RESEARCH PAPER

The Efficacy of Lyophilized Platelet Rich Fibrin in Treatment of Mandibular Grade II Furcation Involvement: Controlled Clinical Trial

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ABSTRACT

Background: There is a great difficulty in treating furcation involvement because of its complicated structure and inaccessibility. Platelet rich fibrin (PRF) has been illustrated to have potential for periodontal regeneration, though it cannot be stored and must be prepared just before use. These limitations could be overcome with the use of lyophilized PRF (Ly-PRF) that retains biological activity.

Aim: To estimate the clinical, radiographic, and biochemical efficiency of Ly-PRF combined with xenograft in treating mandibular grade II furcation defects.

Materials and Methods: Twenty cases (30-45 age) with grade II furcation defects were randomly separated into 2 groups of 10 cases each: Group I – open flap debridement (OFD) using xenograft alone and Group II – open flap debridement using xenograft and ly-PRF (platelet-rich fibrin). The clinical factors (Gingival Index, Plaque Index, Vertical and Horizontal Clinical Attachment Level), radiographic factors (bone density, bone fill, furcation dimensions) and gingival crevicular fluid (GCF) RANK-L levels were evaluated at baseline, 3, 6 and 9 months.

Results: Both groups revealed notable improvement; however, Group II demonstrated statistically superior outcomes. At 9 months, Group II presented notably greater VCAL gain (2.65±0.46 vs. 0.87±0.13 mm, P<0.001), HCAL gain (1.79±0.35 vs. 0.43±0.08 mm, P<0.001), bone fill (58.8±7.6% vs. 38.1±5.9%, P<0.001), and bone density (894±81.3 vs. 640.5±57.9 HU, P<0.001). RANKL levels decreased significantly in Group II at 3 months (84.5±20.6 vs. 414±34.1 pg/ml, P<0.001).

Conclusion: Lyophilized PRF combined with xenograft significantly enhances radiographic and clinical outcomes in mandibular grade II furcation defects, potentially through modulation of RANKL-mediated bone resorption.

Keywords: Periodontal regeneration, Furcation defects, Platelet-rich fibrin, Lyophilized PRF, Xenograft, RANKL

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INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory illness distinguished by progressive destruction of the periodontal tissues and is found in approximately 1.1 billion people worldwide (1). The furcation involvement or periodontal involvement of the trifurcation or bifurcation of multirooted teeth is one of the most difficult clinical situations in periodontology (2). The furcation areas of the roots are very complex, with root concavities, ridges and limited accessibility which hinders effective debridement and makes the regenerative procedures difficult (3).

There are different classifications with Grade II furcation defects (partial involvement more than 1/3 but not all) being ideal candidates for regenerative therapy (4). The therapeutic objective is to regenerate the entire periodontal tissues: periodontal ligament, new cementum and alveolar bone formation (5). There are many different regenerative techniques that have been used (bone grafts, guided tissue regeneration [GTR] and enamel matrix derivatives), but it is not possible to obtain a complete and predictable reconstruction (6).

A 2nd-generation platelet concentrate, platelet-rich fibrin, has emerged in popularity because of its abundance of autologous growth parameters like platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (7_8). These growth parameters are conducive to cell migration, proliferation, angiogenesis and tissue remodeling (9). The fresh PRF (F-PRF) has some limitations, however, such as the requirement for immediate application following preparation, short shelf-life and non-commercialization (10).

To overcome such limitations, lyophilization (freeze-drying) has emerged to be a viable approach. Lyophilized PRF (Ly-PRF) preserves the biological activity of growth parameters and has longer storage stability thus can be considered an "off-the-shelf" product, suitable for multiple applications (11_12). Current researches have illustrated that Ly-PRF enhances expression of important osteogenic markers like type I collagen, osteocalcin, osteopontin and

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bone morphogenetic protein-2 (BMP-2), which are included in the process of osteogenic differentiation of bone marrow-derived MSCs (13).

Deproteinized xenografts, especially bovine deproteinized bone, have been extensively employed in periodontal regeneration (14). They are porous, have a similar structure to human bone and help to promote bone growth and angiogenesis (15). A combination of xenografts and biological agents may improve the regenerative properties, however, xenografts do not possess osteoinductive properties (16).

Receptor activator of nuclear factor-kappa B ligand (RANKL) is a important molecule in bone metabolism, being the main regulator of osteoclast activation, differentiation and survival (17). A higher expression of RANKL is observed in periodontal disease and is related to the severity of the illness, establishing RANKL as a promising biomarker to monitor the bone resorption and treatment outcome of the disease (18).

To date, there are only some preliminary scientific data regarding the use of Ly-PRF and xenografts for treating furcation defects. This randomized controlled clinical trial aimed to compare the clinical, radiographic, and biochemical effects of Ly-PRF and xenograft compared to xenograft alone in the treatment of grade II furcation involvement in the mandible.

