Effect of different platelet rich fibrin matrices for ridge preservation in multiple tooth extractions: a split-mouth randomized controlled clinical trial

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Conflict of interest and founding

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Abstract

Aim: To evaluate dimensional changes in the alveolar ridge and bone structure after tooth extraction when L-PRF or A-PRF+ were used in comparison to unassisted socket healing.

Materials and Methods: Twenty patients in need of at least three tooth extractions in the aesthetic zone were included. L-PRF, A-PRF+ or control were randomly assigned, leaving one empty socket/edentulous site between conditions. CBCT scans were obtained immediately after tooth extraction and after 3 months of healing. Horizontal and vertical dimensional changes of the ridge and socket fill were calculated. Histological and micro-CT analysis of bone biopsies were used to evaluate post-surgical bone structural healing.

Results: Mean horizontal and vertical changes at 1-mm below the crest (buccal and palatal side) were similar for the three sites ($p>0.05$). For the socket fill, L-PRF (85.2%) and A-PRF+ (83.8%) showed superior values than the control (67.9%). The histological and radiological analysis reported more newly formed bone for the PRF groups, without any significant differences between both.

Conclusions: PRF matrices failed to reduce the dimensional changes after multiple tooth extractions in the premaxilla. After 3-months healing, both PRF matrices showed radiographically a significant superiority for the socket fill. Histologically, they seemed to enhance new bone formation.

Key words: CBCT analysis, histology, multiple tooth extractions, platelet rich fibrin, ridge preservation.

Clinical Relevance

Scientific rationale for study: Differences in biological characteristics between platelet rich fibrin (PRF) matrices have been envisaged but their clinical relevance in alveolar ridge preservation (ARP) still needs to be demonstrated.

Principal findings: PRF matrices used for ARP failed to counteract the ridge resorption that occurs after multiple extractions in the anterior maxilla. However, after 3 months of healing, both PRF constructs showed more socket fill and newly formed bone than an unassisted socket.

Practical implications: ARP techniques in multiple tooth extractions when an immediately mucosa-supported prosthesis is also used might jeopardize the final result.

Introduction

The alteration of the hard and soft tissue contour after tooth extraction has been extensively studied (Araujo & Lindhe, 2005; Cardaropoli, Araujo, & Lindhe, 2003; Schropp, Wenzel, Kostopoulos, & Karring, 2003). According to a systematic review of Tan and colleagues (2012), the mean horizontal bone resorption after 6 months was 3.8 ± 0.2 mm at the crest. The vertical resorption was extended to 11-22% after 6 months, whereas the horizontal resorption ranged from 29 to 63%. However, the extension of alveolar bone resorption may vary dependig on tooth type as obseved by Couso-Queiruga and co-workers (2021) (Couso-Queiruga, Stuhr, Tattan, Chambrone, & Avila-Ortiz, 2021) with a higher radiological horizontal bone resorption in molar sites vs non-molar sites (3.61 vs 2.54 mm). An additional bone loss of 2.4 mm in width and of 1.1 mm in height has been reported in sites with compromised buccal bone wall (García-González et al., 2020). Interestingly, these numbers only comprise single tooth extractions, making the additional ridge resorption in cases with multiple extractions less quantifiyable. Consequently, bone and/or soft tissue augmentation procedures would be necessary to overcome the bone deficiency.

Different surgical techniques have been described to overcome this resorption process (Barootchi et al., 2019; Troiano et al., 2018; Vignoletti et al., 2012). Even though different treatments have been attempted to prevent this, studies failed to show a technique that totally compensates that event (Chappuis, Araujo, & Buser, 2017). The use of grafting biomaterials into extraction sockets has been intensively studied (Barone et al., 2017; Botticelli, Berglundh, & Lindhe, 2004; Discepoli et al., 2015; Iorio-Siciliano et al., 2020; Vignoletti & Sanz, 2014). In a recent systematic review with meta-analysis (Avila-Ortiz, Chambrone, & Vignoletti, 2019), alveolar ridge preservation (ARP) procedures were found to be effective in limiting physiologic ridge reduction compared to natural healing. However, a certain degree of bone resorption was detected even if an ARP was used. The effect of ARP procedures is unpredictable, probably due to the influence of local and systemic factors, which are not yet fully understood.

