Clinical and Biochemical Evaluation of The Use of Alb-PRF Versus L-PRF in Mandibular Third Molar Extractions: a Split-Mouth Randomized Clinical Trial

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Abstract

Objective

This study compares the performance of L-PRF and a new activated plasma albumin gel, Alb-PRF.

Materials and Methods

In a controlled, split-mouth study involving ten volunteers, twenty extracted molars were treated with either Alb-PRF (Group 1) or L-PRF (Group 2). Post-extraction, pain, trismus (jaw muscle spasm), infection presence, and swelling were evaluated after one and seven days using the Visual Analogue Scale (VAS) for pain, a trismus measurement method, and Gabka and Matsumura's swelling analysis method. Infection presence was based on any detected suppuration. The concentrations of different analytes in the surgical sites were also examined. The data were statistically analyzed with significance defined at p < 0.05 (t-test).

Results

No significant difference was noted between the groups for pain and trismus, but Alb-PRF showed a significant reduction in swelling on day seven. Interleukin-1 beta (IL-1b) was significantly different between groups. The Alb-PRF group showed lower levels of proinflammatory cytokines (GM-CSF, IL-1b, IL-6, IFNy, IL-8, IL-15, RANTES, and MIP-1a) after seven days, with only higher expressions of MIP-1b, IL-1b, and MCP-1 found in the L-PRF group.

Conclusion

Differences were observed in the release of analytes between L-PRF and Alb-PRF, with Alb-PRF significantly reducing edema after seven days.

Clinical Relevance:

In the first clinical trial using denatured albumin combined with PRF, the results showed that Alb-PRF had better outcomes in reducing swelling and improving post-operative recovery compared to L-PRF. This makes it a promising alternative for dental procedures that require invasive methods.

1. Introduction

Bone tissue, a specialized connective tissue, exhibits a substantial capacity for regeneration and remodeling, which is crucial for maintaining structural and functional integrity[1]. Minor fractures often heal without leaving fibrous scar tissue[2–4]. However, localized bone loss resulting from infections, tooth extraction, fractures, surgical resections, or under certain pathological conditions like vascular compromise or metabolic disorders, may lead to fibrous tissue formation[5, 6], disrupting functionality and aesthetics, and impacting overall quality of life.

To address the challenges in bone tissue bioengineering, researchers have focused on developing new biomaterials that can serve as three-dimensional scaffolds. These innovative biomaterials enable cell migration, angiogenesis, new extracellular matrix deposition, mineralization, and tissue regeneration. Ideally, bone substitute biomaterials should include molecules that promote bone differentiation, but this can be costly.

Platelet concentrates offer a clinically relevant and cost-effective alternative, as a source of cells and growth factors[7]. These concentrates, derived from the patient's peripheral blood through multiple centrifugation protocols, release various growth factors to augment healing[8]. One of the byproducts of blood is called leukocyte platelet-rich fibrin (L-PRF). This autologous biomaterial can be obtained through a simple technique that involves a single centrifugation process without any anticoagulant additives[8−10]. The presence of platelets and leukocytes allows for the continuous production and release of various growth factors, making it a cost-effective option[11]. Previous research has documented the effectiveness of L-PRF in various clinical applications, such as controlling hemostasis in oral procedures[12–14] and treating osteonecrosis of the jaws[15].

The L-PRF is a structure that comprises fibrin, platelets, leukocytes, and plasma proteins. It is often utilized as a safeguarding layer for soft tissues and for techniques that require guided bone regeneration. However, the L-PRF membrane is not suitable for procedures that need prolonged protective barriers due to its high bioabsorption and reduced stability. Although the exact residency time of the membrane post-surgery[15−17] remains uncertain, it has been shown that it can actively release cytokines for up to 28 days in a biological medium[18]. However, after this period, the membrane was found to be partially degraded. It's important to note that this was an in vitro study and did not involve enzymes that could degrade the L-PRF.

To overcome their stability limitations, a new process was introduced that involves adding the liquid portion of L-PRF to denatured platelet-poor plasma. Initially called Alb-CGF (albumin with the presence of concentrate of growth factors)[19], the process was later renamed Alb-PRF (albumin with liquid PRF) in subsequent articles by authors from the same group[20, 21]. This process results in the formation of a malleable membrane made up of dense protein structures encased in fibrin fibers that trap cells and platelets. It has demonstrated impressive structural stability for 21 days in mice subcutaneous tissue[21], along with the capability for gradual cytokine and growth factor release[19, 21]. Furthermore, albumin's proven capacity to carry various drugs due to interactions with its three specific domains makes it a promising candidate for drug delivery functions[22–24].
The use of albumin in tissue engineering has been extensively documented [25]. This is due to its abundance, ease of isolation from blood plasma precipitation, high purity, and homogeneity [26, 27]. Albumin-enriched biomaterials provide an optimal structure for cell proliferation and show minimal reduction over time, suggesting less in vitro degradation [25]. Studies further suggest that the association with albumin can stabilize the fibrin network's ultrastructure [27]. In addition, preliminary data indicate that combining denatured serum albumin significantly enhances the PRF-based scaffold, yielding an autologous, biocompatible material with the potential for enhanced durability and sustained action [20, 21, 24].

