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Evaluation of the Effect of Primary and Secondary Closure on the Use of Leukocyte and Platelet Rich Fibrin in Impacted Lower Third Molar Surgery

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Abstract: The aim of this study was to compare the effect of using L-PRF in patients undergoing impacted lower third molar surgery with either primary or secondary closure techniques. Methods: This prospective, randomised, double-blind, split-mouth clinical trial was conducted in patients with bilateral lower impacted third molars of similar position. Primary closure was performed in group 1 and secondary closure in group 2. Group 1 closure technique was applied to one side of the patients and group 2 closure technique was applied to the other side at different times. Nine patients were excluded from the study due to alveolitis and failure to attend regular control visits out of the 45 patients evaluated. Results: Of the 36 patients included in the study, 23 were female and 13 were male and the mean age was 22.42±3.36 years. The secondary closure group had lower VAS scores at hour 6 ($p<0.05$). Pain decreased more in the primary closure group when comparing changes between the VAS scores at 6 hours and 7 days ($p<0.05$). Conclusions: The results of this study, showing that both secondary and primary closure are effective with similar outcomes in terms of pain, swelling and trismus, should be supported by future clinical trials.

Keywords: leukocyte and platelet rich fibrin; closure techniques; wound healing; regenerative medicine; primary closure; secondary closure; impacted third molar

1. Introduction

Leukocyte and platelet rich fibrin (L-PRF) therapy is currently an area of significant interest in the field of regenerative medicine. This special type of platelet concentrate is now used to accelerate the healing process in oral and maxillofacial surgical procedures involving both soft and hard tissues [1]. L-PRF is a simple and completely natural treatment option that does not require anticoagulants, bovine thrombin or other additives. The procedure involves taking blood before surgery and centrifuging it for 12 minutes. This creates a pliable membrane and a collection of platelets and growth factor-rich clots held together by fibrin. The top layer, known as the red layer, has a high concentration of platelets that release growth factors as the fibrin matrix is formed. Of paramount importance is the layer known as the buffy coat, which consists of white cells, platelets and significant amounts of fibrin. Fibrin plays a crucial role in the body's healing process and in the formation of connective tissue after trauma. The white cells in L-PRF are trapped and stimulated as part of the natural healing mechanism. The final layer is acellular and contains approximately twice the amount of platelets found in peripheral blood. It is worth noting that L-PRF uses the patient's own blood, removing any concerns about disease transmission. This should encourage clinicians to incorporate L-PRF into their practice [1–3]. L-PRF is currently being used in a number of procedures, including the treatment of drug-induced osteonecrosis of the jaw, extraction socket healing and pre-prosthetic bone surgery [4–7].

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It appears that primary closure has advantages over secondary closure in terms of postoperative infection, facial swelling and trismus after lower impacted third molar surgery. This may be due to open flap debridement. However, some studies suggest that primary and secondary closure result in similar postoperative complications. For example, a systematic review by Bailey et al showed a higher incidence of infection with primary wound closure compared with secondary intention to leave the wound to heal. However, the confidence intervals were wide and favoured both methods of healing. It is not clear why this should be, but it is the case that pain and trismus have been shown to be reduced with primary closure compared with second intention healing. On the other hand, Morely et al concluded that there was insufficient evidence to recommend either primary or secondary closure techniques. This is because the nature and quality of the data over the years has been unjustified and imprecise. This makes it difficult to determine the best techniques to use, especially as surgical training becomes more competency-based [8–11].

The use of L-PRF in impacted third molar surgery has shown improved wound healing, reduced incidence of alveolar osteitis and improved postoperative recovery [5]. However, wound closure after surgery is an ongoing debate. When the studies evaluating the closure method of the lower impacted wisdom tooth surgery without L-PRF are examined, it is seen that the primary closure method is recommended in the old studies, while the secondary closure method is recommended in the current studies in terms of complications [12–16]. There are no studies reporting which closure technique is more successful in preventing or reducing postoperative complications after impacted lower third molar surgery when L-PRF is used. We believe that demonstrating the potential benefits of L-PRF on postoperative complications and which type of closure is more effective will ensure that L-PRF is used with the correct technique for this purpose. The aim of this study was to compare the effect of using L-PRF in patients undergoing impacted lower third molar surgery with either primary or secondary closure techniques.

