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HUMAN RANDOMIZED CONTROLLED TRIAL

Efficacy of inorganic bovine bone combined with leukocyte and platelet-rich fibrin or collagen membranes for treating unfavorable periodontal infrabony defects: Randomized non-inferiority trial

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Abstract

Background: Growing evidence shows the efficacy of platelet concentrates in periodontal therapy. This study aimed to demonstrate that an inorganic bovine bone graft (IBB) in combination with a leukocyte and platelet rich fibrin (L-PRF) is non-inferior to a combination with a collagen membrane (CM) when managing unfavorable infrabony defects (IBDs).

Methods: All patients exhibited at least one unfavorable IBD; they were randomly assigned to two groups, 31 treated with L-PRF+IBB and 31 with CM+IBB. A clinical and radiographic examination was performed at baseline and 12 months later. Clinical attachment level (CAL), gingival recession (GR), probing depth (PD), and radiographic defect bone level (DBL) post-therapy changes were compared between the two treatments. A non-inferiority margin = 1 mm was set to determine the efficacy of the test treatment (–1 mm for GR); a second non-inferiority margin = 0.5 mm (–0.5 mm for GR) was chosen for clinical relevance.

Results: Twelve months after surgery a significant improvement of clinical and radiographic parameters was observed at both experimental sites. The 90% confidence intervals of the CM+IBB–L-PRF+IBB mean difference for CAL gain (–0.810 mm [–1.300 to –0.319]) and DBL gain (–0.648 mm [–1.244 to –0.052]) were below the 0.5 mm non-inferiority margin; GR increase (1.284 mm [0.764 to 1.804]) remained above the –0.5 mm, while PD reduction (0.499 mm [0.145 to 0.853]) crossed its 0.5-mm margin.

Conclusions: The L-PRF+IBB treatment of unfavorable IBDs offers non-inferior efficacy for CAL gain, showing less GR and more DBL gain too, while for PD reduction it is inferior to the CM+IBB treatment.

KEYWORDS
bone transplantation, fibrin, randomized controlled trial, regeneration, wound healing

1 | INTRODUCTION

One of the main purposes of periodontal surgery is to correct the tissue anatomy altered by bone resorption.[1] In fact, deep infrabony defects (IBDs) represent a frequent sequela of periodontitis; they have been observed to be viable predictors of tooth loss, and they are high-risk sites for periodontitis progression.[2]

Deep IBDs are treated by different therapies, including bone grafts (BGs), guided tissue regeneration (GTR), treatments with biological mediators, and combinations of these techniques.[3]

GTR makes use of a mechanical barrier by preventing the apical migration of epithelium while stabilizing the blood clot.[4] GTR with non-absorbable and resorbable membranes improves clinical attachment level (CAL) gain and reduces probing depth (PD), also resulting in lower gingival recession (GR) and more radiographic defect bone level (DBL) gain than open flap debridement (OFD) alone.[5] Currently, resorbable membranes, including collagen membranes (CMs), are the most commonly used in clinical practice, as they overcome the many limitations of non-absorbable membranes while maintaining their advantages.[6]

In recent years, a growing amount of evidence has shown the great efficacy of biological mediators, including platelet concentrates, in periodontal reconstructive therapy to stimulate specific cell anabolic activities.[7] Leukocyte- and platelet-rich fibrin (L-PRF) is a low-cost platelet concentrate that is easy to use and has shown great efficacy in promoting periodontal regeneration.[7]

When treating unfavorable IBDs, the combination of BGs and non-rigid membranes or regenerative biological mediators, which lack space-making properties, was shown to offer better results, as this combination sustains the overlying soft tissues, increases coagulum stability, and enhances mesenchymal cell proliferation.[8]

In this regard, meta-analyses of the literature show that both CM and L-PRF, combined with a BG, show particular efficacy in producing periodontal regeneration.[7,9–11]

Among BGs, inorganic bovine bone (IBB) is a xenograft with unlimited supply and proven clinical safety;[12] it is obtained by protein extraction from bovine bone and consists of hydroxyapatite, similar to human bone, with excellent biocompatibility.[12] It was shown in randomized clinical trials that IBB combined with both CM and L-PRF is highly effective in the regenerative treatment of IBDs.[8,13]

GTR and L-PRF combinations with BG have been compared with other therapeutic procedures;[9,11] however, to our knowledge, no studies reporting on L-PRF+BG versus GTR+BG have been published to date.

In this study, we aimed to investigate whether a non-inferior CAL gain can be obtained with IBB and an L-PRF membrane compared with IBB and a CM 1 year after surgery.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This randomized, masked, parallel, two-arm, non-inferior clinical trial evaluated the 12-month clinical and radiographic outcomes after unfavorable IBD treatment by an L-PRF+IBB* combined treatment (new treatment) or by a CM†+IBB combination (active comparator). The CM+IBB combination was reported as the gold-standard therapy for unfavorable IBDs.[8] The surgical treatment of unfavorable IBDs was performed using two combinations of regenerative materials. The graft (IBB) was identical for both therapies; consequently, the study was focused on the hypothesized non-inferiority of L-PRF used in place of a CM. OFD was the historical placebo against which to demonstrate efficacy. Both a per-protocol and an intention-to-treat population were relevant to the results.

The main outcome was CAL gain at 12 months. The secondary outcomes were changes in GR, PD, and DBL.

The case for the null hypothesis was:

$$H_0 : \mu_{CM+IBB} - \mu_{L-PRF+IBB} \geq \Delta_{NI}$$

namely, that the effect of the active comparator was larger than that of the new treatment by at least one Δ_{NI}. The alternative hypothesis was:

$$H_1 : \mu_{CM+IBB} - \mu_{L-PRF+IBB} < \Delta_{NI}$$

2.2 | Non-inferiority margins

An estimate of the CAL gain provided from the CM+IBB treatment of

$$M_{CM+IBB} = 3.30 \pm SD = 1.11 \cdot 95\%CI \ (2.66 to 3.95)$$

was obtained by aggregating all available data from 119 patients reported in a random effects meta-analysis by Parrish et al.,[14] as shown in Figure S1 in online *Journal of Periodontology*.

For OFD surgical treatments, the estimate from the literature[15] was $M_{OFD} = 2.47$ mm.

The differential effect of adding a GTR was 0.83 mm, rounded to 1 mm.

