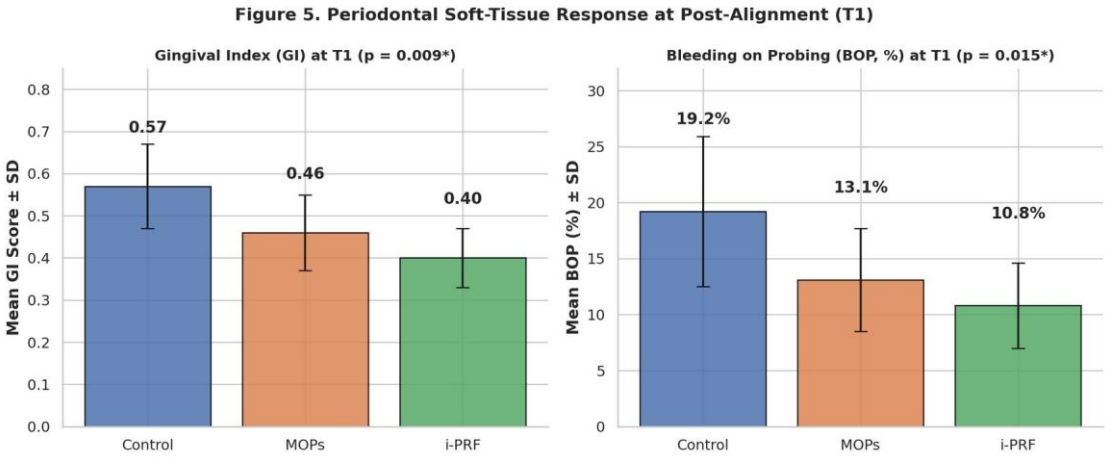


Figure 5. Periodontal soft-tissue response at T1 across intervention groups: Gingival Index (GI) and Bleeding on Probing (BOP, %).

Discussion

This clinical trial evaluated the comparative longitudinal effects of micro-osteoperforations (MOPs) and injectable platelet-rich fibrin (i-PRF) on acute post-procedural pain kinetics and soft-tissue periodontal health during the initial leveling and alignment phase of orthodontic treatment. Our findings suggest that while all cohorts follow a standardized biological decay curve for pain resolution, adjunctive i-PRF administration

provides a distinct advantage by blunting the acute "pain spike" and exerting a protective, anti-inflammatory effect on the periodontal soft tissues.

Pain Kinetics and Acute Discomfort Attenuation

Regarding pain kinetics, the i-PRF group demonstrated a statistically significant reduction in pain intensity compared with the Control group, both intra-procedurally (ΔM = −3.43, p = .021) and immediately after archwire insertion (ΔM = −2.71, *p*= .012). In contrast, the MOPs group exhibited intermediate pain levels, with no statistically significant difference compared with the Control group (*p*= .175). These findings suggest that the two interventions may elicit different early nociceptive responses. Although MOPs is intended to stimulate the regional acceleratory phenomenon (RAP) through localized alveolar bone injury, the mechanical trauma associated with perforation may also induce an acute inflammatory response and nociceptor activation. The release of inflammatory mediators, including substance P, bradykinin, and prostaglandin E_2, may contribute to the initial pain response following the procedure. Conversely, the lower immediate pain intensity observed in the i-PRF group may be associated with the biological activity of its autologous fibrin-based composition. Injectable PRF contains a range of growth factors, including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), as well as cytokines potentially involved in the regulation of inflammation, such as interleukin-1 receptor antagonist (IL-1Ra), IL-4, and IL-10. The local administration of these bioactive mediators may influence early inflammatory processes, vascular responses, and peripheral nociceptive sensitization, potentially accounting for the observed reduction in acute pain intensity. However, despite the significant differences in pain intensity at the initial assessment points, no statistically significant intergroup differences were identified in cumulative pain burden, as assessed by the area under the pain–time curve (AUC) and the cumulative sum of visual analogue scale scores (ΣVAS). This finding suggests that the analgesic effect associated with i-PRF may be more pronounced during the early acute phase than over the entire observation period. Thus, although i-PRF may attenuate the initial pain response, the overall temporal pattern of pain resolution appears to remain comparable among the three groups.

