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Effects of Leukocyte-Platelet-Rich Fibrin (L–PRF) on Pain, Soft Tissue Healing, Growth Factors, and Cytokines after Third Molar Extraction: A Randomized, Split-Mouth, Double-Blinded Clinical Trial

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Abstract: This study assessed the effects of leukocyte-platelet-rich fibrin (L–PRF) on soft tissue healing and the correlation with the local concentration of growth factors (GF) and cytokines in the dental socket of lower third molars. Forty lower-third molars (20 participants) were included in this randomized, double-blinded, split-mouth study. After extractions, randomized sides received alveolar filling with L–PRF on one side and a natural blood clot on the other side. The pain was assessed for up to seven days and soft tissue healing (Landry index) for 14 days post-extraction. Swabs were collected from the surgical sites for GF and cytokine assessment by flow luminometry. Participants reported lower postoperative pain on the sides grafted with L–PRF, which also presented increased tissue healing scores ($p < 0.05$). There were increased levels of all GFs and several cytokines at the L–PRF site at day one, while vascular endothelial growth factor (VEGF), IL–10, and IL1–RA remained higher throughout for seven days ($p < 0.05$). VEGF concentration at L–PRF sites correlated positively with the participants' blood platelet content ($\rho = 0.769$). PDGF correlated negatively with pain experience on days 2 and 3, and positively with soft tissue healing scores, while FGFb presented a weak correlation with a reduction of pain on day 3. The use of L–PRF improves the soft tissue healing process and decreases postoperative pain after the third molar extractions, which correlates with an increase in the local concentration of growth factors such as PDGF and FGFb.

Keywords: platelet-rich fibrin; third molar; clinical trial; growth factors; cytokines

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1. Introduction

Lower third molar tooth extraction is one of the most common surgeries performed by dental surgeons in the daily clinic. However, some postoperative complications, such as pain, trismus, swelling, and alveolitis, are possible after this procedure [1–3]. Therefore, different strategies have been developed to reduce the risk of these complications and improve tissue healing.

One of the current strategies is the implantation of the leukocyte-platelet-rich fibrin (L–PRF) immediately after tooth extraction. Prior studies have demonstrated the effectiveness of this autologous biomaterial in reducing postoperative complications in third molars [4,5]. This second-generation autologous blood-derived biomaterial contains a dense fibrin mesh with increased contents of platelets and leukocytes, as well as increased concentrations of essential growth factors and cytokines that will assist in tissue repair, especially concerning soft tissue healing [6,7]. Among those, the Transforming Growth Factor Beta (TGF–β), the Platelet-Derived Growth Factor (PDGF), the Vascular Endothelial Growth Factor (VEGF), and the basic Fibroblast Growth Factor (FGF–2) are considered some of the essential mediators produced by leukocytes and platelets in L–PRF membranes. There is strong in vitro evidence of continuous production and release of these mediators by the new autologous biomaterials [7–9].

Several studies on post-extraction sockets assessing the contribution of L–PRF have shown evaluations that indicated better postoperative and substantial improvements in soft tissue healing and pain reduction, employing tools such as the Landry index for soft tissue repair, and the Visual Analogue Scale (VAS) for pain [4,5,10,11]. These analyses contribute to a qualitative assessment related to the patient's recovery in the postoperative period. It may be supposed that these effects are mainly related to the continuous release of growth factors, previously described by different in vitro studies [9,12]. However, there is a lack of clinical evidence on the local release of growth factors and cytokines by grafted L–PRF membranes in this model and their correlation with the improvement in pain and tissue healing on the management of third molar extractions. Even though the local release assessment is challenging to transpose from in vitro to clinical settings, surface protein extraction methods using tapes and swabs have been successfully used in clinical studies [13,14]. In this context, the present study aimed to evaluate the tissue repair and postoperative pain in lower third molar extraction sockets treated with L–PRF, assessing the correlation between these outcomes and the presence of growth factors and cytokines quantified at the surface of the surgical site by using a swab extraction method.

2. Materials and Methods

2.1. Study Design and Outcomes

The present prospective study was conducted in a randomized, double-blind, and split-mouth design. The study was conducted at the Associate Laboratory of Clinical Research in Dentistry (LPCO) of the Dentistry School at Fluminense Federal University, Niterói, Rio de Janeiro, Brazil, between March and July 2019. The study adhered to the principles described in the Declaration of Helsinki and approved by the Ethical Committee of the Antonio Pedro University Hospital under the registration no. 2.721.351. All participants were informed of the study procedures/objectives and were included only after providing written informed consent. The Consolidated Standards of Reporting Trials (CONSORT) statement [15] guidelines were followed to ensure the quality and transparency of this trial.

The primary outcome was represented by postoperative pain and soft tissue healing. For the pain assessments, the primary outcome measures included the pain experienced until the 7th day after surgery according to VAS scores (days 1, 2, 3, and 7) and the Sum of Pain Intensity Differences (SPID) from 24 h to 48 h, 72 h, or 196 h after surgical procedures, while for soft tissue healing the measures were the Landry indexes at the 7th and 14th day after surgery. The secondary outcome was the content of tissue-healing and inflammatory mediators present in the mucosa at 24 h and seven days after surgery.

2.2. Sample Calculation and Randomization

This study's sample size calculation was performed using the software SPSS, version 22.0 (IBM Corporation, Armonk, NY, USA). The primary outcomes (pain and tissue healing) were chosen to calculate the sample size based on the availability of supporting data from the literature. The data from previous studies related to third molars extraction and L–PRF placement [5,10] and a pilot study with 5 patients (included in the present research) shows

that a sample size of at least 16 extractions in each group is required to achieve 80% power at a significance level of 5% (α = 0.05) [16]. Therefore, a sample size of 20 extractions for each group was adopted (two extractions per patient, for a total of 20 participants). Randomization was performed through the flipping of a coin, where each side represented an experimental group. The sides—the right or left dental sockets—were distributed into two groups: L–PRF (n = 20) and Clot (n = 20). The allocation sequence was concealed until participants were enrolled and assigned to interventions.

2.3. Inclusion and Exclusion Criteria

The complete medical and dental history of each participant was obtained. Participants between 18 to 30 years old requiring lower third molar extractions with Level A and Class I, according to the Pell–Gregory classification [17], were included. Participants were excluded from the study if they were smokers, presented local infection (e.g., pericoronitis), had motor difficulties that impeded or hampered hygiene, had prior pain in the orofacial region (e.g., joints and/or masticatory muscles issues), were pregnant, had decompensated metabolic diseases or periodontal disease without previous treatment, or had a history of radiotherapy or use of bisphosphonates.

All research participants were previously informed of the physical and psychological criteria required to participate in this project. The psychological criteria for project participation included no history of anxiety, mood, eating, and/or psychotic disorders that could compromise participation and collaboration in the study.

2.4. Participant Selection

Recruitment of research participants was carried out by a trained investigator (R.C.M.-M.), who diagnosed the indication of the lower third molar extractions after the admission of the patients to the School of Dentistry. The initial evaluation and diagnosis of the teeth indicated for extraction was confirmed by two other trained professionals who were not a part of this research team. All the participants were masked to the studied groups.

2.5. Pre-Surgical Procedures

Venipuncture was performed prior to any treatment (median basilica vein, median cubital vein, median cephalic vein). Blood was drawn into two sterile red cover 10 mL tubes without anticoagulant (BD Vacutainer®, Becton Serum Blood Collection Tubes, Dickinson & Company, Franklin Lakes, NJ, USA). This process was performed at room temperature (20 °C).

L–PRF clots were produced using tubes according to the fabricant (IntraSpin™, Biohorizons®, Birmingham, AL, USA), and immediately centrifuged at 2700 rpm for 12 min (~708 g) using a vertical/fixed-angle centrifuge (IntraSpin™, Biohorizons®, Birmingham, Alabama, AL, USA). This centrifugation protocol considered the g-force value referenced at the bottom of the centrifugation tubes (RCF–max) [18]. After centrifugation, each L–PRF clot was removed from the tube and separated from the red element phase at the base using cotton pliers.

A further blood sample was collected in a 4 mL EDTA tube (BD Vacutainer Lavender K2–EDTA Blood Collection Tubes, Dickinson and Company, USA) for hematological analysis. The analysis was performed using the Wiener lab. Counter 19/19 CP (Labinbraz Comercial Ltd.a, Sao Paulo, Brazil), obtaining the platelet and white blood cells (WBC) counts of each participant.

