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Effects of leukocyte- and platelet-rich fibrin alone and combined with hyaluronic acid on early soft tissue healing after surgical extraction of impacted mandibular third molars: A prospective clinical study

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ABSTRACT

Purpose: In this prospective, randomized, double-blind, controlled study, we evaluated the effects of leukocyte- and platelet-rich fibrin (L-PRF) alone and combined with hyaluronic acid (HA) sponge on early healing of soft tissue after mandibular third molar (M3) surgery.
Patients and methods: In total, 60 patients aged 18–30 (mean 22.3), 22 male and 38 female, were included in this study. After surgical extraction of the impacted M3, L-PRF was applied to the socket in the L-PRF group ($n = 20$), and a combination of L-PRF and HA was applied in the L-PRF + HA group ($n = 20$). Nothing was applied in the control group ($n = 20$). The primary outcome variable was healing score for the mucosa over the extraction socket on the 7th, 14th, and 21st days. Secondary outcome variables were frequencies of postoperative complications: hemorrhagic complications, alveolar osteitis (AO), and postoperative wound infection.
Results: Mean healing scores for the mucosa on the 7th, 14th, and 21st days for both the L-PRF group and the L-PRF + HA group were significantly better than those for the control group. The ratio of alveolar osteitis for the control group was 1:20 and the ratio of postoperative wound infection for the control group was 1:20. There were no cases of alveolar osteitis or postoperative wound infection in the L-PRF and L-PRF + HA groups. No hemorrhagic complications were observed in this study.
Conclusion: The results of this study suggest that L-PRF alone and when combined with HA can be an effective way to improve soft tissue healing, and could be used to prevent postoperative alveolar osteitis and infection after M3 surgery. Further studies with larger study groups are necessary.

1. Introduction

Surgical extraction of impacted M3 is one the most frequently performed dental surgical procedures (Goktay et al., 2011). Alveolar osteitis (AO), wound infection, and prolonged or delayed bleeding are the main complications after M3 surgery. Even though these complications are mainly related to the quality of the early blood clot formed in the socket and patients' own healing capacity, postoperative complications can damage patients' trust in their surgeon and lower the surgeons' prestige.

Various surgical techniques and materials designed to decrease postoperative complications and accelerate the healing process

after M3 surgery have been described (Gocmen et al., 2015; Kaul et al., 2012; Goktay et al., 2011; Gönül et al., 2015; Gonul et al., 2015; Koray et al., 2014; Ozgul et al., 2015; Kumar et al., 2015; Alissa et al., 2010; Hoaglin and Lines, 2013; Pasqualini et al., 2005; Chaudhary et al., 2012; Aloy-Prosper et al., 2010; Sammartino et al., 2009).

L-PRF is a concentrate obtained from the patient's own blood, collecting on a single fibrin membrane all the constituents of a blood sample that are favorable to healing and immunity. It contains platelets as well as abundant growth factors and other cytokines, including PDGF (platelet-derived growth factor), TGFb-1 (transforming growth factor beta), and IGF (insulin-like growth factor) (Dohan et al., 2006a, 2006b). Although platelet and leukocyte cytokines play an important part in the biology of this biomaterial, the fibrin matrix supporting them provides the determining element responsible for the overall therapeutic potential of L-PRF (Choukroun et al., 2006).

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Several animal and human studies have shown that platelet concentrates applied to a tooth-extraction socket can decrease postoperative complications and improve healing (Kaul et al., 2012; Zhao et al., 2011; Aloy-Prosper et al., 2010; Ozgul et al., 2015; Antonello Gde et al., 2013; Kumar et al., 2015; Alissa et al., 2010; Farina et al., 2013; Albanese et al., 2013; Hoaglin and Lines, 2013; Sammartino et al., 2009).

Hyaluronic acid (HA), a high-molecular-weight glycosaminoglycan, and one of the major components of extracellular matrix, can be found in numerous tissues, including joint synovial fluid and vitreous humour of the eye (Gocmen et al., 2015; Vindigni et al., 2009).

HA has many properties that make it an ideal molecule for facilitating wound healing, including inducing beneficial early granulation tissue formation, inhibiting destructive inflammation during the healing phase, and promoting re-epithelialisation and angiogenesis (Koray et al., 2014; Vindigni et al., 2009).

