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CLINICAL INNOVATION REPORT

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Leucocyte- and platelet-rich fibrin block for bone augmentation procedure: A proof-of-concept study

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Abstract

Aim: The objective of this proof-of-concept study was to investigate the effects of a new guided bone regeneration technique with a tissue engineering approach.

Materials and Methods: This single cohort observational study evaluated the outcome of the leucocyte- and platelet-rich fibrin (L-PRF) Block for horizontal bone augmentation in the maxilla. The L-PRF Block is prepared by mixing a particulated biomaterial with chopped L-PRF membranes at a 50:50 ratio and adding liquid fibrinogen to glue all together. Horizontal augmentation was assessed linearly and volumetrically immediately after surgery and 5–8 months later by matching consecutive cone beam computed tomography (CBCTs).

Results: Ten patients (mean age of 50.7 years [±17.2]) representing 15 sites with horizontal alveolar deficiencies were included. Superimposition of pre-operative and posthealing CBCT scans showed an average linear horizontal bone gain of 4.6 mm (±2.3), 5.3 mm (±1.2) and 4.4 mm (±2.3), measured at 2, 6 and 10 mm from the alveolar crest, respectively. The volumetric gain was 1.05 cm^3 (±0.7) on average. The resorption rate after 5–8 months was 15.6% (±6.7) on average.

Conclusions: L-PRF Block may be a suitable technique to augment deficient alveolar ridges.

KEYWORDS

bone augmentation, bone substitutes, bone volume, guided bone regeneration, leucocyte- and platelet-rich fibrin, leucocyte- and platelet-rich fibrin block, platelet concentrate, tissue engineering

1 | INTRODUCTION

Alveolar bone resorption after tooth loss or extraction can lead to insufficient bone volume, which negatively affects the prognosis of dental implants (Ashman, 2000; Esposito, Grusovin, Coulthard, & Worthington, 2006). Traditionally, bony defects have been classified according to anatomical deficiency as follows: horizontal, vertical or combinations. Vertical ridge augmentation has been reported to be successful, but with a low degree of predictability and a rather high complication rate (Esposito et al., 2009; Rocchietta, Fontana, & Simion, 2008). More predictable results have been obtained with horizontal bone augmentation (Donos, Mardas, & Chadha, 2008; Kuchler & von Arx, 2014). In addition, similar clinical and radiological results have been reported for implants placed with bone augmentation compared with those completely placed into pristine bone (Benic, Bernasconi, Jung, & Hämmerle, 2017).

Numerous techniques have been described to reconstruct deficient alveolar ridges (Buser et al., 2002; Esposito et al., 2009). In the simultaneous treatment approach, guided bone regeneration (GBR) is associated with superior outcomes when compared to other procedures and has become the treatment of choice to provide optimal bone support for dental implants (Aghaloo & Moy, 2007;

Sanz-Sánchez, Ortiz-Vigón, Sanz-Martín, Figuero, & Sanz, 2015). In the staged treatment approach, autologous bone blocks (ABB) are the most frequently used. However, this technique shows an increased morbidity (due to the presence of a second surgical site) and postoperative complications. Furthermore, varying degrees of graft resorption during healing have been reported (Benic & Hämmerle, 2014; Sanz-Sánchez et al., 2015). Moreover, a composite bone graft combining a xenograft with particulated autogenous bone has also been proposed to increase the osteogenic properties of the graft (Urban, Nagursky, & Lozada, 2011).

In the last few decades, the therapeutic potential of tissue engineering for bone regeneration has gained considerable interest. Recently, various clinical trials have validated the safety and predictability of these approaches (Avila-Ortiz et al., 2016). The use of a second-generation platelet concentrate, leucocyte- and platelet-rich fibrin (L-PRF), to create a graft with high concentration of growth factors, platelets and leucocytes may enhance the development of mature lamellar bone. The clinical capacities and properties of L-PRF have already been reported in two recent systematic reviews (Castro et al., 2017a,b). However, its benefit in GBR has remained unclear.

The use of a fluid form of PRF (i-PRF) has been proposed to agglutinate the particulated bone graft material (de Mourão et al., 2015). i-PRF has been tested with different centrifugation speeds to selectively enrich leucocytes, platelets and growth factors release (Choukroun & Ghanaati, 2018). Recently, a case report described a similar technique using i-PRF (Chenchev, Ivanova, Neychev, & Cholakova, 2017). However, a specific clinical protocol, with radiological results, is still missing.

In this study, a similar fluid, named liquid fibrinogen, was obtained and mixed with L-PRF membranes and particulated biomaterial to obtain a L-PRF Block.

Therefore, the aim of this study was to radiologically assess and clinically investigate the outcome and early resorption of this new GBR technique with a tissue engineering approach.

Clinical Relevance

Scientific rationale for the study: Bone augmentation with autologous bone is often associated with increased morbidity and postoperative complications. A tissue engineering approach with an leucocyte- and platelet-rich fibrin (L-PRF) Block may reduce these disadvantages and enhance bone regeneration. The objective of this proof-of-concept study was to evaluate the use of the L-PRF Block for horizontal ridge augmentation.

Principal findings: Significant horizontal ridge augmentation was obtained with L-PRF Block. The resorption rate of the graft was very low, which allowed implant placement in all cases.

Practical implications: L-PRF Block appears a realistic alternative for horizontal augmentation of deficient alveolar ridges. This procedure is safe, predictable, with a high feasibility and a low morbidity.

2 | MATERIALS AND METHODS

This study was designed as a case study, single cohort trial evaluating the outcome of a L-PRF Block in patients in need of a horizontal bone augmentation before implant placement in the maxilla.

