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HUMAN RANDOMIZED CONTROLLED TRIAL

Added benefit of L-PRF to autogenous bone grafts in the treatment of degree II furcation involvement in mandibular molars

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Abstract

Background: Tissue regeneration within the periodontally involved furcation area is one of the most challenging aspects of periodontal surgery. The aim of this study was to evaluate the additional benefit of leukocyte and platelet-rich fibrin (L-PRF) to autogenous bone grafts (ABGs) in the treatment of mandibular molar degree II furcation involvement, comparing the clinical outcomes with those from open flap debridement (OFD)+ABG and OFD alone treatments.

Methods: Fifty-four patients, exhibiting one buccal or lingual mandibular molar furcation defect, were randomly assigned to three treatment groups: OFD+ABG+L-PRF (n = 18); OFD+ABG (n = 18); and OFD (n = 18). Clinical (probing depth [PD], horizontal clinical attachment level [HCAL], vertical clinical attachment level [VCAL], gingival recession [GR]) and radiographic (vertical bone level [VBL]) parameters were evaluated at baseline and 6 months after treatment. HCAL change was the primary outcome.

Results: No significant differences within each group were reported for GR changes, but statistically significant improvements in HCAL, VCAL, PD, and VBL were observed in all groups, except for VBL in the OFD group. At 6 months, the mean HCAL gain was 2.29 ± 0.18 mm in the OFD+ABG+L-PRF group, which was significantly greater than that in the OFD+ABG (1.61 ± 0.18 mm) and OFD (0.86 ± 0.18 mm) groups. Both OFD+ABG+L-PRF and OFD+ABG therapies produced a significantly greater clinical and radiographic improvement than OFD.

Conclusion: The addition of L-PRF to ABG produces a significantly greater HCAL gain and PD reduction as compared with OFD+ABG treatment in mandibular degree II furcation involvements.

KEYWORDS
clinical trials, furcation involvements, grafts, platelet-rich fibrin, regeneration, wound healing

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1 | INTRODUCTION

The involvement of the molar furcation area is one of the most common consequences of periodontitis; several retrospective studies have suggested that molars with furcation involvement have a compromised prognosis[1] and respond less favorably to periodontal therapy.[2]

Different approaches have been proposed for the treatment of furcation involvement and the healing within the furcation area is one of the most challenging aspects in surgical periodontal therapy.[3]

The consensus report from the 2015 AAP Regeneration Workshop,[4] reporting that periodontal regeneration is a highly predictable therapeutic option for the treatment of class II furcation involvement of lower molars, concluded that a combined therapeutic approach associating bone grafts with membranes/biological agents produces better results than monotherapeutic treatments.[5–8]

However, a recent systematic review by Jepsen et al.,[9] concluded that bone grafts (BGs), without membranes, offer the best results in horizontal bone gain when treating degree II furcation involvements, and that, to date, no gold-standards have been defined in this specific therapy.[9]

For many years scientific evidence has supported the concept that autogenous BG (ABG) is an effective regenerative material in the treatment of infrabony defects,[10,11] as well as in the treatment of class II furcations.[12] More recently, a systematic review[13] of the treatment of periodontal infrabony defects, concluded that, comparing the several biomaterial groups, ABGs revealed the most favorable outcomes. ABG is the only BG that contains living progenitor cells capable releasing osteoinductive growth factors and it provides an osteoconductive surface for cell attachment. ABG has long been considered the ideal grafting material in bone reconstructive bone surgery, possessing clear advantages over other grafting options, and its limitations can be mostly identified in its limited availability and the need for a surgical sampling site.[13]

Several studies[14,15] have suggested that platelet concentrates increase the healing potential of natural blood clot. Among these concentrates, leukocyte and platelet-rich fibrin (L-PRF) is a second-generation platelet concentrate, developed by Choukroun et al.[16] consisting of a slowly polymerized complex fibrin network incorporating leukocytes, glycoproteins and a high concentration of growth factors; this combination facilitates wound healing[17] and has led to the widespread use of L-PRF in periodontal surgery.[18]

The results of a systematic review on the use of L-PRF in the treatment of furcation involvements,[19] concluded that the addition of L-PRF to a BG does not provide an advantage compared to the use of BG alone, the only exception being vertical clinical attachment level gain, at least for the graft materials used in the included studies (bioactive glass and hydroxyapatite, DFDBA, beta-three calcium phosphate). Considering that, compared to allogenic, xenogenic, and alloplastic grafts, ABG is the only graft to contain autologous living cells, and that L-PRF exerts its biological action on living cells,[17,20] the aim of this study was to evaluate whether the adjunct of L-PRF to ABG, could offer a potential additional benefit when treating mandibular molars degree II furcation involvements.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A randomized controlled trial was planned to compare the experimental treatment (OFD+ABG+L-PRF) with two active comparators (Fig. 1).[21] All patients were measured on several outcomes before randomization, to provide independent baseline values to be used as covariates. Three balanced groups were created.

The patients exhibited buccal or lingual mandibular degree II furcation involvement; after surgical access raising a full-thickness flap, L-PRF combined with ABG was applied to the debrided defects in the OFD+ABG+L-PRF group; the first comparator group was treated with OFD+ABG (OFD+ABG group) and the second comparator group was treated with OFD alone (OFD group).

2.2 | Sample size

The primary outcome was horizontal clinical attachment level (HCAL) gain scores. The secondary outcomes were probing depth (PD), vertical clinical attachment level (VCAL), gingival recession (GR), and vertical bone level (VBL) changes. An estimate of the expected gain in HCAL was assumed from a meta-analysis by Troiano et al.[22] The lower bound of the 95% CI for the OFD+ABG treatment effect adopted was $\delta = 1.07$. Fifteen patients per group were required to have a 90% chance of detecting an increase of 1.07 mm in the HCAL measurement in the OFD+ABG+L-PRF group with respect to the first comparator (OFD+ABG), in a two-tailed test, with an $a = 5\%$ when the estimated SD = 0.88 mm. To compensate for possible dropouts or outliers, 18 patients per group were finally recruited.