HA in the solid form is commonly used as a biocompatible, biodegradable, and non-immunogenic wound dressing (Cervelli, De Angelis et al., 2010; Longinotti, 2014; Cervelli, Lucarini, et al., 2010; Vindigni et al., 2009; Zavan et al., 2009).

Various platelet concentrations in combination with different HA forms are applied in many medical fields to accelerate wound healing, reduce scar tissue, protect the wound site from external factors, decrease the number of dressings, and increase postoperative patient comfort (Cervelli, De Angelis et al., 2010; Cervelli, Lucarini, et al., 2010; Longinotti, 2014; Sandhu et al., 2015).

This study explored the clinical effect of L-PRF alone and L-PRF combined with HA sponge (Hyaloss Matrix®, Anika Therapeutics, Bedford, MA) on early wound healing in comparison with a control group after M3 surgery.

2. Material and methods

This prospective, randomized, double-blind, controlled study was designed to assess the hypothesis that L-PRF alone, and especially when combined with HA sponge, will accelerate soft tissue healing and reduce the incidence of postoperative complications after partially impacted M3 extraction.

The G*Power 3.1 Program (Heinrich-Heine-Universität — Düsseldorf, Germany) was used to calculate an appropriate sample size. Each group required 20 (in total $20 \times 3 = 60$) subjects to detect an effect size of 0.24 with a power of 0.8 (alpha error probability = 0.05).

Sixty patients in the Department of Oral and Maxillofacial Surgery, Dentistry Faculty, Marmara University, Istanbul, Turkey were enrolled to the study, which followed the Declaration of Helsinki on medical protocol and ethics. Ethics committee approval was obtained from the appropriate institution (Marmara University Faculty of Dentistry Ethics Committee Protocol Code: 2016-50) for clinical investigations, and all participants signed an informed consent agreement.

To standardize the study, all three groups comprised elective patients with a unilateral, partially erupted, mandibular third molar, who underwent extraction surgery (Fig. 1). The M3 positions were evaluated on preoperative radiographs based on the Pell and Gregory classification. The inclusion criteria were mandibular third molars with class 2, position B vertical impaction and all patients were between 18 and 30 years of age, non-smokers, and classified as ASA I, using the guidelines of the American Society of Anesthesiology. The exclusion criteria were pericoronitis, use of any kind of drug (including oral contraceptives), smoking, and failure to follow oral hygiene instructions.

A custom random number generator was used by the third investigator (OG) to randomly assign patients to three groups.
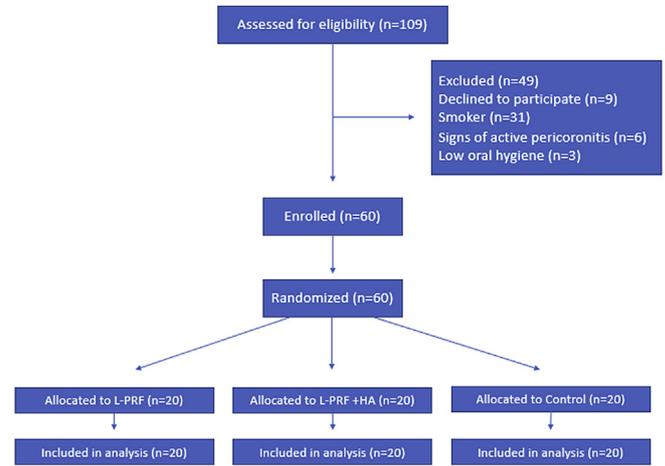

Fig. 1. Flowchart of patient's selection and randomization in three study groups.

Patients did not know their group assignments until the study was completed.