All patients were treated at the University Hospital in Leuven, Belgium. The study protocol was approved by the Ethical Committee of the KU Leuven (reference S60304, UZ Leuven University Hospitals, Belgium) and was in accordance with the Helsinki Declaration of 1975, as revised in 2008.

2.1 | Inclusion and exclusion criteria

The recruited patients had to be able to understand the nature of the proposed surgical procedure and to sign an informed consent. Moreover, the following inclusion criteria had to be fulfilled: (1) in need of one (or more) implant in the maxilla, (2) in need of horizontal bone augmentation, (3) sufficient vertical bone height at the recipient site for implant placement and (4) healthy oral mucosae.

A patient was excluded in the presence of any of the following contraindications: (1) general contraindication for implant placement and/or surgical treatment, (2) ongoing inflammatory and/or autoimmune disease of the oral cavity, (3) immunosuppressant, corticosteroid or bisphosphonate therapy, (4) history of malignancy, radiotherapy or chemotherapy for malignancy within the past 5 years, (5) smoker, (6) insulin-dependent diabetes and (7) blood-related diseases.

2.2 | Outcome variables

The primary outcome measure was defined as the gain in ridge width (mm) at 5–8 months after horizontal bone augmentation using a L-PRF Block. The horizontal width of the alveolar ridge was assessed on cone beam computed tomography (CBCT) considering linear and volumetric measurements.

The secondary outcome measures were the resorption rate of the graft after healing and the occurrence of an adverse event (wound infection, exposure of the graft and soft tissue dehiscence). Adverse event was recorded at week 1 and 2, and at months 1, 2 and 5–8 after surgery. A CBCT was taken immediately after GBR and after 5–8 months of healing.

2.3 | Preparation of L-PRF Block

Before starting the surgery, 8–16 tubes (9 ml) of venous blood were collected from the patients (Figure 1). For six to 14 tubes (red cap, glass coating [BVBCTP-2; IntraSpin, Intra-Lock, FL, USA]), a standard protocol, as reported before (Temmerman et al., 2016), was followed (12 min centrifugation, 2,700 rpm/408 *g* RCF; centrifuge rotor radius 5 cm). Two tubes (white cap, plastic coating [WCT;