* Bio-Oss - Geistlich Biomaterials Italia S.r.l. Thiene, Italy
† Evolution - OsteoBiol, ROEN s.a.s., Torino, Italy

Therefore, a margin $\Delta M_1 = 1$ mm was set to ensure the new treatment efficacy versus OFD (30% of the whole effect), according to the so-called fixed margin approach.[16] A second smaller margin $\Delta M_2 = 0.5$ mm (degree of inferiority) was set to preserve a further 50% of the comparator effect (15% of the whole).

The same non-inferiority margins were set for DBL and PD, while margins of –1 mm and –0.5 mm were set for GR.

2.3 | Sample size

Sixteen patients per group were required to detect the greater margin ΔM_1 in a one-sided test, with $\alpha = 0.05$, $1 - \beta = 0.80$ and an SD = 1.11 mm.[14]

To detect a halved margin $\Delta M_2 = 0.5$ mm the sample size must be increased four-fold. Instead, when the collected data meet the assumptions of an analysis of covariance with the baseline values as covariate, these values account for half the response variance thanks to their theoretical correlation with the gain-scores $P = 0.707$,[17] and thence the required sample size just doubles.[18] Therefore, if there is truly no difference between the standard and experimental treatment, 62 patients are required to be 80% sure that the upper limit of a one-sided 95% confidence interval (CI) of their difference will be below the noninferiority margin of 0.5 mm (and >95% sure that it will be below the non-inferiority margin of 1 mm).

2.3.1 | Study population

Sixty-two patients (26 males; mean age, 55 ± 12 [43 to 66 years]) participated in this study; they were selected from a population of 216 patients diagnosed with stage III-IV periodontitis[19] who presented at the Unit of Periodontology of the "G. D'Annunzio" University between September 2017 and April 2018.

2.3.2 | Patients' inclusion criteria

The inclusion criteria were as follows: 1) systemically healthy; 2) no history of medications that may affect periodontal status in the previous 6 months; 3) not pregnant/lactating; 4) never-smoker/former-smoker ≥10 years; 5) a full-mouth plaque score (FMPS)[20] and full-mouth bleeding score (FMBS)[21] <20% at surgery, 6) no history of periodontal treatment for at least 2 years, 7) ≥20 teeth without dental mobility, 8) at least one site with radiographically detected vertical bone loss (alveolar crest level—defect bottom distance = ≥4 mm, and a $PD \geq 5$ mm 12 weeks after non-surgical treatment, and 9) no periapical lesions at experimental sites.

In this study, we considered unfavorable IBDs only predominantly 1-, combined 1-2- and 2-wall defects/craters, circumferential defects (at least three surfaces involved) or teeth with a wide defect angle (≥36).[22] Circumferential bone probing examined the bony architecture during non-surgical therapy; the anatomy had to be confirmed during surgical intervention. A single experimental site for each patient was considered for the study. The volunteers signed a consent form approved by the ethical committee of G. D'Annunzio University after having received comprehensive written information about the study. This study was approved by the human subject's ethics board of G. D'Annunzio University and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013

The study was performed from September 2017 to November 2019.

All patients received scaling and root planing (SRP) by ultrasonic instruments[‡] and Gracey curets[§] as well as oral home care instructions 4 months before the surgical treatment.

This study is registered at Clinicaltrials.gov as NCT03715374.

2.3.3 | Randomization and blinding protocol

The trial director was responsible for randomly assigning patients to treatment groups after enrollment and was not involved with the clinical interventions or the study measurements. A computer-generated table[**] was used to make the random assignment, which was known only to the trial director. An opaque envelope, which concealed group allocation, was opened just before the intervention surgery. A blood draw, needed for the L-PRF+IBB treatment, was performed for all patients. Patients and examiners were masked to group membership; clinical and radiographic examiners were masked to each other. The study analyst was also masked to group membership. The analyst received the data by groups labeled A and B and returned two 90% CIs for the differences (A minus B and vice versa). The masking was not broken until after study completion, and the correct difference was retained.

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

2.4 | Clinical measurements

Three months after SRP, the patients were examined, and FMPS, FMBS, PD, CAL, and GR were recorded at six sites per tooth. Probing was done with a University of North Carolina no. 15 periodontal probe.[††] Data collection were performed at baseline and 1 year later by the same experienced examiner (LR).

2.5 | Radiographic measurements

Periapical radiographs were taken with a 70-kV intraoral X-ray system[‡‡] with an exposure time of 0.12 seconds and a digital sensor.[§§] Intraoral standardized radiographs were taken with the long-cone technique before and 12 months after SRP using digital sensor holders[***] customized to the selected experimental teeth by a thermoplastic occlusal reference. Specific dental software[†††] was used to measure the distance between the alveolar crest level and defect bottom.

2.6 | Platelet-rich fibrin preparation

The Choukroun et al.[23] protocol was applied to produce L-PRF immediately before surgery. From each patient in both 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/min for 10 minutes.

The fibrin clot (L-PRF) was collected and squeezed in the L-PRF Box[§§§] to obtain three membranes: one of these was cut and mixed with the IBB, while the others were used to cover the graft.

2.7 | Surgical technique

The same experienced surgeon (MP) operated on all patients (Fig. 1). The defects were accessed using the simplified papilla preservation flap technique.[24] At the buccal aspect, an intracrevicular incision obliquely continued across the papilla intrasulcularly at the neighboring teeth; vertical releasing incisions completed the flap design, if necessary. At each tooth, an intrasulcular, palatal incision was limited to the mid-palatal aspect only. After flap elevation and granulation tissue removal, in the new treatment sites, one L-PRF membrane was shredded and mixed with the IBB; the composite graft was then placed within the IBD until complete filling. Two L-PRF membranes in each patient were then placed onto the filled defect.