To obtain L-PRF, immediately before surgery, two blood samples were collected by a surgical nurse into empty 10-mL plastic tubes, spray-coated with silica to act as a clot activator (BD Vacutainer®, New Jersey, USA), and the samples were immediately centrifuged

Fig. 2. Silica coated 10 mL tube shows three different layers after centrifugation. The most upper layer is platelet poor plasma, and the middle layer is fibrin buffy coat layer represented by a very large and dense polymerized leukocyte- and platelet-rich fibrin block. The bottom layer is red blood cell layer.
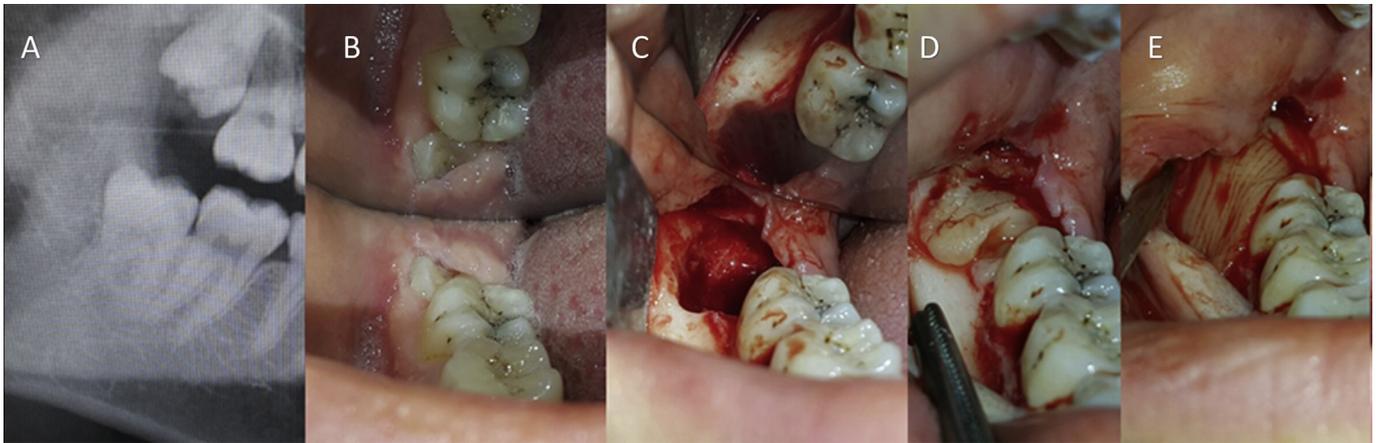
Fig. 3. Preoperative radiograph (A) and photograph (B). After extraction (C), L-PRF from one of the tubes was applied to the socket (D). L-PRF membrane obtained from the other tube was applied under the flap covering the socket (E).

at 3000 rpm (400 g) for 10 min in a table-top centrifuge (Elektro-mag® M 615 P, İstanbul, Turkey) (Fig. 2). L-PRF from one of the tubes was pressed into a membrane between two sterile metal surfaces by the third investigator (OG), as described by Dohan and Choukroun (Dohan et al., 2006a). In the L-PRF + HA group, the serum exudate released during pressing was adsorbed to the HA sponge (Fig. 4B), allowing for the application of HA with its cytokine-binding and carrier molecule properties.

The surgeon who performed the surgeries was blinded to each patient's group until the tooth extraction was completed. All tooth extractions in all groups were performed by the same investigator (MA), using the same procedure and following the same surgical steps. After surgical extraction of the impacted M3, the third investigator (OG) informed the surgeon of the patient's group assignment.