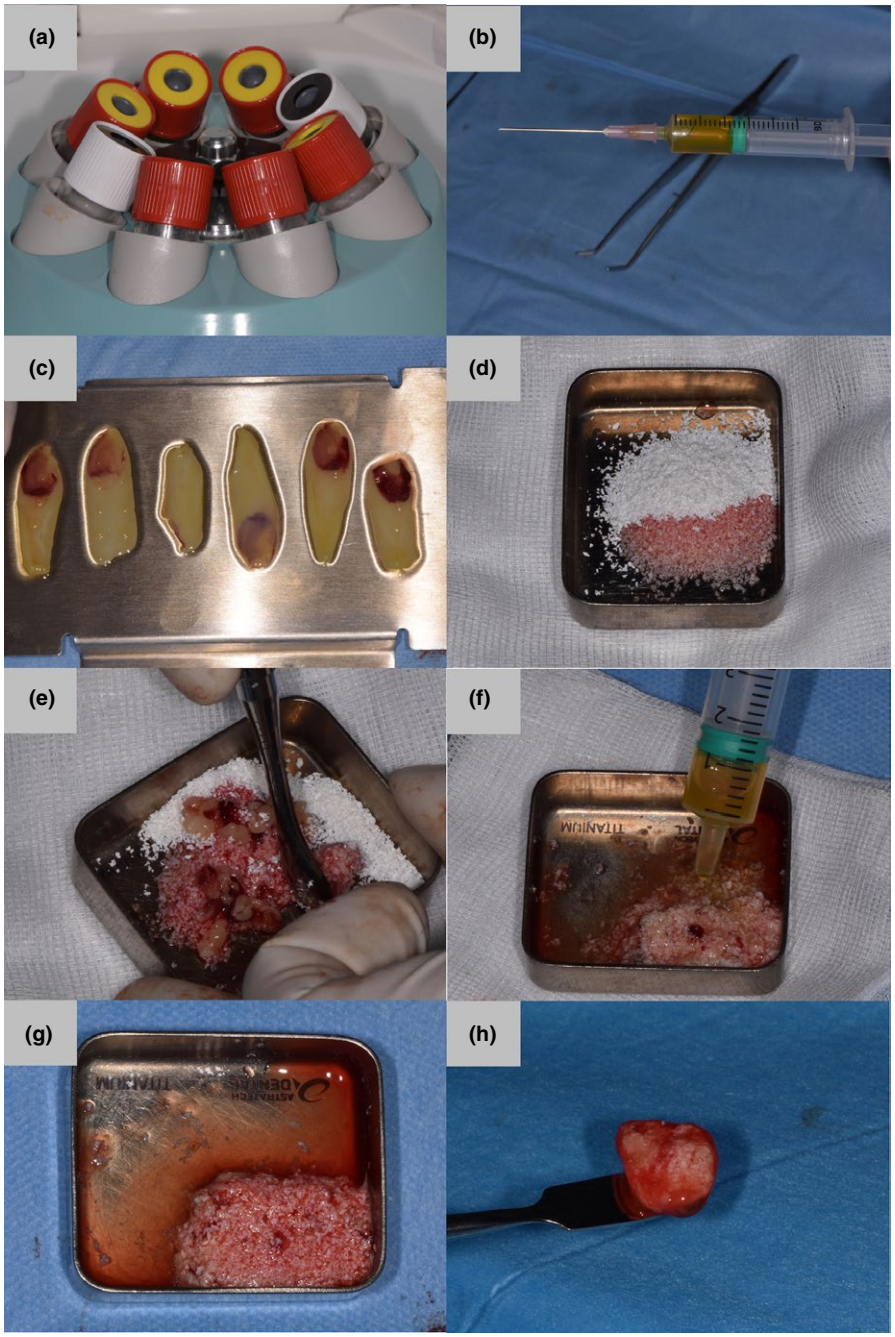

FIGURE 1 Clinical preparation of leucocyte- and platelet-rich fibrin (L-PRF) Block using 0.5 g of biomaterial. (a) collection of six tubes (red cap, glass coated) of blood following standard protocol, and two tubes for liquid fibrinogen (white cap, plastic coating). (b) collection of the liquid fibrinogen with a sterile syringe. (c) L-PRF membranes after compression (Xpression; Intra-Lock, FL, USA). (d) biomaterial slightly wetted with L-PRF exudate only to facilitate the mixing. (e) mixing of membranes and bone substitute. (f): addition of liquid fibrinogen over the homogeneous mix. (g) shaping into the desired form. (h) L-PRF Block after ±5 min

IntraSpin]) were drawn and placed last in the centrifuge (IntraSpin) at 2,700 rpm/408 *g* RCF for 3 min only.

The yellow fluid (liquid fibrinogen) at the top of the white cap tubes was aspirated with a sterile syringe, without the red part.

After full centrifugation of the red cap tubes, the L-PRF clots were removed from the tubes using surgical tweezers. The clots were thereafter gently compressed into membranes using a sterile metal box (Xpression; Intra-Lock, FL, USA).

To prepare the L-PRF Block (Table 1), L-PRF membranes were cut into small pieces and mixed with deproteinized bovine bone mineral (DBBM) (Bio-Oss Small particles; Geistlich AG, Wolhusen, Switzerland) at a ratio of two membranes/0.5 g biomaterial (which provides a 50:50 ratio). The liquid fibrinogen was added to the homogeneous mix and stirred gently for ±10 s while shaping it to the desired form. The fibrinogen will be polymerized into fibrin (by the activated platelets of the chopped membranes) within a few minutes and trap the biomaterial into a fibrin mesh containing platelets and leucocyte, forming the L-PRF Block.

2.4 | Treatment procedures

All surgical procedures were performed under local anaesthesia and strict sterile conditions (Figure 2). A midcrestal incision was made in the gingiva and, for adequate surgical access, intrasulcular incisions at adjacent teeth and one or two divergent vertical releasing incisions were performed a tooth away from the defect.

TABLE 1 Protocol for the preparation of leucocyte- and platelet-rich fibrin block

Protocol for preparation of L-PRF Block using 0.5 g of biomaterial (BioOss):
-Venipuncture: collect 6 tubes (red cap, glass coating) of blood following standard protocol and 2 tubes (white cap, plastic coating), the latter is drawn last and is placed last in centrifuge (2,700 rpm/408 *g* RCF).
-After 3 min interrupt centrifugation, remove both white cap tubes.
-Immediately restart the centrifuge with rem\aining red cap tubes for another 9 min.
-Immediately aspirate the yellow fluid (= liquid fibrinogen) in white cap tube with a sterile syringe, get as close as possible to the red cells, but do not aspirate them; the liquid can be kept in the syringe up to 20–30 min.
-After full centrifugation of the remaining tubes, remove L-PRF clots and compress gently into membranes.
Preparation of "block"
-Chop membranes in very small pieces.
-Mix chopped membranes and bone substitute in Ti-dish (with a 50:50 ratio), if the mix is too dry, one can add some L-PRF exudate. Get a uniform mix.
-Spray 1cc of liquid fibrinogen over the homogeneous mix, and stir gently for ±10 s while shaping it to the desired form.
-Fibrinogen will clot into fibrin within a few minutes and trap the biomaterial to form a L-PRF Block.

A mucoperiosteal flap was elevated to expose the alveolar crest at least 5 mm beyond the bone defect. On the recipient site, multiple cortical perforations were made to expose the medullary space. In case of a simultaneous approach (two patients), bone level implants (Astra EV; Dentsply Implants, Mölndal, Sweden) were inserted, following manufacturers protocol. A periosteal releasing incision was performed to mobilize the flap. A collagen membrane (Bio-Gide; Geistlich AG, Wolhusen, Switzerland) was fixed on the vestibular side with titanium tacks (Frios; Dentsply Implants). Then, the L-PRF Block was placed on the recipient site and the membrane was fixed in place on the palatal side with additional titanium tacks. The grafted area was covered with the remaining L-PRF membranes to protect the graft/membrane in case of exposure. A primary tension-free closure was obtained, and the flap was sutured in two layers with horizontal mattress and single interrupted sutures (Cytoplast, Osteogenics Biomedical, USA).

The patients were provided with antibiotics (amoxicillin + clavulanic acid 500/125 mg for 7 days) and analgetics (600 mg ibuprofen, for at least 3 days). They were instructed to rinse twice a day with chlorhexidine (Perio Aid 0.12%; Dentaid, Spain) mouth rinse and not to brush the surgical area until suture removal. Sutures were removed at day 14.

A CBCT was taken after surgery to determine the initial volume of the augmented area. After 5–8 months, another CBCT was taken to evaluate the augmented site after healing and to plan the implants for a staged approach.

2.5 | Radiographic recordings

Following the clinical treatment protocol of our institution, CBCT (NewTom VGi evo; QR Verona, Verona, Italy) scans were acquired at three time points: pre-operatively (T0), immediately postoperatively (T1) and at the 5–8 months follow-up (T2) to allow an accurate surgical planning and reliable postoperative evaluation of the bone healing at the level of the augmented site (Van Dessel et al., 2017). A high-resolution scanning protocol was used with fixed exposure parameters: 0.2 mm voxel size, 110 kV, 360° rotation and 10 × 5 cm field of view. According to this particular CBCT system, the tube current was dynamically adjusted for each patient, allowing a significant dose reduction (in average effective dose of 126 μSV).