2. Materials and Methods

This prospective, randomised, double-blind, split-mouth clinical study was conducted at Van Yüzüncü Yıl University, Faculty of Dentistry, Oral and Maxillofacial Surgery Clinic between October 2022 to May 2023. The study was conducted in accordance with the current version of the Declaration of Helsinki. The study was approved by Van Yüzüncü Yıl University Faculty of Medicine Clinical Research Ethics Committee (decision number: 07 dated 01.09.2022). Among the volunteers, those who met the inclusion criteria were informed about the study and signed informed consent was obtained. Medical and dental history was taken at the first clinic visit. The presence or absence of bilateral impacted third molars was assessed by clinical and radiological examination. Demographic information, facial and mouth opening measurements, pain scores and group numbers were recorded on the anamnesis forms.

Inclusion criteria were 18 years of age or older, no systemic disease, bilateral and similar (vertical or mesioangular) position, asymptomatic, partially mucosa and bone retained lower impacted third molars.

Exclusion criteria were as follows: smokers, pregnant or breastfeeding women, those not attending follow-up visits, those allergic to the materials or drugs used in the study, smokers, those with alveolitis, and those taking additional or different drugs to the study drugs.

Randomisation in the trial was achieved using the envelope method. From the envelopes given to the patients by the support staff, the patients chose which side to operate on first and which group to use first. Only the sessional staff knew the numbers on the envelopes. To ensure blinding of the study authors, the suturing phase, i.e. closure, including L-PRF placement, was performed by an independent experienced surgeon outside the study. Surgeries were performed on Mondays, Tuesdays and Wednesdays to allow for post-operative day 2 controls. The second operations were performed at least 4 weeks later.

Facial measurements for swelling assessment (The reference points for the facial measurements used in the swelling score were the angulus-lateral canthus, angulus-lateral corner of the nose and angulus-pogonion distances. A single value was obtained by averaging these 3 distances in the

 doi:10.20944/preprints202404.0094.v2

swelling score) and mouth opening measurements for trismus assessment were performed preoperatively, on postoperative day 2 and on postoperative day 7 with the patients sitting upright in the unit. Pain was assessed by VAS and patients rated their postoperative pain at 6, 12, 18 and 24 hours and on days 2, 3, 4, 5, 6 and 7.

2.1. Study Groups

Patients underwent two different closure procedures. 10 ml of blood was taken from the patients and L-PRFs were centrifuged at 2700 rpm for 12 minutes in 1 tube without activator, and prepared by forming their final shapes into plugs in the L-PRF set for 5 minutes. The same surgical protocol was used in both groups and 1 L-PRF plug was placed in the extraction sockets. At the suture stage with 3/0 silk suture, primary closure was performed in group 1 with periosteal release to achieve tension-free closure, and secondary closure in group 2 of the approximately 5-7 x 5-7 mm space that existed prior to surgery adjacent to the second molar (partial mucosa retention) (Figure 1). Amoxicillin 1 g (2x1), naproxen sodium 550 mg (2x1) and 0.15% benzidamine hyrochloride + 0.12% chlorhexidine digluconate (3x1) were used routinely in the postoperative period. Patients were advised to follow a soft diet and were instructed on oral care and the use of medications. Controls were performed on the 2nd and 7th postoperative day, and sutures were removed after the controls on the 7th postoperative day. Surgery was performed by one of the authors. Pre- and post-operative assessments were performed by another author surgeon.

(**a**) (**b**)

Figure 1. (**a**) Primary closure in group 1; (**b**) Secondary closure in group 2.