Numerous studies in the medical field have shown that L-PRF is highly effective in promoting soft tissue healing [12, 13, 15, 28] and as a supplementary treatment for pain and swelling reduction [29]. However, despite extensive research on popular databases such as Web of Science and MEDLINE, there is currently no clinical study available that utilizes this innovative autologous biomaterial in humans.

The aim of the research was to assess Alb-PRF and L-PRF, both clinically and biochemically, after being placed in dental sockets following the removal of mandibular third molars.

2. Materials and Methods

2.1 Ethical Considerations

This study was a randomized, controlled, double-blind, split-mouth study. It was conducted according to the principles described in the Helsinki declaration regarding experiments on human beings and following Normative Resolution n. 466 of 2012 of the National Health Council (CNS). The Ethics Committee approved this study (no. 5,072,786). In addition, this study followed the CONSORT-statement guidelines [30] to ensure the present randomized study's quality and transparency. Subject volunteers were recruited after they agreed to participate in the study and signed an informed consent form (ICF) agreeing to follow the proposed guidelines and schedule. The sample consisted of 22 post-extraction sockets (11 subjects). All teeth with no treatment possibilities, verified by clinical and radiological examination by another professional not involved in the study were recommended for extraction.

2.2 Eligibility Criteria

The present clinical trial was designed to encompass a specific demographic group which, upon meeting certain criteria, was essential to the validity of the study. The following criteria outlined the ideal profile of a prospective participant.

The study targeted individuals who were aged over 18 and who exhibited an indication of mandibular third molar exodontia. This cohort should have either erupted or partially erupted mandibular third molars. It was also required that participants expressed their willingness to cooperate with the study and had already signed the informed consent form. Moreover, a platelet count above 150,000 mm$^2$ was a crucial health parameter that needed to be satisfied.

On the other hand, the trial also defined a strict set of exclusion criteria. These criteria were established to control the variable factors that could have interfered with the results of the study. For instance, participants who had mandibular third molars that were unerupted, impacted, or in a horizontal, mesio- or distal-angled position were not considered for the study. Additionally, individuals with conditions such as diabetes, carriers of blood dyscrasias, or metabolic bone diseases (including osteomalacia, hypocalcemia, hypercalcemia, and osteoporosis) were also excluded from participation.

Medication usage was another area of concern; individuals using drugs that could have altered or compromised the bone healing response, such as prolonged use of bisphosphonates or corticoids, were deemed ineligible. The same rule applied to those with a history of anxiety, mood, eating, or psychotic disorders, given that these conditions could have affected their ability to participate and collaborate in the study.

Furthermore, any motor dysfunction that inhibited the performance of oral hygiene led to the exclusion of the candidate from the study. Those who smoked, unless they had been without smoking for at least six months, were not eligible. Pregnant women or infants, as well as participants who had undergone radiotherapy, chemotherapy, or any other cancer treatment, also fell into the category of exclusion due to the potential risks these situations could have posed to the study or the individual's health.

2.3 Sample size calculation, Randomization, and Blinding

Sample size calculation for the present study was conducted using Sealed Envelope software (London, UK). The test power revealed a sample size of at least eight subject volunteers in each group with 90% strength and a 5% significance level. Considering a 20% dropout rate in advance, a sample size of 10 subject volunteers for each group becomes necessary. The method of randomization of subject volunteers was intra-participant by coin system (heads and tails). For the present study, the evaluator and the subject volunteers were blinded to the type of platelet concentrate used inside the socket, thus characterizing a double-blind study.

2.4 Preparation of platelet concentrates

2.4.1 L-PRF Preparation (Control Group)

Initially, blood was collected in two sterile 9 ml red cap tubes without the presence of anticoagulant (BD Vacutainer®, Becton Serum Blood Collection Tubes, Dickinson & Company, Franklin Lakes, NJ, USA) at room temperature 22°C. L-PRF membranes were produced using tubes according to the manufacturer, with centrifugation at 2700 rpm for 12 min (~ 708 g) using a fixed angle/vertical centrifuge (IntraSpin ™, Biohorizons®, Birmingham, Alabama, AL, USA). This centrifugation protocol considers the g-force value referenced to the bottom of the centrifuge tubes (RCF - max) [31, 32]. After centrifugation, each L-PRF membrane was removed from the tube and separated from the red phase at the base using sterile forceps.