In the L-PRF group ($n = 20$), PRF from one of the tubes was applied to the socket, and L-PRF membrane obtained from the other
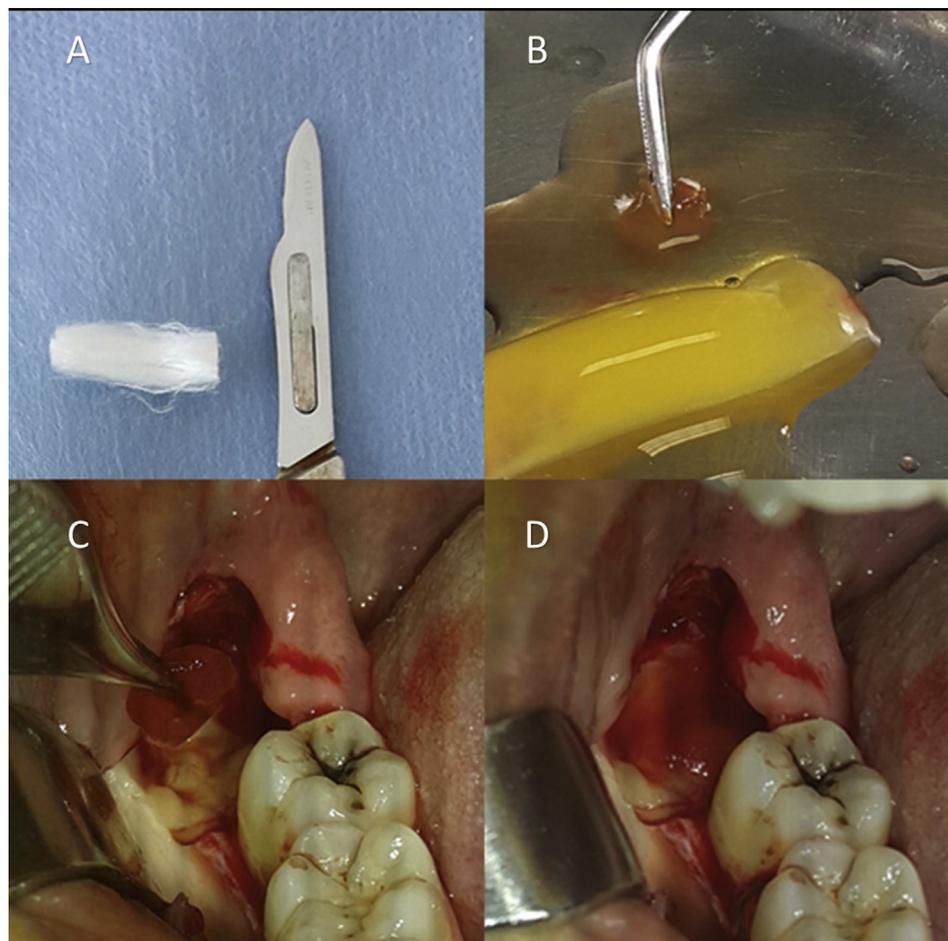

Fig. 4. (A) Hyaloss Matrix. (B) Serum exudate released during pressing of L-PRF into the membrane was adsorbed to the HA sponge. (C) Application of HA sponge on the L-PRF-filled extraction socket. (D) After application of the HA sponge and before L-PRF membrane application.

tube was applied under the flap covering the socket (Fig. 3). In the L-PRF + HA group (*n* = 20), the HA sponge was placed between the two layers of L-PRF (Fig. 4). Nothing was applied to the socket in the control group (*n* = 20).

In all groups, flap closure was performed with 3/0 silk sutures, leaving the gingiva over the socket for secondary healing, as described by Pasqualini (Pasqualini et al., 2005). Standard post-operative medication, comprising amoxicillin 1000 mg (Alfoxil 1gr; Fako, Istanbul, Turkey) twice daily, paracetamol 500 mg (Parol 500 mg; Atabay, Istanbul, Turkey) thrice daily if needed, and chlorhexidine mouthwash thrice daily, was administered to all patients for a week, and sutures were removed on day 7.

The primary outcome variables were the healing scores for the mucosa over the extraction socket on the 7th, 14th, and 21st days.

Secondary outcome variables were frequencies of the following postoperative complications: postoperative hemorrhagic complications, alveolar osteitis, and postoperative wound infection.

All postoperative assessments were performed by the second investigator (TA), who was blinded to the patients' group assignments until the study was completed, and did not participate in randomization or operations.

2.1. Evaluation of the mucosa healing over the extraction socket

A new socket wound healing scale was introduced in this study to assess the status of post extraction wounds in terms of wound dehiscence, epithelialization, quality of granulation tissue filling the post-extraction socket, and depth between early granulation tissue and wound margin. This new scale is a combination of the gingival healing index of Landry et al. (Varghese et al. 2017) and the wound healing scale used by Cervelli et al. (Cervelli et al., 2010).