The postoperative scans were spatially matched to the preoperative CBCT based on selected areas where no changes had taken place during healing. A voxel-based registration method was applied, which maximizes the joint histogram intensity pattern of the entire 3D volume via correlation metrics (Maes et al., 1997). All CBCT scans were positioned in the same coordinate system by computing the rigid transformation that spatially aligns each postoperative CBCT scan with the corresponding pre-operative scan using registration software based on mutual information. Subsequently, standardized linear measurements were made on cross-sectional images generated perpendicular to the occlusal plane using the same reference points and lines (Schindelin et al., 2012). A vertical reference line was defined at the mid-point of the bone graft. Three horizontal reference lines were drawn at 2, 6 and 10 mm.

The aligned scans were imported into MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) for automatic volumetric assessment. Afterwards, an implant surgeon was trained for particular image analysis as such to apply the semi-interactive livewire boundary extraction tool to extract 3D augmented area (Barrett and Mortensen, 1997). The outer borders of the initial bone graft (T1) and bone graft after 5–8 months healing (T2) were separately selected using livewire segmentation, and the total volume of the bone graft was registered.

2.6 | In vitro micro-CT

A L-PRF Block was created following the described procedure, and a micro-CT (SkyScan 1172; Bruker, Belgium) was taken to analyse the composition and biomaterial volumetric distribution of the block. The measurements were performed with CT Analyser (version 1.11.5.1; Bruker) (Figure 3).

2.7 | Statistical analysis

The data were exported into SPSS software for Mac OS X (version 22.0; SPSS Inc., USA) for the statistical analysis.

Descriptive analysis was performed for numeric parameters using means ± standard deviations. Because the data (volumetric/linear) were not normally distributed, comparisons between pre-, postaugmentation and posthealing measurements were made by a Wilcoxon signed-rank test. The patient was always the statistical unit.

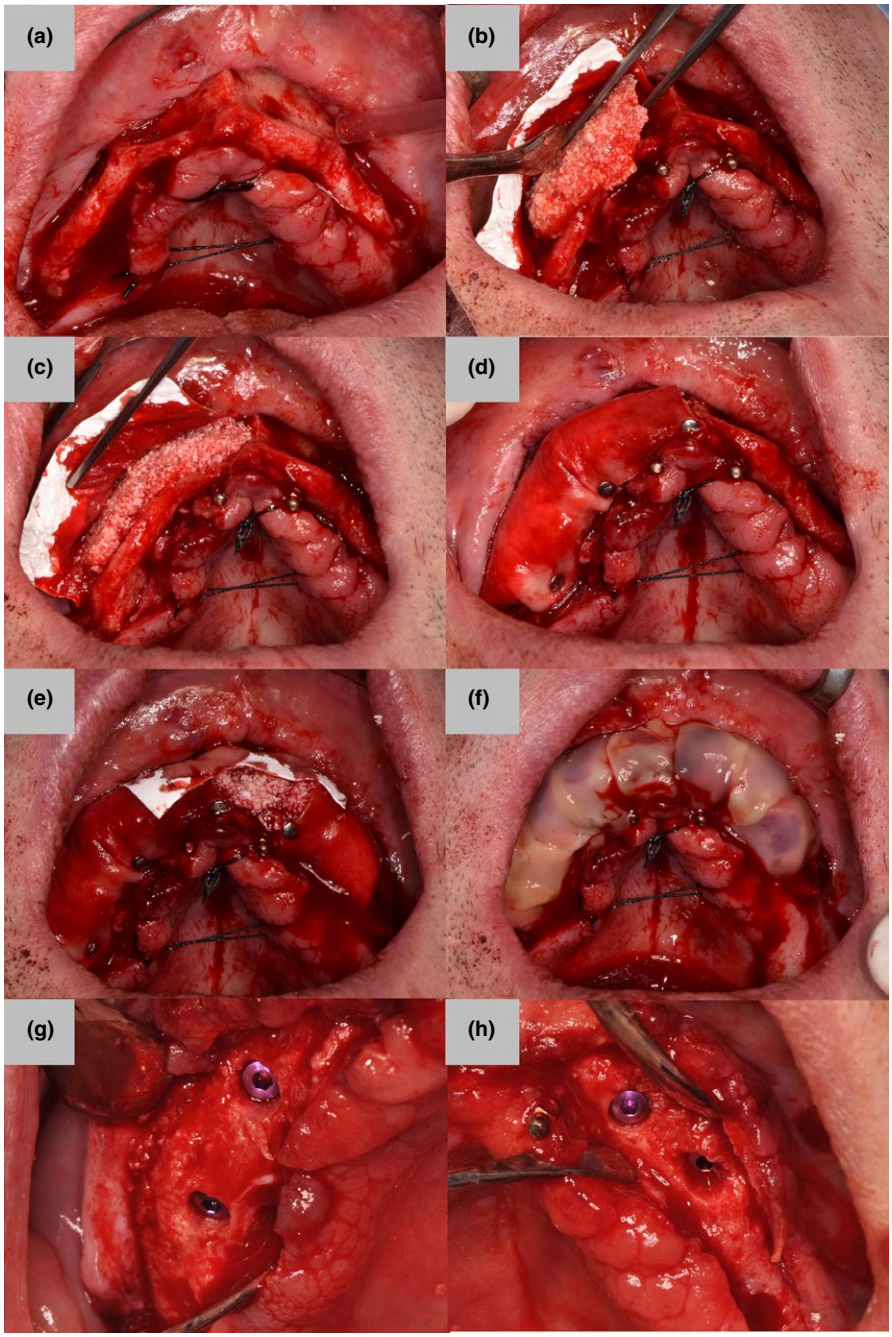

FIGURE 2 Clinical application of leucocyte- and platelet-rich fibrin (L-PRF) Block for horizontal bone augmentation in one of the study patients with bilateral augmentation and staged procedure. (a) knife-edge alveolar ridge in the upper jaw. (b & c): after buccal fixation of a collagen membrane, the L-PRF Block is placed on the recipient site in the right upper jaw. (d) palatal fixation of the collagen membrane to stabilize the graft. (e): same procedure applied to the left upper jaw. (f) coverage of the collagen membrane with L-PRF membranes. (g & h) augmented sites at re-entry after 9 months for implant placement