2.6. Surgical Procedures

Panoramic radiographs were taken by the same professional operator and with the use of the same machine (i–CAT, Kavo, Brazil). The participants were informed to avoid the use of any anti-inflammatory drugs before the surgery, or any other medication to prevent the influence on the healing process. All surgeries were performed by the same operator (C.F.M.) with the same pre-, trans-, and postoperative protocol. The examiner

responsible for postoperative evaluations (M.T.S.) was not present in any surgery, blinding the study for the evaluation. The participants were also not informed about the location of the sockets that received the L–PRF or the sockets that were clot-filled.

Local asepsis was performed by rinsing with 0.12% chlorhexidine digluconate (Periogard® Colgate, São Paulo, Brazil) for one minute and with extraoral use of 4% chlorhexidine soap (Riohex Rioquímica®, Rio de Janeiro, Brazil). The local anesthesia was administered using a Carpule syringe (Quinelato®, Schobell Industrial Ltd.a., São Paulo, SP, Brazil) to block the inferior alveolar lingual and buccal nerves, using 2% alphacaine with 1:100,000 epinephrine (DFL Indústria e Comércio®, Rio de Janeiro, RJ, Brazil). Both sides were blocked before the third molar extractions. Soft tissue release around the tooth was performed using a no. 3 scalpel handle (Bard Park, Quinelato®, Sao Paulo, SP, Brazil) and a no. 15 blade (Solidor, Lamelid®, SãoPaulo, Brazil) to test the success of deep anesthesia and for better apical positioning of the elevators and forceps. Tissue detachment was performed using a Molt no. 9 detacher (Quinelato®, São Paulo, SP, Brazil) around the tooth, followed by elevator and forceps dislocation (Quinelato®, Sao Paulo, SP, Brazil) for later removal. After the extraction was completed, the dental socket was gently explored with a Lucas no. 4 Curette (Quinelato®, Sao Paulo, SP. Brazil) and irrigated with 0.9% physiological saline solution (~20 mL).

For each participant, one dental socket was left as is to be filled with blood as a physiological condition after tooth removal. A few minutes after extraction, the clot could be noticed at the level of the alveolar border. In the absence of a blood clot, a gentle curettage of the alveolar socket was performed to cause slight bleeding. For the sockets receiving the L–PRF, after removing the third molar, four L–PRF clots were placed directly in the dental socket with the aid of an alveolar curette. The tissue was sutured using the traditional single stitch suturing technique with a Johnson 4–0 silk thread (Johnson & Johnson, Ethicon®, São Paulo, Brazil). The number of stitches was determined by the surgeon's criteria, who performed a primary closure in all cases. It is essential to mention that after tooth extraction, there was a simulated manipulation of the tissue to hide to the research participant the side in which the L–PRF grafting occurred.

Paracetamol (500 mg, Tylenol, Johnson & Johnson, Brazil) was prescribed every six hours for pain. In the case of severe pain reported, a protocol was adopted, including an emergency medication (Ibuprofen 400 mg, Advil, Pfizer, USA) every eight hours for three days. Oral hygiene instructions were provided to avoid the accumulation of bacterial plaque above the suture. Participants were instructed to use 0.12% chlorhexidine digluconate rinse (Periogard®, Colgate, São Paulo, Brazil) twice daily for seven days. All sutures were removed after seventy days.

2.7. Clinical Measurements

The participants were scheduled for postoperative evaluation after 1, 2, 3, 7, and 14 days. During those appointments, the evaluation of clinical measurements was performed by the same examiner masked to the study, including parameters such as soft tissue healing, pain, and the number of analgesics consumed. Postoperative pain was measured in all participants on days 1, 2, 3, and 7, while the healing index was measured on days 7 and 14 (even though the routine clinical examination was performed on the first days).

The assessment of soft tissue healing around the sockets was performed using the healing index system described by Landry et al. [19]. The following parameters were used to assess the level of healing: the color of tissues, epithelialization of wound margins, the presence of bleeding on palpation, granulation, and suppuration. The level of healing was scored as 1–very poor, 2–poor, 3–good, 4–very good, or 5–excellent. As an example, very poor healing was attributed to sites with tissue color that was more than 50% red, and the presence of bleeding, tissue granulation, and suppuration. On the other hand, excellent healing was attributed to sites with a healthy pink color and without bleeding, tissue granulation, or exposed connective tissue.

Pain analysis was performed according to the VAS on a 0–10 scale, where 0 indicates the absence of pain and 10 was attributed to the most severe pain ever felt, along with the

graphic classification scale [20]. The participants were previously oriented regarding the pain assessment and asked to report any difficulty in distinguishing between sites. The number of painkillers consumed was also recorded. In this study, a score ≥ 5 was considered as an indicator of severe pain after the third molar extraction [21], resulting in the use of emergency medication for the patients. A derived time-weighted endpoint, the Sum of Pain Intensity Differences (SPID), was also calculated from the available times of data collection, considering the first measure (24 h) as a baseline, and its difference score calculated at each subsequent time point, in hours (SPID24–48, SPID24–72, and SPID24–196).

2.8. Quantification of Cytokines and Growth Factors

On the first and seventh postoperative days, the participants submitted to a swab collection in the surgical region on both sides in order to collect the surface cytokines and growth factors present in the site. The alveolar ridge was isolated with cotton rollers to avoid saliva collection, and after that it was dried with an air jet for 10 s. The samples were collected through friction in the central portion of the socket, in addition to the buccal, lingual, distal, and mesial portion of the gingival border of the operated region. Sterile swabs were used for this procedure (Plastic-Cotton Tipped Applicators, MedLine Industries, IL, USA). The swabs were then placed on 15 mL falcon tubes containing 1.5 mL phosphate-buffered saline solution (PBS) with 0.2% sodium dodecyl sulfate (SDS) and 0.5% propylene glycol and sonicated for 30 min on an ultrasonic bath maintained at 4 °C with ice, for the extraction of proteins. The liquid was collected and stored in different aliquots in cryovials and kept in an ultra-freezer at −80 °C until the quantification assays. The quantification was performed by a masked operator.

For the detection of the biomolecules, a multiparametric immunoassay based on XMap-labeled magnetic microbeads (LuminexCorp, Blvd Austin, TX, USA) was used, through a commercial kit (27–plex panel, Biorad Inc., USA) capable of quantifying IL–1β, IL1-RA, IL-4, IL-6, IL8, IL–10, IL–12 (p70), IL–13, IL–15, IL–17, CCL11, FGF–b, CSF3, CSF2, IFN–γ, CXCL10, CCL2, CCL3, CCL–4, PDGF, CCL5, TNFα and VEGF, and a 1–plex kit containing TGF–β1 beads. Quantification of the magnetic beads and dosages was performed with a BioPlex MAGPIX system (Biorad Inc., Hercules, CA, USA). Results were analyzed using the Xponent v. 3.0 software (Luminexcorp, USA).

2.9. Statistical Analysis

Wilcoxon nonparametric two-tailed tests were used for the comparison of the clinical data in the postoperative period. Data from the cytokine and growth factor assessment for the control and L–PRF sites were analyzed by nonparametric, paired Mann-Whitney U tests. The correlation between the molecule concentrations and the clinical parameters or the blood cell count (WBC and platelet) was investigated through a two-tailed Spearman's rank correlation matrix, where coefficients between 0.3–0.5 indicated weak correlations, 0.5–0.7 indicated moderate correlations, and coefficients above 0.7 indicated strong correlations, according to a rule of thumb proposed previously [22]. The data was also evaluated through multiple regression analyses using the least squares method, where each outcome measure (VAS, CI, SPID) was considered to be the independent variable on a test. The normality of residuals was assessed by the D'Agostino-Pearson omnibus and Kolmogorov–Smirnov tests. For all tests, an alpha error of 5% was considered. The tests were performed with the help of the GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Clinical Evaluation

The sample of recruited participants (March–July 2019) was composed of fourteen females and six males, with a mean age of ~23 years old (range 18–29) (Table 1). The follow-up during the postoperative period indicated a good recovery in all cases without serious complications, intolerance to the medication used, or side effects. There was no

alveolar osteitis, tissue necrosis, abscess, or delayed healing. The CONSORT chart of participant flow is shown in Figure 1.

Table 1. Socio-demographic data of the patients included in the study.