Assessments were performed at 7, 14, and 21 days following surgery using a blunt periodontal probe with millimetric markers (Fig. 5), and scored as a number between 0 (best) and 4 (worst) (Fig. 6):

- 0 = wound covered with keratinized gingiva (tissue color — all tissues pink; response to palpation — no bleeding). Continuous with healthy tissue.
- 1 = socket filled with organized granulation tissue (response to blunt palpation — no bleeding). Collapsed to a depth of 0–2 mm from the buccal gingival margin.
- 2 = socket filled with organized granulation tissue (tissue color — ≥ 25% and <50% of gingiva red; response to blunt palpation —
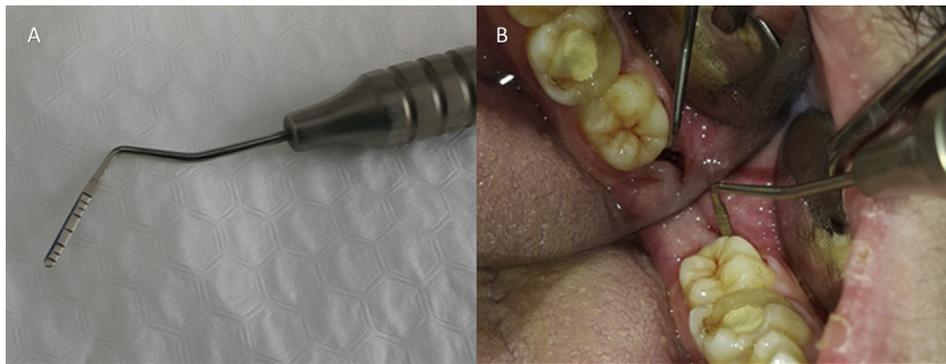

Fig. 5. (A) Blunt periodontal probe with millimetric markers. (B) Evaluation of socket wound healing with periodontal probe.
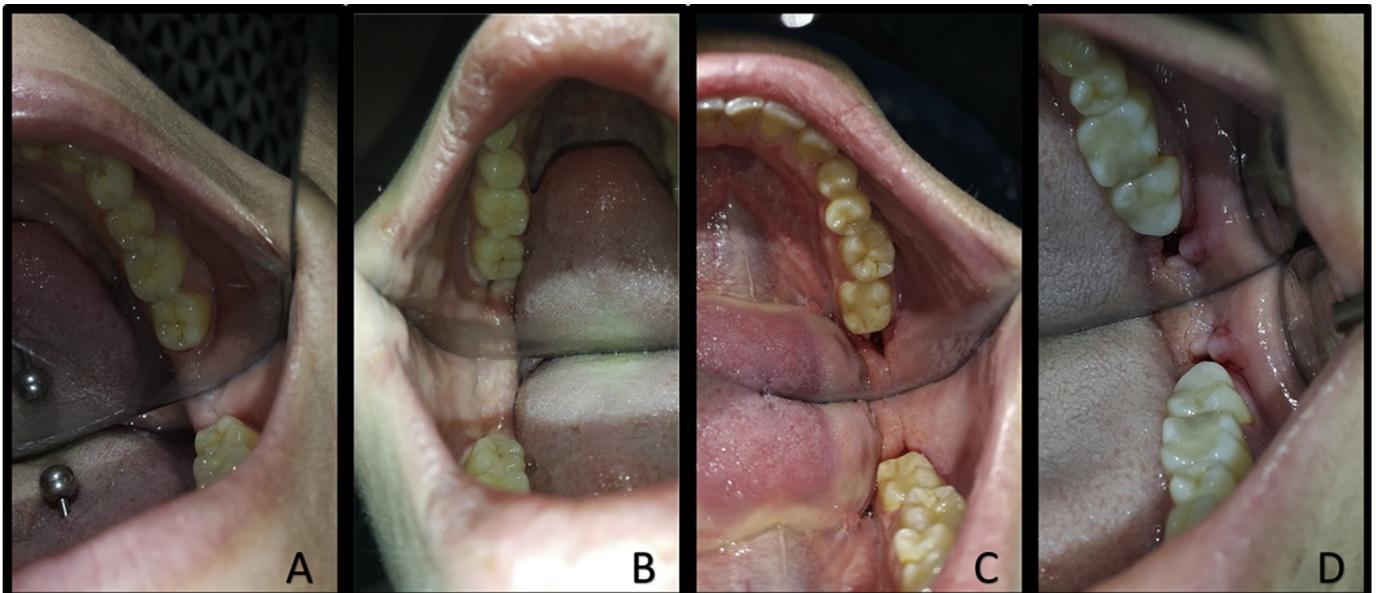

Fig. 6. Clinical images of the different scores: (A) score 0; (B) score 1; (C) score 2; (D) score 3.

no bleeding). Collapsed to a depth of 2–4 mm from the buccal gingival margin.