3 | RESULTS

3.1 | Patient characteristics

Ten patients (partially or fully edentulous; mean age [50.7 ± 17.2 years]) were included, representing 15 defect sites (five patients needed a bilateral augmentation and contributed with two sites each). Two patients were treated with a simultaneous approach and eight with a staged approach. No dropouts were registered. The mean healing time was 6.5 ± 1.0 months (Table 2).

One patient showed a partial wound closure failure during the second week. However, due to the use of the coverage with L-PRF membranes, no collagen membrane exposure was observed. All other patients showed uneventful postoperative wound healing, without adverse events.

Radiological and clinical examination at the time of re-entry revealed integration of the grafts with the surrounding bone, often without bone substitute loosening and/or particles in the flap. For all staged approach subjects, the gain in ridge dimension allowed a successful implant placement.

3.2 | Linear measurements

A statistical significant gain in alveolar ridge width was achieved at the crest, midcrest and apical levels (Table 3).

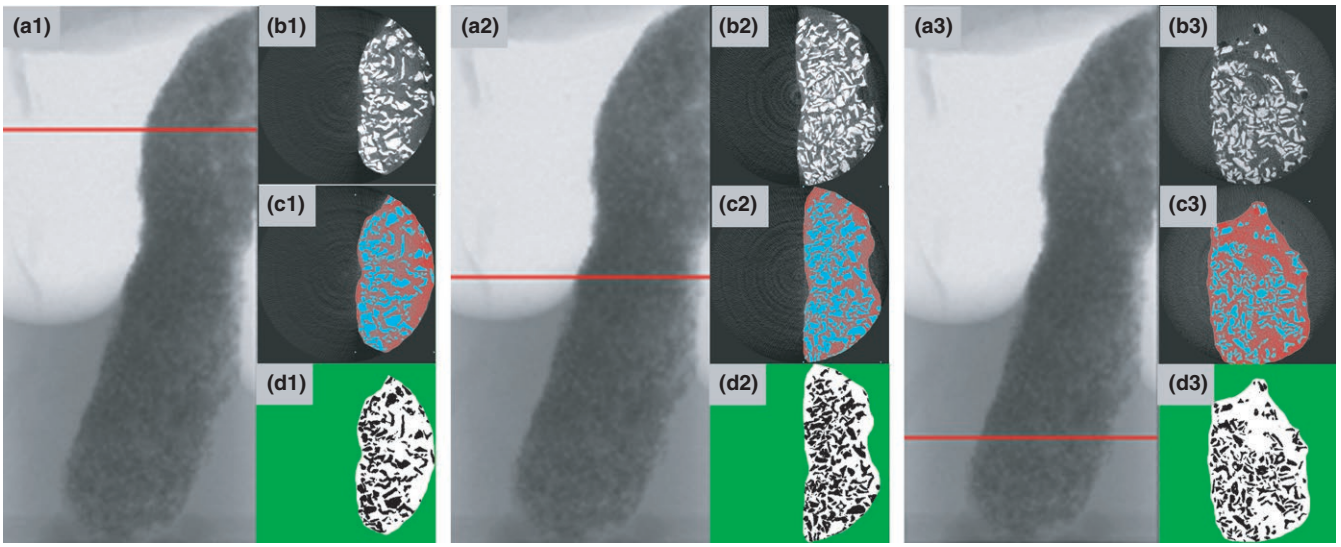

FIGURE 3 Micro-CT analyses of a leucocyte- and platelet-rich fibrin (L-PRF) Block. Three segmentations are reported. 1a, 2a, 3a: L-PRF Block and segmentation heights. 1b, 2b, 3b: cross-sectional image of segmentations. 1c, 2c, 3c: cross-sectional image of segmentations after selecting the region of interest. 1d, 2d, 3d: cross-sectional image of segmentations after separating the biomaterial from the L-PRF membranes and liquid fibrinogen to calculate the volumetric distribution

TABLE 2 Subject characteristics at baseline and timing of posthealing cone beam computed tomography (CBCT) and implant placement

Number (site no.)	Gender	Age (year)	CBCT healing (after … months)	Implant placement (after … months)	Implant timing
1 (1)	F	61	6	8	Staged
2 (2, 3)	F	61	7	9	Staged
3 (4, 5)	F	57	8	10	Staged
4 (6, 7)	M	49	7	10	Staged
5 (8)	F	57	6	7	Staged
6 (9, 10)	F	20	6	9	Staged
7 (11)	F	72	6	8	Staged
8 (12)	M	23	10	14	Staged
9 (13, 14)	M	63	6	–	Simultaneous
10 (15)	M	44	5	–	Simultaneous
Mean (±*SD*)		59.7 (±17.2)	6.7 (±1.4)	9.4 (±2.1)	
Median		57	6	9	
Range		20–72	5–10	7–14	

The mean horizontal gain measured at 2, 6 and 10 mm from the alveolar crest was 4.6 ± 2.3 mm, 5.3 ± 1.2 mm and 4.4 ± 2.3 mm, respectively.