Biological additives, for instance platelet concentrates, have also been proposed as adjunctive for bone regeneration. A second generation of platelet concentrates, leukocyte and platelet rich fibrin (L-PRF) was introduced to eliminate the drawbacks of the first generation (Dohan et al., 2006). L-PRF is a 100% autologous fibrin matrix containing platelets and leucocytes obtained after blood centrifugation without anticoagulants at high spin. The three-dimensional fibrin matrix in L-PRF serves as a scaffold for the cells entrapped in it but also for the growth factors produced by these cells, resulting in a slow and gradual releasing rate (Castro et al., 2019; Hachim, Whittaker, Kim, & Stevens, 2019). Moreover, the absence of bone substitute remnants has been also seen as advantage for the use for this biomaterial for ARP (Dragonas et al., 2019; Temmerman et al., 2016). It

has been shown that the use of L-PRF accelerates neoangiogenesis (Ratajczak et al., 2018), stimulates the local environment for differentiation and proliferation of surrounding cells (Dohan Ehrenfest, Doglioli, de Peppo, Del Corso, & Charrier, 2010), and continuously releases growth factors over a period of 7-14 days (Schär, Diaz-Romero, Kohl, Zumstein, & Nesic, 2015).

Recently, new PRF protocols [advanced platelet rich fibrin (A-PRF), and advanced platelet rich fibrin+ (A-PRF+)] have been proposed reducing the g force and duration of the centrifugation (El Bagdadi et al., 2017; Ghanaati et al., 2014). By reducing the relative centrifugal force, an increase in the release of growth factors and in the concentration of leucocytes and platelets was envisaged. However, the clinical relevance of these differences still needs to be demonstrated.

The aim of this study was to evaluate the dimensional changes in the alveolar ridge after tooth extraction when L-PRF or A-PRF+ were used in comparison to unassisted socket healing (control). Primary outcome variables were defined as the changes in horizontal width at crest-1 mm levels. Secondary outcome variables were established as the changes in horizontal width at crest-3 mm and -5 mm, and vertical resorption at the buccal and palatal side. The socket fill was defined as tertiary outcome variable. Finally, the % of bone volume/tissue volume (BV/TV) and bone microstructure were considered as quaternary outcome.

Material and Methods

The study was approved by the Ethical Committee of the KU Leuven, UZ Leuven University Hospitals (S-57938, B322201525149) and conducted in accordance with the requirements of the Helsinki Declaration of 1975 (revised in 2008) and with the CONSORT statements (Moher et al., 2012) (www.consort-statement.org). The study was registered in Clinicaltrials.gov with the number NCT03268512.

Study population

From August 2015 to October 2018, patients in the need of at least three tooth extractions in the aesthetic region (premaxilla, single-rooted teeth) were evaluated for initial study eligibility at the Department of Periodontology (University Hospitals Leuven, Belgium). Patients fulfilling all criteria were invited to participate in the study and provided written informed consent prior to inclusion (Table S1).

L-PRF and A-PRF+ preparation

Eight blood tubes per participant were collected prior to any treatment. Four sterile 9-ml silica-coated plastic tubes without anticoagulant (BVBCTP-2, Intra-Spin, Intra-Lock, Florida, USA) were used to prepare L-PRF, and another 4 sterile 10-ml glass tubes without anticoagulant (DUO, Nice, France) for A-PRF+. These were immediately centrifuged at 2,700 rpm (RCF_{clot}: 408 *g*) for 12 min to prepare L-PRF (IntraSpin, Intra-Lock, Florida, USA) (Temmerman et al., 2016) and at 1,300 rpm (RCF_{clot}: 145 *g*) for 8 min (DUO Process, Nice, France) to prepare A-PRF+ (Fujioka-Kobayashi et al., 2017). After centrifugation, each L-PRF/A-PRF+ clot was collected from the tube and carefully separated from the red blood cells with a spatula. All L-PRF/A-PRF+ clots were then transformed into 1-mm thick membranes by gentle compression using especially designed boxes for each protocol (Xpression box, Intra-Lock, Florida, USA or A-PRF+ box, DUO Process, Nice, France).