In the active control sites, after defect filling with IBB, a CM was placed to cover the grafted defect. Then, after periosteal fenestration, the flap was repositioned, and internal horizontal mattress sutures+interrupted

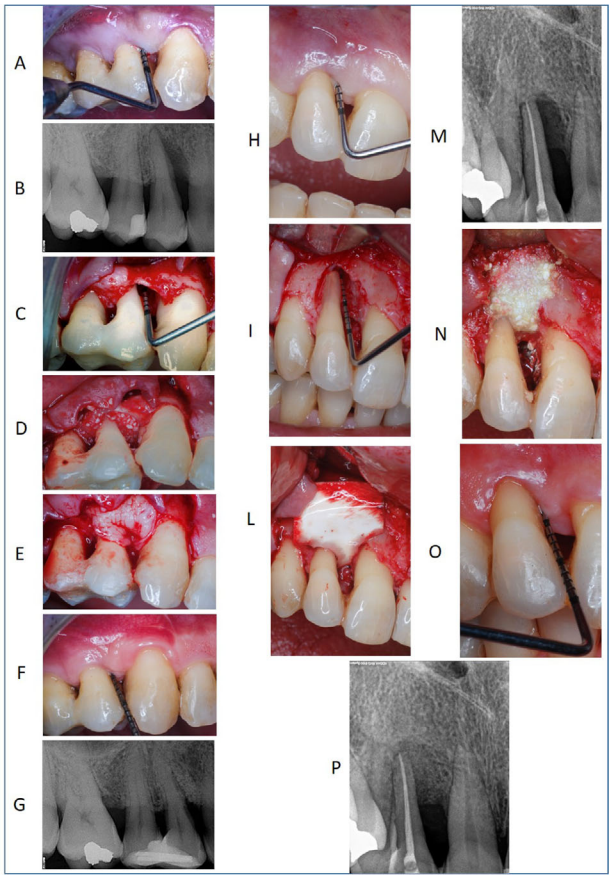

FIGURE 1 **A**) Test site. Deep periodontal pocket associated with an unfavorable infrabony defect. **B**) Periapical radiography of the test site at baseline. **C**) The wide, two-walled crater-like infrabony defect after debridement. **D**) The infrabony defect is filled by the inorganic bovine bone graft. **E**) The graft is covered by the L-PRF membrane. **F**) Clinical aspect of the test site 1 year after surgical treatment. **G**) Periapical radiography of the test site 12 months after surgical treatment. **H**) Control site. Deep periodontal pocket associated with an unfavorable infrabony defect. **I**) Periapical radiography of the control site at baseline. **L**) 1- to 2-walled infrabony defect at debridement. **M**) The defect is filled by IBB. **N**) A collagen membrane covers the graft. **O**) Clinical aspect of the control site 1 year after surgical treatment. **P**) Periapical radiography of the control site 12 months after surgical treatment

[††] UNC-15, Hu-Friedy, Chicago, IL
[‡‡] Carestream Dental, Atlanta, GA
[§§] Carestream CS 2200, Carestream Dental, Atlanta, GA
[***] RINN XCp-ds, DENTSPLY Italia, Rome, Italy
[†††] Carestream RVG 5200, Carestream Dental, Atlanta, GA
[‡‡‡] IntraSpin, Intra-Lock System Europa, Salerno, Italy
[§§§] Xpression Fabrication Kit, Intra-Lock System Europa, Salerno, Italy

sutures[****] were placed to ensure tension-free flap primary closure. Finally, a local injection of 4 mg[††††] betamethasone was performed for a better postoperative course and to minimize the effect of edema on the sutures.

2.8 | Postoperative care

All patients received 2 g/d amoxicillin+clavulanic acid[‡‡‡‡] for 6 days for postoperative infection prevention, not to improve the clinical outcome but to prevent possible postoperative infections and to reduce postoperative discomfort.[25]

Moreover, the patients were prescribed 400 mg of oral ibuprofen,[§§§§] twice daily for pain control when needed and 0.12% chlorhexidine[*****] rinses twice daily for 3 weeks. Sutures were removed after 14 days. Two weeks after suture removal, cautious brushing with a soft toothbrush was allowed; after 4 weeks, interdental brushing was recommended; in the meantime, the patients used a 1% chlorhexidine gel[†††††] twice daily. Weekly supragingival professional hygiene and motivational reinforcement were administered to the patients for 6 weeks. Patients were maintained by monthly professional cleaning up to the 1-year evaluation.

2.9 | Statistical analysis

To find evidence of the new treatment non-inferiority, multiple univariate analyses[26] of single outcomes were performed. The difference between the treatment averages of the CAL gain was estimated using an analysis of covariance adjusted for baseline, and the 90% CI was obtained. Non-inferiority was claimed if its upper bound was less than $M_2 = 0.5$ mm. Secondary outcomes were analyzed similarly, controlling the per-family error rate with the Bonferroni adjustment for the effective independent end point number computed according to Nyholt.[27,28]

The assumption of no covariate-by-treatment interaction was verified on all the outcomes. For CAL gain, a sensitivity analysis, which compared the full sample results with those obtained both excluding two suspected outliers and using a set of robust estimation methods (Hampel, Huber, Tukey bisquare, and Yohai MM estimator), was performed to assess the robustness of the primary analysis findings. The resulting graph is available as Figure S2 in online *Journal of Periodontology*.

The 3.6.1 R software[‡‡‡‡‡] package was used.

3 | RESULTS

3.1 | Study population

Figure 2 shows the CONSORT[29] flow diagram. All 62 patients completed the trial fully complying with the specifications, so patients were analyzed in the group to which they had been randomized. The study thus provides evidence for both the per-protocol and the intention-to-treat population.

3.2 | Clinical and radiographic outcomes

All IBDs met the anatomical inclusion criteria after confirmation by intrasurgical inspection; IBD anatomy is described in Table 1. After 12 months, none of the experimental sites showed bleeding on probing. Accordingly, the FMPS and FMBS remained <20% throughout the study without significant differences within and between groups (Table 2). No postoperative complications were reported by the patients.

Clinical and radiographic parameter scores are shown in Table 2; they significantly improved in both test and control defects (Table 3 and Fig. 3).

Figure 3 shows the 90% CI for the difference CM+IBB–L-PRF+IBB between the treatment averages for all the parameters. The 95% upper bound for CAL gain was −0.319 mm (unadjusted value −0.437), proving non-inferiority to the M_2 margin. The 95% CI upper bound for GR was 0.764 mm, while for DBL, it was −0.052 mm; both of them were non-inferior to the respective M_2. The 95% upper bound for the PD difference was 0.85 mm: it was inferior to M_2 but non-inferior to M_1.

4 | DISCUSSION

4.1 | Principal findings

Our results, while showing the efficacy of both treatments, indicate that the new treatment produces CAL gain improvements non-inferior to the active comparator. Actually, since the CI upper bound for the intention-to-treat analysis set stays below the zero

[****] Ethicon, Johnson & Johnson, Pomezia, Italy
[††††] Bentelan, Alfasigma S.p.a., Bologna, 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.