Gender	*n* = 20 (Participants)
Male	6
Female	14
Age (years), mean ± SD	23 ± 3.28
Education Level	
High school diploma	14 (9 female and 5 male)
Bachelor's degree	6 (5 female and 1 male)
Extraction indication	*n* = 40 (dental extractions)
Previous pericoronitis	17
Dental caries	9
Orthodontic treatment	14

SD = Standard deviation.

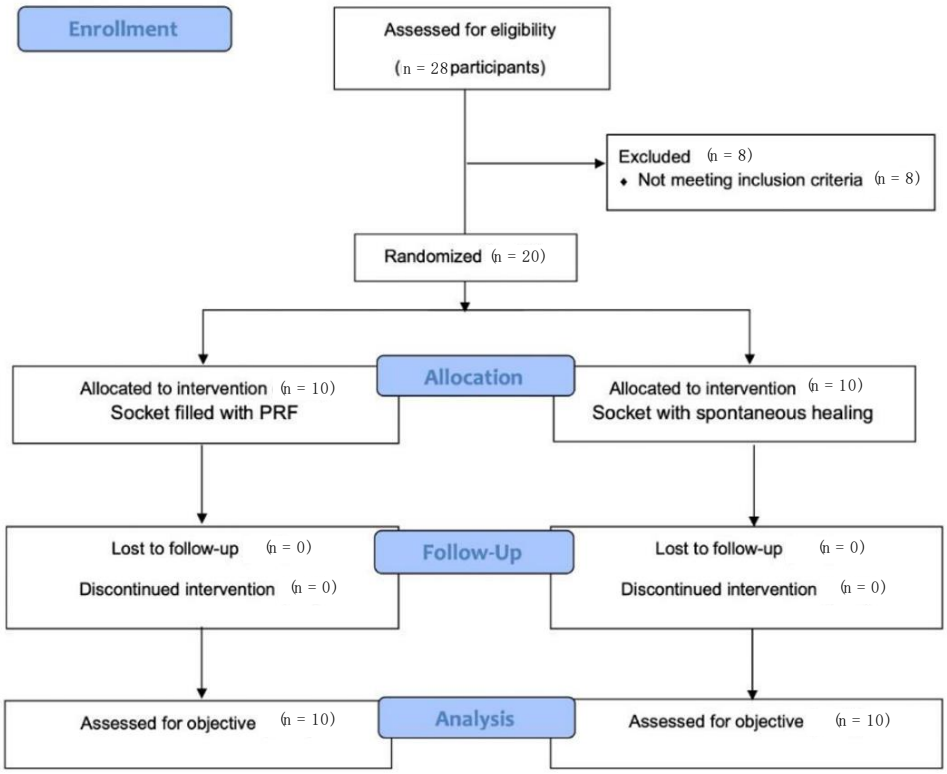

Figure 1. Flowchart illustrating the procedure of the clinical trial (CONSORT) [15].

No participant reported difficulties in differentiating the pain between both surgical sites. On the first postoperative day, a total of 70% of the participants reported severe pain on the site without the use of L–PRF, while 30% experienced severe pain in the L–PRF site. On the second day, the results were 30% and 10%, respectively. Only 10% of the patients reported severe pain in the clot site on the third day, while no severe pain was reported in both groups on the seventh day. Figure 2A shows that the mean score on VAS was significantly lower on the L–PRF sites in all the assessed days ($p < 0.05$). Overall, the average use of Paracetamol per participant was 1.4 tablets per day in the first three days. Of the 20 participants, six made use of emergency medication (Ibuprofen) for three days in the same period and the pain was controlled after that. When the pain was compared using the time-weighted endpoint SPID, there was no significant difference ($p < 0.05$) between control and L–PRF groups from 24 h after surgery to either 48 h (26 ± 26 for

L–PRF versus 39 ± 36 for Control), 72 h (84 ± 46 for L–PRF versus 90 ± 56 for Control), or 196 h (439 ± 150 for L–PRF versus 565 ± 160 for Control). Figure 2B shows the assessments for the cicatrization index.

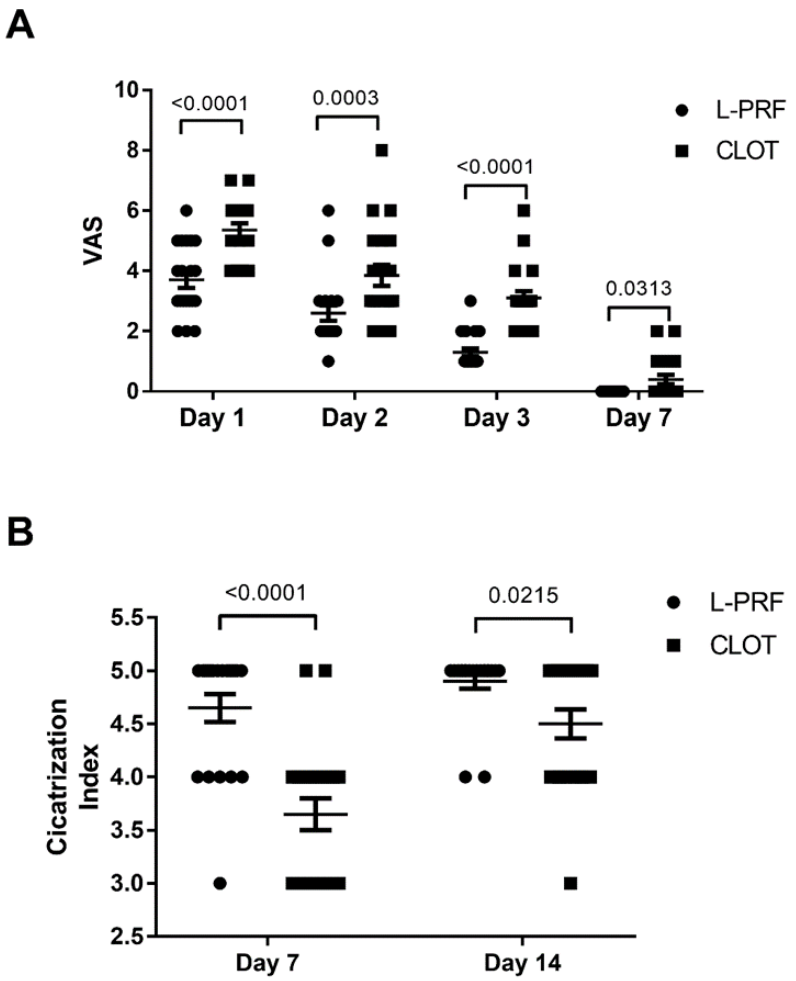

Figure 2. (**A**) Pain evaluation by Visual Analog Scale (VAS) for 1, 2, 3, and 7 days in the postoperative period. (**B**) Comparison of the soft tissue healing, as measured by the Landry´s index, at seven and 14 days of the postoperative period. Squares indicate the score for the clot site of each participant, while circles indicate the scores for the leukocyte-platelet-rich fibrin (L–PRF) sites. Lines indicate the mean ± SD. Connecting lines indicate statistical difference (p value indicated on the figure).

On the seventh day, it was possible to find a highly significant difference after the comparison to the Clot group ($p < 0.0001$). After 14 days, a significant difference was also observed, showing a better healing process to the L–PRF group ($p = 0.0215$). It is possible to observe the healing follow-up in Figure 3.

3.2. Biochemical Analysis

Figure 4 shows the mean content of growth factors and cytokines measured on the surface of the surgical sites (control and L–PRF), on the first and seventh days after the procedure. From the 28 analytes initially assessed, twelve had detectable levels after extraction from the swabs. From those, all growth factors (VEGF, FGF–2, PDGFbb, TGF–β1) and the majority of the inflammatory cytokines (IL–1β, IL–4, TNF–α, IL1–RA, IL–10, and IFN–gamma) were present at significantly higher levels in the L–PRF sites, as compared to Clot at day 1 ($p < 0.05$). However, by the end of one week most of the analytes were present at similar levels at the L–PRF and Clot sites, with the exceptions of VEGF, which attained a

two-fold increase as compared to control, and the anti-inflammatory cytokines IL–10 and IL–1RA, which were around 1.5 × increased at the L–PRF sites ($p < 0.05$).