2.4.2 Alb-PRF Preparation
Blood samples were collected using 9 ml plastic PET tubes (BD Vacutainer®, Becton Serum Blood Collection Tubes, Dickinson & Company, Franklin Lakes, NJ, USA). To produce each membrane, two tubes were inserted into a centrifuge (IntraSpin ™, Biohorizons®, Birmingham, AL, USA), and the protocol for L-PRF was applied to obtain the liquid-phase PRF (plasma + cell-rich portion) was centrifugation at 2700 rpm for 12 min (~ 708 g) using a fixed angle/vertical centrifuge (IntraSpin ™, Biohorizons®, Birmingham, Alabama, AL, USA). After processing, it was possible to visualize the plasma and the remaining blood containing red blood cells. Approximately 2 ml of the initial portion of plasma was collected with a syringe of 3 ml and 18 G needle (Injex®, São Paulo, Brazil), while the rest of the blood (cell-rich portion and RBCs) was preserved at room temperature (22°C).

The syringes containing platelet-poor plasma (PPP) were inserted into a device for denaturing human protein plasma-activated plasma albumin gel (APAG®, Silfraden, Italy). After 10 min at an operating temperature of 75°C. After 10 minutes at a temperature of 70°C, the syringes were stored at room temperature for another 10 minutes to allow cooling.

Subsequently, using a 5 ml syringe with an 18 G needle (Injex®, Brazil), the 4 ml of the rich portion of the buffy coat was collected, added to the heated PPP layer in the glass container, and mixed gently. After the fibrin polymerization, the process was completed in about five minutes with the formation of the membrane.

The procedures to obtain peripheral blood to produce the L-PRF and Alb-PRF were performed on the day of the surgeries immediately before the beginning of the dental extractions. Furthermore, five subjects were aleatorily chosen to donate a blood sample to prepare the blood byproducts for assessing the in vitro release of biological mediators.

### 2.5 Surgical Procedures

Medical and dental anamneses were performed on all participants. Initially, the participants were diagnosed and selected by clinical examination to evaluate the need for dental extractions confirmed by periapical/panoramic radiography of the face. Intraoral photographs were taken of all participants pre- and post-treatment after they agree and sign the Informed Consent Form. Site asepsis was performed by rinsing the mouth with 0.12% chlorhexidine digluconate (Periogard® Colgate New York, New York, USA) for one minute and extraoral with 4% chlorhexidine soap (Riohex Rioquímica® Duque de Caxias - RJ).

Then, local anesthesia was administered (using Carpule syringe, Dowell®, Rancho Cucamonga California, USA ) for inferior alveolar, lingual, and buccal nerves, using alphacaine 2% with epinephrine 1:100,000 (DFL Indústria e Comércio®, Rio de Janeiro, RJ, Brazil). The surgical approach was the same in all surgeries, following the technique described by Hupp (25) for closed extractions of mandibular molars. The release of the soft tissue around the tooth was performed using scalpel handle #3 (Bard Park, Dowell®, Rancho Cucamonga California, USA) and blade # 15c (Solidor, Lamelid®, Osasco, SP Brazil) to test the success of deep anesthesia and for better apical positioning of the lever and forceps. The detachment of the tissue was performed using the Molt detacher nº 9 (Dowell®, Rancho Cucamonga California, USA) around the tooth, and then luxation using an elevator and forceps (Dowell®, Rancho Cucamonga California, USA) for subsequent tooth removal. After finishing the exodontia, the socket was delicately curetted with Lucas curette nº 4 (Dowell®, Rancho Cucamonga California, USA) and rinsed with 0.9% saline solution.

After tooth removal, each socket was filled with L-PRF (G1) or Alb-PRF (G2) according to randomization for each side in the same research participant. Then the socket was sutured with Johnson 4 - 0 silk thread (J&J Ethicon®, Jardim das Indústrias, São José dos Campos, SP, Brazil) using an “X” stitch.

In both groups, the suture was performed without tension, and the research participants received Azithromycin 500 mg (Astro, Eurofarma Laboratórios S.A.®, Itapevi, SP, Brazil) starting on the day of surgery and maintained for four days. The research participants were also instructed to perform oral hygiene using Chlorhexidine gel 0.2% (Perioxidin gel, Laboratório Lacer, S.A ®, - Sardenya, Barcelona, Spain) twice a day, starting on the day of surgery and maintained for 14 days, and analgesia with 500 mg of Paracetamol (Medley®, Campinas, SP, Brazil) only in case of pain.

### 2.6 Postoperative evaluation

One trained evaluator (M.T.) examined all subject volunteers after 7 and 14 days. The consultations were performed by the same examiner and always at the same time. Parameters such as pain, trismus, swelling, presence or absence of infection, and soft tissue healing were evaluated. The pain was analyzed according to the Visual Analogue Scale (VAS), with 0 being no pain and 10 being the most severe pain, together with the graphic rating scale. (26) The research participants received the printed scale and were instructed on how to fill it in before and after the surgical procedure. The subject volunteers filled out daily during the first seven days after surgery and returned the document at the last appointment for recording and tabulation of the data. The number of analgesics consumed during this period was also recorded.