2. Materials and Methods

2.1. Study Design and Setting

This research has been executed as a randomized, controlled, parallel-group clinical trial executed at the Department of Oral Medicine and Periodontology, Faculty of Dental Medicine, Al-Azhar University, Assiut Branch, Egypt. The research protocol has been accepted by the Institutional Ethical Committee (Approval Number: [Insert Number]) and registered at the Clinical Trials Registry ([Insert Registration Number]). Prior to enrollment, each participant gave a written informed consent form.

2.2. Participants

Twenty patients (16 males, 4 females; age range 30-45 years, mean 41.5±3.4 years) with mandibular first molar grade II furcation defects were enrolled. Sample size was detected depending on preceding research with α=0.05 and β=0.20 (power 80%).

Inclusion Criteria:

· Systemically healthy patients
· Grade II furcation defects (Glickman classification, 1972) (19)in vital, asymptomatic mandibular first molars
· Horizontal clinical attachment loss (HCAL) ≥ 3 mm following phase I therapy
· Radiographic radiolucency in the furcation area

Exclusion Criteria:

· Unacceptable oral hygiene (Plaque Index > 1.5) following phase I therapy (20)
· Interproximal intrabony defects
· Gingival recession
· Endodontic involvement
· Tooth mobility ≥ Miller's Grade II (21)
· Smoking or pregnancy
· History of periodontal treatment within 6 months
· Antibiotic or anti-inflammatory use within 3 months
· Systemic diseases affecting periodontal health

2.3. Randomization and Blinding

Case were randomly subdivided into 2 groups employing online randomization software (www.randomizer.org) with allocation concealed in sealed envelopes:

· Group I (Control, n=10): OFD + Xenograft alone
· Group II (Test, n=10): OFD + Xenograft + Ly-PRF

The clinician conducting the clinical and radiographic evaluations was blinded to the assigned group.

2.4. Phase I Therapy

All cases underwent initial periodontal treatment comprising:

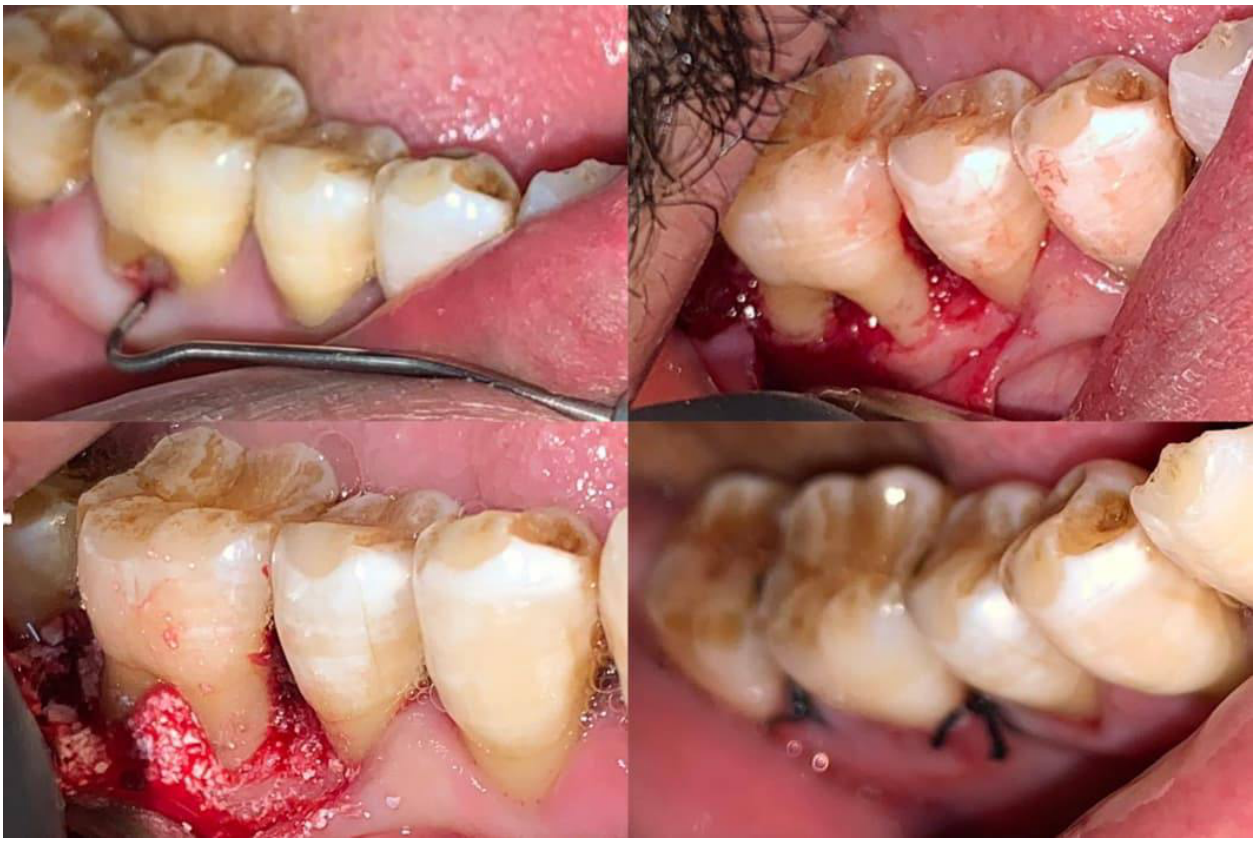
· Oral hygiene instructions and motivation
· Full-mouth scaling and root planning (2-4 sessions within 2 weeks) using manual and ultrasonic instruments under local anesthesia
· Correction of restorative and prosthetic irritants
· Dietary modifications