Surgical procedure

Tooth extractions were performed under local anaesthesia and sterile conditions with a flapless approach. After extraction, the sockets were carefully cleaned and randomized as control or test site by means of a computer program (Research Randomizer, version 4.0). L-PRF, A-PRF+ or control were randomly assigned, leaving one empty socket/edentulous site between conditions. The position of the extraction sites is presented in Figure 1S. At the test sites (L-PRF or A-PRF+) 2-3 membranes, depending on the size of the socket, were inserted and compressed with an amalgam plunger. A 3-4 mm full-thickness envelope was created at the buccal and palatal side to create space for 1-2 folded membrane placed into this envelope to seal the socket. A crossed horizontal mattress

suture (Vicryl 4.0, Ethicon™, Johnsson & Johnsson®, New Jersey, USA) was used to stabilize the L-PRF/A-PRF+ without any attempt for primary wound closure, followed by individual sutures for better adaptation of the material when needed. At the control site, a cross-suture was applied to stabilize the coagulum (Figure 1). Patients were unaware of the allocation of each treatment option. Immediately after tooth extractions, a first cone beam-CT (CBCT) (T0) was taken in order to record the baseline conditions.

All patients were asked to take an analgesic three times a day (Paracetamol 1 g) for 3 days, and use of an antiseptic mouth rinse (Perio-Aid™ 0.12%, Dentaid®, Barcelona, Spain). All patients received an immediate prosthesis and were advised not to remove it the first 24 hours after surgery.

Follow-up

One week after tooth extraction, patients were scheduled for suture removal and the prosthesis was adapted. After three months of healing, a second CBCT (T1) was taken (Figure 2). This second CBCT was also used for the planning of the implant surgery when desired.

Sample size calculation

The minimum required sample size was estimated using the results of a previous study comparing L-PRF and unassisted healing for ARP (Temmerman et al., 2016). The power sample size was calculated to detect a difference in horizontal bone resorption of 15-20% amongst treatments and control. A power analysis in G*Power suggested a sample size of 16 participants for a split-mouth design with three groups (L-PRF, A-PRF+, control) assuming 90% power with an α=0.05. The sample size was, however, increased to 21 patients due to a potential drop-out during follow-up.

CBCT acquisition

To assess the alterations in the alveolar ridge, CBCT was taken immediately after tooth extraction (T0) and another after a 3 months' interval (T1) using a NewTom VGi evo CBCT (Cefla, Imola, Italy). Clinical scanning protocol was fixed to a 10x10 cm field-of-view, a voxel size of 200 mm, 360°rotation, at 110kVp and using tube current modulation, which adapts emission according to the patient and thus eliminates any risk of exposure to an unnecessarily high dose (Stratis et al., 2017) (Stratis, Zhang, Jacobs, Bogaerts, & Bosmans, 2019).

Radiological analysis

Both post-operative CBCT scans were spatially aligned using a rigid computer-assisted registration (MeVisLab, MeVis Medical Solutions AG, Bremen, Germany) based on selected areas of the dataset where no anatomical changes had taken place during follow-up (Van Dessel et al., 2016)

(Van Dessel et al., 2017). Data from each patient were encoded and all measurements were performed blinded without knowing treatment allocation.

2D- Analysis

The matched post-operative CBCT scans were imported into Fiji software (Schindelin et al., 2012) and measurements were performed using the same reference points and lines according to Temmerman and co-workers (Temmerman et al., 2016). The following distances were measured from the centre of the socket according to Jung and co-workers (Jung et al., 2013) and Temmerman and co-workers (Temmerman et al., 2016) (Figure 3):

- the thickness of the buccal bone at baseline (T0) at 1, 3, and 5 mm below the crest
- the horizontal ridge width at crest-1mm (HW-1mm), crest-3mm (HW-3mm), and crest-5mm (HW-5mm) at the buccal/palatal side, in millimeters and later transformed to percentages
- the vertical resorption on both buccal and palatal side, in millimeters
- the socket fill defined as the highest point of viewable mineralized bone at the middle of the socket; absolute values (in mm) and percentages were calculated by comparing the initial depth of the socket and the depth after three months of healing

Biopsy collection

When patients wanted replacement of the missing teeth, implant surgery was planned. During implant surgery, a bone core biopsy was collected from each preserved socket with a 2.0 mm trephine bur (3 samples per patient, 1 bone core per preserved socket). In the case of an edentulous ridge, a customized stent was prepared from a dental cast before extractions as reference to determine the centre of the initial socket. After collection, the biopsies were immediately frozen in liquid nitrogen and kept at -80°C.

Micro-CT scanning

Micro-CT scanning of bone biopsies was performed on a SkyScan 1172 high-resolution desktop system (Bruker, Kontich, Belgium). The 3D bone (micro)structure was evaluated in terms of ratio between bone volume (newly formed bone) and total tissue volume (%BV/TV), trabecular thickness, trabecular number, and trabecular separation. Data from each patient was encoded and all measurements were performed blinded for the treatment allocation.