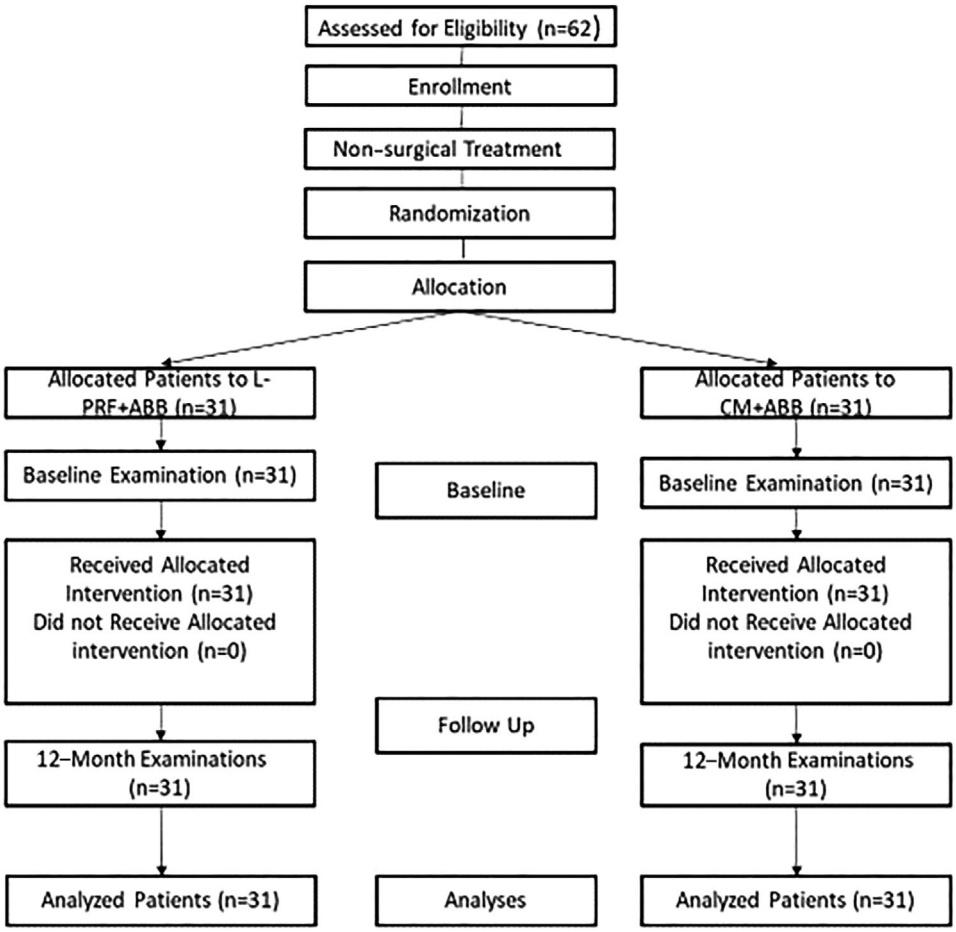

FIGURE 2 CONSORT flow diagram

line, it would prove the superiority of the L-PRF+IBB treatment ($P = 0.0003$). However, an essential condition for the validity of an Noninferiority trial (NIT) is the assumption that the active comparator actually shows its effect in the trial: lacking the OFD arm, we have to check the comparator effect by historical data.

In our study, the effect of CM+IBB was 2.77 mm (2.46 to 3.09) against the estimate in the literature[14] of 3.30 mm (2.66 to 3.95, $I^2 = 0.9$). It was somewhat poor even though within the expected range, which however might be too wide being the result of a limited number of small heterogeneous studies. Somehow a difference was expected, since the reference population of our study, unfavorable IBDs, was not perfectly comparable with our literature reference, not specific for unfavorable defects. Another possible explanation is that the standards of care, in particular of the OFD intervention, have improved over time.[17] However, the rough estimate of the OFD pre-post effect we inferred from the Stoeklin-Wasmer et al.[10] data (2.06; 1.64 to 2.48) tends to exclude this cause, as our point estimate (2.47) is on the upper boundary. Regarding the risk of bias, the differences at baseline should have been suitably addressed by randomized allocation, even though the comparability of randomized groups cannot be taken as granted in small samples.

This result might question our setting of margins. Conversely, our choice seems again fully confirmed by the literature,[10] which provides an estimate of the CM+IBB–OFD difference of 1.71 (1.26 to 2.15), the CI lower bound of which is even greater than M_1. L-PRF+IBB indirectly demonstrated efficacy compared with OFD, as even their difference was greater than M_1 (3.58 to 2.47 = 1.11 >1 mm). Therefore, the CM+IBB performance remains the only data which might be inconsistent with the literature.

The assay sensitivity, undermined by the smaller than expected effect of the active comparator in this study, is outweighed by the superiority of the new treatment over the active comparator, whose effect was greater than our placebo estimate. With this reasoning, however, the generalizability of this superiority is questioned in turn. In fact, had the comparator performed as reported in the literature, the non-inferiority result would still hold, while the superiority would be questionable. This adds up to the poor clinical relevance of the difference, at best smaller than 1 mm,