Four to six weeks following phase I therapy, reassessment was executed to verify eligibility for surgical intervention.

2.5. Surgical Procedure

All surgical operations were undertaken by the same experienced periodontist.

1. Pre-operative Preparation: 0.12% chlorhexidine digluconate mouth rinse was administered immediately before surgery.
2. Anesthesia: Local anesthesia (4% articaine with 1:100,000 epinephrine) was administered.
3. Flap Reflection: Full-thickness sulcular incisions were made and extended to interproximal line angles. A full-thickness mucoperiosteal flap was raised to offer adequate surgical access.
4. Debridement: Complete defect and root surface debridement was executed using ultrasonic and hand instrumentation. The surgical field was irrigated with sterile normal saline (0.9%).
5. Graft Application:
   · Group I: Bovine xenograft (Bony-Tite®) was applied to the furcation defect.
   · Group II: Bovine xenograft was mixed with Ly-PRF granules and applied to the defect.(fig1)
6. Suturing: Flaps were coronally repositioned and sutured employing 3-0 or 4-0 silk interrupted sutures. Gentle pressure was applied with saline-soaked gauze.



(Fig1)

2.6. Lyophilized Platelet-Rich Fibrin (Ly-PRF) Preparation

Ly-PRF was produced depending on the Choukroun's protocol (22) with modifications:

1. Venous blood (10 milliliters) was obtained in glass tubes without anticoagulant and immediately centrifuged at 400×g (approximately 3000 rpm) for ten minutes.
2. Following centrifugation, the middle PRF layer (fibrin buffy coat) was gently isolated from the red blood cell layer employing sterile forceps and scissors.
3. Fresh PRF matrices were compressed utilizing a sterile PRF compression device.
4. For lyophilization, fresh PRF was frozen at -80°C for thirty minutes, then freeze-dried overnight at -51°C.
5. Dried samples were ground into granules utilizing a sterile pestle and mortar and stored at 4°C until use.

2.7. Post-Operative Care

All patients received:

· Systemic antibiotics: Amoxicillin-clavulanic acid (625 milligrams three times per day for seven days)
· Chlorhexidine mouthwash (0.12%, two times per day for 4 weeks)
· Analgesic/anti-inflammatory: Diclofenac potassium (50 milligrams twice daily for 7 days)
· Post-operative instructions including avoidance of brushing the surgical site for 10 days, soft diet, and ice pack application

2.8. Clinical Evaluation

Clinical parameters were estimated at baseline (pre-surgery), 3, 6, and 9 months following surgery:

1. Plaque Index (PI): Silness and Löe Index (1964) (20)
2. Gingival Index (GI): Löe and Silness Index (1963) (23)
3. Vertical Clinical Attachment Level (VCAL): Distance from cemento-enamel junction to pocket base using Williams graduated periodontal probe
4. HCAL: Horizontal probe penetration into the furcation using Naber's probe

All measurements were executed at six sites per tooth, with furcation measurements recorded at the furcation entrance.

2.9. Radiographic Evaluation

Cone-beam computed tomography (CBCT) was executed at baseline, 3, 6, and 9 months using a standardized protocol (Dentsply Sirona, Finland; 90 kV, 10 mA, 13 sec exposure, 0.25 mm^3 isotropic voxel size). Images were analyzed employing Blue Sky Plan 4 software (Version 4.13.31).

The following parameters were assessed:

· Bone Density (Hounsfield Units, HU): Measured in the region of interest (ROI) at the furcation area
· Bone Fill (%): Percentage of defect filled with new bone
· Furcation Dimensions: Height, width, and depth measured in millimeters

2.10. Biochemical Assessment

Gingival Crevicular Fluid (GCF) Collection:

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GCF samples were attained at baseline, 1 week, 1 month, and 3 months post-surgery.
1. Selected teeth were isolated with cotton rolls.
2. Supragingival plaque was carefully removed without touching the marginal gingiva.
3. The site was gently dried using an air syringe.
4. Sterile paper points (#30) were inserted into the furcation defect to maximum depth for thirty seconds.
5. Contaminated samples (blood or saliva) were excluded.
6. Paper points were immediately positioned in 1.5 mL Eppendorf tubes comprising 1 mL phosphate buffer solution (pH 7.3).
7. Samples were vortexed (20 min), centrifuged (4000 rpm, 15 min), and frozen at -80°C until analysis.