- 3 = socket filled with unorganized granulation tissue (tissue color — > 50% of granulation tissue red; response to blunt palpation — bleeding). No evidence of infection.
- 4 = socket shows signs of infection or alveolitis; socket filled with foreign materials (food, etc.).

2.2. Evaluation of postoperative complications

Every patient was assessed for signs of prolonged and delayed postoperative bleeding, alveolar osteitis (AO), and postoperative wound infection, and incidents were recorded.

2.3. Statistical analyses

When the study data were evaluated, the IBM SPSS Statistics 22 program (SPSS IBM, Istanbul, Turkey) was used for statistical analysis. The overall significance of the study was evaluated at $p < 0.05$.

3. Results

Mean healing scores for the mucosa over the extraction socket on the 7th, 14th, and 21st days for both the L-PRF group and the L-PRF + HA group were significantly better than those of the control group (Table 1, Fig. 7). The L-PRF + HA group showed better scores than the L-PRF group on the 7th and 21st days, but this difference was not statistically significant. The ratio of alveolar osteitis for the control group was 1:20 and the ratio of postoperative wound infection for the control group was 1:20. There were no cases of alveolar osteitis or postoperative wound infection in the L-PRF and

Table 1
Healing scores for the mucosa over the extraction socket on the 7th, 14th, and 21st days.

Healing scores	L-PRF group	L-PRF + HA group	Control group	[a]p-value
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
7th day	1.6 ± 0.5 (2)	1.5 ± 0.51 (1.5)	2.75 ± 0.55 (3)	0.001*
14th day	1.1 ± 0.64 (1)	1.1 ± 0.64 (1)	2.25 ± 0.55 (2)	0.001*
21st day	0.35 ± 0.49 (0)	0.3 ± 0.47 (0)	1.7 ± 0.47 (2)	0.001*
[b]p-value	0.001*	0.001*	0.001*	

$p < 0.05$.
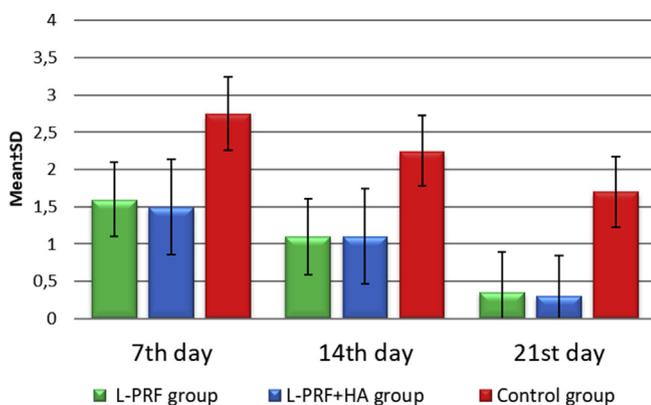[a] Kruskal–Wallis Test.
[b] Friedman Test.

Fig. 7. Graphic of the extraction socket healing scores.

Table 2
Incidence of postoperative complications: prolonged and delayed postoperative bleeding, alveolar osteitis (AO), and wound infection.

Complications	L-PRF group	L-PRF + HA group	Control group
Alveolar osteitis	None	None	1 (5%)
Wound infection	None	None	1 (5%)
Bleeding	None	None	None

Table 3
Patient demographics: age, gender, and surgery duration.

Demographics	L-PRF group	L-PRF + HA group	Control group	p-value
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
Mean age	22.3 ± 1.78 (22)	22.15 ± 2.62 (22)	22.7 ± 2.94 (22.5)	0.810
Mean surgery duration	21.1 ± 3.07 (20)	19.9 ± 4.42 (18)	19.2 ± 4.18 (18)	0.333
Gender distribution	8M:12F	7M:13F	7M:13F	

One-factor Anova.