Power analysis was performed using G*Power (v 3.1.7) to determine sample size. As a result of the calculation based on the reference study, the effect size was calculated as d = 0.512 and it was calculated that at least 32 people should be included in the study to have 80% power at the $\alpha = 0.05$ level [17]. A total of 45 patients were evaluated during the study period. Five of these patients were excluded from the study because of alveolitis and four patients were excluded because they did not attend the controls regularly. Finally, data from 36 patients were analysed.

2.2. Statistical Analysis

Our analyses were carried out using SPSS 26.0, with a confidence level of 95%. In our analyses, mean and standard deviation values were given for the measurements, and frequency and percentage values for the gender distribution. Examination of measurements taken at different times with respect to side of surgery and gender was analysed by independent groups t-test. Repeated ANOVA test was used to analyse the change in measurements over time according to the operated side and gender.

3. Results

Of the 36 patients included in the study, 23 were female and 13 were male and the mean age was 22.42±3.36 years (Table 1).

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Table 1. Age and Gender Distribution.

Age	**min-max: (18-39)**	**Mean:22,42±3,36**	
		n	**%**
Gender	Female	23	63,9
	Male	13	36,1

VAS scores at hour 6 showed a significant difference between groups. VAS scores were higher in group 1 ($p<0.05$). The differences between the groups in the VAS, except at the 6th hour, in the use of rescue analgesics, in mouth opening and in the facial measurements at other times were not significant ($p>0.05$) (Table 2).

Table 2. Comparison of mouth opening, face measurement, VAS scores and rescue analgesic number according to groups.

	Groups				
	Primary closure		**Secondary closure**		P2
	Mean	Sd	Mean	sd	
Preopative mout opening	45,56	6,51	44,23	6,37	0,547
2nd postoperative day mouth opening	26,47	8,26	25,56	6,93	0,612
7th postoperative day mouth opening	38,61	8,00	36,39	6,20	0,192
P1 =0,528					
Preoperative face measurements	109,67	7,73	110,21	7,47	0,179
2nd postoperative day face measurements	114,72	8,10	111,78	7,51	0,114
7th postoperative day face measurements	111,00	8,65	109,22	6,89	0,338
P1 =0,157					
6th hour VAS	5,83	2,48	4,50	2,72	**0,033***
12th hour VAS	4,56	3,09	3,64	2,89	0,198
18th hour VAS	4,50	2,91	3,53	2,61	0,141
24th hour VAS	3,83	2,26	2,94	2,35	0,107
2nd day VAS	3,14	2,11	3,50	2,78	0,537
3rd day VAS	1,86	1,62	2,61	2,51	0,138
4th day VAS	1,31	1,53	2,06	2,12	0,090
5th day VAS	0,86	1,31	1,56	2,09	0,096
6th day VAS	0,81	1,51	1,17	1,54	0,318
7th day VAS	0,61	1,23	1,06	1,58	0,187
P1 **=0,000***					
Rescue analgesic number	8,81	5,59	7,39	4,92	0,257

p1<0,05 Repeated ANOVA, p2<0,05 Independent groups t test .

It was found that the change in mouth opening and facial measurements over time did not cause a significant difference between the groups ($p>0.05$) (Table 2).

When the change in VAS scores was analysed according to time, a difference was observed between the 6th hour and the 7th day. It was found that the decrease was greater in group 1 than in group 2 ($p<0.05$). There was no difference between the groups in terms of change at other times ($p>0.05$) (Table 2).

 doi:10.20944/preprints202404.0094.v2

The preoperative, 2nd and 7th postoperative facial scores differed according to gender. It was found that the facial scores of males were higher than those of females ($p<0.05$). Day 4 VAS scores differed by gender. Male VAS scores were higher than female VAS scores ($p<0.05$). There was no statistically significant difference in VAS scores and rescue analgesic use between the genders at other times ($p>0.05$) (Table 3).

Table 3. Comparison of mouth opening, face measurement, VAS scores and rescue analgesic number according to gender.