Trismus was evaluated from the interincisal distance measured from the mesial edges of the upper and lower right incisors during maximum mouth opening, as described by UStün et al. [33]. The presence of infection in the dental sockets was clinically evaluated and recorded on postoperative days 1, 7, and 14. Soft tissue healing evaluation was performed using the Landry index, which classifies the healing process as very poor, poor, good, very good, and excellent according to the presence of granulation tissue, bleeding at the slightest touch, exposure of connective tissue at the margin of the incision, and the presence or absence of suppurition.

The swelling was evaluated using a modification of Gabka and Matsumara's tape measure method [29, 34], with pre-and postoperative measurement in the following areas: from the tragus to the pogonion, from the tragus to the labial commissure, from the external palpebral commissure to the mandible angle. The sum of the three preoperative measurements was used for each side. They were measured again after 7 and 14 postoperatively, and the difference between the preoperative and postoperative values established the swelling value of the day.

### 2.7 Assessment of the in vitro release of biological mediators by Alb-PRF and L-PRF membranes
To compare the ability of cytokine and growth factor release from Alb-PRF and L-PRF membranes, blood samples were prepared as described above and cultured (n = 5) for 7 days in 6-well culture plates (TPP, USA), in the presence of 4 ml of DMEM (Dulbecco's Modified Eagle's Medium, GIBCO, USA), without the use of antibiotics, in a humid atmosphere at 37°C and 5% CO2. The conditioned media were collected and stored in a freezer at -80°C. A multiparametric immunoassay based on XMap-labeled magnetic microbeads (LuminexCorp, USA) was employed through a commercial kit (27-plex panel, Biorad Inc., USA) capable of quantifying IL-1β, IL-10, IL-12 (p70), IL-13, IL-15, IL-10, IL-8, IL-17, CCL11, FGF-b, CSF3, CSF2, IFN-γ, CXCL10, CCL2, CCL3, CCL-4, PDGF, CCL5, TNFα and VEGF. Quantification of the magnetic beads was performed with a BioPlex MAGPIX system (Biorad Inc., USA), and the results were analyzed using Xponent v. 3.0 software (Luminexcorp, USA).

2.8 Evaluation of cytokines and growth factors in the surgical sites

On days 1 and seven after surgery, a swab collection was performed in each operated region of the participants to perform the quantification of cytokines and growth factors present in the site over time, as previously described. The swabs were immersed in 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, sonicated for 30 minutes on an ultrasonic bath with ice maintained at 4°C, for protein extraction. The liquid was collected and stored on an ultrafreezer at ~ 80°C. The cytokines and Growth factors from the surgical sites were then detected using the same multiparametric assay described above for the in vitro release of mediators. Quantification was performed with a BioPlex MAGPIX system (Biorad Inc., USA), and the results were analyzed using Xponent v. 3.0 software (Luminexcorp, USA).

2.9 Statistical analysis

In the variables swelling, analgesic consumption, and pain (Visual Analog Scale), the data obtained were expressed as mean with a 95% confidence interval. After applying the D'Agostino & Pearson normality test (p < 0.05) and removal of outliers by the ROUT method (robust regression and outlier removal, Q = 1%), the Tukey test for multiple comparisons (mixed effect analysis) and paired (p < 0.05) was applied. Calculations and graphs were done in Prism 9.0 (GraphPad Software Inc., USA).

Also, in the trismus variable, after applying the D'Agostino & Pearson normality test (p < 0.05) and removing outliers by the ROUT method (robust regression and outlier removal, Q = 1%), data were expressed with mean and confidence interval. Paired Student's t-test was applied to identify differences between groups (p < 0.05).

Data from the cytokine and growth factor assessment for the control and PRF sites were analyzed by nonparametric, paired Mann-Whitney U tests. The correlation between these results and the blood cell counting (platelet and lymphocytes) was investigated through Spearman's Rank Correlation Test, where only strong correlations (coefficient above 0.7) were considered. For all tests, an alpha error of 5% was considered. The tests were performed with help of the GraphPad Prism 9.0 (GraphPad Software Inc., USA).

3. Results

The sample of subjects’ volunteers consisted of three men and seven women, with a mean age of 22.1 ± 3.14 years. Of the 20 teeth, two had extensive carious lesions, and 16 had a previous history of pericoronitis. Regarding the position of the teeth, three presented mesioangular impaction, and 17 with horizontal impaction according to Winter's classification. Regarding education, one research participant had a complete college degree, and nine had a full high school degree.

Postoperative follow-up indicated a good recovery in all cases, with no severe complications, and no research participant presented intolerance to the prescribed medications or side effects/adverse effects.

The CONSORT flowchart is shown in Fig. 1.