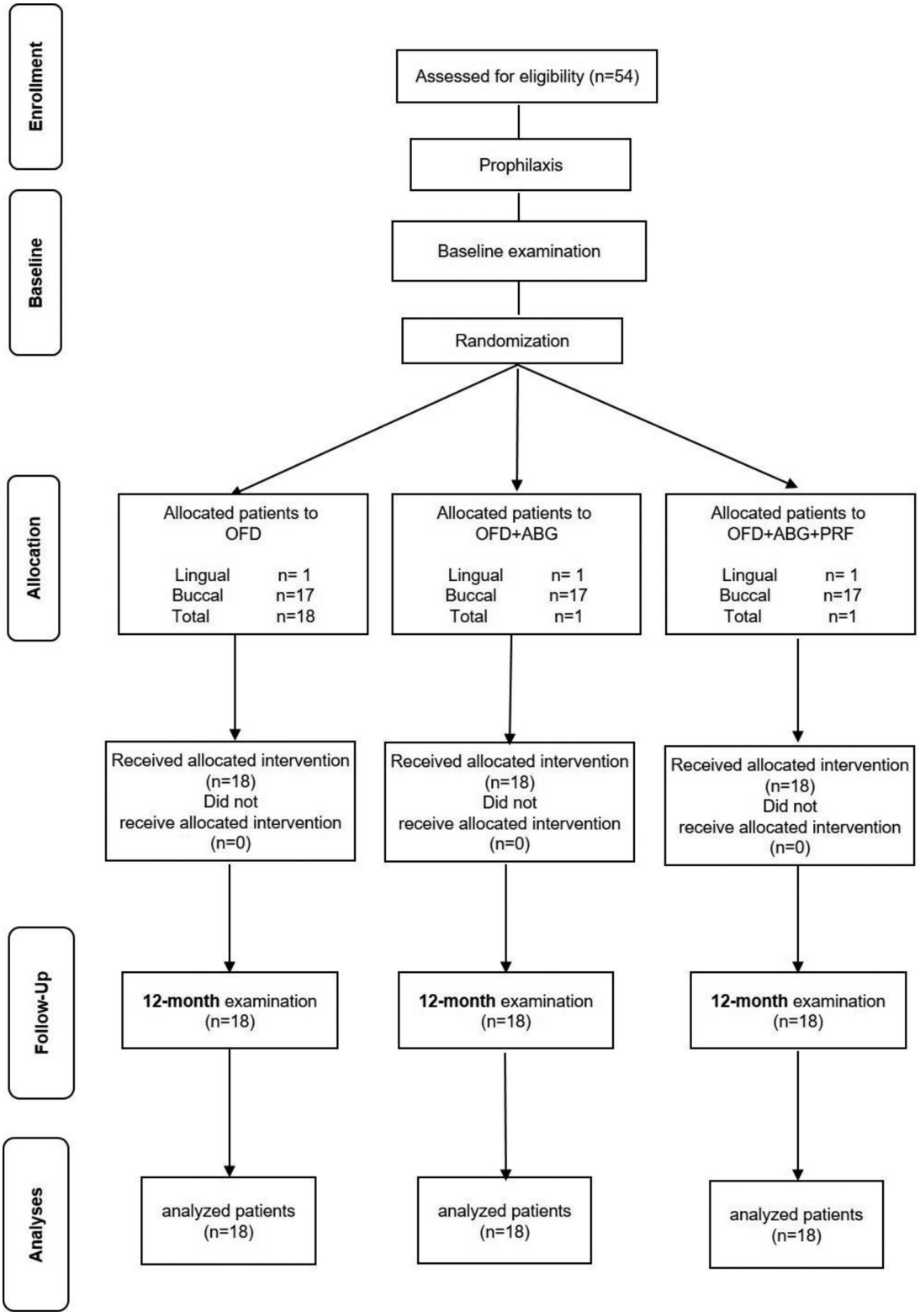

FIGURE 1 CONSORT diagram showing the study layout

2.3 | Study population

Fifty-four patients (22 males) aged 39 to 65 years (mean: 54 ± 14 years) participated in this study, 51 of whom had buccal and 3 of whom had lingual furcation involvement; they were selected among 278 patients diagnosed affected by Stage III–Stage IV periodontitis,[23] after having visited the Unit of Periodontology of the G. D'Annunzio University of Chieti-Pescara, Italy, between July 2017 and October 2018.

2.4 | Patients' inclusion criteria

The inclusion criteria: 1) being systemically healthy; 2) not having taken any medications that could affect the periodontal status over the previous 6 months; 3) not being pregnant/lactating; 4) being never-smoker/former-smoker ≥10 years; 5) having a full-mouth plaque score (FMPS)[24] and full-mouth bleeding score (FMBS)[25] <20% at surgery; 6) not having been subjected to periodontal treatment for 2 years; 7) having ≥20 teeth without dental mobility; and 8) having a buccal or lingual degree II furcation defect in periapical-lesion free and not mobile first or second mandibular molars with HCAL >3 mm and PD ≥5 mm in the mid-buccal or mid-lingual sites when evaluated 12 weeks after non-surgical periodontal therapy. Each patient participated in the study with a single experimental site.

The volunteers signed a consent form approved by the ethical committee of G. D'Annunzio University after having received comprehensive information. This study was in accordance with the Declaration of Helsinki of 1975, as revised in 2013. The present study was conducted from December 2018 to May 2020. All patients received scaling and root planing, by ultrasonic instruments[*] and hand curettes[†] and oral home care instructions three months before the surgical treatment. This study is registered at ClinicalTrials.gov as NCT03756493.

2.5 | Blinding protocol, randomization, and examiner reliability

Random allocation of defects to groups was accomplished by a computer-generated table;[‡] lingual furcation involvements were assigned one to each group. Opaque envelopes were used to conceal group allocation and were opened during surgery after defect debridement. Matching between group and treatment was known only to a person not involved in the study (coordinator) who was also responsible even for keeping and breaking the blinding.

Three blinding levels were envisaged. The first was related to one examiner and data collector (LR), trained with a previous intra-examiner calibration exercise (20 patients measured twice 24 hours apart to assure a Fleiss-Cohen's $Kappa_w \geq 0.6$).

The second level included two expert clinicians (MS and BF), who had to evaluate the radiographs. To improve interrater reliability, two measurements were independently obtained by MS and BF, who had finally to agree upon the optimal interradicular alveolar crest level (IACL) and furcation fornix (FF) locations.

At the third level was the surgeon (MP), who was masked until the envelopes were opened. The statistician analyzed the data in generically labeled groups, which the coordinator eventually re-associated to the undergone treatments after breaking the blinding.

2.5.1 | Clinical measurements

Patients underwent complete oral and periodontal examinations 3 months after scaling and root planing (SRP). These examinations included FMPS, FMBS, PD, VCAL, and GR at six sites per tooth using a UNC-15 probe.[§] At furcation mid-buccal/mid-lingual experimental sites, HCAL was also measured with a ZA2 probe[**] tacking the initial fluting of the furcation entrance as the fixed reference level.[26] In cases of subgingival furcation entrance, the gingival margin was gently dislocated by a second straight probe. All readings were performed with the help of 6× magnifying glasses.[††] The clinical measurements were rounded to the nearest millimeter and were repeated 6 months after surgery.