L-PRF + HA groups. No prolonged or delayed postoperative bleeding was observed in this study (Table 2). No adverse events or side-effects for L-PRF and HA were observed. Age, gender distribution, and total surgery duration in the control and case groups were comparable, and no significant differences were noted among groups (Table 3).

4. Discussion

Surgical extraction of a partially impacted M3 leaves a large bone and soft tissue defect behind. Efforts to form a primary closure of the mucosa over the socket wound in the hope of protection of the bone defect from the intraoral environment generally result in wound dehiscence and wound edges collapsing into the socket because of the lack of support under the suturation line.

In their study, which compared primary and secondary closure of the surgical wound after removal of impacted mandibular third molars, Pasqualini et al. reported that secondary healing of the surgical wound produces less postoperative swelling and pain than occurs with primary healing (by hermetically suturing the socket). At the check-up on day 7 after surgery, 33% of primary closure group patients had dehiscence distal to the second molar (Pasqualini et al., 2005).

Secondary healing has its own disadvantages. If a loosely organized blood clot is resorbed early and leaves the post extraction socket unprotected, that can lead to complications such as delayed hemorrhage, AO, and wound infection. The literature suggests that both PRF and HA could be effective materials in preventing postoperative complications and enhancing healing.

Various platelet concentrates have been applied to decrease postoperative complications and enhance healing after M3 surgery, and the results have generally been promising for these autogenous materials (Albanese et al., 2013; Alissa et al., 2010; Aloy-Prosper et al., 2010; Antonello Gde et al., 2013; Farina et al., 2013; Gülnihal and ATILLA, 2014; Hoaglin and Lines, 2013; Kaul et al., 2012; Kumar et al., 2015; Ozgul et al., 2015; Varghese et al. 2017; Zhao et al., 2011; Sammartino et al., 2009).

L-PRF has obvious advantages over platelet rich plasma (PRP) and other platelet concentrates, including ease of preparation and lack of biochemical treatment of blood, resulting in a preparation that is strictly autologous (Varghese et al. 2017). Also, as described by Dohan Ehrenfest et al. and Dohan et al. a PRF clot can be pressed

between two sterile surfaces to obtain an autologous fibrin membrane (Dohan Ehrenfest et al., 2010; Dohan et al., 2006b).

In this study, the L-PRF was applied to the socket in clot form (Fig. 3D), and an L-PRF membrane was placed under the flap (Fig. 3E) to protect the socket wound from the oral cavity. Thus, L-PRF was used as both a barrier membrane and as a graft that filled the extraction socket and stabilized the early blood clot. The fibrin network in L-PRF is better organized than a normal blood clot. The more tightly organized fibrin network resists disintegration and acts as a scaffold for forming granulation tissue in the alveolar socket; it also provides structural support for epithelial cells migrating over the socket wound.

Proteins such as vitronectin, fibronectin, and growth factors found in the serum exudate are expressed from the PRF clot. This exudate can be used to hydrate graft materials, rinse the surgical site, and store autologous grafts (Dohan et al., 2006b). Thus, in the L-PRF + HA group, exudate that was released through pressing the PRF into membrane was adsorbed to the HA sponge (Fig. 4) as a result of HA's cytokine-binding and carrier molecule properties. Zavan et al. used a similar form of HA as a carrier for growth factors in order to treat ulcers (Zavan et al., 2009).

Longinotti et al. used HA matrix as a scaffold for PRP to treat open tendon wounds, and described HA as an ideal scaffold for promoting rapid remodeling and improved healing, thus facilitating early rehabilitation and return to function (Longinotti, 2014).

HA is reported to be a very promising candidate for the mediation of periodontal tissue regeneration and wound healing. Gontiya and Galgali reported that subgingival placement of 0.2% HA gel, along with scaling and root planning, led to a significant improvement in gingival parameters and reduced inflammatory infiltrate at experimental sites evaluated histologically (Gontiya and Galgali, 2012).

Sandhu et al. presented a case report that assessed a patient with a grade II furcation defect, treated with an HA gel and PRF combination. They reported significant furcation defect fill on re-evaluation through surgical re-entry and radiographic follow-up at 6 months (Sandhu et al., 2015).