3.1 Pain

No research participant reported difficulty distinguishing pain and its intensity between the right and left sides. Postoperative assessment using the Visual Analog Scale (VAS) was performed on days 0, 1, 2, 3, 4, 5, and 6 in both groups (L-PRF and Alb-PRF). In the Alb-PRF group, days 5 and 6 reduced pain according to the VAS compared to day 0. In addition, on day six, the pain reduced compared to day 2 (p < 0.05). In the L-PRF group, day six reduced pain compared to days 0, 1, and 2 (p < 0.05). No significant difference was observed between the L-PRF and Alb-PRF groups in the same experimental period (p < 0.05) (Fig. 2).

3.2 Swelling

All research participants were evaluated on the day of surgery and seven days after for quantification of postoperative edema. All research participants allowed the measurements to be taken without difficulty. Figure 3 shows both groups’ swelling results 1 and 7 days after surgery. The Alb-PRF group showed a significant reduction in edema in the 7-day experimental period (0.61 mm; C.I. 0.08–1.13) when compared to the one-day (2.67 mm; C.I. 1.86–3.47) (p = 0.007). There was no statistical difference between the L-PRF and Alb-PRF groups in the same experimental period (p > 0.05).

3.3 Trismus

Figure 4. Postoperative trismus assessment 1 and 7 days after surgery. The graph expresses the medians and confidence intervals through the points (n = 10). Comparison between experimental periods identifies a significant difference represented by the bar (Paired Student T Test, p < 0.05).

3.4 Analgesic consumption
Analgesic consumption (tablets a day) was evaluated on days 0, 1, 2, 3, 4, 5, and 6 (0.08; CI 0.14–1.45). There was a significant reduction in analgesic consumption between the groups Day 0 (2.50; CI 1.89–3.1), Day 1 (3.80; CI 2.41–5.18), Day 2 (3.60; CI 2.28–4.91) and Day 3 (3.22; CI 1.55–4.88) (p < 0.05). The reduction was also observed on Day 5 (2.10; CI 0.81–3.38) when compared to Day 2 (p = 0.04) (Fig. 5).

3.5 Biochemical analysis

A comparison was made between the ability of L-PRF and Alb-PRF membranes produced from donors to release tissue repair and inflammatory mediators through multiparametric in vitro assessment, as shown in Table 1. After 7 days of incubation, L-PRF presented an increased release of the cytokines GM-CSF, IL-1β, IL-6, TNFα, IFNγ, IL-8, IL-15, IL-4, RANTES, MIP-1a, and MCP-1 (p < 0.05). Alb-PRF presented an increased release of the growth factor PDGF (p = 0.038).

Table 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Alb-PRF</th>
<th>L-PRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>810 ± 153</td>
<td>1104 ± 298</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>551 ± 39</td>
<td>426 ± 24 *</td>
</tr>
<tr>
<td>bFGF</td>
<td>28 ± 6</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>G-CSF</td>
<td>381 ± 58</td>
<td>278 ± 74</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>12 ± 6</td>
<td>57 ± 22 *</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2 ± 2</td>
<td>162,7 ± 78 *</td>
</tr>
<tr>
<td>IL-6</td>
<td>268 ± 45</td>
<td>4564 ± 946 *</td>
</tr>
<tr>
<td>TNFα</td>
<td>39 ± 9</td>
<td>98 ± 24 *</td>
</tr>
<tr>
<td>IFNγ</td>
<td>85 ± 29</td>
<td>246 ± 37 *</td>
</tr>
<tr>
<td>IL-8</td>
<td>6954 ± 782</td>
<td>120589 ± 756 *</td>
</tr>
<tr>
<td>IL-13</td>
<td>2 ± 1.4</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>IL-15</td>
<td>12 ± 8.9</td>
<td>96 ± 26 *</td>
</tr>
<tr>
<td>IL-7</td>
<td>0,46 ± 0.46</td>
<td>0,634 ± 0.46</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>5 ± 4</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>IL-17A</td>
<td>29 ± 8</td>
<td>37 ± 7</td>
</tr>
<tr>
<td>IL-9</td>
<td>4 ± 2</td>
<td>5 ± 0.1</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.31 ± 0.1</td>
<td>0,3 ± 0.38</td>
</tr>
<tr>
<td>IL-2</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>267 ± 70</td>
<td>262 ± 64</td>
</tr>
<tr>
<td>IL-4</td>
<td>0,9 ± 0</td>
<td>8,7 ± 0.7 *</td>
</tr>
<tr>
<td>IL-10</td>
<td>5 ± 4</td>
<td>19 ± 13</td>
</tr>
<tr>
<td>RANTES</td>
<td>593 ± 55</td>
<td>1084 ± 162 *</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>133 ± 3</td>
<td>136 ± 9</td>
</tr>
<tr>
<td>IP-10</td>
<td>20 ± 7</td>
<td>49 ± 22</td>
</tr>
<tr>
<td>MIP-1b</td>
<td>170 ± 57</td>
<td>155 ± 59</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>0 ± 0</td>
<td>39 ± 5 *</td>
</tr>
<tr>
<td>MCP-1</td>
<td>356 ± 22</td>
<td>155 ± 38 *</td>
</tr>
</tbody>
</table>

(*) Values were statistically different between such experimental groups (p < 0.05)

Regarding the release of inflammatory mediators in the surgical sites, Fig. 6 shows a heatmap of the surface quantification of several detectable molecules on the first and seventh day after surgery. It is possible to observe that very similar levels were detected for the main growth factors investigated (PDGF, bFGF, GM-CSF, G-CSF) in both experimental groups. While VEGF had a higher mean concentration in the L-PRF group (525 pg/mL versus 389 for Alb-PRF), there was no significant statistical difference between both experimental sites (p > 0.05).