The resorption rate of the graft was analysed in nine patients, presenting 14 sites. One patient did not receive a postaugmentation CBCT due to technical problems. The mean linear graft resorption during healing was 16 ± 11.8% (Figure 4).

3.3 | Volumetric measurements

From T0 to T2, the alveolar crest was increased in average 1.05 ± 0.7 cm^3, presenting an average grafted surface area of 7.0 ± 3.3 cm^2 (Table 4).

The mean volumetric graft resorption during healing was 15.6 ± 6.7% (Figure 5).

3.4 | Micro-CT

The volumetric analysis on micro-CT indicated a volume of 39% for the particulated biomaterial and 61% for the L-PRF and liquid fibrinogen.

4 | DISCUSSION

To the best of our knowledge, this is the first report on tissue engineering with the application of the L-PRF Block, an approach to GBR

TABLE 3 Linear radiographic alveolar ridge width measured at 2, 6 and 10 mm below alveolar crest. The gain was calculated comparing the pre-operatively and posthealing cone beam computed tomography (CBCTs). The resorption of the graft was calculated comparing the immediately postoperatively and posthealing CBCTs

Site	T0	T1	T2	Gain (T2-T0)	Resorption % (T1-T2)
2 mm					
Mean (±*SD*)	2.7 (±1.3)	8.7 (±1.5)	7.3 (±1.7)	4.6 (±2.3)	16 (±11.8)
Median (25–75%)	2.8 (1.7–3.7)	8.8 (7.4–9.9)	6.9 (6.1–8.6)	4.8 (2.9–6.6)	15.8 (3–27)
Range	1–4.8	6–10.9	5–10.1	0.3–7.6	1.2–39.2
6 mm					
Mean (±*SD*)	4.2 (±1.7)	10.7 (±1.5)	9.6 (±1.4)	5.3 (±1.2)	10.8 (±8.3)
Median (25–75%)	4 (3–5.1)	10.6 (9.7–11.8)	9.2 (8.3–10.7)	5.5 (4.6–6.2)	11 (3.5–15)
Range	1.1–7.5	8.2–12.9	7.9–11.6	3.2–7.3	0.9–29.7
10 mm					
Mean (±*SD*)	7.2 (±2.9)	12.5 (±1.4)	11.6 (±1.5)	4.4 (±2.3)	7.2 (±5.4)
Median (25–75%)	6.9 (5.3–9.4)	12.5 (11.5–13.3)	11.6 (10.6–12.8)	4.8 (2.7–5.8)	4.3 (3.5–10.5)
Range	1.4–11.6	9.3–14.9	8.1–13.7	0–8.9	1.7–17.5

T0: pre-operatively; T1: immediately postoperatively; T2: posthealing.

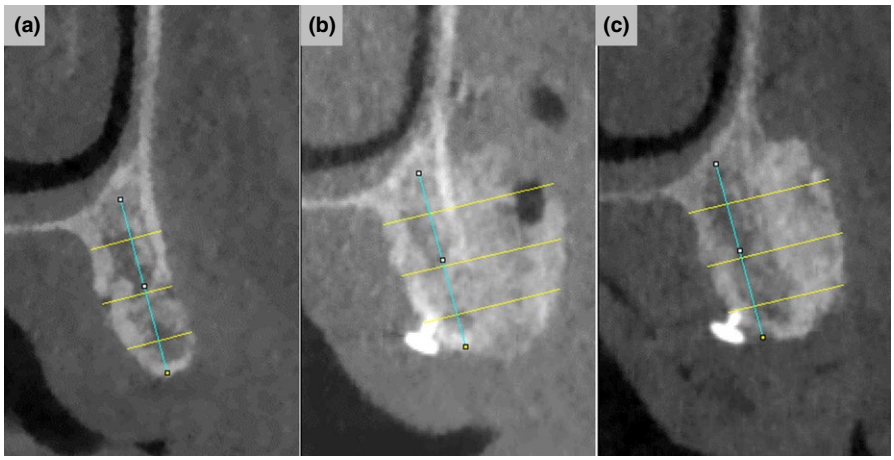

FIGURE 4 Linear measurements of the same study patient. Pre-operatively T0 (a), postoperatively T1 (b) and posthealing T2 (c). The blue standard line is perpendicular to the occlusal plane. The yellow lines are positioned at 2, 6 and 10 mm from the alveolar crest

TABLE 4 Volumetric radiographic resorption (T1-T2) of the graft during healing

Site	T1 (cm^3)	T2 (cm^3)	Resorption (%)	Resorption (cm^3)
Mean (±*SD*)	1.23 (±0.81)	1.05 (±0.66)	15.6 (±6.7)	0.19 (±0.16)
Median (25–75%)	1.17 (0.53–1.84)	0.93 (0.45–1.55)	13.5 (10–22)	0.13 (0.07–0.22)
Range	0.33–2.87	0.25–2.35	7–26	0.06–0.55

T1: immediately postoperatively; T2: posthealing.

without the use of autologous bone. This case series demonstrates that the L-PRF Block can be used safely and effectively for horizontal augmentation of resorbed alveolar ridges. A mean horizontal bone gain of 4.7 ± 2 mm was achieved. Some sites gained up to 7–8 mm.

Autologous bone blocks are still considered as the gold standard to reconstruct resorbed alveolar ridges. However, the need for a second surgical site evokes a higher patient morbidity. This morbidity further increases when bone is harvested outside the oral cavity (Nkenke & Neukam, 2014). A second drawback is the varying degree of graft resorption during healing (Benic & Hämmerle, 2014).