Histological processing and morphometric analysis

After the micro-CT scanning, bone biopsies were fixed in 4% paraformaldehyde and decalcified in 0.5M EDTA (pH 7.4)/phosphate buffered-saline at 4°C for 14 days, followed by dehydration and embedding in paraffin. Three serial sections (4-µm) were made starting from the

central region of the sample and perpendicular towards the long axis, and stained with haematoxylin and eosin for general morphological analysis.

Histological sections were captured by an automated slice scanner (Axioscan, Carl Zeiss, Oberkochen, Gemany), matched with the corresponding 2D micro-CT slices, and further processed in CTAn (Bruker). Newly formed bone was automatically determined within the same region of interest for micro-CT and histological coupes and bone volume fraction (%BV/TV) was calculated. Data from each patient was encoded and all measurements were performed blinded for the treatment allocation.

Statistical analysis

Treatments were compared by creating a linear mixed model with patient as random factor and treatment as fixed factor was applied. Differences between treatments were corrected for simultaneous hypothesis testing according to Tukey. Descriptive analyses expressed data as mean values with standard deviations. All data were tested for normality using the Shapiro-Wilk test. A second mixed-model was constructed for histological and micro-CT analysis. Non-parametric comparisons between groups (Kruskal-Wallis, pairwise with automatic Bonferroni correction) were used to explore significant interaction effects. Significant differences were noted a $p < 0.05$.

Results

Demographic data

Twenty-one patients were included in this study (15 women, 6 men) (Table S1). Three patients were smokers of <10 cig/day. One patient did not return for the second CBCT after 3 months of healing, so he was excluded from the study (drop-out= 1). All sockets healed uneventfully. A total of 60 teeth in the premaxilla were included for analysis (central incisors: 25, lateral incisors: 16, canines: 19). The CONSORT flow chart is shown in Figure S1. Five out of the 20 included patients asked for teeth replacement with dental implants. Those patients underwent implant surgery and bone samples could be collected from the preserved sites (Table S2). No additional bone augmentation during implant surgery was needed at any locations. The rest of the participants were provided with a definitive and well-adapted full denture.

Radiological analysis

Thickness buccal bone

The mean thickness of the buccal plate at baseline at 1 mm below the crest was 1.1 ± 0.3 mm, 0.9 ± 0.3 mm, and 1.1 ± 0.4 mm for L-PRF, A-PRF+, and control sites, respectively. Overall, no

statistically significant differences could be found at any level below the crest amongst groups (p>0.05). Data is shown in Table S3.

Horizontal resorption

The mean ridge width differences between baseline (immediately after extraction) and three months of healing were measured at three levels below the crest (HW-1mm, HW-3mm, and HW-5mm) on both the buccal and palatal sides (Table 1).

Buccally:

No statistically significant differences at any of the levels below the crest (HW-1mm, HW-3mm, and HW-5mm) were observed amongst groups (p>0.05). For the L-PRF, A-PRF+, and unassisted sites, the values 1 mm below the crest were -1.6 ± 0.8 mm, -1.6 ± 0.7 mm, -1.7 ± 1.0, respectively.

Palatally:

At the palatal side, a similar horizontal bone resorption was observed for all groups (L-PRF: -0.6 ± 0.7 mm, A-PRF+: -0.6 ± 0.8 mm, control: 0.5 ± 0.7 mm). No statistically significant differences at any of the levels below the crest (HW-1mm, HW-3mm, and HW-5mm) were seen amongst groups (p > 0.05).

Changes in the total ridge (width)

The overall mean changes in the width at crest-1 mm were -28.1% ± 13.5, -28.1% ± 11.8, and -26.4% ± 12.3 for L-PRF, A-PRF+, and control, respectively. No statistical significant differences were reached amongst groups (p>0.05) at any level below the crest (Table 2). Bone resorption was less pronounced in all groups towards the apical part (crest-1mm > crest-3mm > crest-5mm).

The fact that one socket had an unassisted socket at one or both sides or an edentulous ridge or a remnant tooth did not influence the alveolar bone resorption. No statistically significant differences could be found between positions (p>0.05).