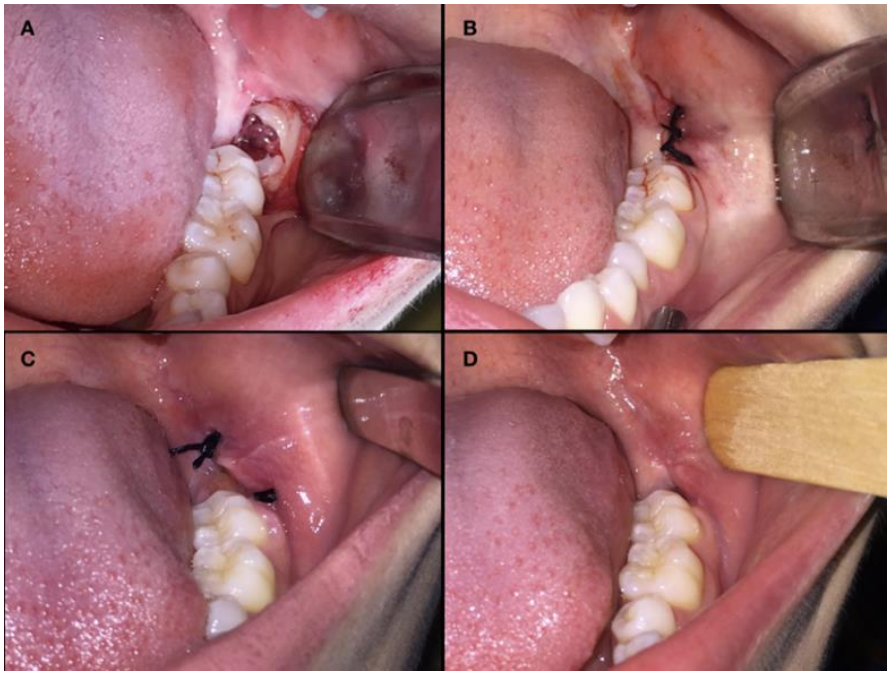

Figure 3. (**A**) L–PRF placed at the dental socket; (**B**) Immediate postoperative area, after suturing; (**C**) Evaluation of soft tissue healing after seven days, socket showing good healing (Landry's Index 3) (**D**) After 14th day with excellent healing (Landry's Index 5) of soft tissues.

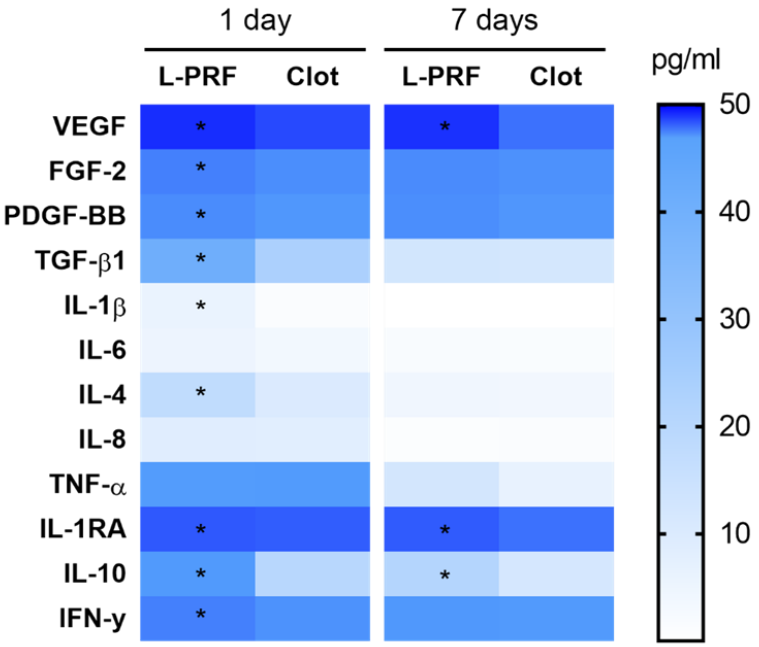

Figure 4. Heatmap of the cytokines extracted from swabs of the L–PRF and Clot sites at 1 and 7 days after surgery. The intensity of the blue color indicates the mean concentration (n = 20) of each analyte, as detected by flow luminometry. An asterisk indicates a significant difference between L–PRF and Clot at the same experimental time ($p < 0.05$).

In order to observe if the content of growth factors and cytokines could correlate with hematological parameters (WBC and platelets) of each participant, an analysis was

performed with blood samples collected before the production of L–PRF. The hematological analysis showed that the WBC (6.99 ± 1.30) and platelets (262.7 ± 14.90). The WBC count (×103/microL) were within normal ranges in all participants. A Spearman's rank correlation test was performed between the WBC and platelet contents, and the values of analytes detected for each participant (Table 2). From all interactions, a significant positive correlation was detected between the blood platelet count of the participants and the surface concentration of VEGF ($p = 0.04$, rho = 0.769). TGF–β1 presented a coefficient comparable to a moderate correlation with platelet content, even with a non-significant borderline p value ($p = 0.05$, rho = 0.634).

In order to identify relationships between the outcome measurements and the detected biological mediators, several multiple regression analyses were performed, as shown in Table 3 for the control group and Table 4 for the L–PRF sites. The multiple r^2 from the tests varied from 0.596 to 0.741, and the residuals passed normality in all tests ($p < 0.05$). However, no significant association was identified between any pain or cicatrization index and the concentrations of growth factors and cytokines in both surgical sites ($p > 0.05$). However, when a correlation matrix was produced between the local growth factor/cytokine concentrations and the clinical evaluation scores through a two-tailed analysis, significant correlations were observed between some endpoints and growth factors, as shown in Table 5. FGFb presented a weak negative correlation with the pain score on the third day (Rho = −0.479, $p = 0.033$), while PDGF negatively correlated with pain scores on both the second and third days (Rho = 0.524, $p = 0.018$ and Rho = −0.709, $p = 0.009$, respectively) and with the cicatrization indexes on the seventh (Rho = 0.516, $p = 0.020$) and 14th days (Rho = 0.522, $p = 0.018$).

Table 2. Analysis of correlation between cell counts and surface cytokine/growth factor concentration on each site of the participants (Clot and L–PRF), at day one after surgery.

		VEGF	TGF–β1	FGFb	PDGF–bb	TNF–α	IL–10	IL–6	IL–4	IL–1β	IL–8	IL–1RA	IFN–γ
Clot Leucocytes (WBC)	Spearman ρ	−0.432	0.0365	0.335	0.054	−0.032	−0.275	0.450	−0.481	0.098	−0.429	−0.372	−0.243
	p value	0.212	0.924	0.341	0.884	0.933	0.435	0.191	0.159	0.789	0.216	0.286	0.494
Platelets	Spearman ρ	0.079	0.382	−0.468	−0.395	0.110	0.176	0.123	0.175	0.132	0.129	0.1963	0.060
	p value	0.899	0.272	0.173	0.256	0.764	0.620	0.734	0.620	0.713	0.719	0.578	0.871
L–PRF Leucocytes (WBC)	Spearman ρ	−0.561	0.465	−0.226	−0.287	−0.440	−0.440	0.000	−0.142	−0.218	−0.258	0.017	0.278
	p value	0.096	0.177	0.529	0.420	0.202	0.203	1.009	0.694	0.553	0.467	1.000	0.431
Platelets	Spearman ρ	**0.769 ***	0.634	0.012	0.049	0.430	0.021	−0.389	−0.135	−0.359	0.051	−0.638	−0.490
	p value	**0.040**	0.050	0.980	0.899	0.212	0.957	0.264	0.708	0.308	0.889	0.067	0.154

Bold numbers with an asterisk indicate a significative correlation ($p < 0.05$, Rho > 0.3).

Table 3. Results for the multiple regression analysis between the different outcomes and the detected growth factors and cytokines on the Control site.