RANKL Analysis:

RANKL concentrations were detected with a commercially accessible highly sensitive enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's recommended protocol. The optical density of the samples was read at 450 nm using an ELISA reader, and the corresponding RANKL concentrations were obtained from the generated standard curves.

2.11. Statistical Analysis

Data were evaluated applying IBM SPSS Statistics for Windows (Version 23.0). Normality was measured employing Kolmogorov-Smirnov and Shapiro-Wilk tests: · Parametric data (VCAL, HCAL, bone density, bone fill, furcation dimensions, RANKL): Presented as mean ± standard deviation; Repeated measures ANOVA with Bonferroni post-hoc test for within-group changes; Independent t-test for between-group evaluations.

· Non-parametric data (PI, GI): Presented as median (range); Friedman's test with Dunn's post-hoc for within-group changes; Mann-Whitney U test for among-group evaluations.

Significance level was set at $P \leq 0.05$.

3. Results

All 20 cases finished the 9-month follow-up period without complications. Baseline characteristics showed no notable distinctions among groups (Table 1).

Table 1: Baseline Attributes of the Examined Groups.

Parameter	Group I (Xenograft)	Group II (Xenograft+Ly-PRF)	P-value
Age (years)	41.2 ± 3.6	41.8 ± 3.2	0.698
Gender (M/F)	8/2	8/2	1.000
PI	2.2 (0.35)	2.18 (0.48)	0.910
GI	2.4 (0.35)	2.37 (0.46)	0.910
VCAL (mm)	6.91 ± 0.9	6.88 ± 1.19	0.950
HCAL (mm)	4.53 ± 0.65	4.51 ± 0.86	0.954
Bone Fill (%)	10.2 ± 3.6	11.2 ± 4.4	0.602
Bone Density (HU)	339.5 ± 52.5	360.0 ± 60.4	0.429

Values expressed as mean ± SD or median (range)

3.1. Clinical Parameters

Plaque Index (PI) and Gingival Index (GI):

Both groups displayed notable improvement in PI and GI scores over time ($P < 0.001$). However, Group II demonstrated notably lower scores at 3, 6, and 9 months relative to Group I ($P \leq 0.039$ for PI; $P \leq 0.039$ for GI) .

Vertical Clinical Attachment Level (VCAL):

Within-group changes: Both groups showed significant improvement ($P < 0.001$).

Between-group comparison: At 6 and 9 months, Group II showed significantly lower VCAL (better attachment) than Group I ($P = 0.004$ and $P < 0.001$, respectively).

VCAL Gain: Group II demonstrated significantly greater VCAL gain at all time points ($P \leq 0.022$), with the variation becoming more pronounced over time (Table 2).

Horizontal Clinical Attachment Level (HCAL):

· Within-group changes: Significant improvement was noted in both groups ($P < 0.001$).

· Between-group evaluation: At 6 and 9 months, Group II showed significantly lower HCAL (better horizontal attachment) than Group I ($P = 0.002$ and $P < 0.001$, respectively).

· HCAL Gain: Group II showed significantly greater HCAL gain at all time points ($P \leq 0.003$) (Table 2).

Table 2: Clinical Attachment Level Gain Over Time

Parameter	Time Point	Group I (Xenograft)	Group II (Xenograft+Ly-PRF)	P-value
VCAL Gain (mm)	3 months	1.30 ± 0.15	1.60 ± 0.35	0.022*
	6 months	1.09 ± 0.13	2.29 ± 0.44	<0.001*
	9 months	0.87 ± 0.13	2.65 ± 0.46	<0.001*
HCAL Gain (mm)	3 months	0.73 ± 0.11	1.04 ± 0.26	0.003*
	6 months	0.61 ± 0.09	1.52 ± 0.34	<0.001*
	9 months	0.43 ± 0.08	1.79 ± 0.35	<0.001*

*Values expressed as mean ± SD; Significant at P ≤ 0.05

3.2. Radiographic Parameters

Bone Fill Percentage:

· Peak at 6 months: Both groups showed maximal bone fill at 6 months, followed by a slight decrease at 9 months (remodeling phase).

· Between-group comparison: Group II showed significantly higher bone fill at all time points (P < 0.001 at 3, 6, and 9 months) (Table 3).

Bone Density (HU):

· Peak at 6 months: Both groups showed maximal bone density at 6 months.

· Between-group comparison: Group II showed notably greater bone density at all time points (P < 0.001 at 3, 6, and 9 months) (Table 3).

Furcation Dimensions:

· Furcation Height: Group II showed significantly greater height (more bone formation) at 3, 6, and 9 months (P ≤ 0.008) (Table 3).