Vertical resorption

The mean vertical height changes at the buccal side were 0.2 ± 1.2 mm, 0.2 ± 1.1 mm, and -0.2 ± 0.8 mm for L-PRF, A-PRF+, and control, respectively (Table 1). Differences amongst groups were however not statistically significant (L-PRF vs A-PRF+ p=0.9, tests vs control p=0.3). The mean vertical height changes at the palatal aspect were -1.1 ± 0.9 mm, -1.0 ± 0.8 mm, and -1.0 ± 0.9 mm, for L-PRF sites, A-PRF+ sites, and control sites, respectively. Also here, no statistical differences were reached (p=0.8).

Socket Fill

Statistically significant differences were found for the percentage of socket fill between L-PRF (85.2% ± 22.9) vs. control (67.9% ± 19.2) (p=0.005), and A-PRF+ (83.8% ± 18.4) vs. control (67.9% ± 19.2) (p=0.01) (Table 3).

Morphometrical bone analysis

Histological evaluation

L-PRF and A-PRF+ presented a mean %BV/TV of 47.7 ± 7.9% and 54.5 ± 5.6%, respectively (Figure 4). No statistical significant differences could be found between test groups (p>0.05). Both test groups showed statistically significant more %BV/TV than the control group (34.7 ± 6.9%) (p<0.05).

Micro-CT: 2D and 3D analysis

For the 2D and 3D analysis of %BV/TV, no statistical significant differences could be found between test groups (p=0.18 and p=0.71, respectively). When compared to the control group, A-PRF+ showed statistical significant higher newly formed bone in both 2D and 3D analysis (p<0.001 and p=0.04, respectively), whereas the differences between L-PRF and control did not reach the significance (p=0.09 and p=0.64) (Figure 4 and S2).

Discussion

The present split-mouth randomized clinical trial analysed the effect of two different platelet concentrates derivatives (L-PRF and A-PRF+) on ridge preservation after tooth extraction in comparison to unassisted socket healing (blood clot). In the CBCT analysis, no statistically significant differences in the horizontal and vertical dimension could be observed amongst the 3 groups after 3 months of healing. However, both PRF matrices showed radiographically on the CBCT measurements a significant superiority for the socket fill. Histologically, PRF matrices seemed to promote higher percentage of newly formed bone in comparison to the control, after a 3-months healing period.

Over the last 20 years, the global burden of complete edentulism has diminished on average. However, in contrast to high-income countries where the prevalence of edentulism is decreasing, an opposite trend is observed in low- and middle-income countries, mainly as the result of increments in periodontal diseases and caries (Kassebaum et al., 2014; Tyrovolas et al., 2016). Campbell and co-workers (Campbell, 1960) reported that edentulous patients wearing dentures had, on average, smaller residual ridges than those not wearing dentures. Increased ridge resorption was attributed to

the pressure form the prostheses. Similar conclusions had been stated in the literature (Ozan et al., 2013; Wyatt, 1998).

The fact that in this study the resorption of both the buccal and palatal side barely differed amongst groups, and that the vertical bone resorption at the palatal side was more extensive than at the buccal side led us to hypothesize that the use of the full prostheses may have act as a co-founding factor, masking the effect of the platelet rich fibrin (PRF) matrices. Recently it has been hypothesized that L-PRF membranes might have the capacity to supress the catabolic events that are caused by osteoclastic bone resorption, but that they cannot reverse the process once osteoclastogenesis has started (Kargarpour et al., 2020; Strauss, Nasirzade, Kargarpoor, Stähli, & Gruber, 2020). After tooth extraction, osteoclasts start to resorb the bundle bone (Araujo & Lindhe, 2005; Trombelli et al., 2008). Osteoclastic activity can be intensified by different factors such as mechanical pressure. In the present study, all patients enrolled were provided with a full immediate removable prosthesis. Mechanical pressure transmitted continuously and/or intermittently through the prosthesis has been considered one of the causative factors for bone resorption in denture-supporting tissues (Lytle, 1959; Sato, Hara, Mori, Shirai, & Minagi, 1998). Moreover, Alrajhi and co-workers (Alrajhi, Askar, Habib, & Elsyad, 2020) concluded that anterior maxillary areas had higher bone loss compared to posterior areas. One needs to keep in mind that this is a common protocol in daily practice after multiple tooth extractions. Consequently, ARP techniques in multiple tooth extractions when a mucosa-supported prosthesis is also used might not have the same results as in single tooth extractions. However, the evidence around the bone resorption pattern after multiple tooth extraction is scarce, what makes the comparison of our results with others, at this moment, not possible.