Hammad et al. stated that, in their clinical and histomorphometric study on rat models, topical application of HA significantly improved the healing of oral wounds, and that HA is an alternative biomaterial for use in accelerating and enhancing oral wound healing (Hammad et al., 2011).

The authors believe that instead of using HA in a gel or spray form, a solid HA sponge, which slowly biodegrades and releases only biocompatible HA chains under a protective barrier like a PRF membrane, ensures that an effective amount of HA stays in the wound during the critical first weeks of healing.

In the literature, different parameters, such as wound dehiscence, gingival parameters, and probing depth at the distal surface of the adjacent tooth, were used to evaluate postoperative soft tissue healing after M3 surgery. But these parameters were inadequate for assessing the quality of the granulation tissue filling the post-extraction socket in the early weeks of healing. In this study, a new socket healing scale, which is a combination of the skin wound healing scale used by Cervelli et al. (Cervelli, De Angelis et al., 2010) and the healing index after periodontal surgery described by Landry (Varghese et al. 2017), was used to assess post-extraction soft tissue healing of the socket. This new scale assesses not only dehiscence but also the depth of the defect, which is an important parameter for wound healing and for assessing the risk of complications such as AO and wound infection.

The clinical presentation of AO is well known. Most commonly, 2–3 days after tooth removal, the patient returns complaining of intense pain in the alveolus of the extraction site. The alveolus is empty, with apparent disintegration of the normal blood clot (Fridrich and Olson, 1990).

The reported occurrence of AO ranges from 0.5% to 68.4%, with most studies reporting less than 15%. Surgical site infections are also reported to occur across a wide range (1.2–27%), with most studies citing an incidence of less than 5%. Reiland et al. in their study with 1,020 patients aged age 14 and 30, who received post-operative oral antibiotics for 5 days, reported that 6.4% developed AO an average of 5.7 days after the procedure, and 2.6% developed a surgical site infection an average of 23.2 days after the procedure (Reiland et al., 2017).

In their study with 952 surgical third molar extraction sites, Fridrich and Olson reported that older patients had higher incidence rates of AO than younger patients (age <20 11.0%; age 20–30 20.9%; age >30 30.0%). They also reported a higher rate of AO for females who used oral contraceptives: 21.5% compared with 11.4% for females not using oral contraceptives (Fridrich and Olson, 1990).

Low AO and infection results in our study can be explained by strict postoperative medication of 1 g amoxicillin twice a day and chlorhexidine mouthwash three times a day. Results can also be attibuted to the study's inclusion criteria, which included being non-smoking, young (mean of 22 years of age), and healthy (ASA 1) adults, who did not use any drugs, including oral contraceptives, and who maintained a high level of oral hygiene.

Sammartino et al. evaluated the use of L-PRF as a safe filling and hemostatic material after dental extractions for the prevention of hemorrhagic complications in heart surgery patients, without modification of the anticoagulant oral therapy. Patients were treated with L-PRF clots placed into 168 post-extraction sockets (the mean international normalized ratio (INR) value was 3.16 ± 0.39). Only two patients (4%) reported hemorrhagic complications, which were resolved a few hours after the surgery by compression and topical hemostatic agents. They also reported that soft tissue healing was quick, and that wound closure was always complete at the time of suture removal 1 week after surgery (Sammartino et al., 2011).

In our study, each patient remained in the clinic for 1 h after extraction, under observation for hemorrhagic complications. A sterile gauze sponge was placed over the socket wound immediately after wound closure with sutures, and the patient bit on it for 30 min. Then the wound was observed for postoperative bleeding, and a second sponge was applied for another 30 min, after which a second assessment was carried out to measure postoperative bleeding, as described by Sammartino et al., (2011). The authors believe that this long and strict compression explains why there were no hemorrhagic complications observed in this study.

The main limitation of our study was sample size, which was adequate for assessment of wound healing but small for the assessment of postoperative complications. The authors believe that, despite the small numbers, the results of this study regarding the incidence of complications should be included in the literature.

5. Conclusion

The results of this study suggest that L-PRF alone, and when combined with HA, can be an effective way to improve soft tissue healing, and could be used to prevent postoperative alveolar osteitis and infection after M3 surgery. Further studies with larger study groups are necessary.

Conflicts of interest

All the authors declare that they have no conflict of interest.

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