2.5.2 | Radiographic measurements

Periapical radiographs were obtained using a 70-kV intraoral X-ray system[‡‡] with an exposure time of 0.12 seconds and a digital sensor.[§§] Intraoral standardized radiographs were obtained with the "long-cone technique" before SRP and 6 months after surgery using digital sensor holders[***] customized to the selected experimental teeth by a thermoplastic occlusal reference. VBL was evaluated by dedicated

[*] Cavitron Select, DENTSPLY, Rome, Italy.
[†] Hu-Friedy, Milan, Italy.
[‡] R Core Team (2019), Vienna, Austria.

[§] Hu-Friedy, Chicago, IL.
[**] Deppeler SA, Rolle, Switzerland.
[††] UNIVET Optical Technologies, Rezzato (BS), Italy.
[‡‡] Carestream CS 2200, Carestream Dental, Atlanta, GA.
[§§] Carestream RVG 5200, Carestream Dental, Atlanta, GA.
[***] RINN XCp-ds, Dentsply Italia, Rome, Italy.

dental software[†††] measuring the linear distance between the IACL and the FF.

2.5.3 | Platelet-rich fibrin preparation

The Choukroun et al. protocol[16] was applied to produce L-PRF immediately before surgery. From each patient in all groups, to avoid unblinding, 30 mL of blood was collected in three 10-mL sterile tubes without anticoagulant, and it was quickly centrifuged[‡‡‡] at 3,000 revolutions/minute for 10 minutes.

The fibrin clot (L-PRF) was collected and squeezed in the L-PRF Box[§§§] to form three membranes for each OFD+ABG+L-PRF site: one of these membranes was cut and mixed with the ABG acting as a grafting material while the others were used to cover the graft.

2.5.4 | Surgical technique

The same experienced surgeon (MP) operated on all patients within 7 days from baseline measurements (Fig. 2). Before surgery, the patient rinsed with 0.2% chlorhexidine solution. Following local anesthesia, in all groups buccal or lingual continuous intrasulcular incisions were made, preserving as much interdental soft tissue as possible, without a conventional papilla preservation flap design. Releasing incisions were made to increase accessibility, if needed, and full-thickness buccal or lingual flaps were elevated, depending on whether the furcation involvement was buccal or lingual; the granulation tissue was removed and SRP was performed by ultrasonic and hand instruments. In addition, an Arkansas stone burr mounted on a low-speed handpiece was used[27] to remove any residual calculus and any anatomical irregularities in the furcation area. ABG was collected close to the experimental teeth using bone scrapers.

In the OFD group, furcation involvement underwent mechanical debridement only using an ultrasonic scaler, curettes, and Arkansas burrs.

After debridement, in the OFD+ABG group the defects were filled with ABG only, while, in the OFD+ABG+L-PRF group, one L-PRF membrane was cut into small pieces and mixed with the ABG to fill the furcation defect. Two L-PRF membranes were adapted to cover each grafted defect and were secured in place using a 5-0 Vicryl suture.[****]

Finally, in all groups, the flap was put back in its original position and sutured by 3-0 non-absorbable silk suturing material.[††††]

2.5.5 | Postoperative care

All patients received 2 g/day amoxicillin+clavulanic acid[‡‡‡‡] for 6 days to prevent possible postoperative infections and to reduce postoperative discomfort.[28]

The patients were prescribed 400 mg of oral ibuprofen,[§§§§] twice daily, for pain control and 0.12% chlorhexidine,[*****] twice daily for 3 weeks. Sutures were removed after 14 days. Only 2 to 4 weeks after suture removal, respectively, cautious brushing using a soft toothbrush and interdental brushing were recommended; the same time, the patients used 1% chlorhexidine gel[†††††] twice daily. Weekly supragingival professional hygiene and motivational reinforcement were administered to the patients for 6 weeks.

2.6 | Data analysis

Multiple univariate analyses were planned to perform pairwise comparisons regarding single outcomes. The data were analyzed with baseline adjusted ANCOVAs using Huber-White robust covariance matrices; post-hoc pairwise comparisons were adjusted for multiplicity according to the single-step method. The robustness of the results of the main outcome HCAL to hypothesis test assumption violations was checked by a sensitivity analysis comparing ANCOVA with a set of distribution-free tests—the $\chi 2$ test, Armitage's trend test, and Logistic Ordinal Regression (LOR) Proportional Odds. The effect size was estimated by comparison of a set of robust tests—an M-estimator with Hampel's ψ function, Yohai's MM- estimator with the deterministic initial Peña-Yohai estimator and a Theil-Sen regression with the Harrell-Davis estimator. Eventually, ANCOVA was compared with a non-parametric case resampling bootstrap test of the model. In the regression analyses, 95% CIs were reported to assess the treatment effect. An $\alpha = 0.05$ level was chosen as significance threshold.

R statistical software, version 3.5.2[‡‡‡‡‡] [29–33] was used.

[†††] Carestream Dental, Atlanta, GA.
[‡‡‡] IntraSpin, Intra-Lock System Europa SpA, Salerno, Italy.
[§§§] Xpression Fabrication Kit, Intra-Lock System Europa SpA, Salerno, Italy
[****] Ethicon Vicryl 5-0, Johnson & Johnson Medical Spa, Pomezia, Italy.
[††††] Ethicon Perma-Hand Suture 3-0 Silk, Johnson & Johnson Medical Spa, Pomezia, Italy.
[‡‡‡‡] Augmentin, SmithKline Beecham, Milan, Italy.
[§§§§] Nurofen Express 400 mg, Reckitt Benckiser Group, Slough, Berkshire, UK.
[*****] Dentosan 0.12 Trattamento Mese, Johnson & Johnson, Pomezia, Italy.
[†††††] Corsodyl dental gel, GlaxoSmithKline Consumer Healthcare S.p.A., Baranzate, Italy.
[‡‡‡‡‡] R Core Team (2019), Vienna, Austria.