While a similar pattern was observed for several proinflammatory (IL-6, IL-5, IL-12) and anti-inflammatory (IL-4, IL-10) cytokines, L-PRF sites presented significantly higher levels (p < 0.05) of the proinflammatory cytokine IL-1β, approximately 20-fold higher than Alb-PRF, at both 1 and 7 days, and the
A correlation analysis was performed between each detected cytokine and the main clinical outcomes were investigated both one day (Table 2) and seven days (Table 3) after surgery. The analysis identified a few mediators that strongly correlated to the observed levels of clinical outcomes observed in the participants. In the L-PRF surgical sites, the levels of IL-1β at day one presented a correlation with the presence of trismus, while the report of pain (VAS at 0 and 3 days) was positively correlated to the levels of this cytokine at day seven (p < 0.05, Rho > 0.7). The IL-4 levels also presented a direct correlation with swelling at seven days (Rho = 0.8667, p < 0.05) and pain (VAS at 3 and 5 days, p < 0.05, Rho > 0.7) for L-PRF sites, and with initial pain (VAS at day zero) in Alb-PRF sites (Rho = 0.8359, p < 0.05).

In the L-PRF sites, the growth factor GM-CSF positively correlated with trismus observed at both 1 and 7 days (p < 0.05, Rho > 0.7). On the other hand, in the Alb-PRF sites, RANTES levels detected on day seven were negatively correlated with pain (VAS at two and five days), while the levels of PDGF were inversely correlated to swelling (Rho = -0.7545, p < 0.05).

|                        | VAS 0 | VAS 1D | VAS 2D | VAS 3D | VAS 4D | VAS 5D | VAS 6D | Swelling 1d | Swelling 7d | Trismus 1d | Trismus 7d | Alb-PRF 0 | Alb-PRF 1D | Alb-PRF 2D | Alb-PRF 3D | Alb-PRF 4D | Alb-PRF 5D | Alb-PRF 6D | Swelling 1d | Swelling 7d | Trismus 1d | Trismus 7d |
|------------------------|-------|--------|--------|--------|--------|--------|--------|------------|------------|------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|-----------|-----------|
| **MIP-1b**             | 0.0741| -0.3706| 0.7856 | 0.2546 | 0.1149 | 0.1853 | 0.4637 | 0.2275     | 0.4458     | 0.3615     | 0.3615     |
| **IL-6**               | 0.0124| -0.4751| 0.2578 | 0.3516 | 0.1916 | 0.2347 | 0.1364 | 0.3713     | 0.7470     | 0.5784     | 0.3415     |
| **IL-5**               | 0.1125| -0.1684| 0.1180 | -0.0546| -0.4459| -0.0938| 0.0828 | 0.6606     | 0.5658     | 0.3415     | 0.3415     |
| **GM-CSF**             | 0.3488| 0.0621 | 0.4865 | 0.7013 | 0.7365 | 0.3368 | 0.0591 | 0.5904     | 0.5637     | 0.5784     | 0.5784     |
| **Rantes**             | 0.1243| 0.0621 | 0.6298 | 0.4607 | 0.3052 | 0.6649 | 0.7683 | 0.2275     | 0.3212     | 0.0667     | 0.0667     |
| **IL-1b**              | 0.1359| -0.1853| 0.7013 | 0.3758 | 0.1532 | 0.2965 | 0.7683 | 0.4311     | 0.3212     | 0.4097     | 0.4097     |
| **bFGF**               | 0.2347| -0.3336| 0.4637 | 0.3031 | 0.3065 | 0.0988 | 0.6627 | -0.0240    | 0.5543     | 0.4097     | 0.4097     |
| **VEGF**               | 0.2718| -0.1482| 0.3548 | 0.1037 | 0.0306 | 0.0249 | 0.5576 | 0.3313     | 0.5576     | 0.4097     | 0.4097     |
| **PDGF**               | 0.1367| -0.2610| 0.3448 | -0.1646| -0.2955| -0.1802| 0.5785 | 0.4096     | 0.5578     | 0.5784     | 0.5784     |
| **IL-4**               | 0.0124| -0.2610| 0.3854 | -0.1646| -0.2955| -0.1802| 0.5785 | 0.4096     | 0.5578     | 0.5784     | 0.5784     |
| **MCP-1**              | 0.1989| -0.2983| 0.3052 | -0.1646| -0.2955| -0.1802| 0.5785 | 0.4096     | 0.5578     | 0.5784     | 0.5784     |
| **MIP-1a**             | -0.1359| -0.3356| 0.0306 | -0.1646| -0.2955| -0.1802| 0.5785 | 0.4096     | 0.5578     | 0.5784     | 0.5784     |
| **IL-10**              | 0.1359| -0.2594| 0.0306 | -0.1646| -0.2955| -0.1802| 0.5785 | 0.4096     | 0.5578     | 0.5784     | 0.5784     |