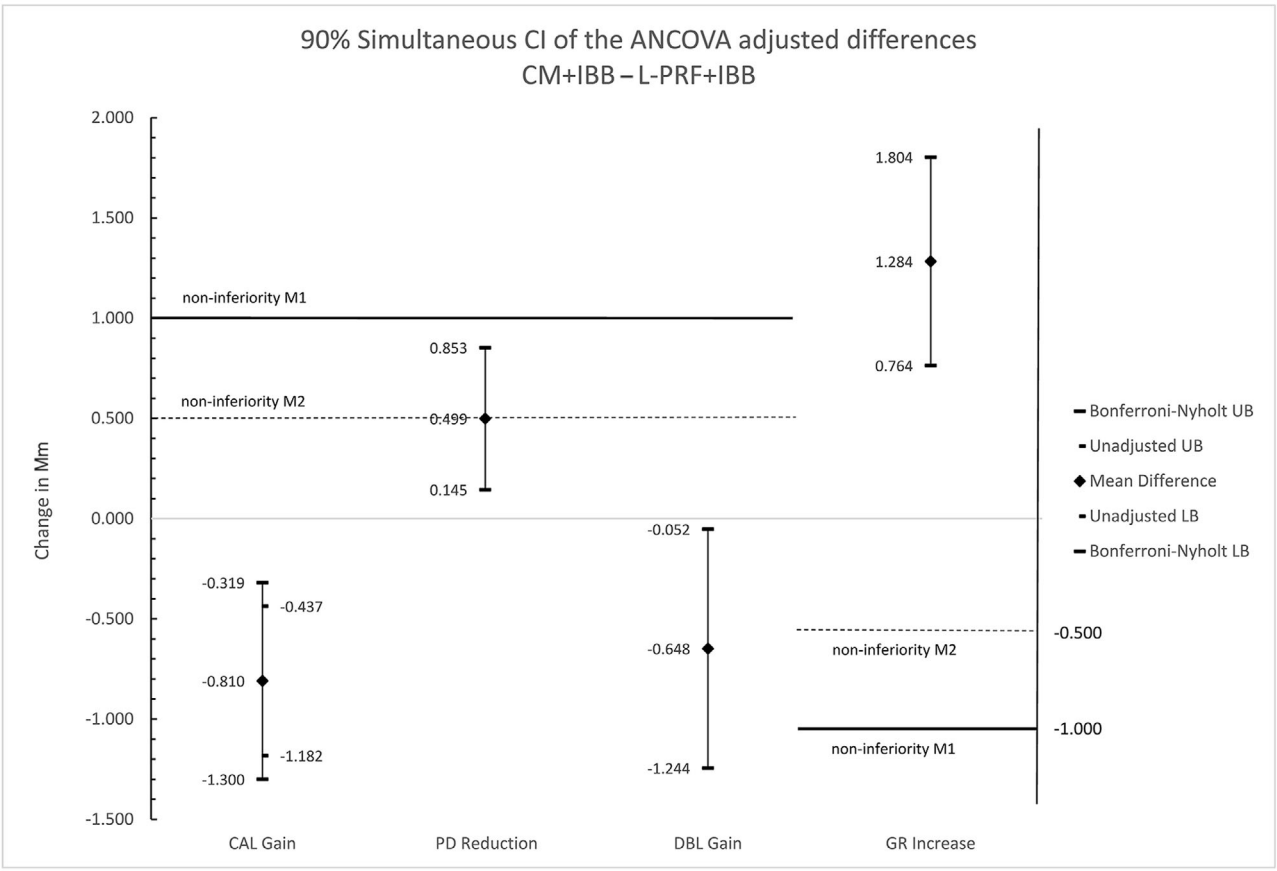

FIGURE 3 Simultaneous 90% CIs of adjusted differences between new treatment and active comparator. PD, probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level; UB, upper bound; DIFF: Point estimate of the Difference; LB, lower bound

which is why we chose a non-inferiority hypothesis. All considering, we believe that claiming non-inferiority is the fairest and most balanced decision.

4.2 | Agreements and disagreements with previous findings

Currently, in the periodontal literature, no papers specifically comparing L-PRF and GTR outcomes in IBD treatment, with or without BG, are available; therefore, our results cannot be compared with others. Only recently was it shown that an L-PRF+BG combination yields non-inferior CAL gain in unfavorable IBDs when compared with another regenerative combination: the enamel matrix derivative+BG treatment.[30] Likewise, in maxillary sinus augmentation, Bosshardt et al.[31] and Gassling et al.[32] studied the effects of L-PRF membranes versus absorbable CMs to cover the lateral window, again reporting no differences in vital bone formation.

Our results for CAL gain scores for each individual treatment are within those from previous reviews: L-PRF+BG (2.97 to 3.9 mm)[7] and GTR+BG (1.39 to 4.70).[5]

In the treatment of non-unfavorable IBDs, previous studies[13,33,34] suggest that the L-PRF+BG combination improves CAL gain compared with L-PRF alone. This was observed with different fillers: demineralized bone matrix[33] and porous hydroxyapatite.[34] Lekovic et al.[13] compared L-PRF alone and in association with IBB, showing an increased effectiveness of L-PRF in case of the association.

On the contrary, the addition of a BG to a GTR technique has not always been considered more effective.[35] Only in the presence of unfavorable IBDs, in fact, a superiority of the combined therapy was shown.[8] Moreover, in the treatment of two-wall defects, associating a BG to a membrane produces greater histological bone regeneration than using a membrane alone.[11]

L-PRF+IBB and CM+IBB are surgical techniques based on different principles. L-PRF increases specific anabolic cellular activities; it releases polypeptide growth factors for at least 7 to 28 days,[36] stimulating and activating stem cells, fibroblasts, cementoblasts, osteoprogenitor cells, and endothelial cells to promote angiogenesis.[36,37]

TABLE 1 Anatomical characteristics of experimental bony defects

Treatment	Predominantly (>50%) 1-wall defects	Combined 1- to 2-wall defects	1- to 2-wall defects with buccal and/or lingual extension	Circumferential defects	2-wall crater-like defects
CM+IBB	9	11	4	5	2
L-PRF+IBB	8	12	3	6	2

CM+IBB, defects treated by collagen membrane + inorganic bovine bone combination; L-PRF+IBB, defects treated by L-PRF + inorganic bovine bone combination.

Originally it was believed that the biological principle underlying GTR was to exclude the epithelium at the early stages of periodontal healing to avoid junctional epithelium downgrowth.[38] Subsequently, the need for a complete seal to epithelial cells has been questioned,[39] and currently the GTR clinical effects are believed to be referable mainly to the greater wound stability produced by the membrane rather than by the physical obstruction. This suggests that maintenance of an undamaged fibrin clot at the interface between the tooth and the flap is of primary importance for periodontal regeneration.[40]

It is reasonable that the L-PRF membrane placement onto the BG may have favored graft and clot stabilization, retaining them into the IBD[41] in the early healing phases, and may have favored regeneration independently from L-PRF–related growth factors.[30] However, the effectiveness of an L-PRF membrane as a cell barrier might be questioned considering its too short reabsorption time (1 to 2 weeks).[41]

4.3 | Discussion of secondary outcomes

In this study, the secondary outcomes were only investigated in relationship to the main outcome CAL gain to qualitatively describe its quantitative effect; therefore, their analyses are not intended to make specific confirmatory non-inferiority claims. As an aside, note that, if the study aim were to do confirmatory claims for secondary outcomes too, the M_1 and M_2 margins would still be suitable for DBL, while they should be somewhat shrunk for PD and GR (both of them or just one).