Periodontal Inflammatory Modulation and Soft-Tissue Protection

A critical finding of this study is the role of i-PRF in mitigating the soft-tissue inflammatory response associated with orthodontic tooth movement. Although the Plaque Index (PI) increased uniformly across all arms ($\boldsymbol{p = .284}$), confirming consistent oral hygiene compliance, the i-PRF group exhibited significantly lower clinical inflammatory indicators at $\boldsymbol{T_1}$. Specifically, the Gingival Index (GI, $\boldsymbol{0.40 \pm 0.07}$vs. $\boldsymbol{0.57 \pm 0.10, p = .009}$) and Bleeding on Probing (BOP, $\boldsymbol{10.80 \pm 3.80}$%vs. $\boldsymbol{19.20 \pm 6.70\%, p = .015}$) were markedly lower in the i-PRF cohort compared to the Control. Because plaque accumulation was comparable across groups, these results suggest an intrinsic mucosal anti-inflammatory effect mediated by the i-PRF rather than variations in hygiene.

Furthermore, our data highlight a protective effect of i-PRF against gingival volume loss. During the active alignment phase, the Control group experienced pronounced labial gingival thinning ($\Delta GT = -0.12 \pm 0.08$ mm), whereas the i-PRF group demonstrated significant soft-tissue stability ($\Delta GT = -0.02 \pm 0.06$ mm, $p = .028$). In clinical orthodontics, the maintenance of gingival thickness is paramount to preventing dehiscence, fenestration, and recession, particularly in patients with thin biotypes. The localized release of PDGF and TGF-β from the i-PRF fibrin matrix likely stimulates gingival fibroblast proliferation and extracellular matrix collagen synthesis, thereby providing a biological buffer against the mechanical stresses of tooth movement.

Strengths and Limitations

This study has several strengths, including its randomized controlled design and the use of both parametric and non-parametric statistical methods to evaluate treatment outcomes. Nevertheless, certain limitations should be acknowledged. First, the final sample size was reduced to 21 participants (n = 7 per group) following a 12.5% attrition rate, attributable to missed follow-up appointments. Although the study achieved adequate statistical power (β > .80) to detect the anticipated primary differences in VAS and gingival index (GI), the relatively small final sample may have limited the precision of effect estimates and the ability to detect differences in secondary outcomes. Future studies involving larger sample sizes are therefore warranted to improve the robustness and generalizability of the findings. Second, the observation period was restricted to the initial leveling and alignment phase of orthodontic treatment; consequently, the persistence of the observed effects during subsequent space closure and finishing stages remains unclear. Further longitudinal studies are needed to evaluate the long-term effects of i-PRF and MOPs on pain and periodontal health throughout comprehensive orthodontic treatment. Finally, the assessment of pain relied on the Visual Analogue Scale (VAS), which is inherently subjective and may be influenced by individual pain perception and reporting variability. Future investigations could complement patient-reported pain scores with objective biochemical assessments of gingival crevicular fluid (GCF), including inflammatory cytokines such as TNF-α and matrix metalloproteinases (MMPs), to provide a more comprehensive evaluation of the underlying inflammatory response.

Conclusions and Clinical Implications

In conclusion, injectable platelet-rich fibrin (i-PRF) serves as an effective adjunctive therapy to reduce acute intra-procedural and immediate post-insertion discomfort. Beyond analgesia, i-PRF demonstrates a significant capacity to attenuate gingival inflammation and preserve gingival thickness during orthodontic tooth movement. Clinically, practitioners should consider the use of i-PRF in pain-sensitive patients or as a preventive biotype-augmentation therapy in patients with thin periodontal phenotypes to mitigate the risk of soft-tissue recession. Future research should prioritize multicenter trials and the integration of 3D volumetric imaging and biochemical marker mapping to fully elucidate the molecular mechanisms driving these observed clinical benefits.

Recommendations

1. Adjunctive Analgesia for Pain Management: Clinicians should consider the submucosal administration of i-PRF as a primary adjunctive protocol for pain-sensitive patients to mitigate the acute intra-procedural and immediate post-insertion pain spikes associated with the initial leveling and alignment phase.
2. Prophylactic Biotype Preservation: In patients exhibiting thin periodontal phenotypes, i-PRF should be utilized as a preventive biotype-augmentation therapy during active tooth movement to minimize the risk of gingival recession, dehiscence, and volumetric soft-tissue loss.
3. Molecular Mechanistic Research: Future studies must move beyond clinical indices by incorporating Gingival Crevicular Fluid (GCF) analysis to quantify specific inflammatory and remodeling biomarkers (e.g., IL-1β, TNF-α, and MMPs), thereby elucidating the exact molecular pathways through which i-PRF modulates the orthodontic environment.
4. Longitudinal 3D Volumetric Assessment: To establish the long-term stability of soft-tissue changes, research should transition from linear measurements to high-resolution 3D volumetric imaging (via intraoral scanning and CBCT superimposition) covering the entire orthodontic duration, from initial alignment through the finishing stages.