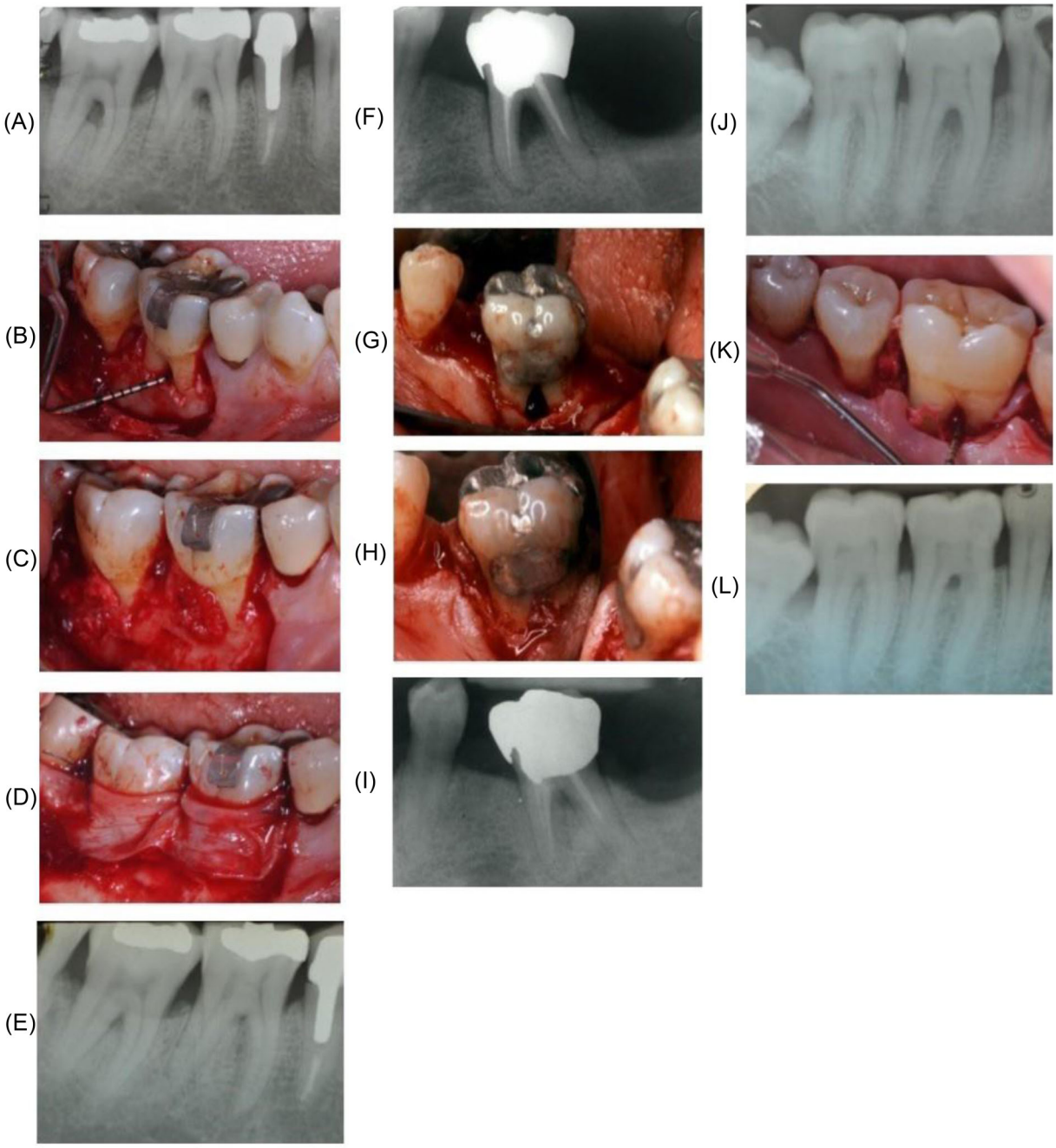

FIGURE 2 (**A**) Periapical radiography of the OFD+ABG+L-PRF site at baseline. (**B**) Buccal degree II furcation involvement after debridement. (**C**) The furcation defect is filled by autogenous bone graft. (**D**) The graft is covered by the L-PRF membrane. (**E**) Periapical radiography of the OFD+ABG+L-PRF site at 6 months after surgical treatment. (**F**) Periapical radiography of the OFD+ABG site at baseline. (**G**) Buccal degree II furcation defect after debridement. (**H**) The furcation defect is filled by autogenous bone graft. (**I**) Periapical radiography of the OFD+ABG site at 6 months after surgical treatment. (**J**) Periapical radiography of the OFD site at baseline. (**K**) Lingual degree II furcation defect after debridement. (**L**) Periapical radiography of the OFD site at 6 months after surgical treatment

3 | RESULTS

3.1 | Performed analysis

For the HCAL gain outcome, the unadjusted and baseline-adjusted model provided different results,[34] since ANOVA, as well as all tests lacking covariate adjustment, failed to detect the well-known difference favoring the OFD+ABG over the OFD group.

ANCOVA, in restoring results agreeing with the literature, greatly improved the explained variance ($R^2 = 0.47$) compared with ANOVA ($R^2 = 0.25$; $P = 0.00003$) and

was therefore preferred. Unfortunately, its assumptions regarding sustainability were questioned by the presence of recursive suspect outliers (although excluding 3 and 8 patients, some global assumption tests[35] were passed).

Both the proportional odds-logistic ordinal regression (PO-LOR) and the bootstrap tests were sufficiently powered to confirm the parametric test results, but their sample size was smaller than the 40 to 50 patients per group required by the rules of thumb. Therefore, a sensitivity analysis comparing the results with those provided by tests relying on minimal model assumptions was in order. Note that these tests answer varying research questions, so, it is their overall agreement that provides the definitive grounds for the study conclusions.

Firstly, ANOVA and ANCOVA hypothesis tests were compared with their distribution-free categorical data homologous tests (Fig. 3). The multinomial omnibus Fisher exact test and Armitage linear-by-linear test (Fig. 4), followed by Bonferroni-Holm adjusted pairwise comparisons, and the proportional odds, performed on the response categorized straightforwardly, consisting of integers in few modalities (Fig. 4). Second, to obtain a sense of the effect-size, ANOVA and ANCOVA 95% confidence intervals were compared with those coming from the two cleaned samples and from some robust estimators. Finally, a non-parametric case resampling BCa bootstrapping of the ANCOVA model was performed (Fig. 3).

Neither the OLS ANCOVA nor the and robust regression 95% simultaneous confidence intervals included zero, so all of the pairwise comparisons in all tested methods were significant at the $\alpha = 0.05$ level. The CI lower bounds among matching tests were very similar—clinically hardly distinguishable—to the bootstrap ANCOVA but were greater than those with the OLS full ANCOVA, while their width appeared just slightly smaller. The -3 and -8 samples showed slightly smaller effects but slightly narrower intervals.