L-PRF+IBB treatment produced significantly less GR than CM. This may be related to the trophic effects exerted by growth factors from L-PRF, and it is a desirable feature when surgery needs to be performed in esthetically sensitive areas. Anabolic effects from L-PRF may explain the significantly greater DBL gain. The PD reduction in L-PRF is significantly inferior to CM as a consequence of the lower post-surgical GR. Although this is a less favorable result, it has questionable clinical relevance in quantitative terms (difference in means: 0.49 mm); in fact, the mean PD at follow-up for L-PRF+IBB was 3.35 ± 0.755, a value similar to what in the literature is reported as closed pocket (4 mm).[42]

4.4 | Clinical implications

In our study, both regenerative techniques were applied in unfavorable defects: only 1-, 1-2- and 2-wall defects, circumferential defects or teeth with a wide defect angle were categorized as unfavorable IBDs and involved in the study.

TABLE 2 Observed and estimated marginal means in mm (means ± SD) of the clinical and radiographic parameters

Parameter	Treatment	Baseline Mean ± SD (95% CI) Observed	12 months Mean ± SD (95% CI) Observed	Baseline to 12 months Mean ± SD (95% CI) Observed	Baseline to 12 months Mean ± SD (95% CI) Estimated marginal
PD	L-PRF+IBB	7.61 ± 1.407(7.10 to 8.13)	3.35 ± 0.755(3.08 to 3.63)	4.26 ± 1.182(3.82 to 4.69)	4.28 ± 0.634(4.05 to 4.51)
	CM+IBB	7.68 ± 1.447(7.15 to 8.21)	2.87 ± 0.670(2.63 to 3.12)	4.81 ± 1.327(4.32 to 5.29)	4.78 ± 0.634(4.55 to 5.01)
	Diff	*P = 0.9*	*P = 0.01*	*P = 0.09*	*P = 0.003*
CAL	L-PRF+IBB	8.55 ± 1.234(8.10 to 9.00)	5.06 ± 0.772(4.78 to 5.35)	3.48 ± 1.262(3.02 to 3.95)	3.58 ± 0.876(3.27 to 3.90)
	CM+IBB	8.84 ± 1.508(8.29 to 9.39)	5.97 ± 1.140(5.55 to 6.36)	2.87 ± 1.284(2.40 to 3.34)	2.77 ± 0.876(2.46 to 3.09)
	diff	*P = 0.4*	*P = 0.0001*	*P = 0.06*	*P = 0.0006*
GR	L-PRF+IBB	0.94 ± 0.512(0.75 to 1.12)	1.71 ± 0.643(1.47 to 1.95)	0.77 ± 0.669(0.53 to 1.02)	0.713 ± 0.924(0.381 to 1.05)
	CM+IBB	1.16 ± 0.779(0.88 to 1.45)	3.10 ± 1.193(2.66 to 3.53)	1.94 ± 1.209(1.49 to 2.38)	1.997 ± 0.924(1.665 to 2.33)
	diff	*P = 0.2*	*P = 0.0001*	*P = 0.0001*	*P = 0.000001*
DBL	L-PRF+IBB	9.48 ± 1.262(9.02 to 9.95)	6.71 ± 0.973(6.35 to 7.07)	2.77 ± 1.431(2.25 to 3.30)	2.81 ± 1.066(2.42 to 3.19)
	CM+IBB	9.58 ± 1.523(9.02 to 10.14)	7.39 ± 1.283(6.92 to 7.86)	2.19 ± 1.447(1.66 to 2.72)	2.16 ± 1.066(1.78 to 2.54)
	diff	*P = 0.8*	*P = 0.02*	*P = 0.1*	*P = 0.02*
FMBS	L-PRF+IBB	0.12 ± 0.144(0.068 to 0.164)	0.11 ± 0.085(0.080 to 0.143)	0.009 ± 0.159(−0.049 to 0.068)	0.0141 ± 0.215(−0.0136 to 0.0418)
	CM+IBB	0.13 ± 0.114(0.089 to 0.173)	0.11 ± 0.067(0.089 to 0.138)	0.017 ± 0.130(−0.030 to 0.065)	0.0124 ± 0.215(−0.0153 to 0.0401)
	diff	*P = 0.8*	*P = 0.9*	*P = 0.8*	*P = 0.93*
FMPS	L-PRF+IBB	0.13 ± 0.120(0.087 to 0.175)	0.14 ± 0.076(0.115 to 0.170)	−0.012 ± 0.135(−0.061 to 0.038)	0.0015 ± 0.769(−0.0261 to 0.0292)
	CM+IBB	0.16 ± 0.103(0.121 to 0.197)	0.13 ± 0.077(0.105 to 0.161)	0.026 ± 0.125(−0.020 to 0.072)	0.0124 ± 0.769(−0.0152 to 0.0401)
	diff	*P = 0.3*	*P = 0.6*	*P = 0.3*	*P = 0.58*

PD, probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level; FMBS, full mouth bleeding score; FMPS, full mouth plaque score; CM+IBB, defects treated by collagen membrane + inorganic bovine bone combination; L-PRF+IBB, defects treated by L-PRF + inorganic bovine bone combination.

NB, note well. All *P* values in observed scores columns refer to two-tail ANOVAs analyses (based on observed means). The baseline–12 months follow-up Estimated marginal column reports two-tail ANCOVA analyses. No *P* value was adjusted for multiplicity.

TABLE 3 Differences between treatments in clinical and radiographic parameter changes in mm (means ± SE) from baseline to 12 months

		ANCOVA		ANOVA	
Parameter	**Treatment**	**Estimated mean ± SE**	**90% CI**	**Observed mean ± SE**	**90% CI**
CAL gain	L-PRF+IBB	3.582 ± 0.157	3.319 to 3.845	3.484 ± 0.229	3.102 to 3.866
	CM+IBB	2.773 ± 0.157	2.510 to 3.035	2.871 ± 0.229	2.489 to 3.253
	Treatment Difference				
cAL gain	CM+IBB–L-PRF+IBB	–0.810 ± 0.223	–1.300 to -0.319	–0.613 ± 0.323	–1.324 to 0.098
PD reduction	CM+IBB–L-PRF+IBB	0.499 ± 0.161	0.145 to 0.853	0.548 ± 0.319	–0.153 to 1.250
GR increase	CM+IBB–L-PRF+IBB	1.284 ± 0.236	0.764 to 1.804	1.133 ± 0.255	0.616 to 1.707
DBL gain	CM+IBB–L-PRF+IBB	–0.648 ± 0.271	–1.244 to -0.052	–0.581 ± 0.366	–1.384 to 0.233

PD, probing depth; CAL, clinical attachment level; GR, gingival recession; DBL, defect bone level; CM+IBB, defects treated by collagen membrane + inorganic bovine bone combination; L-PRF+IBB, defects treated by L-PRF + inorganic bovine bone combination.