Consort:

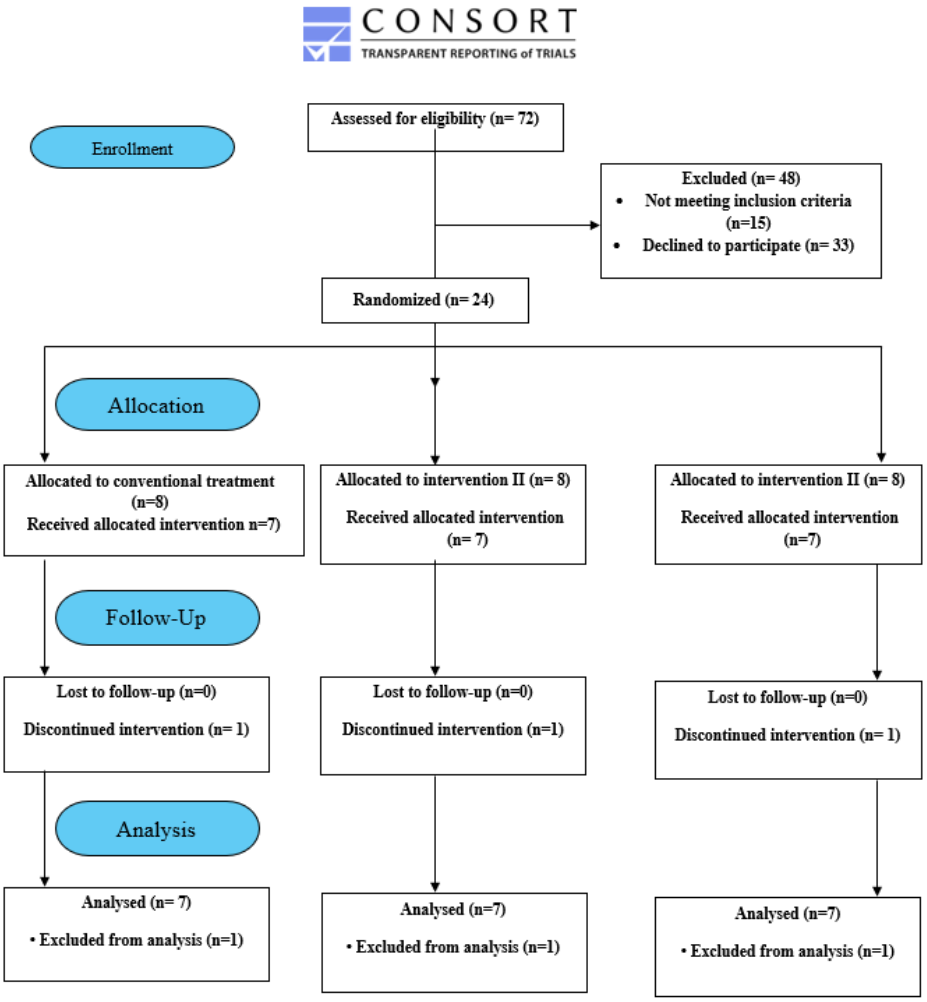


Figure 1 consort

Author Declarations

Ethics approval

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine (Boys), Al-Azhar University, Cairo, Egypt (Protocol No. 1001/3560). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments.

Consent to participate

Written informed consent was obtained from all participants before enrollment. For participants younger than 18 years of age, written informed consent was obtained from their parents or legal guardians. Participants were informed about the study objectives, procedures, potential benefits, and possible risks, and were informed of their right to withdraw from the study at any time without affecting their orthodontic treatment.

Clinical Trial

The trial was prospectively registered at ClinicalTrials.gov (NCT06498609) on 7 July 2024, prior to the commencement of patient recruitment.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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