3.2 | Study population

All 54 enrolled patients completed the study with full compliance of specifications; therefore, although the evaluation criterion of interest was the intention-to-treat, the results are also valid to a per-protocol analysis too. The experimental groups were balanced by age and sex, as well as lingual furcation proportions.

3.3 | Clinical and radiographic outcomes

The FMPS and FMBS remained <20% throughout the entire study without significant differences among the groups at each time point or between the time points within each group.

Parameter comparability at baseline was assured using ANCOVA models whenever needed. Table 1 shows that statistically significant differences in HCAL, VCAL, residual PD and VBL were observed at 6 months within the 3 groups, with the OFD group showing the smallest improvements. However, GR scores did not show significant differences between baseline and the 6-month examination in any of the three groups. Table 2 shows that changes in clinical parameter scores were significantly different among the groups, except for GR.

Six months postoperatively, the HCAL and VCAL gains and the PD and VBL changes were significantly greater in the OFD+ABG+L-PRF and OFD+ABG groups. Furthermore, the OFD+ABG+L-PRF group showed significantly greater HCAL gains and PD reductions than the OFD+ABG group.

The VCAL and VBL gains in the two experimental groups using ABG were almost identical without significant differences.

Finally, at 6 months, 12 of 18 (66.6%) furcations in the OFD+ABG+L-PRF group and 11 of 18 (61.1%) in the OFD+ABG group changed from degree II to degree I involvement, according to Hamp et al. classification.[36] Seven patients in the OFD+ABG+L-PRF group and 6 patients in the OFD+ABG group, presented significant HCAL gains and maintained degree II furcation involvement.

In the OFD group, only 1 of 18 (5.5%) improved from degree II to degree I. No furcation evolved toward complete closure or degree III involvement.

4 | DISCUSSION

4.1 | Principal findings

To verify whether the combination of the osteoconductive, osteoinductive, osteogenic, and space-maintaining properties of ABG with the biological properties of L-PRF, could produce better clinical and radiographic effects when treating degree II furcation involvements of mandibular molars, we designed a study with two active comparators (OFD+ABG and OFD alone).

We used HCAL measurement as the main outcome to assess furcation healing.

While confirming that all 3 surgical treatments can significantly improve the clinical conditions, our results showed that the ABG+L-PRF combination leads to a greater HCAL gain than in the other experimental groups, offering a significant added benefit (Table 1).

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TABLE 1 Clinical parameter scoring (n = 18 in each)

Variable	Treatment	Observed at baseline Mean ± SD (95% confidence interval)	Observed at 6 months Mean ± SD (95% confidence interval)	Estimated changes baseline–6 months Mean ± SE (95% confidence interval)	Within groups difference
PD	OFD+ABG+L-PRF	4.61 ± 1.378 (3.93 to 5.30)	2.33 ± 1.029 (1.82 to 2.85)	2.515 ± 0.174* (2.165 to 2.865)	ANCOVA P <0.001 $\eta^2 = 0.907$
	OFD+ABG	5.17 ± 0.618 (4.86 to 5.47)	3.00 ± 0.343 (2.83 to 3.17)	2.150 ± 0.169* (1.809 to 2.490)	ANCOVA P <0.001 $\eta^2 = 0.929$
	OFD	5.61 ± 1.614 (4.81 to 6.41)	4.39 ± 1.335 (3.73 to 5.05)	1.002 ± 0.174* (0.653 to 1.351)	ANCOVA P <0.001 $\eta^2 = 0.498$
	Among groups difference	ANOVA NS	ANOVA P < 0.001 $\eta^2 = 0.441$	ANCOVA P < 0.001 $\eta^2 = 0.442$	
HCAL	OFD+ABG+L-PRF	5.50 ± 1.043 (4.98 to 6.02)	3.22 ± 1.003 (2.72 to 3.72)	2.299 ± 0.180* (1.938 to 2.660)	ANCOVA P <0.001 $\eta^2 = 0.841$
	OFD+ABG	5.11 ± 0.900 (4.66 to 5.56)	3.67 ± 0.97 (3.18 to 4.15)	1.613 ± 0.183* (1.245 to 1.981)	ANCOVA P <0.001 $\eta^2 = 0.816$
	OFD	6.06 ± 1.731 (5.19 to 6.92)	5.00 ± 1.283 (4.36 to 5.64)	0.866 ± 0.184* (0.496 to 1.236)	ANCOVA P <0.001 $\eta^2 = 0.573$
	Among groups difference	ANOVA NS	ANOVA P <0.001 $\eta^2 = 0.335$	ANCOVA P <0.001 $\eta^2 = 0.381$	
VCAL	OFD+ABG+L-PRF	6.56 ± 2.455 (5.33 to 7.78)	4.50 ± 2.595 (3.21 to 5.79)	2.139 ± 0.278* (1.580 to 2.698)	ANCOVA P <0.001 $\eta^2 = 0.836$
	OFD+ABG	6.83 ± 2.093 (5.79 to 7.87)	4.89 ± 2.324 (3.73 to 6.04)	1.994 ± 0.276* (1.439 to 2.549)	ANCOVA P <0.001 $\eta^2 = 0.861$
	OFD	8.33 ± 2.951 (6.87 to 9.80)	7.39 ± 2.570 (6.11 to 8.67)	0.811 ± 0.284* (0.241 to 1.381)	ANCOVA P <0.027 $\eta^2 = 0.255$
	Among groups difference	ANOVA NS	ANOVA P <0.002 $\eta^2 = 0.217$	ANCOVA P <0.003 $\eta^2 = 0.203$	
GR	OFD+ABG+L-PRF	1.94 ± 1.798 (1.05 to 2.84)	2.17 ± 2.256 (1.04 to 3.29)	-0.225 ± 0.199 (-0.626 to 0.175)	ANOVA NS
	OFD+ABG	1.67 ± 0.970 (0.69 to 2.65)	1.89 ± 2.298 (0.75 to 3.03)	-0.230 ± 0.200 (-0.633 to 0.172)	ANOVA NS
	OFD	2.72 ± 3.495 (0.98 to 4.46)	3.00 ± 3.430 (1.29 to 4.71)	-0.267 ± 0.201 (-0.671 to 0.138)	ANOVA NS
	Among groups difference	ANOVA NS	ANOVA NS	ANOVA NS	
VBL	OFD+ABG+L-PRF	4.22 ± 2.625 (2.92 to 5.53)	2.50 ± 2.307 (1.35 to 3.65)	1.758 ± 0.254* (1.247 to 2.268)	ANCOVA P <0.001 $\eta^2 = 0.847$
	OFD+ABG	3.78 ± 1.060 (3.25 to 4.31)	2.17 ± 1.339 (1.50 to 2.83)	1.724 ± 0.257* (1.208 to 2.240)	ANCOVA P <0.001 $\eta^2 = 0.792$
	OFD	5.28 ± 3.025 (3.77 to 6.78)	5.33 ± 2.828 (3.93 to 6.74)	-0.204 ± 0.259 (-0.725 to 0.317)	ANCOVA NS
	Among groups difference	ANOVA NS	ANOVA P <0.001 $\eta^2 = 0.298$	ANCOVA P <0.001 $\eta^2 = 0.423$	

GR, gingival recession; HCAL, horizontal clinical attachment level; OFD, furcation involvements treated by OFD alone; OFD+ABG, furcation involvements treated by autogenous bone graft*; OFD+ABG+ L-PRF, furcation involvements treated by L-PRF + autogenous bone graft combination; PD, probing depth; VBL, vertical bone level; VCAL, vertical clinical attachment level.