		VEGF	TGF–b1	FGFb	PDGF–bb	TNF–alfa	IL–10	IL–6	IL–4	IL–1b	IL–8	IL–1RA	IFN–Gamma
VAS (day one)	Coefficient	−0.002922	0.3448	−2.08	0.3448	0.3501	0.0003067	−2.669	0.9531	−5.03	2.771	0.02966	−0.1704
Multiple R2 = 0.6538	SE	1.74609×10^{11}	8.689×10^{14}	1.352×10^{14}	1.071×10^{15}	7.551×10^{14}	3.43×10^{10}	7.609×10^{15}	2.202×10^{14}	$2{,}514 \times 10^{15}$	$4{,}265 \times 10^{15}$	$7{,}81449 \times 10^{12}$	$1{,}59 \times 10^{14}$
	95% CI	-4.12×10^{11} to 4.12×10^{12}	-2.055×10^{15} to 2.055×10^{15}	-3.196×10^{14} to 3.196×10^{14}	-2.533×10^{15} to 2.533×10^{15}	-1.785×10^{15} to 1.785×10^{15}	-8.11×10^{10} to 8.11×10^{10}	-1.799×10^{16} to 1.799×10^{16}	-5.207×10^{14} to 5.207×10^{14}	-5.946×10^{15} to 5.946×10^{15}	-1.009×10^{16} to 1.009×10^{16}	-1.84×10^{12} to 1.84×10^{12}	-3.760×10^{14} to 3.760×10^{14}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
VAS (day 2)	Coefficient	0.01323	−0.916	−1.279	−0.916	0.8711	0.0001924	−2.471	2.224	−10.74	4.307	0.006912	−0.2313
Multiple R2 = 0.5957	SE	2.98372×10^{11}	1.485×10^{15}	2.31×10^{14}	1.83×10^{15}	1.29×10^{15}	58,631,858,055	1.3×10^{16}	3.763×10^{14}	4.297×10^{15}	7.288×10^{15}	1.33534×10^{13}	2.717×10^{14}
	95% CI	−70,553,7947,738 to 705,537,947,738	-3.511×10^{15} to 3.511×10^{15}	-5.462×10^{14} to 5.462×10^{14}	-4.328×10^{15} to 4.328×10^{15}	-3.051×10^{15} to 3.051×10^{15}	−138,642,313,474 to 138642313474	-3.074×10^{16} to 3.074×10^{16}	-8.898×10^{14} to 8.898×10^{14}	-1.016×10^{16} to 1.016×10^{16}	-1.723×10^{16} to 1.723×10^{16}	−315,75,789,661,657 to 31,575,789,661,657	-6.424×10^{14} to 6.424×10^{14}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
VAS (day 3)	Coefficient	−0.008301	−0.3741	−2.558	−0.3741	0.6477	−0.000004932	−4.217	3.077	−9.134	2.942	0.02786	−0.2079
Multiple R2 = 0.5957	SE	1.78067×10^{11}	8.862×10^{14}	1.378×10^{14}	1.092×10^{15}	7.7×10^{14}	34,991,205,207	7.759×10^{15}	2.246×10^{14}	2.564×10^{15}	4.35×10^{15}	7.96925×10^{12}	1.621×10^{14}
	95% CI	-4.21×10^{11} to 4.21×10^{11}	-2.095×10^{15} to 2.095×10^{15}	-3.259×10^{14} to 3.259×10^{14}	-2.583×10^{15} to 2.583×10^{15}	-1.821×10^{15} to 1.821×10^{15}	-8.27×10^{10} to 8.27×10^{10}	-1.835×10^{16} to 1.835×10^{16}	-5.310×10^{14} to 5.310×10^{14}	-6.063×10^{15} to 6.063×10^{15}	-1.028×10^{16} to 1.028×10^{16}	-1.88×10^{13} to 1.88×10^{13}	-3.834×10^{14} to 3.834×10^{14}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
CI (day 7)	Coefficient	−0.003013	0.02395	−0.06562	0.02394	0.2534	0.00002793	−1.158	2.91	−5.612	−0.6447	−0.006342	−0.0268
Multiple R2 = 0.6824	SE	1.25912×10^{11}	6.266×10^{14}	9.74692×10^{13}	7.724×10^{14}	5.445×10^{14}	24742518484	5.487×10^{15}	1.588×10^{14}	1.813×10^{15}	3.076×10^{15}	5.63511×10^{12}	1.147×10^{14}
	95% CI	-2.97×10^{11} to 2.97×10^{11}	-1.482×10^{15} to 1.482×10^{15}	-2.305×10^{14} to 2.305×10^{14}	-1.826×10^{15} to 1.826×10^{15}	-1.287×10^{15} to 1.287×10^{15}	-5.85×10^{10} to 5.85×10^{10}	-1.297×10^{16} to 1.297×10^{16}	-3.755×10^{14} to 3.755×10^{14}	-4.287×10^{15} to 4.287×10^{15}	-7.273×10^{15} to 7.273×10^{15}	-1.3×10^{13} to 1.3×10^{13}	-2.711×10^{14} to 2.711×10^{14}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
CI (day 14)	Coefficient	0.002542	−0.1547	0.03387	−0.1547	−0.226	0.00008631	−0.1141	−2.935	6.486	−0.4847	0.009534	0.0623
Multiple R2 = 0.6667	SE	1.10432×10^{11}	5.496×10^{14}	8.54861×10^{13}	6.774×10^{14}	4.775×10^{14}	21,700,624,254	4.812×10^{15}	1.393×10^{14}	1.59×10^{15}	2.697×10^{15}	4.94232×10^{12}	1.006×10^{14}
	95% CI	-2.61×10^{11} to 2.61×10^{11}	-1.300×10^{15} to 1.300×10^{15}	-2.021×10^{14} to 2.021×10^{14}	-1.602×10^{15} to 1.602×10^{15}	-1.129×10^{15} to 1.129×10^{15}	-5.13×10^{10} to 5.13×10^{10}	-1.138×10^{16} to 1.138×10^{16}	-3.293×10^{14} to 3.293×10^{14}	-3.760×10^{15} to 3.760×10^{15}	-6.378×10^{15} to 6.378×10^{15}	-1.16×10^{13} to 1.16×10^{13}	-2.378×10^{14} to 2.378×10^{14}
	p-value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
SPID 24–48	Coefficient	−0.3877	30.26	−19.21	30.26	−12.5	0.002744	−4.739	−30.49	137	−36.86	0.5459	1.462
Multiple R2 = 0.7039	SE	7.68153×10^{12}	3.823×10^{16}	5.946×10^{15}	4.712×10^{16}	3.322×10^{16}	1.50947×10^{12}	3.347×10^{17}	9.688×10^{15}	1.106×10^{17}	1.876×10^{17}	3.438×10^{14}	6.995×10^{15}
	95% CI	-1.82×10^{13} to 1.82×10^{13}	-9.039×10^{16} to 9.039×10^{16}	-1.406×10^{16} to 1.406×10^{16}	-1.114×10^{17} to 1.114×10^{17}	-7.855×10^{16} to 7.855×10^{16}	-3.56×10^{12} to 3.56×10^{12}	-7.915×10^{17} to 7.915×10^{17}	-2.291×10^{16} to 2.291×10^{16}	-2.616×10^{17} to 2.616×10^{17}	-4.437×10^{17} to 4.437×10^{17}	-8.129×10^{14} to 8.129×10^{14}	-1.654×10^{16} to 1.654×10^{16}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
SPID 24–72	Coefficient	−0.2586	47.51	−7.727	47.51	−19.65	0.01022	32.42	−81.46	235.5	−40.96	0.5892	2.362
Multiple R2 = 0.7411	SE	1.26831×10^{13}	6.312×10^{16}	9.818×10^{15}	7.78×10^{16}	5.485×10^{16}	2.49231×10^{12}	5.527×10^{17}	1.6×10^{16}	1.826×10^{17}	3.098×10^{17}	5.676×10^{14}	1.155×10^{16}
	95% CI	-2.99×10^{13} to2.99×10^{13}	-1.492×10^{17} to 1.492×10^{17}	-2.322×10^{16} to 2.322×10^{16}	-1.840×10^{17} to 1.840×10^{17}	-1.297×10^{17} to 1.297×10^{17}	-5.89×10^{12} to 5.89×10^{12}	-1.307×10^{18} to 1.307×10^{18}	-3.782×10^{16} to 3.782×10^{16}	-4.319×10^{17} to 4.319×10^{17}	-7.326×10^{17} to 7.326×10^{17}	-1.342×10^{15} to 1.342×10^{15}	-2.731×10^{16} to 2.731×10^{16}
	p value	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999

Table 3. *Cont.*

		VEGF	TGF–b1	FGFb	PDGF–bb	TNF–alfa	IL–10	IL–6	IL–4	IL–1b	IL–8	IL–1RA	IFN–Gamma
SPID 24–168	Coefficient	−0.1601	69.64	−28.6	69.64	−15.58	0.0581	57.51	−133.7	136.4	116.9	1.578	−4.764
Multiple R2 = 0.6888	SE	2.91903×10^{13}	1.453×10^{17}	2.26×10^{16}	1.791×10^{17}	1.262×10^{17}	5.73606×10^{12}	1.272×10^{18}	3.681×10^{16}	4.203×10^{17}	7.13×10^{17}	1.306×10^{15}	2.658×10^{16}
	95% CI	-6.9×10^{13} to 6.9×10^{13}	-3.435×10^{17} to 3.435×10^{17}	-5.343×10^{16} to 5.343×10^{16}	-4.234×10^{17} to 4.234×10^{17}	-2.985×10^{17} to 2.985×10^{17}	-1.35×10^{12} to 1.35×10^{12}	-3.008×10^{18} to 3.008×10^{18}	-8.705×10^{16} to 8.705×10^{16}	-9.939×10^{17} to 9.939×10^{17}	-1.686×10^{18} to 1.686×10^{18}	-3.089×10^{15} to 3.089×10^{15}	-6.285×10^{16} to 6.285×10^{16}
	p value												

VAS—Visual analog scale of pain; CI—Cicatrization Index; SPID—Sum of Pain Intensity Difference for the determined interval, in hours; 95% CI—95% confidence interval; SE—standard error.