In single tooth extraction studies where no provisional mucosa-supported prostheses were provided, the use of PRF matrices showed promising results in the preservation of the alveolar ridge (Castro et al., 2017; Strauss, Stähli, & Gruber, 2018). Temmerman and co-workers (Temmerman et al., 2016) reported a mean change in horizontal dimension at 1 mm below the crest of 1.4 mm (23%) and 5.0 mm (51%) for L-PRF group and control group, respectively. A recent study (Canellas et al., 2020) showed a mean change in width at 1 mm below the crest of 0.9 mm and 2.2 mm for L-PRF group and control group, respectively. Similar benefits have been reported in the literature (Alzahrani, Murriky, & Shafik, 2017; Anwandter et al., 2016; Hauser et al., 2013; Zhang et al., 2018). However, the outcome seems to be very technique-sensitive (Areewong, Chantaramungkorn, & Khongkhunthian, 2019; Suttapreyasri & Leepong, 2013).

Another hypothesis that might contribute to explain the findings of this article is related to the resistance of the L-PRF membranes to external forces and their stability under certain conditions. Angiogenesis is a delicate process driven by surrounding tissue's need for oxygen and nutrients,

which incites production of vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), and other pro-angiogenic stimuli (Eelen et al., 2018). Recently, Ratajczak and co-workers (Ratajczak et al., 2018) described the angiogenic potential of L-PRF in an *in-vitro* study by inducing key steps of the angiogenic process such as endothelial proliferation, migration, and tube formation. They also demonstrated that L-PRF was able to induce blood vessel formation *in vivo* with a chorioallantoic membrane assay. However, all this processes require wound stability to allow vessel sprouting to occur (Ghiasi, Chen, Vaziri, Rodriguez, & Nazarian, 2017). In the present study, the use of an immediate prosthesis may have also jeopardized the angiogenic capacity of the L-PRF and A-PRF+ by decreasing their stability inside the socket.

Hard and soft tissue alterations after tooth extraction have been well documented by Cardaropoli and co-workers (Cardaropoli et al., 2003). Healing of an extraction socket was characterized by a sequence of histological events, where the buccal bone was more extensively resorbed than the palatal/lingual plate (Araujo & Lindhe, 2005). This can be explained by the fact that the vestibular bone plates are generally thinner (Araújo, Silva, Misawa, & Sukekava, 2015). In the present study, there were no differences in width of the buccal bony plates amongst test and control groups and they were in average around 1 mm thick for all groups. Given the mean values reported in literature for the aesthetic zone (0.8 ± 0.4 mm) (Huynh-Ba et al., 2010), we can consider that the buccal bone plates in our study were rather thick.

Socket fill was found to statistically differ between both PRF matrices and the control group in this present study (L-PRF: 85%, A-PRF+: 83%, control: 67%). These results are similar from those already reported in the literature for single tooth extractions (Alzahrani et al., 2017; Temmerman et al., 2016). So despite the limited effect on the horizontal dimension, the use of both L-PRF and A-PRF+ seemed to partially counteract bone resorption in the vertical dimension (mean bone gain of 0.2 mm) and showed radiographically significant greater socket fill in comparison to the control. Both parameters are crucial when considering the replacement of the missing tooth by an oral implant.

The biological characteristics of L-PRF and A-PRF+ have been extensively evaluated in vitro studies (Dohan Ehrenfest et al., 2018; Fujioka-Kobayashi et al., 2017; Pitzurra, Jansen, de Vries, Hoogenkamp, & Loos, 2020). The A-PRF+ protocol reduced the *g* force and the duration of the centrifugation. Some studies have reported an increase in growth factors release from A-PRF+ clots as well as a more homogenous cellular distribution throughout the membranes (El Bagdadi et al., 2017, Ghanaati et al., 2014). However, no differences could be found in the present study between L-PRF and A-PRF+ groups. It is of utmost importance to understand the properties of the biomaterials that are currently used in the clinical setting. However, it should be stressed that anatomical, functional and host factors may have a strong influence in bone resorption/regeneration patterns. Therefore, care should be taken when extrapolating in vitro results to the clinical practice.