· Furcation Width: Group II showed significantly narrower width (defect reduction) at all follow-up points (P ≤ 0.006).

· Furcation Depth: Group II showed significantly shallower depth (defect reduction) at all follow-up points (P ≤ 0.005).

Table 3: Radiographic Parameters at 9-Month Follow-up

Parameter	Group I (Xenograft)	Group II (Xenograft+Ly-PRF)	P-value
Bone Fill (%)	38.1 ± 5.9	58.8 ± 7.6	<0.001*
Bone Density (HU)	640.5 ± 57.9	894.0 ± 81.3	<0.001*
Furcation Height (mm)	4.05 ± 0.41	4.87 ± 0.37	<0.001*
Furcation Width (mm)	3.60 ± 0.40	2.77 ± 0.39	<0.001*
Furcation Depth (mm)	4.00 ± 0.50	3.07 ± 0.43	<0.001*

Clinical and Radiographic Interpretation: · The group II – Ly-PRF, showed higher bone fill (58.8% vs. 38.1%), bone density (894 vs. 640.5 HU) and better furcation dimensions (decreased width and depth, increased height) than the group of xenograft alone.

· For the progressive improvement in VCAL and HCAL by 9 months in Group II, it is suggested that the activity is continuing to improve, while in Group I, there may have been some relapse following 6 months.

3.3. Biochemical Parameters (RANKL)

RANKL Levels in Gingival Crevicular Fluid:

· Post-surgical spike: Both groups presented a significant rise in RANKL levels at 1 week post-surgery (surgical trauma response).

· Decline phase: RANKL levels decreased notably in both groups from 1 week to 1 month and 3 months.

· Between-group comparison: At 1 and 3 months, Group II revealed notably lower RANKL levels than Group I (P < 0.001 at both time points) (Table 4).

· Sustained reduction: In Group II, RANKL levels at 3 months (84.5 ± 20.6 pg/ml) were notably lower than baseline (419.5 ± 36.6 pg/ml), indicating sustained anti-resorptive effect. In Group I, RANKL levels at 3 months (414 ± 34.1 pg/ml) returned to near-baseline levels, suggesting limited long-term modulation of bone resorption.

Table 4 : RANKL Level Changes Over Time

Time Point	Group I (Xenograft)	Group II (Xenograft+Ly-PRF)	P-value
Baseline	419.0 ± 34.0	419.5 ± 36.6	0.975
1 Week	729.0 ± 34.1	729.5 ± 36.6	0.975
1 Month	439.0 ± 34.1	196.0 ± 38.9	<0.001*
3 Months	414.0 ± 34.1	84.5 ± 20.6	<0.001*

*Values expressed as mean ± SD (pg/ml); Significant at P ≤ 0.05

4. DISCUSSION

This randomized controlled clinical trial illustrated that the addition of lyophilized platelet-rich fibrin (Ly-PRF) to xenograft significantly enhanced clinical, radiographic, and biochemical outcomes in the treatment of mandibular grade II furcation defects relative to xenograft alone. The results are highly favorable and provide good proof of the potential of Ly-PRF as an effective adjunct in periodontal regenerative therapy.

Superior Clinical Outcomes:

The finding of a larger decrease in PI and GI scores in Group II indicates that Ly-PRF could help with the healing of soft tissues and decrease inflammation. This result is consistent with preceding investigations that showed anti-inflammatory effect of PRF by regulating the secretion of cytokines and inducing angiogenesis (24).The significantly better VCAL and HCAL gains in Group II indicate that Ly-PRF not only enhanced the quantity of tissue regeneration but also the stability of clinical attachment over time.

Notably, in Group I (xenograft alone), initial attachment gains at 3 months were followed by partial relapse at 6 and 9 months. This pattern suggests limited long-term maintenance of regenerative outcomes when using xenografts alone. In contrast, Group II demonstrated progressive improvement through 9 months, indicating sustained regenerative activity provided by the growth factors in Ly-PRF. This sustained release of PDGF, TGF-β, and VEGF from Ly-PRF may have promoted continuous cellular recruitment, proliferation, and differentiation necessary for long-term tissue regeneration (25-26).

Enhanced Radiographic Regeneration:

The radiographic findings strongly support the clinical observations. Group II showed significantly greater bone fill (58.8% vs. 38.1%) and bone density (894 vs. 640.5 HU) at 9 months. The peak bone fill observed at 6 months in both groups, followed by a slight decrease at 9 months, is consistent with the normal bone remodeling process where initial woven bone is partially resorbed and replaced by more mature lamellar bone (27). The much higher values obtained in Group II however indicate that Ly-PRF stimulated more vigorous initial formation of bone and preservation of newly formed bone during the remodeling stage.