The use of particulated bone grafts instead of bone blocks has been supported in the literature. However, graft instability in particulated grafts can lead to integration failure. To overcome this problem, a rigid fixation of the membrane on both the vestibular

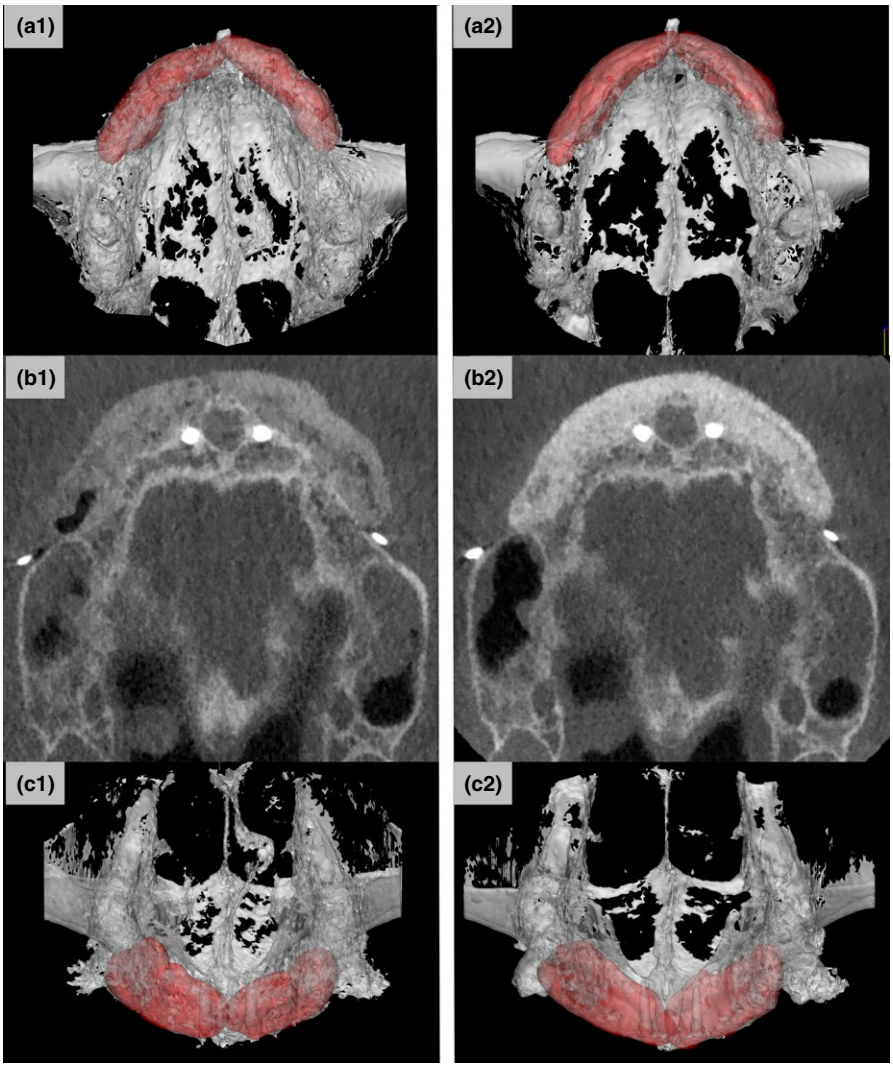

FIGURE 5 3D and volumetric analysis of cone beam computed tomography (CBCT) images and respective 3D models of the same study patient. Postoperatively T1 (1) and posthealing T2 (2): 3D reconstruction from a caudal view (1a, 2a), axial slice from CBCT (1b, 2b), 3D reconstruction from a frontal view (1c, 2c). The knife-edge alveolar ridge in the upper jaw (grey) was treated bilaterally with two leucocyte- and platelet-rich fibrin (L-PRF) Blocks (red)

and palatal/lingual side to immobilize the graft was proposed (Urban et al., 2011). The stability of the graft is also enhanced when using bone blocks instead of particulated grafts (Mir-Mari, Benic, Valmaseda-Castellón, Hämmerle, & Jung, 2017).

The novel technique described in this study combines the properties of bone blocks and particulated grafts (Figure 6) reducing the disadvantages of both. Comparable results have also been reported in the literature with a similar GBR surgical approach without the use of platelets concentrates (Sanz-Sánchez et al., 2015). However, the combination with liquid fibrinogen to form the L-PRF Block may increase ease in handling and predictability of the augmentation procedure. It provides a block made out of particulated graft, with increased stability of the augmented area. Furthermore, it excludes the discomfort inherent to the secondary harvesting site. The composition and properties of the liquid fibrinogen will be reported in another paper.

It has already been suggested in the literature that PRF membranes can be cut into small pieces and added to graft material, functioning as a "biological matrix" which may promote the migration of osteoprogenitor cells to the centre of the graft and induce neoangiogenesis (Simonpieri, Del Corso, Sammartino, & Dohan Ehrenfest, 2009).

The properties of this technique are based on a tissue engineering approach. Successful tissue engineering relies on two fundamental principles: a space-maintaining scaffold and a matrix that permits cell recruitment and neovascularization and delivers morphogenetic, regulatory and growth factors (Avila-Ortiz et al., 2016). (1) The DBBM provides a scaffold, which is embedded in a fibrin matrix, creating more space between the graft particles, and therefore allowing cell ingrowth from surrounding tissue. (2) The L-PRF in the block is a matrix rich in activated platelets secreting a wide range of bioactive molecules and growth factors including the following: bone morphogenetic protein (BMP), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1) and transforming growth factor-β2 (TGF-β2). These play key roles in bone healing and regeneration (Choukroun et al., 2006). Not only will L-PRF stimulate the in vitro proliferation and differentiation of human oral bone mesenchymal stem cells in a dose-dependent way, but it also induces mesenchymal stem cell migration as a response to the factors released