NB Correlated outcomes multiple comparisons computed by mean of simultaneous 90% Bonferroni-Nyholt CIs.

NB, note well. The estimated marginal means are evaluated at the following average values of covariates:

$Baseline_{CAL}$ = 8.69.

$Baseline_{PD}$ = 7.68.

$Baseline_{GR}$ = 1.05.

$Baseline_{DBL}$ = 9.53.

Anatomy is a paramount characteristic of angular IBDs, and it is related to their potential for healing. In particular, the number of residual bony walls and the width of the angle root-bone surface were shown to influence the extent to which bone responds to therapy.[43]

This seems to be true in conventional surgery as in regenerative procedures making use of enamel matrix derivative. In contrast, Tonetti et al.[22] and Trombelli et al.[44] reported that defect morphology did not affect the amount of bone fill following GTR. However, this observation was obtained using non-resorbable membranes and may be due to the wound stabilizing and space-making effects[45] of the ePTFE membranes used by the authors. In contrast, when using CMs, which lack stiffness when soaked in biological fluids, the supporting architecture of the IBD helps avoid membrane collapse into the defect, ensuring the space-maintaining effect needed for regeneration.[8] When the defect anatomy is not helpful, the presence of BGs contributes to ensuring that the membrane maintains its position under pressure by the sutured gingival flap.[8,10]

We treated unfavorable IBDs, and a graft was used in both groups to ensure blood clot stability. This overcomes the scarce consistency of L-PRF and CMs, unable to guarantee a sufficient space, crucial for regeneration.[46]

In this study, we used IBB as a graft material. The best BG would most likely be the patient's autogenous bone.[12] However, when there is a limited availability of tissue close to the IBD area, the withdrawal of autologous bone represents an invasive procedure that requires a second surgical site. Bone substitutes are possible alternatives, and among them, IBB is a widely used xenograft[12] that has also demonstrated histologic evidence of regeneration in human infrabony defects.[47]

4.5 | Limitations of the study

In our study, we did not use a stent-assisted probing methodology that would have somewhat reduced probing variability, nor did we use a split-mouth protocol that eliminates much of the intersubject variability. However, this study design has a number of recently highlighted drawbacks,[48,49] so it is more complex to apply, and its use must have a valid justification.

This study investigated unfavorable defects, as defined in the literature.[24] In this category, we included different IBDs, very heterogeneous in their architecture, but due to the small sample size, we could not use a randomized block experimental design that would have addressed heterogeneity more effectively, thus reducing the risk of bias.

Finally, although an important factor favoring L-PRF is its negligible cost, a particularly important characteristic in countries where a National Health System bears the cost

of public periodontal health, future papers are needed to exactly evaluate the economic advantages of the use of L-PRF while confirming our clinical results.

5 | CONCLUSIONS

Despite its limitations, our study suggests that when treating unfavorable IBDs L-PRF+IBB offers non-inferior CAL and DBL gain compared with CM+IBB, along with a significantly lower GR, which is a particularly desirable feature when the clinician works in anterior sextants. However, a slightly greater PD was observed.

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AUTHOR CONTRIBUTIONS

Imena Rexhepi, Paolo De Ninis, Michele Paolantonio, and Beatrice Femminella are co-first authors having designed the work, written, revised, and edited it; Bruna Sinjari designed the study, interpreted the data, and revised the paper; Lorenzo Secondi and Giulia Paolantonio collaborated in the design of the study, its writing and editing; they cooperated in the interpretation of the data; Matteo Serroni and Pasquale Santamaria cooperated in the study design, article editing, and critical revising of the manuscript; Luigi Romano collaborated in the study design, interpretation of data, and article editing. He also performed the data collection with Imena Rexhepi and Beatrice Femminella. Paolo De Ninis did the statistical analysis.

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REFERENCES

1. Papapanou P, Tonetti MS. Diagnosis and epidemiology of periodontal osseous lesions. *Periodontol*. 2000;22:8-21.
2. Nieri M, Muzzi L, Cattabriga M, Rotundo R, Cairo F, Pini Prato GP. The prognostic value of several periodontal factors measured as radiographic bone level variation: a 10-year retrospective multilevel analysis of treated and maintained periodontal patients. *J Periodontol*. 2002;73:1485-1493.
3. Figueira EA, de Assis AO, Montenegro SCL, et al. Long-term periodontal tissue outcome in regenerated infrabony and furcation defects: a systematic review. *Clin Oral Investig*. 2014;18:1881-1892.
4. Gottlow J, Nyman S, Lindhe J, Karring T, Wennström J. New attachment formation in the human periodontium by guided tissue regeneration. Case reports. *J Clin Periodontol*. 1986;13:604-616.
5. Kao RT, Nares S, Reynolds MA. Periodontal regeneration—intrabony defects: a systematic review from the AAP Regeneration Workshop. *J Periodontol*. 2015;86:S77-S104.
6. Cortellini P, Pini Prato G, Tonetti MS. Periodontal regeneration of human intrabony defects with bioresorbable membranes. A controlled clinical trial. *J Periodontol*. 1996;67:217-223.
7. Miron RJ, Zucchelli G, Pikos MA, et al. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clin Oral Investig*. 2017;21:1913-1927.
8. Paolantonio M. Combined periodontal regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A Controlled Clinical Study. *J Periodontol*. 2002;73:158-166.
9. Del Fabbro M, Karanxha L, Panda S, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev*. 2018;11:CD011423.
10. Stoecklin-Wasmer C, Rutjes AWS, da Costa BR, Salvi GE, Jüni P, Sculean A. Absorbable collagen membranes for periodontal regeneration: a systematic review. *J Dent Res*. 2013;92:773-781.
11. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials—biological foundation and preclinical evidence: a systematic review. *J Clin Periodontol*. 2008;35(8 Suppl):106-116.
12. Baldini N, De Sanctis M, Ferrari M. Deproteinized bovine bone in periodontal and implant surgery. *Dent Mater*. 2011;27:61-70.
13. Lekovic V, Milinkovic I, Aleksic Z, et al. Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intrabony periodontal defects. *J Periodontal Res*. 2012;47:409-417.
14. Parrish LC, Miyamoto T, Fong N, Mattson JS, Cerutis DR. Non-bioabsorbable vs. bioabsorbable membrane: assessment of their clinical efficacy in guided tissue regeneration technique. A systematic review. *J Oral Sci*. 2009;51:383-400.
15. Graziani F, Gennai S, Cei S, et al. Clinical performance of access flap surgery in the treatment of the intrabony defect. A systematic review and meta-analysis of randomized clinical trials. *J Clin Periodontol*. 2012;39:145-156.
16. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for Industry, Non-Inferiority Clinical Trials, November 2016, Washington, DC.
17. Altman DG. *Practical Statistics for Medical Research*. London: Chapman & Hall; 1991:285.
18. Julious SA. Sample sizes for clinical trials with normal data. *Stat Med*. 2004;23:1921-1986.
19. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):S159-S172.
20. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol*. 1972;43:38.
21. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975;25:229-235.
22. Tonetti MS, Pini-Prato G, Cortellini P. Periodontal regeneration of human intrabony defects. IV. Determinants of healing response. *J Periodontol*. 1993;64:934-940.