The enhanced dimensions of the fuctions – higher, narrower and shallower – suggest better defect resolution and architectural restoration in Group II. The results are in line with the reported effect of the growth factors produced by PRF to stimulate osteoblast proliferation and activity (28). Further, the porous nature of Ly-PRF might have served as an ideal scaffold for cellular infiltration and vascularization, which, along with the osteoconductive properties of the xenograft, was suitable for the healing process.In addition, the porous nature of Ly-PRF may have served as an ideal scaffold for cellular infiltration and vascularization, besides the osteoconductive properties of the xenograft, which would be suitable for the healing process (29).

Biochemical Evidence: RANKL Modulation

The differential expression of RANKL between groups may have been the most mechanistically relevant finding. Both groups had a similar immediate increase in RANKL at 1 week following surgery, which is consistent with a transient surgical trauma and acute inflammatory response.An expected surge in RANKL was observed at 1 week post-surgery in both groups, which was consistent with transient surgical trauma and acute inflammatory response (30). The RANKL level in Group II, however, remained significantly reduced (84.5 ± 20.6 pg/ml) suggesting effective modulation of the osteoclast activity, in contrast to Group I, where near baseline RANKL level was achieved at 3 months.

RANKL is the primary mediator of osteoclast differentiation, activation, and survival (31).Elevated RANKL/OPG ratio is associated with periodontal disease severity and bone resorption (32). The continued decrease of RANKL in Group II may indicate that Ly-PRF has anti-resorptive activity via several mechanisms:

1. Direct inhibition of RANKL expression by osteoblasts and inflammatory cells
2. Upregulation of osteoprotegerin (OPG), the natural RANKL inhibitor (33)
3. Reduction of pro-inflammatory cytokine production (IL-1β, TNF-α, IL-17) that stimulate RANKL expression (34)
4. Promotion of osteoblastic differentiation, altering the local bone metabolic balance toward formation rather than resorption (35)

The significant correlation between reduced RANKL levels and improved clinical/radiographic outcomes in Group II supports the role of RANKL as a valuable biomarker for monitoring periodontal regeneration and underscores the biological basis for the observed therapeutic benefits.

Advantages of Lyophilized PRF

This study addresses several limitations of fresh PRF by using its lyophilized form. Fresh PRF requires immediate use after preparation, limiting its clinical applicability (36).Ly-PRF overcomes this limitation through (37-38):

· Extended storage stability without loss of biological activity
· Ability to be produced as an "off-the-shelf" product
· Continuous release of growth factors
· Reduced immunogenicity and improved handling characteristics

Furthermore, recent studies have demonstrated that the lyophilization process may actually enhance the biological properties of PRF by increasing growth factor concentration and modifying the three-dimensional fibrin network to promote better cell adhesion and proliferation (39-40).In vitro studies have revealed that Ly-PRF significantly increases osteogenic differentiation of mesenchymal stem cells via upregulation of BMP-2, osteopontin, and osteocalcin expression (41-42).

Comparison with Previous Studies

Our outcomes are in line with preceding studies investigating PRF in furcation defects. Agarwal et al. (34) reported significant improvement in clinical attachment levels and bone fill when PRF was employed with bone graft in furcation defects. Likewise, Pradeep et al. (44).demonstrated superior outcomes with PRF and allograft compared to allograft alone. However, this research is among the first to estimate lyophilized PRF in this context, providing evidence for its clinical efficacy and potential for wider clinical application.

Some studies have reported limited or comparable outcomes with PRF and bone grafts (45).which may be ascribed to variations in preparation protocols, defect characteristics, and follow-up periods. Our standardized lyophilization protocol and comprehensive evaluation—including biochemical assessment—provide additional confidence in the observed outcomes.

Clinical Implications

The outcomes of this research have several practical implications:

1. Ly-PRF can be used as an important additional material in regenerative periodontal therapy in furcation defects.
2. The continuous release of growth factors might help to improve long-term durability of regeneration.
3. Being able to prepare and store Ly-PRF may increase its clinical uses and make it applicable for multiple applications and emergency procedures.
4. The level of RANKL in GCF may serve as a biochemical marker for treatment response and predict treatment response and regenerative outcomes.

Study Limitations

Several limitations should be acknowledged:

1. Sample Size: The results presented should be viewed with some caution as they were obtained from a relatively small sample size (n=10 per group) and thus are not representative.
2. Follow-up Duration: 9 months is enough to assess the outcomes of early regeneration, but longer follow-up (≥ 2 years) is required to assess the durability of the results.
3. Histological Evaluation: Without histologic evaluation, it is difficult to document complete regeneration rather than repair of periodontal tissues.
4. Biomarker Assessment: OPG measurement in combination with the measurement of RANKL would have given more complete information about the ratio of RANKL to OPG.
5. Single-Blind Design: Surgeon and patients were not blinded, there may be some bias in the clinical assessors.