Table 4. Results for the multiple regression analysis between the different outcomes and the detected growth factors and cytokines on the L–PRF surgical site.

		VEGF	TGF–b1	FGFb	PDGF–bb	TNF–alfa	IL–10	IL–6	IL–4	IL–1b	IL–8	IL–1RA	IFN–Gamma
VAS (day one)	Coefficient	0.02764	−0.1627	0.1696	−2.716	−0.04526	0.04117	2.914	−0.4218	−3.725	0.8876	0.0283	−0.0944
Multiple R2 = 0.6538	SE	2.26727×10^{12}	6.864×10^{14}	1.84×10^{14}	4.094	6.66499×10^{13}	3.52915×10^{12}	6.25×10^{15}	2.292×10^{15}	3.893×10^{15}	2.153×10^{15}	5.89863×10^{12}	7.88918×10^{13}
	95% CI	-5.36×10^{12} to 5.36×10^{12}	-1.62×10^{15} to 1.623×10^{15}	-4.351×10^{14} to 4.351×10^{14}	−12.40 to 6.965	-1.576×10^{14} to 1.576×10^{14}	-8.34×10^{12} to 8.34×10^{12}	-1.478×10^{16} to 1.478×10^{16}	-5.421×10^{15} to 5.421×10^{15}	-9.205×10^{15} to 9.205×10^{15}	-5.091×10^{15} to 5.091×10^{15}	-1.39×10^{12} to 1.39×10^{12}	-1.865×10^{14} to 1.865×10^{14}
	p value	>0.9999	>0.9999	>0.9999	0.5283	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
VAS (day 2)	Coefficient	0.001511	0.03459	2.165	−2.875	0.2745	−0.01392	−0.4092	−0.05736	−0.1734	0.4868	0.00836	−0.03179
Multiple R2 = 0.5957	SE	2.34162×10^{12}	7.089×10^{14}	1.901×10^{14}	4.175	6.88358×10^{13}	3.64489×10^{12}	6.455×10^{15}	2.368×10^{15}	4.02×10^{15}	2.224×10^{15}	6.09207×10^{12}	8.14791×10^{13}
	95% CI	-5.54×10^{12} to 5.54×10^{12}	-1.676×10^{15} to 1.676×10^{15}	-4.494×10^{14} to 4.494×10^{14}	−12.75 to 6.998	-1.628×10^{14} to 1.628×10^{14}	-8.62×10^{12} to 8.62×10^{12}	-1.526×10^{16} to 1.526×10^{16}	-5.599×10^{15} to 5.599×10^{15}	-9.507×10^{15} to 9.507×10^{15}	-5.258×10^{15} to 5.258×10^{15}	-1.44×10^{12} to 1.44×10^{12}	-1.92×10^{14} to 1.92×10^{14}
	p value	>0.9999	>0.9999	>0.9999	0.5133	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
VAS (day 3)	Coefficient	0.0009442	0.04703	0.2057	−1.876	0.131	−0.01446	0.5434	−0.2135	−0.776	0.9408	0.002328	−0.03558
Multiple R2 = 0.5957	SE	1.17081×10^{12}	3.544×10^{14}	9.50296×10^{13}	1.64	3.44179×10^{13}	1.82244×10^{12}	3.228×10^{15}	1.184×10^{15}	2.01×10^{15}	1.112×10^{15}	3.04604×10^{12}	4.07395×10^{13}
	95% CI	-2.77×10^{12} to 2.77×10^{12}	-8.381×10^{14} to 8.381×10^{14}	-2.247×10^{14} to 2.247×10^{14}	−5.755 to 2.003	-8.13×10^{14} to 8.13×10^{14}	-4.30×10^{12} to 4.30×10^{12}	-7.632×10^{15} to 7.632×10^{15}	-2.799×10^{15} to 2.799×10^{15}	-4.753×10^{15} to 4.753×10^{15}	-2.629×10^{15} to 2.629×10^{15}	-7.20×10^{12} to 7.20×10^{12}	-9.63×10^{14} to 9.63×10^{14}
	p value	>0.9999	>0.9999	>0.9999	0.2903	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
CI (day 7)	Coefficient	−0.01202	−0.4186	−2.129	1.224	−0.1411	0.01456	4.754	0.4722	−2.011	0.8682	0.0071	−0.05192
Multiple R2 = 0.6824	SE	1.09519×10^{12}	3.315×10^{14}	8.8892×10^{13}	2.042	3.2195×10^{13}	1.70474×10^{12}	3.019×10^{15}	1.107×10^{15}	1.88×10^{15}	1.04×10^{15}	2.84931×10^{12}	3.81083×10^{13}
	95% CI	-2.59×10^{12} to 2.59×10^{12}	-7.840×10^{14} to 7.840×10^{14}	-2.102×10^{14} to 2.102×10^{14}	−3.604 to 6.052	-7.62×10^{13} to 7.62×10^{13}	-4.03×10^{12} to 4.03×10^{12}	-7.139×10^{15} to 7.139×10^{15}	-2.619×10^{15} to 2.619×10^{15}	-4.446×10^{15} to 4.446×10^{15}	-2.459×10^{15} to 2.459×10^{15}	-6.73×10^{12} to 6.730×10^{12}	-9.0×10^{13} to 9.0×10^{13}
	p value	>0.9999	>0.9999	>0.9999	0.567795	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
CI (day 14)	Coefficient	−0.009527	−0.2259	0.3207	0.7161	−0.04191	0.0129	2.573	0.5418	−0.2613	−0.1487	−0.006896	0.009277
Multiple R2 = 0.6667	SE	5.85406×10^{11}	1.772×10^{14}	4.75148×10^{13}	1.046	1.72089×10^{13}	9.11222×10^{11}	1.614×10^{15}	5.919×10^{14}	1.005×10^{15}	5.56×10^{14}	1.52302×10^{12}	2.03698×10^{13}
	95% CI	-1.38×10^{11} to 1.38×10^{11}	-4.190×10^{14} to 4.190×10^{14}	-1.124×10^{14} to 1.124×10^{14}	−1.757 to 3.189	-4.06×10^{13} to 4.06×10^{13}	-2.15×10^{12} to 2.15×10^{12}	-3.816×10^{15} to 3.816×10^{15}	-1.400×10^{15} to 1.400×10^{15}	-2.377×10^{15} to 2.377×10^{15}	-1.315×10^{15} to 1.315×10^{15}	-3.60×10^{12} to 3.60×10^{12}	-4.81×10^{13} to 4.81×10^{13}
	p value	>0.9999	>0.9999	>0.9999	0.5156	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
SPID 24–48	Coefficient	0.627	−4.734	−47.89	3.795	−7.674	1.322	79.75	−8.746	−85.24	9.621	0.4785	−1.502
Multiple R2 = 0.7039	SE	4.65977×10^{13}	1.411×10^{16}	3.782×10^{15}	103.1	1.37×10^{15}	7.25323×10^{13}	1.285×10^{17}	4.712×10^{16}	8.001×10^{16}	4.425×10^{16}	1.212×10^{14}	1.621×10^{15}
	95% CI	-1.102×10^{14} to 1.102×10^{14}	-3.336×10^{16} to 3.336×10^{16}	-8.943×10^{15} to 8.943×10^{15}	−240.1 to 247.7	-3.239×10^{15} to 3.239×10^{15}	-1.715×10^{14} to 1.715×10^{14}	-3.038×10^{17} to 3.038×10^{17}	-1.114×10^{17} to 1.114×10^{17}	-1.892×10^{17} to 1.892×10^{17}	-1.046×10^{17} to 1.046×10^{17}	-2.867×10^{14} to 2.867×10^{14}	-3.834×10^{15} to 3.834×10^{15}
	p value	>0.9999	>0.9999	>0.9999	0.9717	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999
SPID 24–72	Coefficient	1.268	−9.767	−48.76	−16.37	−11.9	2.658	136.6	−13.74	−156	8.344	1.102	−2.914
Multiple R2 = 0.7411	SE	7.94774×10^{13}	2.406×10^{16}	6.451×10^{15}	175.2	2.336×10^{15}	1.237×10^{14}	2.191×10^{17}	8.036×10^{16}	1.365×10^{17}	7.548×10^{16}	2.068×10^{14}	2.765×10^{15}
	95% CI	-1.879×10^{14} to 1.879×10^{14}	-5.689×10^{16} to 5.689×10^{16}	-1.525×10^{16} to 1.525×10^{16}	−430.6 to 397.8	-5.525×10^{15} to 5.525×10^{15}	-2.925×10^{14} to 2.925×10^{14}	-5.181×10^{17} to 5.181×10^{17}	-1.900×10^{17} to 1.900×10^{17}	-3.227×10^{17} to 3.227×10^{17}	-1.785×10^{17} to 1.785×10^{17}	-4.889×10^{14} to 4.889×10^{14}	-6.539×10^{15} to 6.539×10^{15}
	p value	>0.9999	>0.9999	>0.9999	0.9282	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999