23. Choukroun J, Adda F, Schoeffer C, Vervelle A. PRF: an opportunity in perio-implantology. *Implantodontie*. 2000;42:55-62.
24. Cortellini P, Prato GP, Tonetti MS. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *Int J Periodontics Restorative Dent*. 1999;19:589-599.
25. Abu-Ta'a M. Adjunctive Systemic antimicrobial therapy vs asepsis in conjunction with guided tissue regeneration: a randomized, controlled clinical trial. *J Contemp Dent Pract*. 2016;17:3-6.
26. Huberty CJ, Morris D. Multivariate analysis versus multiple univariate analyses. *Psychological bulletin*. 1989;105:302-308.
27. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet*. 2004;74:765-769.
28. Derringer J, A simple correction for non-independent tests. osf.io/re5w2. Published October 19, 2018. https://doi.org/10.17605/OSF.IO/F2TYW
29. The CONSORT Group. CONSORT. Available at http://www.consort-statement.org/. Accessed May 2, 2013.
30. Paolantonio M, Di Tullio M, Giraudi M, Romano L, Secondi L, Paolantonio G, Graziani F, Pilloni A, De Ninis P, Femminella B. Periodontal regeneration by leukocyte and platelet-rich fibrin with autogenous bone graft versus enamel matrix derivative with autogenous bone graft in the treatment of periodontal intrabony defects: A randomized non-inferiority trial. *J Periodontol*. 2020;91:1595-1608.
31. Bosshardt DD, Bornstein MM, Carrel J-P, Buser D, Bernard J-P. Maxillary sinus grafting with a synthetic, nanocrystalline hydroxyapatite-silica gel in humans: histologic and histomorphometric results. *Int J Periodontics Restorative Dent*. 2014;34:259-267.
32. Gassling V, Purcz N, Braesen J-H, et al. Comparison of two different absorbable membranes for the coverage of lateral osteotomy sites in maxillary sinus augmentation: a preliminary study. *J Cranio-Maxillo-fac Surg Off Publ Eur Assoc Cranio-Maxillo-fac Surg*. 2013;41:76-82.
33. Chandradas ND, Ravindra S, Rangaraju VM, Jain S, Dasappa S. Efficacy of platelet rich fibrin in the treatment of human intrabony defects with or without bone graft: a randomized controlled trial. *J Int Soc Prev Community Dent*. 2016;6(Suppl 2):S153-159.
34. Pradeep AR, Bajaj P, Rao NS, Agarwal E, Naik SB. Platelet-Rich fibrin combined with a porous hydroxyapatite graft for the treatment of 3-wall intrabony defects in chronic periodontitis: a randomized controlled clinical trial. *J Periodontol*. 2017;88:1288-1296.
35. Garrett S. Periodontal regeneration around natural teeth. *Ann Periodontol*. 1996;1:621-666.
36. Tsai CH, Shen SY, Zhao JH, Chang YC. Platelet-rich fibrin modulates cell proliferation of human periodontally related cells in vitro. *J Dent Sci*. 2009;4:130-135.
37. Arunachalam M, Pulikkotil SJ, Sonia N. Platelet rich fibrin in periodontal regeneration. *Open Dent J*. 2016;10:174-181.
38. Listgarten MA, Rosenberg MM. Histological study of repair following new attachment procedures in human periodontal lesions. *J Periodontol*. 1979;50:333-344.
39. Wikesjö UM, Nilvéus RE, Selvig KA. Significance of early healing events on periodontal repair: a review. *J Periodontol*. 1992;63:158-165.
40. S Medikeri R, Meharwade V, M Wate P, V Lele S. Effect of PRF and allograft use on immediate implants at extraction sockets with periapical infection -clinical and cone beam CT findings. *Bull Tokyo Dent Coll*. 2018;59:97-109.
41. Kawase T, Kamiya M, Kobayashi M, et al. The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation. *J Biomed Mater Res B Appl Biomater*. 2015;103:825-831.
42. Wennström JL, Tomasi C, Bertelle A, Dellasega E. Full-mouth ultrasonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontitis. *J Clin Periodontol*. 2005;32:851-859.
43. Ellegaard B, Löe H. New attachment of periodontal tissues after treatment of intrabony lesions. *J Periodontol*. 1971;42:648-652.
44. Trombelli L, Kim CK, Zimmerman GJ, Wikesjö UM. Retrospective analysis of factors related to clinical outcome of guided tissue regeneration procedures in intrabony defects. *J Clin Periodontol*. 1997;24:366-371.
45. Haney JM, Nilvéus RE, McMillan PJ, Wikesjö UM. Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. *J Periodontol*. 1993;64:883-890.
46. Wikesjö UM, Nilvéus R. Periodontal repair in dogs: effect of wound stabilization on healing. *J Periodontol*. 1990;61:719-724.
47. Nevins ML, Camelo M, Lynch SE, Schenk RK, Nevins M. Evaluation of periodontal regeneration following grafting intrabony defects with bio-oss collagen: a human histologic report. *Int J Periodontics Restorative Dent*. 2003;23:9-17.
48. Qin D, Hua F, He H, Liang S, Worthington H, Walsh T. Quality of split-mouth trials in dentistry: 1998, 2008, and 2018. *J Dent Res*. 2020;3:22034520946025.
49. Chung B, Pandis N, Scherer RW, Elbourne D. CONSORT extension for within-person randomized clinical trials. *J Dent Res*. 2020;99:121-124.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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