In bone structural analysis, the present study showed more newly formed bone for both L-PRF and A-PRF+ (around 50%) compared to the control group. However, these results need to be interpreted with caution given the limited sample size (n=5). Limited evidence is reported in the literature on the qualitative/quantitative histological and radiological analysis after the use of these biomaterials in ARP techniques. For instance, Canellas and co-workers published higher values of %BV/TV for L-PRF compared to a control (L-PRF: 55.9 ± 11.9%, control: 36.7 ± 11.1%) in a histomorphometrical analysis (Canellas et al., 2020). They reported similar percentages in terms of newly formed bone as the ones presented in the current study. On a radiological analysis with micro-CT, Hauser and co-workers (2013) (Hauser et al., 2013) also observed a tendency for higher value %BV/TV in the L-PRF group (+12.9%) compared to the control. Analysis of the trabecular morphology also revealed the superiority of L-PRF versus the control group, although in that study trabecular thickness did not reach statistical significance.

Clinicians should be aware of the limitations in the present study when using PRF matrices in multiple tooth extractions with an immediate denture. However, up to now there is no evidence about the use of PRF matrices in multiple extractions without the mechanical influence of a dental prosthesis. Moreover, one does not have to forget the trauma and post-operative pain after multiple tooth extractions. The use of L-PRF may reduce patient's discomfort and fasten soft tissue healing (de Almeida Barros Mourão, de Mello-Machado, Javid, & Moraschini, 2020; Dragonas et al., 2019).

Conclusions

Within the limitations of this study, it can be concluded that PRF matrices used for ARP failed to counteract ridge resorption that occurs after multiple extractions in the anterior maxilla after 3 months of healing. However, both PRF matrices showed radiographically a significant superiority for the socket fill compared to an unassisted socket. Histologically, they seemed to enhance new bone formation. ARP techniques in multiple tooth extractions with the use of immediately mucosa-supported prosthesis might jeopardize the final result.

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Tables

Table 1. Dimensional changes at the buccal and palatal site after three months of healing for L-PRF group, A-PRF+ group, and control group. Horizontal width reduction measured at 1 mm (HW-1 mm), 3 mm (HW-3 mm), and 5 mm (HW-5 mm) below the crest. Vertical changes measured in the middle of the extraction socket both buccally and palatally. *sd*: standard deviation. Values are shown in millimetres.

	L-PRF			**A-PRF+**			**Control**		
Buccal									
	mean	median	*sd*	mean	median	*sd*	mean	median	*sd*
HW-1 mm	-1.6	-1.5	*0.8*	-1.6	-1.5	*0.7*	-1.7	-1.6	*1.0*
HW-3 mm	-1.5	-1.4	*0.8*	-1.2	-1.1	*0.6*	-1.4	-1.5	*0.8*
HW-5 mm	-1.0	-1.0	*0.7*	-0.9	-0.8	*0.6*	-1.0	-1.0	*0.6*
Vertical	0.2	0.3	*1.2*	0.2	0.1	*1.1*	-0.2	-0.2	*0.8*
Palatal									
	mean	median	*sd*	mean	median	*sd*	mean	median	*sd*
HW-1 mm	-0.6	-0.5	*0.7*	-0.6	-0.3	*0.8*	-0.5	-0.3	*0.7*
HW-3 mm	-0.4	-0.4	*0.4*	-0.4	-0.2	*0.7*	-0.3	-0.2	*0.4*
HW-5 mm	-0.2	0.0	*0.4*	-0.3	0.0	*0.6*	-0.4	-0.1	*0.6*
Vertical	-1.1	-0.9	*0.9*	-1.0	-1.0	*0.8*	-1.0	-0.9	*0.9*

Table 2. Total horizontal width (HW) reduction for L-PRF group, A-PRF+ group, and control group. Measurements performed at 1 mm, 3 mm, and 5 mm below the crest. Values are shown in millimetres (mm) and in percentages (%). HW-1mm: horizontal width reduction at 1 mm below the crest; HW-3mm: horizontal width reduction at 3 mm below the crest; HW-5mm: horizontal width reduction at 5 mm below the crest; *sd*: standard deviation.

	L-PRF		**A-PRF+**		**CONTROL**	
Millimeters (mm)						
	mean	*sd*	mean	*sd*	mean	*sd*
HW-1 mm	-2.2	*1.0*	-2.2	*0.9*	-2.2	*1.1*
HW-3 mm	-1.8	*-1.7*	-1.6	*0.9*	-1.7	*0.8*
HW-5 mm	-1.2	*0.8*	-1.2	*0.8*	-1.4	*0.8*
Percentage %						
	mean	*sd*	mean	*sd*	mean	*sd*
HW-1 mm	*-28.1*	*13.5*	*-28.1*	*11.8*	-26.4	*12.3*
HW-3 mm	*-22.2*	*9.7*	*-19.4*	*10.1*	-20.8	*9.0*
HW-5 mm	*-14.4*	*10.1*	*-14.6*	*9.6*	-16.3	*8.1*

Table 3. Socket fill measurements in L-PRF group, A-PRF+ group, and control group. Values are shown in millimetres (mm) and in percentages (%). *: statistically significant; *sd*: standard deviation.