*The between means difference is significant at the level $\alpha = 0.05$ (Sidak correction).

Sensitivity Analysis

Hypothesis tests

Hypothesis Test	Omnibus	OFD+ABG vs OFD	OFD+ABG+L-PRF vs OFD	OFD+ABG+L-PRF vs OFD+ABG
ANOVA	p = 0.000549	p = 0.154	p = 0.0009	p = 0.005
Fisher's exact test	p = 0.00032	p = 0.300	p = 0.014	p = 0.008
Armitage's linear-by-linear	p = 0.00032	p = 0.165	p = 0.001	p = 0.009
Cliff's Δ		p = 0.189	p = 0.0001	p = 0.010
ANCOVA	p = < 0.0001	p = 0.0037	p = < 0.001	p = 0.010
Proportional Odds	p = 0.0001	p = 0.021	p = < 0.001	p = 0.0467

NB. The pairwise comparisons by means of the Fisher, Armitage, Proportional Odds and Cliff's tests along with all the confidence intervals were adjusted for multiplicity according to the Bonferroni-Holm method. ANOVA and ANCOVA model tests were adjusted using the Westfall's method.

Confidence Intervals

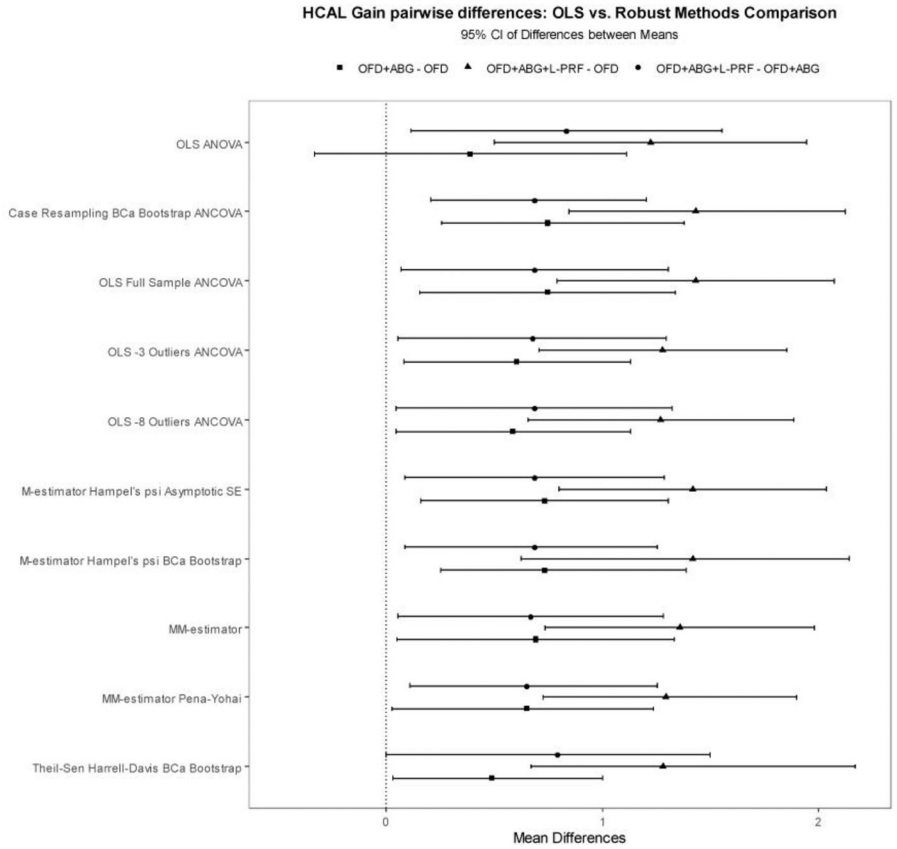

FIGURE 3 Sensitivity analysis: comparison between OLS ANCOVA and several robust or relying on minimal assumptions methods

4.2 | Agreements and disagreements with previous findings

Currently, there are no studies evaluating the efficacy of L-PRF+ABG in the regenerative treatment of degree II mandibular molar furcation involvements; however, several studies have investigated the combined use of different autogenous platelet concentrates (APCs) with other BGs. Lohi et al.[37] studied bioactive ceramic composite granules in combination with L-PRF and reported a 2.5 mm