Results represented as Spearman’s Rho. VAS—Visual analog scale of pain; Bold numbers with an asterisk indicate a significative relevant correlation (p < 0.05, Rho > 0.7).
Correlation has been identified between trismus (day 1) and pain (day 7) parameters with IL-1β PRF membranes both in vitro and in samples collected from surgical sites.

The surgery involving lower-third molars is recognized as one of the most frequent procedures in clinical practice [35]. Adverse effects such as pain, trismus, infection, and edema often ensue post-extraction [36]. This research was conducted with the objective of linking the concentration of cytokines and growth factors at the extracted site, implanted with a new platelet concentrate Alb-PRF and L-PRF, to clinical findings such as pain, trismus, and swelling. Increasing evidence indicates the beneficial utilization of platelet concentrates in post-extraction sites, predominantly to enhance soft tissue healing and diminish postoperative symptoms [29, 37, 38].

The information concerning the concentrations of growth factors and pro- and anti-inflammatory delivered cytokines on the surgical sites implanted with platelet concentrates remains limited and contentious. Prior systematic reviews have assessed and compared L-PRF, PRGF, and PRP with natural healing [38–40]. This research provides a first-time comparison between denatured plasma combined with platelet-rich fibrin (Alb-PRF) and L-PRF in dental sockets from both clinical and in vitro perspectives. The relation between clinical parameters and analytes from the surgical site could be tied to the different cytokine profiles detected in Alb-PRF and L-PRF. The enhanced expression profile of pro-inflammatory molecules can be seen in the measurements of eluates from L-PRF membranes both in vitro and in samples collected from surgical sites.

Correlation has been identified between trismus (day 1) and pain (day 7) parameters with IL-1β levels, noticeable in sites that received L-PRF. This correlation can be grounded on the pro-inflammatory action of this cytokine. Alongside direct participation in inflammation orchestration, IL-1β plays a vital role in...
initiating nociceptive events [41]. GM-CSF, another pro-inflammatory cytokine, exhibited a correlation with trismus in L-PRF surgical sites (days 1 and 7), data that can be validated by the nature of its roles in the inflammatory process.

GM-CSF participates in the proliferation and differentiation processes of hematopoietic cells, specifically macrophages, and granulocytes. During inflammation, various cells can generate GM-CSF, primarily tissue-residing cells and T and B lymphocytes. This cytokine contributes to the survival, adhesion, and traffic of neutrophils, and it boosts the antimicrobial functions of macrophages through enhanced phagocytosis and the generation of reactive oxygen species [42]. However, its elevated expression is linked to degenerative diseases such as rheumatoid osteoarthritis and spondyloarthritis [43]. Even though a correlation was noted between trismus and sites with L-PRF, no significant variations were observed in the dosage of GM-CSF between sites with L-PRF and Alb-PRF.

RANTES, defined in literature as a pro-inflammatory cytokine, primarily recruits and activates leukocytes, monocytes, granulocytes, and dendritic cells in areas of tissue injury. It also contributes to angiogenesis events and potentially affects the differentiation of osteoblasts [44]. An overexpression of RANTES was connected to the initiation of atypical facial pain and trigeminal neuralgia, which contrasts with the negative correlation found between Alb-PRF and RANTES (day 7).

Interleukin 4 (IL-4) is a versatile multipotential cytokine secreted by mast cells, eosinophils, basophils, and Th2 cells [45]. It is widely documented for its anti-inflammatory properties. Nonetheless, some evidence suggests a role of IL-4 in instigating inflammatory conditions such as dermatitis, asthma, and Kawasaki disease. The correlation between IL-4 and pain (Alb-PRF day 1) and swelling (L-PRF day 7) could be linked to this cytokine's propensity to increase vascular permeability, contributing to extracellular fluid accumulation and consequent edema formation [46].

However, a comprehensive understanding of the correlations between specific analytes and symptomatology necessitates the consideration of these cytokines' individual actions and the expression of the interaction of different substances and the inflammation microenvironment [47].

In past studies, the use of L-PRF has been underlined as a valid method in promoting and accelerating soft and hard tissue regeneration due to its effects on wound healing improvement, pain reduction, and bone density increase [29, 37–39]. In this research, a significant difference in the Alb-PRF group from day 1 to day 7 for swelling was observed. These findings are not widely accepted in literature due to variations in the third molar position and surgical trauma during the operation, which directly impact postoperative pain, swelling, and trismus.