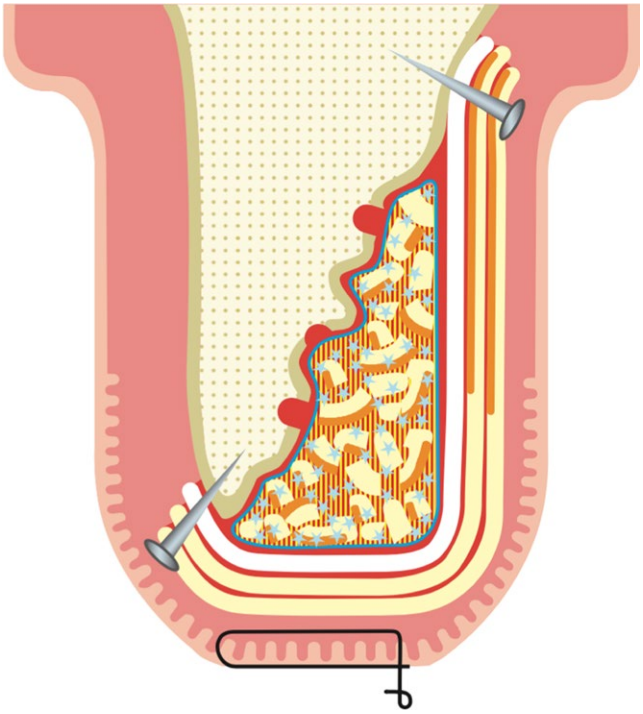

FIGURE 6 Graphic representation of leucocyte- and platelet-rich fibrin (L-PRF) Block for horizontal bone augmentation. The small holes in cortical bone guarantee optimal blood supply. The L-PRF Block is adapted to the bony defect and covered with a collagen membrane fixed via membrane tacks. L-PRF membranes are used to cover the augmented site

(Dohan Ehrenfest, Doglioli, de Peppo, Del Corso, & Charrier, 2010). L-PRF has also shown beneficial properties for neovascularization (Schär, Diaz-Romero, Kohl, Zumstein, & Nesic, 2015). This could lead to faster maturation of the augmented area and a reduced amount of biologically inactive scaffold. The latter is just speculative, and histological comparisons will be needed to confirm this hypothesis. The scaffold and matrix are fixed with the liquid fibrinogen. This starts the coagulation cascade when in contact with the chopped L-PRF membranes. This process takes place in <5 min and traps the biomaterial into a form-retaining block. The block has a form-proof consistency, with light elasticity to adapt it to the recipient site.

One of the aims of this study was to investigate the volumetric stability of the matrix during healing and the possible collapse of the scaffold, endangering the space between the graft particles. A stable matrix would preserve the space between the scaffold particles, allowing cell and vascularization ingrowth from the surrounding tissue. In the literature, scarce data are reported about the early resorption rate of augmented sites after GBR. A resorption rate of 50% for cancellous allografts and collagen membranes after 6 months was described in a multicentre prospective clinical trial (Sterio, Katancik, Blanchard, Xenoudi, & Mealey, 2013). Another study showed a resorption of 37% for a 60:40 DBBM and autogenous bone mixture after 7.5 months (Mordenfeld, Johansson, Albrektsson, & Hallman, 2014). However, neither study fixed the membrane. Studies using fixation reported more favourable results. A resorption rate around 12% was described with a composite graft with collagen or non-resorbable titanium-reinforced membranes (Gultekin, Cansiz, & Borahan, 2017), whereas another study with a composite graft and titanium mesh reported 15.1% (Proussaefs & Lozada, 2006). Similar to these results, in the present study, the mean volumetric resorption rate of the graft was 15.6%. The limited resorption rate indicates that the integrity of the block was maintained during the integration process. The composite graft and the L-PRF Block are made from a mixture of a particulated graft material with autogenous bone or with L-PRF and liquid fibrinogen, respectively. The most important difference between the two techniques is that for the L-PRF Block no autologous bone has to be harvested from a second site.

The initial composition of the block was estimated visually to be 50:50, but after adding liquid fibrinogen, it seems to be 60:40, as analysed with micro-CT. It has already been demonstrated that DBBM has a very slow resorption rate (Galindo-Moreno et al., 2013), which means that after healing (T2) most of the DBBM particles will still be in place, filling the initial 40% of the graft composition. Considering that the average graft resorption was around 15%, it could be interpreted that the remaining 45% of the healed graft is generated from the L-PRF. L-PRF cannot survive by itself. It integrates and differentiates according to the surrounding tissues (Dohan Ehrenfest et al., 2009). Therefore, it is plausible to assume (also based on the high density observed on the T2 CBCTs) that it has been replaced by osseous tissue. Again, histological analysis will be needed to confirm this hypothesis. An ongoing study will histologically evaluate the L-PRF Block.

In the present study, one patient showed partial wound closure failure, with a soft tissue dehiscence of 2 mm. Exposure of the membrane is reported in the literature as a common complication in bone augmentation procedures (Machtei, 2001; Soldatos et al., 2017). In our clinical experience, it seemed beneficial to cover the augmented site with L-PRF to protect the collagen membrane and prevent contamination of the graft. L-PRF acts as a barrier and, in time, integrates with the surrounding soft tissues.

Within the limitations of this clinical innovation report, the L-PRF Block seems a successful new protocol for horizontal alveolar bone augmentation. This procedure is safe, predictable, with a high feasibility and a low morbidity. This is the first study to report this kind of results without the use of autogenous bone (particulated or blocks).

The regenerated bone allowed implant placement in all the cases, with only slight resorption of the graft. However, the stability of the newly formed hard tissue should be histologically estimated and needs further investigation. Therefore, randomized controlled clinical trials and histological analysis are necessary to confirm these results.

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CONFLICT OF INTEREST

The authors declare no competing interests related to this study.

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