	L-PRF			**A-PRF+**			**Control**			***p-values***		
	mean	median	*sd*	mean	median	*sd*	mean	median	*sd*	*L-PRF vs. A-PRF+*	*L-PRF vs. control*	*A-PRF+ vs. control*
Socket fill (%)	85.2	86.5	*22.9*	83.8	88.9	*18.4*	67.9	71.8	*19.2*	*0.9*	*<0.005*	*0.01*
Socket fill (mm)	7.0	6.7	3.0	7.0	6.6	2.7	5.4	5.1	2.3	0.9	<0.05	<0.05

Figures

Figure 1. Surgical procedure for ridge preservation. Occlusal view of the upper jaw after tooth extraction (A) L-PRF clots (B1) and A-PRF+ clots (B2) before compression into membranes. Envelope preparation for socket sealing by insertion of the L-PRF (C1) and A-PRF+ (C2) membranes. Placement of the L-PRF membranes (D1) and A-PRF membranes (D2) into the sockets. Modified horizontal mattress suture to keep the L-PRF (E1) and A-PRF+ (E2) membranes in place without intention of primary closure.

Figure 2. Representative cone-beam computed tomography of one patient at baseline (immediately after tooth extraction, T0) and after 3 months of healing (T1). (A1): CBCT T0 L-PRF group, (A2): CBCT T1 L-PRF group, (B1): CBCT T0 A-PRF+ group, (B2): CBCT T1 A-PRF+ group, (C1): CBCT T0 control group, (C2): CBCT T1 control group.

Figure 3. Linear measurements of the sockets. (A) The middle of the socket was determined at the axial view of the CBCT (T0) based on the width of each socket. A 90° line was drawn in the middle of the socket defining the cross sectional slide where the rest of the measurements were executed. (B) Cross-sectional view of a socket after tooth extraction. The apex of the extraction socket at T0 was marked, and a vertical reference line passing the apex in the centre of the socket was also drawn. Perpendicular to the vertical reference, a horizontal reference line was defined at the level of the crest, buccal and palatal HW-1 mm, HW-3 mm, HW-5 mm are representing the measurements performed at three levels below the bone crest. The depth of the socket was measured as the deepest point of the socket to the bone crest. (C1) Measurement of the initial socket depth at the middle of the socket (vertical yellow line), perpendicular to the crest. (C2) Final socket depth by measuring the distance between the bone crest and the highest bone level at the middle of the socket (blue dotted line). Socket fill is calculated by comparing the initial depth of the socket and the depth after three months of healing.

Figure 4. Histological and micro-CT 2D analysis of the bone samples. (A) L-PRF group; (B) A-PRF+ group; (C) control group, and (D) summary of results. (1) haematoxylin and eosin staining overview, (2) segmentation of bone (green) vs. soft tissue (blue), (3) micro-CT reconstruction. *sd*: standard deviation.

Supplementary information

Table S1. Inclusion and exclusion criteria. ASA-score: American Society of Anesthesiologist (ASA)-score. CI: central incisor, LI: lateral incisor, C: canine. Extracted teeth in () after drop-out.

Table S2. Distribution of implant placement and biopsy collection. The locations where an implant was placed in the same position of a preserved socket are marked in bold. Pat: patient, GBR: guided bone regeneration.

Table S3. Baseline radiographic measurements of the thickness of the buccal bone plate. *sd*: standard deviation. No statistically significant differences were computed amongst groups ($p>0.05$).

Figure S1. CONSORT flow diagram of the progress of the study (randomized controlled clinical trial). n= number of patients.

Figure S2. Micro-CT 3-D analysis of the bone samples. (A) L-PRF group; (B) A-PRF+ group; (C) control group (unassisted healing), and (D) summary of results. In the 3D reconstructions from the micro-CT, soft tissue is colored in blue, and bone in green. Tb.Th: Trabecular thickness, Tb.N: trabecular number, and Th.Sp: trabecular separation.