Table 4. *Cont.*

		VEGF	TGF–b1	FGFb	PDGF–bb	TNF–alfa	IL–10	IL–6	IL–4	IL–1b	IL–8	IL–1RA	IFN–Gamma
SPID 24–168	Coefficient	3.921	−25.38	−32.48	−277.1	−16.25	6.61	416.4	−54.23	−513.6	93.56	3.819	−11.98
Multiple R2 = 0.6888	SE	2.767×10^{14}	8.378×10^{16}	2.246×10^{16}	536.4	8.135×10^{15}	$4{,}308 \times 10^{14}$	7.629×10^{17}	2.798×10^{17}	4.752×10^{17}	2.628×10^{17}	7.2×10^{14}	9.63×10^{15}
	95% CI	-6.544×10^{14} to 6.544×10^{14}	-1.981×10^{17} to 1.981×10^{17}	-5.312×10^{16} to 5.312×10^{16}	−1546 to 991.3	-1.924×10^{16} to 1.924×10^{16}	-1.019×10^{15} to 1.019×10^{15}	-1.804×10^{18} to 1.804×10^{18}	-6.617×10^{17} to 6.617×10^{17}	-1.124×10^{18} to 1.124×10^{18}	-6.215×10^{17} to 6.215×10^{17}	-1.703×10^{15} to 1.703×10^{15}	-2.277×10^{16} to 2.277×10^{16}
	p value	>0.9999	>0.9999	>0.9999	0.6213	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999

VAS - Visual analog scale of pain; CI—Cicatrization Index; SPID—Sum of Pain Intensity Difference for the determined interval, in hours; 95% CI—95% confidence interval; SE—standard error.

Table 5. Analysis of correlation between pain/tissue healing indicators and the surface concentration of cytokine and growth factors on each side of the participants (clot and L–PRF) at day one after surgery.

Outcome Measure		VEGF	TGF–β1	FGFb	PDGF–bb	TNF–α	IL–10	IL–6	IL–4	IL–1β	IL–8	IL–1RA	IFN–γ
Clot													
VAS (day 1)	Spearman ρ	−0.013	0.317	0.299	0.317	−0.316	0.068	−0.273	−0.209	−0.050	−0.387	0.046	−0.300
	p value	0.957	0.173	0.200	0.173	0.175	0.776	0.244	0.378	0.836	0.092	0.847	0.199
VAS (day 2)	Spearman ρ	0.074	0.042	0.107	0.042	−0.274	−0.107	−0.191	−0.152	−0.044	−0.055	−0.133	−0.299
	p value	0.758	0.859	0.655	0.859	0.243	0.653	0.421	0.521	0.853	0.819	0.576	0.200
VAS (day 3)	Spearman ρ	−0.020	−0.127	−0.077	−0.127	−0.268	−0.069	−0.037	−0.002	0.191	−0.008	0.008	−0.292
	p value	0.933	0.594	0.748	0.594	0.254	0.773	0.876	0.994	0.420	0.975	0.972	0.211
CI (day 7)	Spearman ρ	−0.182	−0.045	−0.010	−0.045	0.234	0.222	−0.036	−0.027	−0.197	−0.146	0.098	0.234
	p value	0.443	0.850	0.967	0.850	0.321	0.346	0.882	0.910	0.405	0.540	0.682	0.321
CI (day 14)	Spearman ρ	−0.349	0.091	0.172	0.091	0.404	0.326	0.163	0.057	0.089	−0.076	0.442	0.366
	p value	0.132	0.703	0.469	0.703	0.077	0.161	0.492	0.810	0.710	0.752	0.051	0.113
$SPID_{24-48}$	Spearman ρ	−0.136	0.116	0.105	0.116	−0.002	0.146	−0.031	−0.060	0.021	−0.194	0.090	−0.017
	p value	0.567	0.626	0.659	0.626	0.992	0.538	0.895	0.803	0.930	0.413	0.707	0.942
$SPID_{24-72}$	Spearman ρ	−0.096	0.214	0.212	0.214	−0.081	0.143	−0.129	−0.144	−0.055	−0.260	0.035	−0.095
	p value	0.686	0.365	0.368	0.365	0.734	0.547	0.588	0.545	0.819	0.268	0.884	0.691
$SPID_{24-196}$	Spearman ρ	−0.159	0.276	0.320	0.276	−0.062	0.197	−0.162	−0.191	−0.065	−0.299	0.120	−0.086
	p value	0.502	0.238	0.168	0.238	0.797	0.405	0.495	0.421	0.784	0.200	0.613	0.720
L-PRF													
VAS (day 1)	Spearman ρ	−0.228	−0.122	0.147	−0.403	−0.199	0.278	−0.300	−0.250	−0.138	−0.379	0.031	−0.240
	p value	0.333	0.609	0.536	0.078	0.400	0.235	0.198	0.288	0.560	0.100	0.896	0.309
VAS (day 2)	Spearman ρ	−0.050	−0.243	−0.302	**−0.524 ***	−0.154	0.207	−0.165	−0.138	0.093	−0.223	0.072	−0.301
	p value	0.833	0.301	0.195	**0.018**	0.516	0.381	0.488	0.563	0.697	0.344	0.763	0.198
VAS (day 3)	Spearman ρ	0.120	−0.208	**−0.479 ***	**−0.709 ***	−0.091	−0.003	−0.249	−0.216	−0.135	−0.215	0.013	−0.172
	p value	0.615	0.378	**0.033**	**0.009**	0.704	0.989	0.289	0.360	0.570	0.363	0.957	0.468
CI (day 7)	Spearman ρ	−0.171	0.202	−0.328	**0.516 ***	0.040	−0.105	0.106	0.042	−0.067	0.025	0.042	0.247
	p value	0.470	0.392	0.159	**0.020**	0.868	0.660	0.655	0.861	0.778	0.915	0.862	0.294
CI (day 14)	Spearman ρ	−0.348	0.362	−0.174	**0.522 ***	−0.150	−0.098	0.309	0.117	0.214	0.261	0.166	0.261
	p value	0.133	0.117	0.463	**0.018**	0.529	0.681	0.184	0.622	0.365	0.265	0.485	0.265
$SPID_{24-48}$	Spearman ρ	−0.229	0.134	−0.133	0.078	−0.185	0.073	−0.069	−0.086	−0.104	−0.153	0.076	0.068
	p-value	0.332	0.572	0.575	0.743	0.434	0.759	0.774	0.719	0.662	0.520	0.750	0.776
$SPID_{24-72}$	Spearman ρ	−0.261	0.043	−0.110	−0.003	−0.174	0.204	−0.133	−0.112	−0.080	−0.250	0.076	−0.053
	p value	0.267	0.856	0.646	0.990	0.464	0.388	0.575	0.637	0.738	0.287	0.750	0.824
$SPID_{24-196}$	Spearman ρ	−0.258	−0.083	0.073	−0.288	−0.179	0.277	−0.309	-0.256	−0.188	−0.382	−0.008	−0.215
	p value	0.273	0.727	0.759	0.218	0.451	0.238	0.185	0.275	0.428	0.096	0.975	0.363

VAS—Visual analog scale of pain; CI—Cicatrization Index. SPID—Sum of Pain Intensity Differences. Bold numbers with an asterisk indicate a significative relevant correlation ($p < 0.05$, Rho > 0.3). The Spearman´s correlation rank test could not be performed for VAS at day 7 since all results were null.