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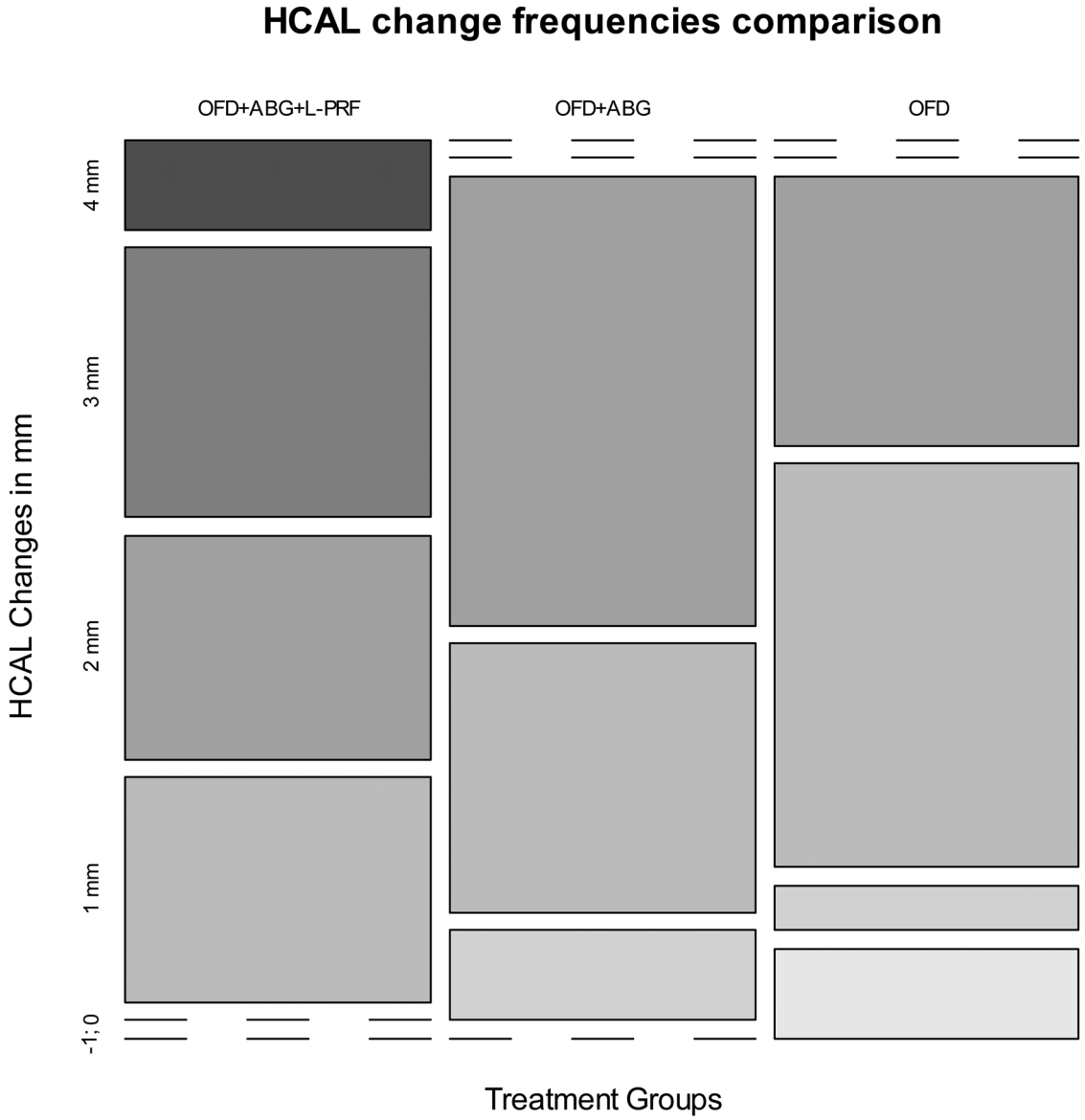

FIGURE 4 Mosaic plot: treatments comparison on horizontal CAL gain, CAL: Clinical Attachment Level

HCAL gain at 6 months. Lafzi et al.[38] used plasma rich in growth factors (PRGF) associated with ABG, reporting an HCAL gain of 2.14 mm, while Mansouri et al.[39] used PRGF+bovine bone mineral and observed a 1.30 mm HCAL gain.

The magnitude of these improvements in HCAL is comparable with the data that we obtained in the OFD+ABG+L-PRF group, and only Basireddy et al.[40] reported better results using L-PRF associated with DFDBA. This difference could be due to various factors such as the anatomical features of the furcation involvement, patient characteristics, and surgeon skill.

However, in 2020, a systematic review and meta-analysis[19] concluded that, with the exception of VCAL, the addition of L-PRF to allografts and alloplasts did not lead to significant clinical benefits. We can hypothesize that the significant advantage in HCAL improvement reported in our study in the OFD+ABG+L-PRF group could be attributed to the autogenous nature of the BG. ABG, in fact, incorporates osteogenic cells into its bone matrix.[41] Furthermore, cell viability and the release of molecules affecting bone formation are particularly high in grafts harvested by bone scrapers from cortical bone,[41] as in the present study. ABG could represent the ideal substrate for the biological action of L-PRF.

Indeed, L-PRF releases a number of polypeptide growth factors for 7 to 28 days,[20] and the overall effect of these biological mediators leads to stimulation of mitosis, differentiation and biosynthetic activity of stem and progenitor cells in periodontal tissues, with increased angiogenesis and matrix synthesis.[20]

TABLE 2 Estimated clinical parameters in millimeters (mean ± SE): pairwise treatment differences in baseline—6 months changes (n = 18 in each group)

Variable	Treatment comparison	Differences in changes baseline—6 months	95% confidence interval
PD	OFD+ABG—OFD	1.148 ± 0.268, P <0.000	0.509 to 1.787
	OFD+ABG+L-PRF—OFD	1.513 ± 0.303, P <0.000	0.792 to 2.235
	OFD+ABG+L-PRF—OFD+ABG	0.365 ± 0.161, P <0.065	-0.018 to 0.749
	ANCOVA	P <0.000 *** $adj\ R^2$ = 0.505	
HCAL	OFD+ABG—OFD	0.747 ± 0.245, P <0.0037	0.156 to 1.338
	OFD+ABG+L-PRF—OFD	1.433 ± 0.266, P <0.001	0.792 to 2.074
	OFD+ABG+L-PRF—OFD+ABG	0.686 ± 0.256, P <0.01	0.067 to 1.304
	ANCOVA	P <0.001 *** $adj\ R^2$ = 0.440	
VCAL	OFD+ABG—OFD	1.183 ± 0.467, P <0.036	0.064 to 2.302
	OFD+ABG+L-PRF—OFD	1.328 ± 0.496, P <0.025	0.140 to 2.515
	OFD+ABG+L-PRF—OFD+ABG	0.145 ± 0.301 P <0.877 NS	-0.575 to 0.865
	ANCOVA	P <0.007 $adj\ R^2$ = 0.164	
GR	OFD+ABG—OFD	0.056 ± 0.253, P <0.974 NS	-0.555 to 0.665
	OFD+ABG+L-PRF—OFD	0.056 ± 0.287, P <0.980 NS	-0.637 to 0.748
	OFD+ABG+L-PRF—OFD+ABG	0.000 ± 0.273, P <1 NS	-0.660 to 0.659
	ANOVA	NS $adj\ R^2$ = -0.038	
VBL	OFD+ABG—OFD	1.928 ± 0.442, P <0.000	0.873 to 2.984
	OFD+ABG+L-PRF—OFD	1.962 ± 0.408, P <0.000	0.987 to 2.937
	OFD+ABG+L-PRF—OFD+ABG	0.034 ± 0.245, P <0.137 NS	-0.552 to 0.620
	ANCOVA	P <0.000 $adj\ R^2$ = 0.434	

GR, gingival recession; HCAL, horizontal clinical attachment level; NS, not significant; OFD, furcation involvements treated by OFD alone; OFD+ABG, furcation involvements treated by autogenous bone graft; OFD+ABG+ L-PRF: furcation involvements treated by L-PRF + autogenous bone graft combination; PD, probing depth; VBL, vertical bone level; VCAL, vertical clinical attachment level.