5. CONCLUSIONS

1. Results illustrated better radiographic and clinical results with open flap debridement using lyophilized platelet-rich fibrin (Ly-PRF) with xenograft than with xenograft alone for treating grade II mandibular furcation defects.
2. Improvements in clinical attachment, bone fill, bone density and the favorable changes in furcation dimension were significantly higher after 9 months with the addition of Ly-PRF.
3. A prolonged decrease in RANKL levels was seen in the Ly-PRF group, indicating that modulation of osteoclast mediated bone resorption may be one explanation for the better regenerative responses.
4. The lyophilization process of PRF allows to preserve its biological activity and eliminate the drawbacks of fresh PRF, thus making it a clinically viable "off-the-shelf" adjunctive treatment.

6. RECOMMENDATIONS

1. Longitudinal Studies: Conduct larger, multi-center randomized controlled trials with extended follow-up periods (≥ 2 years) to evaluate long-term stability of regenerative outcomes.
2. Histological Research: Use the histologic and histomorphometric methods to verify the genuine regeneration of the periodontal tissue including the formation of the periodontal ligament, new cementum and alveolar bone.
3. Biomarker Assessment: Research other markers such as OPG, bone morphogenetic proteins and inflammatory cytokines in order to gain a more complete picture of the regeneration process.
4. Protocol Optimization: Pursue lyophilization optimizations for maximum growth factor preservation and investigate various ways to prepare PRFs (A-PRF and i-PRF) for furcation defects.
5. Comparative Studies: Compare head-to-head the efficacy of Ly-PRF versus other biologic agents (GTR membranes, enamel matrix derivatives, recombinant growth factors), to determine the best treatment modality.
6. Cost-Effectiveness Analysis: Compare the cost effectiveness of Ly-PRF combination therapy with other regenerative techniques.

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REFERENCES

1. Mehriz BM, Atteya MA, Skipina TM, Mostafa MA, Soliman EZ. Association between periodontitis and diabetes mellitus in the general population. J Diabetes Metab Disord. 2022;21(2):1249-1254.
2. Goldman MJ, Ross IF, Goteiner D. Effect of periodontal therapy on patients maintained for 15 years or longer. J Periodontol. 1986;57:347-353.
3. Al-Shammari KF, Kazor CE, Wang H-L. Molar root anatomy and management of furcation defects. J Clin Periodontol. 2001;28(8):730-740.
4. Avila-Ortiz G, De Buitrago JG, Reddy MS. Periodontal regeneration - furcation defects: a systematic review from the AAP Regeneration Workshop. J Periodontol. 2015;86(2 Suppl):S108-130.
5. Karring T, Isidor F, Nyman S, Lindhe J. New attachment formation on teeth with a reduced but healthy periodontal ligament. J Clin Periodontol. 1985;12(1):51-60.
6. Trombelli L. Which reconstructive procedures are effective for treating the periodontal intraosseous defect? Periodontol 2000. 2005;37:88-105.
7. Dohan D, Choukroun J, Diss A, Dohan S, Dohan A, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:37-44.
8. Temmerman A, Cleeren GJ, Castro AB, Teughels W, Quirynen M. L-PRF for increasing the width of keratinized mucosa around implants: a split-mouth, randomized, controlled pilot clinical trial. J Periodontal Res. 2018;53(5):793-800.
9. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:e56-60.
10. Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials. 2007;28(31):4551-4560.
11. Haugh MG, Murphy CM, O'Brien FJ. Novel freeze-drying methods to produce a range of collagen-glycosaminoglycan scaffolds with tailored mean pore sizes. Tissue Eng Part C Methods. 2010;16(5):887-894.
12. Shi L, Li R, Wei S, Zhou M, Li L, Lin F, et al. Effects of a protective agent on freeze-dried platelet-rich plasma. Blood Coagul Fibrinolysis. 2019;30(2):58-65.
13. Wang Z, Han L, Sun T, Wang W, Li X, Wu B. Preparation and effect of lyophilized platelet-rich fibrin on the osteogenic potential of bone marrow mesenchymal stem cells in vitro and in vivo. Heliyon. 2019;5:1-9.
14. Kao ST, Scott DD. A Review of Bone Substitutes. Oral Maxillofac Surg Clin North Am. 2007;19:513-521.
15. Oliveira G, Pignaton TB, de Almeida Ferreira CE, Peruzzo LC, Marcantonio E Jr. New bone formation comparison in sinuses grafted with anorganic bovine bone and B-TCP. Clin Oral Implants Res. 2019;30:483.
16. Rosen PS, Froum SJ, Reynolds MA. Is the use of biologic additions necessary to optimize periodontal regenerative efforts? Clin Adv Periodontics. 2013;3:180-186.
17. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegrin/osteoclastogenesis inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA. 1998;95:3557-3562.
18. Bostanci N, İlgenli T, Emingil G, Afacan B, Han B, et al. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. J Clin Periodontol. 2007;34:370-376.
19. Glickman I. Clinical periodontology: the periodontium in health and disease. Philadelphia: WB Saunders; 1972.
20. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22:121-135.
21. Miller SC. Textbook of Periodontia. 3rd ed. Philadelphia: Blakiston; 1950. p. 125.
22. Dohan D, Choukroun J, Diss A, Dohan S, Dohan A, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:e37-44.
23. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand. 1963;21:533-551.
24. Blanton MW, Barden W, Koke J, Ramakrishna H, Fonseca RJ, Abreu O, et al. The effect of platelet-rich plasma on the clinical outcomes of periodontal regeneration: A systematic review and meta-analysis. J Periodontol. 2023;94(2):153-168.
25. Li Q, Reed D, Min L, Gopinathan G, Li S, Dangaria S, et al. Lyophilized platelet-rich fibrin (PRF)