	Gender				p
	Female		Male		
	Mean	Sd	Mean	sd	
Preopative mouth opening	44,48	6,70	47,46	5,94	0,191
2nd postoperative day mouth opening	24,96	6,69	29,15	10,24	0,146
7th postoperative day mouth opening	36,96	6,95	41,54	9,15	0,100
Preoperative face measurements	106,74	6,68	114,85	6,85	**0,001***
2nd postoperative day face measurements	111,74	6,33	120,00	8,42	**0,002***
7th postoperative day face measurements	108,48	8,04	115,46	8,13	**0,018***
6th hour VAS	5,83	2,64	5,85	2,27	0,982
12th hour VAS	4,09	2,73	5,38	3,62	0,232
18th hour VAS	4,22	2,81	5,00	3,14	0,447
24th hour VAS	3,65	2,25	4,15	2,34	0,530
2nd day VAS	3,13	1,71	3,15	2,76	0,975
3rd day VAS	1,61	1,53	2,31	1,75	0,220
4th day VAS	0,87	1,14	2,08	1,85	**0,020***
5th day VAS	0,61	0,94	1,31	1,75	0,201
6th day VAS	0,48	0,85	1,38	2,18	0,172
7th day VAS	0,35	0,78	1,08	1,71	0,166
Rescue analgesic number	8,17	5,20	9,92	6,28	0,375

*p<0,05 Independent groups t test.

4. Discussion

The wound healing process is a remarkably complex phenomenon that is still not fully understood. In oral surgery, the natural healing process, also known as first intention healing, is achieved by two main techniques, primary and secondary closure. Primary closure refers to the close approximation of the skin or mucosa for the purpose of healing. On the other hand, secondary closure involves allowing the wound to heal through granulation and re-epithelialisation [8,9,15].

The benefits of L-PRF in impacted third molar surgery are numerous and range from decreasing the amount of pain and swelling the patient experiences after surgery to increasing the speed of soft tissue and bone healing. L-PRF provides a scaffold for hard and soft tissue to grow into and around, which is particularly important in impacted third molar surgery where there is a large bony defect that needs to heal. The slow release of growth factors over the first week of healing are thought to greatly increase the speed of the healing process as growth factors are chemicals in the body that promote quicker, more effective tissue repair. As a result, conventional surgical extraction in such cases often leads to potential complications, including severe pain, swelling and trismus. Fortunately, with the development of platelet concentrate techniques, surgeons now have the opportunity to use L-PRF in their daily practice. This technique speeds up the healing process and significantly reduces

 doi:10.20944/preprints202404.0094.v2

the incidence of inflammatory complications. However, despite the promising results of the use of L-PRF, there is currently a lack of studies comparing primary and secondary closure techniques associated with its usage [18–22].

This is the first study to compare the primary and secondary closure methods for L-PRF in impacted lower third molar surgery. We compared primary and secondary closure techniques to determine which would have a greater effect on postoperative complications, or which would mask the potential benefits of L-PRF. There was no difference between the 2 closure methods in terms of mouth opening, facial measurements and use of rescue analgesics. At 6 hours, more pain was observed on the side with primary closure. On the other hand, the decrease in postoperative pain at the end of the first week was greater on the primary closure side. In the present study there was only a difference in the facial measurements when compared by gender, and it was observed that the facial measurements of men were higher on preoperative, 2nd postoperative and 7th postoperative days. The main reason for this difference, apart from the gender difference causing swelling, is that men have a longer face. The fact that men's facial measurements were higher when comparing preoperative values supports this idea.