In the present study, a selection was made for a uniform set of lower third molars regarding location, type, extraction cause, adoption of minimally traumatic extraction procedures, precautions about aseptic chain during surgeries, and postoperative hygiene care instructions, in order to reduce study bias.

Concerning the time points selected for outcome measurements, a period of 1 and 7 weeks for evaluating pain, swelling, and trismus appears reasonable. It has been shown that a few weeks are sufficient to achieve complete wound closure or at least complete re-epithelization, even if a second intention of soft tissue healing occurs [29, 48].

5. Conclusion

Statistical differences were observed in the in vitro release of various analytes between the membranes, highlighted by the significant release of pro-inflammatory cytokines by L-PRF compared to Alb-PRF. Clinically, Alb-PRF demonstrated a significant reduction in edema after seven days. As this represents the first clinical trial using this autologous biomaterial, future studies are suggested to further elucidate the potential of Alb-PRF for dental socket healing and other surgical procedures.

Declarations

Author Contribution: K.J. contributed to the design and execution of the study and the writing of the manuscript. C.F.M. was involved in conceptualizing the study, data interpretation, and manuscript revision. S.C.S. contributed to the experimental design and carried out the surgical procedures. R.C.M. was involved in the design of the study, data collection, and data analysis. M.T. participated in data collection, patient management, and the writing of the manuscript. E.S.L. contributed to data analysis, interpretation, and the writing of the manuscript. P.E.C.L. participated in biochemical analysis, data interpretation, and manuscript revision. J.M.G. was involved in study conceptualization, overseeing surgical procedures, and manuscript revision. G.G.A. participated in the design of the study, data interpretation, and the writing of the manuscript. M.D.C.M. contributed to the design and oversight of the study, data interpretation, and the critical review of the manuscript.

All authors read and approved the final manuscript.

Ethics and consent to participate: The Ethical Committee of the Brazilian government approved the current study with registration number 5,072,786. All subjects who volunteered to participate in the study agreed to do so and signed an informed consent form.

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Conflict of Interests: The authors declare no conflict of interest.

References


24. Mourão CF, Lowenstein A. The Use of Alb-PRF as a Drug Delivery System for Malignant Lesion Treatment. Revista Brasileira de Cancerologia. 2023;69(2).


Figures
CONSORT 2010 Flow Diagram

Enrollment

Assessed for eligibility (n=31)

Excluded (n=20)
• Not meeting inclusion criteria (n=20)

Randomized (n=11 research participants, 22 sockets)

Allocation

Allocated to intervention (n=11)
Socket filled with L-PRF

Allocated to intervention (n=11)
Socket filled with AOB-PRF

Follow-Up

Lost to follow-up (give reasons) (n=1)
Discontinued intervention (withdrawal) (n=0)

Lost to follow-up (give reasons) (n=1)
Discontinued intervention (withdrawal) (n=0)

Analysis

Analysed (n=10)

Analysed (n=10)

Figure 1

Flowchart of the process of inclusion, allocation, and analysis of the research participants recruited according to CONSORT.
Postoperative evaluation of pain according to the visual analog scale. The graph expresses the mean and confidence interval across points (n=10) on days 0, 1, 2, 3, 4, 5, and 6 for both groups. Comparison between experimental periods identified a significant difference represented by the letters a (≠ Day 0 within the same group), b (≠ Day 1 within the same group), and c (≠ Day 2 within the same group). Multiple Tukey's tests (mixed effect analysis) and paired comparisons (p< 0.05).

Evaluation of swelling. Graph expressed as mean and confidence interval showing the points (n=10) in the experimental periods of 1 day and seven days evaluated for the L-PRF and Alb-PRF groups. The comparison between groups did not identify significant differences. The comparison between periods...
showed a reduction of edema in the Alb-PRF group. Tukey's test for multiple comparisons (mixed effect analysis) and paired (p< 0.05).

Figure 4

Postoperative trismus assessment 1 and 7 days after surgery. The graph expresses the medians and confidence intervals through the points (n=10). Comparison between experimental periods identifies a significant difference represented by the bar (Paired Student T Test, p< 0.05).
Figure 5

Evaluation of analgesic consumption (pills a day). The graph expresses the mean and confidence interval through the points (n=10) on days 0, 1, 2, 3, 4, 5, and 6. Comparison between experimental periods identifies a significant difference represented by the letters a ($\neq$ 0D), b ($\neq$ 1D), c ($\neq$ 2D), and d ($\neq$ 3D). Tukey’s test of multiple (mixed effect analysis) and paired comparisons (p< 0.05).
Heatmap of the variation of concentrations of different analytes identified in the surface of the surgical sites implanted with L-PRF or Alb-PRF, collected by swab after 1 or 7 days after surgery. (*) Values were statistically different between such experimental groups (p < 0.05).