4. Discussion

The purpose of the present study was to assess the effects of L–PRF on the healing process of the lower third molars extraction socket and its relationship with the local concentration of growth factors and cytokines. For this, a randomization unit was employed consisting of the participant's extraction socket and not that of a different participant. Such a study design, besides reducing inter-individual and demographic variables such as age and gender, usually provides relevant data without the necessary use of larger samples [13,14]. Another important fact about the present study design was the double blinding at the levels of both participants and data evaluation (clinical and laboratory parameters). These are essential steps to reduce the risk of bias and improve the quality of data from clinical trials. Blinding was a topic of particular concern in the study design in order to ensure that both participants and researchers who assessed the outcomes remained unaware of the treatment on each surgical site. Special care was taken in the dental socket's primary closure, and the local anesthesia by regional block was performed so the participants would not feel the introduction of the L–PRF membranes. Furthermore, the membranes' compressing and natural shrinkage contributed to reducing differences between sites, as shown in Figure 4. With such a model, the present findings indicate that the use of L–PRF impacts the local surface concentration of growth factors, with repercussions on clinical parameters of recovery, such as the perception of pain and the quality of soft tissue repair.

Regarding the local concentration of cytokines and growth factors, the present study employed a method of extraction based on previous reports for epithelial surfaces [23,24]. The current findings show that the use of surface swabs may provide an alternative approach to compare the local release of growth factors by L–PRF. However, the data should also be considered with care, as the method may also be subjected to diverse sources of bias, since some methodological steps may interfere with the final amount of collected analytes, including the time of pressing, area of collection, and previous removal of saliva, which represents another well-known source of cytokines [25]. Besides, the examiner's experience was essential to avoid contamination, e.g., saliva and food debris in the suture region. Therefore, careful steps were taken in this study during sample collection, and the examiner guided the oral hygiene of all study participants to reduce the risk of bias during the evaluations performed. The description of this care may help to increase reproducibility and minimize the risk of bias on this kind of clinical/molecular assessment.

The results show that the method of collection was adequate to detect significant differences between grafted and control sites for at least nine molecules, including all four growth factors investigated. FGF–2 or basic FGF (FGFb) is a potent mitogen, promoting angiogenesis and inducing connective tissue remodeling by the production of the extracellular matrix (ECM) by fibroblasts [26]. VEGF, besides its well-known angiogenic properties, also induces collagen deposition and epithelization through mitogenic and chemotactic actions [27]. PDGF is another GF that promotes mitogenesis, angiogenesis, cellular differentiation, and even the upregulation of other growth factors that promote fibroblastic function [28]. TGF–β1 is a multifunctional mediator that acts mainly by chemotaxis and mitogenesis of fibroblasts and macrophages, as well as stimulation of collagen deposition for connective tissue wound healing [29]. Through these mechanisms, these growth factors are expected to be directly associated with the regenerative properties of L–PRF [12,30]. Remarkably, the data indicate that the presence of L–PRF may also be linked to increased local levels of some pro-inflammatory mediators such as IFN–gamma and IL–1beta, which have already been reported as produced and released by L–PRF preparations [9]. While these cytokines mediate inflammatory changes of the gingival tissues, including angiogenesis and edema [31] that could affect the soft tissue healing improvement, the present data suggest that the supraphysiological concentrations released by L–PRF on dental sockets of the third molars are not sufficient to increase postoperative discomfort. On the other hand, the concentrations of most cytokines decreased to levels similar to the control sides within seven days, with the persistent difference found only to VEGF and the anti-inflammatory

cytokines IL–10 and the antagonist of the receptor of IL–1 (IL–1RA), which might contribute to an immunomodulatory role for L–PRF, as previously proposed by Dohan et al. [8].

Another interesting finding was the identification of a strong positive correlation between the platelet count of the samples used to produce L–PRF and the local content of VEGF and, to a lower extent, TGF–β1. The relatively small sample from the present study may have impaired the identification of possible correlations for other analytes present at smaller concentrations. Still, this finding is in agreement with the idea that individual traits may impact biological factors related to the materials effectivity; in this case, the local release of growth factors, reinforcing the need for point-of-care quality assurance of individual L–PRF preparations, including platelet counts of whole blood and prepared membranes [32,33].

Concerning the analysis of pain and healing of soft tissue, the present findings are in agreement with previous reports on good outcomes related to the use of L–PRF on extracted dental sockets [5,10,34]. L–PRF implanted in the third molar socket improved postoperative discomfort ($p < 0.05$) and significantly enhanced soft tissue healing on the seventh and fourteenth day, a timeframe where several other studies also report good clinical results of L–PRF [5,10,34]. Furthermore, the present study provides direct evidence of the connection between released growth factors and clinical improvement by different outcome measures, represented mainly by the strong correlation of PDGF–bb concentration at the L–PRF site with the reduction of pain on the third day after surgery, and the moderate correlation with the cicatrization index at the 7th and the 14th days after surgery. It is essential to notice that PDGF is considered one of the essential mediators released by platelet aggregates. Its proven efficacy in the regeneration of both soft and hard tissue has even led to its approval by the FDA for clinical uses, such as periodontal therapy [31]. Together with the weak-to-moderate negative correlation of FGFb with pain scores on the third day, these findings should encourage further studies on the release of this growth factor by L–PRF, including larger samples and other biomolecular, histological, and histomorphometric analyses at different times of tissue repair.

One of the main limitations of the present study is related to the fact that the sample size was calculated based on the primary clinical outcomes. While the resulting small sample indeed has proven sufficient for the detection of differences between L–PRF and blood clot, as well as a correlation between PDGF and clinical improvement at the L–PRF site, it might have been insufficient for the identification of direct relationships between the clinical and biochemical outcomes through the multiple regression analysis. We were also unable to identify L–PRF treatment's effects through the time-weighted endpoint of pain assessment SPID, employed mainly in clinical trials of analgesics. In this case, rather than a small sample, the very nature of L–PRF mechanisms, which are possibly anti-inflammatory rather than analgesic, may explain the results. Therefore, the participants experienced lower pain from the baseline (24 h) to the last time of follow-up (7 days). On the other hand, they reported high levels of pain at the baseline, with a significant reduction until the 7th day after surgery, thus producing better time-weighted scores. Therefore, the comparison of the pain scores at each time of follow-up proved more sensitive to the effects of L–PRF treatment, as shown in the present results using VAS, a reliable, valid, sensitive, and appropriate scale commonly used in dental, oral, and maxillofacial surgery [35].

Finally, another important limitation of the study was the choice for a split-mouth design, which makes it difficult to compare some variables such as trismus and the number of painkillers consumed and increased the difficulty of achieving symmetrical patterns of dental position. Furthermore, there is always the risk of the patients experiencing difficulties distinguishing between the left and right sides when assessing pain. Nevertheless, we chose to employ this widely used methodology since it avoids other biases, such as in memory of pain, and avoids the need to submit the control group to a venipuncture (to simulate L–PRF production). In this research, the participants were previously oriented regarding the pain assessment, and none of them reported difficulties differentiating the source of pain. This occurred most likely because the pain after surgical removal of the mandibular

third molar could originate from the periodontal ligament or the adjacent tooth's bone and usually does not surpass the midsagittal plan [35]. Split-mouth designs also have been successfully employed on oral pain assessments with low risk of "carry-across" effects [36].

Regardless of these limitations, the present study adds to the body of evidence on the effectiveness of L–PRF in the recovery of third molar extractions by indicating that clinical parameters may improve after L–PRF implantations and provide firsthand clinical evidence that these effects are correlated with the local increase on growth factor concentration. These results suggest that the collection of local growth factors and mediators from the surface of surgical sites may be generalized for other clinical studies of platelet aggregates and may encourage further studies of the correlation between clinical and molecular parameters to enhance the understanding of the mechanisms of action of this autologous biomaterial on the recovery of soft tissues.

5. Conclusions

The use of L–PRF improves the soft tissue healing process and decreases postoperative pain after the third molar extractions, which correlates with an increase in the local concentration of growth factors such as PDGF and FGFb.

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