Daugela et al. compared L-PRF with a control group using a normal clot after lower impacted third molar surgery. In their study, in which primary closures were performed, they found that there was faster healing and less pain in the first postoperative week on the side where L-PRF was used. They also reported that there was less swelling on the 1st and 3rd postoperative day in the L-PRF group [18]. In another study using primary closure, Dar et al. compared PRF with a normal empty extraction socket on postoperative days 1, 3, 7 and 14. They reported less pain and swelling in the L-PRF group at all postoperative assessments [23]. Tadic et al. compared the effect of L-PRF on periodontal pocket formation in the adjacent tooth and on new bone density and bone volume in the extraction socket of impacted lower third molars with an empty extraction socket left to heal normally. They reported that the parameters evaluated in both groups were similar after 8 weeks [24]. Caymaz and Uyanık compared the effect of L-PRF and A-PRF on complications after impacted lower third molar surgery. In their study using the primary closure method, they found that A-PRF was more effective than L-PRF on pain [25]. da Silva et al studied the effects of L-PRF on post-operative pain and wound healing in people undergoing extraction of lower wisdom teeth and showed that pain was less on the side where L-PRF was used and wound healing was faster. The authors suggested that these results were related to the growth factors released by L-PRF [5]. In their study, Ritto et al. reported that there was no difference in pain and soft tissue healing on the side treated with L-PRF with primary closure after mandibular wisdom tooth surgery compared to the control group, contrary to the studies by Dauga et al., Dar et al. and Caymaz and Uyanık Ritto et al. reported that L-PRF was effective in bone healing in the extraction socket, contrary to the results of Tadic et al. [26].

de Almeida Barros Mourao et al used L-PRF with secondary closure in lower molar extraction sockets and found that the side with L-PRF had less pain and better wound healing than the side left to heal normally [27]. In their study, Afat et al. left the extraction sites to secondary healing after L-PRF following lower impacted third molar surgery. They showed that the groups treated with L-PRF had better wound healing scores than the control group at 1, 2 and 3 weeks [28]. When comparing the effects of primary and secondary closure methods in routine lower impacted wisdom tooth surgery without L-PRF, it is generally reported in the literature that the secondary closure method has fewer postoperative complications, especially pain and swelling [12,14–16]. In contrast to our study, studies using L-PRF have compared primary and secondary closure methods with the control group. The better wound healing and less pain and swelling in the primary and secondary closed extraction sockets with L-PRF compared to the control groups demonstrate the positive postoperative effects of L-PRF [18,23,25,27,28]. These data are the main reason why we preferred L-PRF in this study. In this first study to evaluate the primary and secondary closure methods described in the literature in submerged wisdom tooth extraction sockets using L-PRF, it was observed that the effects of both methods on trismus and swelling were similar. In terms of pain, the side left to secondary healing was less painful during the first 24 hours, and this difference was statistically significant at 6

 doi:10.20944/preprints202404.0094.v2

hours. From day 2, VAS scores were lower on the primary closure side, although not statistically significant. At the end of the first week there was no difference in pain, swelling and trismus. The fact that the side with primary closure reduced pain more than the side with secondary closure when the change in VAS scores was analyzed from hour 6 to day 7 (no difference between the VAS scores on day 7) shows that the primary closure method also has an effect on pain. At 6 hours, we think that the reason for less pain on the side left to secondary healing is due to the lower degree of inflammatory reaction in the initial stage of this type of closure compared to primary closure. Individuals with partially mucosa and bone-retained impacted third molars were included in this study. In the primary closure group, some periosteal release was performed to ensure tension-free closure. This procedure may have had a negative effect on postoperative complications in the primary closure group. The silk suture, which was preferred to avoid patient discomfort, may have influenced the results by increasing the inflammatory response in the region. Although bleeding was controlled in the groups, the differences in postoperative bleeding due to the different closure methods may have influenced the results. In addition, the anti-inflammatory analgesic and mouthwash used in the trial may have influenced the results. These were the limitations of the trial.

5. Conclusions

This was the first study to evaluate the effect of primary and secondary closure methods in extraction sockets with L-PRF after lower impacted third molar surgery. It was found that less pain was observed on the secondary closure group at the sixth hour. Pain decreased more in the primary closure group when comparing changes between the VAS scores at 6 hours and 7 days. The results of this study, showing that both secondary and primary closure are effective with similar outcomes in terms of pain, swelling and trismus, should be supported by future prospective clinical trials.

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Informed Consent Statement: Informed consent was obtained from all subjects included in the study. Written informed consent was obtained from patients for the publication of this paper.

Data Availability Statement: The dataset used in this study is available on request. The data is not publicly available as it contains information that could compromise the privacy of research participants.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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