Estimated clinical parameters in millimeters (mean ± SE): Pairwise treatment differences in baseline–6-month changes (n = 18 in each group).

It should be noted, however, that some studies[42,43] that used L-PRF in furcation involvements without adding any grafts, obtained results quantitatively comparable to ours. This observation might appear surprising if we consider that regenerative procedures with membranes,[44] enamel matrix derivative[45] and platelet concentrates[8] have been shown to be positively affected by the addition of a graft, particularly in unfavorable periodontal defects. In this regard, we should consider that combined treatment has been shown to be superior in non-supportive bony defects primarily due to its ability to guarantee the space maintenance, necessary for regeneration.[46] Conversely, in class II furcation involvements, the space for regeneration is provided by the anatomy of the defect itself, while the greater amount of APC placed in the furcation could compensate for the lack of a graft, exerting its biological action on the cells of the surrounding residual tissue.

4.3 | Discussion of secondary outcomes

For PD reduction, a mean of 2.5 mm was obtained in our OFD+ABG+L-PRF group; this datum is in accordance with those from others[40,38] using APCs+BGs. Similarly, Basireddy et al.[40] and Lafzi et al.[38] observed small and non-significant benefit in PD reduction from APC being added to BGs; conversely, Lohi et al.[37] reported a significant added benefit in PD reduction.

In our study, VCAL improvement in the OFD+ABG+L-PRF group was not significantly better than that in the OFD+ABG group, in agreement with other authors[38,40] who used different APCs and BGs.

The VBL gain that we obtained (1.7 mm) in the OFD+ABG+L-PRF and OFD+ABG groups was slightly greater than that in the other groups.[37,40] This outcome could be due to the use of intraoral autogenous BG, which

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was recently reported to be the biomaterial offering the most favorable outcomes in infrabony defects.[13]

4.4 | Study design

This study aimed to investigate whether adding L-PRF to ABG could lead to an added benefit compared to ABG alone in improving the clinical outcomes of class II furcation involvement treatment. While the better clinical results from the OFD+ABG+L-PRF and OFD+ABG groups compared to the OFD group might be related to the presence of the graft and the APC, our study design does not allow us to attribute the advantage of the OFD+ABG+L-PRF group in improving the primary outcome to the biological activity of L-PRF only. In fact, in the OFD+ABG+L-PRF group, L-PRF not only was used as a graft mixed with ABG but also was used to cover the furcation entrance, as a membrane, similar to the GTR technique. In this regard, the literature has reported the great effectiveness of the GTR technique when treating degree II furcation involvement in mandibular molars,[4] particularly when this technique is used in combination with fillers.[6] These findings might suggest that the application of the L-PRF membrane onto the furcation entrance might have contributed to graft and blood clot stabilization, favoring lesion healing through a mechanism independent of the biological properties of L-PRF. However, the real effectiveness of the L-PRF membrane in representing a physical barrier at the furcation entrance is questionable, considering its short resorption time (1 to 2 weeks).[47]

4.5 | Clinical implications

In this study, the L-PRF+ABG combination was used in the reconstructive treatment of a type of periodontal defect generally considered a challenging[3] lesion responsible for poor prognosis of the affected teeth.[1] Indeed, the literature[1] reports that the persistence of furcation involvement is associated with an increased risk of tooth loss. The regeneration of class II furcation involvement, although possible, is not considered a fully predictable procedure, especially in terms of complete bone fill.[12] In fact, furcation involvement represents a periodontal lesion mainly bordered by non-vascularized walls, which cannot provide an adequate cell and blood supply for regeneration.[48] This fact justifies the additional use of a BG, promoting coronal migration of cells on which regeneration depends.

The results we obtained in the OFD+ABG+L-PRF group confirmed the theoretical assumptions, speculating that ABG favored the coronal migration of cells from the residual periodontium while APC acted by increasing cellular migration, proliferation, and differentiation, while offering a significant added benefit to bone grafting alone from the clinical point of view.

4.6 | Limitations of the study

Some limitations should be emphasized in our research. First, we did not use a stent-assisted probing methodology; this procedure would have been particularly useful in a study on furcations, which, in themselves, present great problems of measurability.[49,50] To reduce this limitation, instead of the classic Nabers Q2N probe, we used a curved probe graduating from 2 millimeters to 2 millimeters limiting the examiner's need for individual interpretation, and improving the identification of the reference point and the detection of measures by gently opening the gingival margin and using 6X magnifying glasses. Second, in our groups, we neglected to evaluate the presence of an infrabony component in the furcation area; in fact, the combination of an infrabony defect with furcation involvement may lead to more favorable results.[5]

This study was not a split-mouth study, and in the study design, there was not a group treated with L-PRF only; this fact did not allow us to evaluate the effectiveness of L-PRF+ABG compared with L-PRF only, as well as to ABG and OFD only. Furthermore, we did not investigate the furcation bone fill three-dimensionally (i.e., CT-cone beam) as other researchers did.[40]

Finally, it should be recognized that patient-centered outcome evaluations are lacking in research.

5 | CONCLUSIONS

Within the limitations of this study, our results suggest that OFD+ABG+L-PRF treatment produces a significantly greater HCAL gain compared to both OFD+ABG and OFD treatments.

This study was a clinical study; therefore, it does not allow us to discuss "regeneration" of the interradicular defects. The lack of histological evidence indicates that healing might be solely defined as tissue repair.

The L-PRF+ABG combination is completely autogenous, avoiding the risk of transmitting known or potentially unknown infectious agents. Furthermore, it is a biomaterial-combination that does not use products derived from other living beings, avoiding possible problems of ethical nature and treatments that are culturally unacceptable in some countries. Finally, this treatment has very low costs, although not quantified in the present research.

No other studies in the literature have evaluated the L-PRF+ABG combination in the treatment of class II furcation involvement; therefore, further investigations are needed to confirm our results.

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The authors report no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

MS, BF, PdN, and MP are co-first authors having designed the work, written, revised, and edited it. BS designed the study, interpreted the data, and revised the paper. GP and LS collaborated in the design of the study and its editing; they also cooperated in the interpretation of the data. IR and PS cooperated in the study design, article editing, and critical revision of the manuscript. LR collaborated in the study design, interpretation of data, and article editing; he also performed the data collection with MS and BF. PdN did the statistical analysis.

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