promotes craniofacial bone regeneration through Runx2. Int J Mol Sci. 2014;15:8509-8525.
26. Ngah N, Dias G, Tong D, Noor S, Ratnayake J, Cooper P, et al. Lyophilised platelet-rich fibrin: Physical and biological characterisation. Molecules. 2021;26:1-14.
27. Roberts WE, Garetto LP, Arbuckle GR, Simmons KE, DeCastro C. What are the long-term effects of orthodontic treatment? Dent Clin North Am. 2010;54(3):407-435.
28. Zhu L, Liu Y, Wang A, Zhu Z, Li Y, Zhu C, et al. Application of BMP in bone tissue engineering. Front Bioeng Biotechnol. 2022;10:1-20.
29. Xu F, Zou D, Dai T, Xu H, An R, Liu Y, et al. Effects of incorporation of granule-lyophilised platelet-rich fibrin into polyvinyl alcohol hydrogel on wound healing. Sci Rep. 2018;8:1-10.
30. Graves D, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol. 2003;74(3):391-401.
31. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegrin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93:165-176.
32. Liu D, Xu JK, Figliomeni L, Huang L, Pavlos NJ, Rogers M, et al. Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. Int J Mol Med. 2003;11(1):17-21.
33. Kwan Tat S, Padrines M, Théoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15(1):49-60.
34. Taubman MA, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. J Periodontol. 2005;76(11 Suppl):2033-2041.
35. Sun J, Han L, Liu C, Ma J, Li X, Sun S, Wang Z. Effect of autologous lyophilized platelet-rich fibrin on the reconstruction of osteochondral defects in rabbits. Exp Ther Med. 2023;26(6):569.
36. Pansare S, Patel S. Lyophilization process design and development: A single-step drying approach. J Pharm Sci. 2019;108:1423-1433.
37. Anthraper M, Chandramouli A, Srinivasan S, Rangasamy J. Lyophilized platelet rich fibrin and gelatin incorporated bioadhesive bone cement composite for repair of mandibular continuity defects. Int J Biol Macromol. 2024;258:1-13.
38. Nie J, Zhang S, Wu P, Liu Y, Su Y. Electrospinning with lyophilized platelet-rich fibrin has the potential to enhance the proliferation and osteogenesis of MC3T3-E1 cells. Front Bioeng Biotechnol. 2020;8:1-10.
39. Wang Z, Han L, Sun T, Wang W, Li X, Wu B. Preparation and effect of lyophilized platelet-rich fibrin on the osteogenic potential of bone marrow mesenchymal stem cells in vitro and in vivo. Heliyon. 2019;5:1-9.
40. Khanbazi MH, Bigham-Sadegh A, Oryan A, Meimandi-Parizi A, Jannesar AM. The effects of allogeneic and xenogeneic lyophilized leukocyte-and platelet-rich fibrin on bone healing in rat. Injury. 2024;55(3):111396.
41. Zhu L, Liu Y, Wang A, Zhu Z, Li Y, Zhu C, et al. Application of BMP in bone tissue engineering. Front Bioeng Biotechnol. 2022;10:1-20.
42. Hong CLN, Pham TAV. Efficacy of platelet-rich fibrin for the treatment of grade II furcation defects in mandibular molars. J Adv Oral Res. 2024;15(2):131-139.
43. Agarwal A, Gupta ND, Jain A. Platelet rich fibrin combined with bone graft in the treatment of furcation defects: A systematic review. J Indian Soc Periodontol. 2020;24(5):402-409.
44. Pradeep AR, Rao NS, Agarwal E, Bajaj P, Kumari M, Naik SB. Comparative evaluation of autologous platelet-rich fibrin and platelet-rich plasma in the treatment of 3-wall intrabony defects in chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2012;83(12):1499-1507.
45. Mitra S, Kamath DG, Shetty ND, Natarajan S. Comparative clinical evaluation of xenograft (Cerabone) versus allograft combined with platelet-rich fibrin for treatment of grade II mandibular furcation defects. Acta Marisiensis Ser Medica. 2023;69(2).