



EVALUATION OF ALVEOLAR RIDGE PRESERVATION USING PLATELET-RICH FIBRIN WITH LEUKOCYTES

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ABSTRACT

Objective: To evaluate the alveolar ridge preservation using platelets-rich fibrin with leukocytes covered by collagen membrane. **Subjects and Methods:** Forty patients have one of maxillary premolars indicated for extraction were selected and divided into two groups Study group: consisted of 20 extraction sockets that received L-PRF covered by collagen membrane. Control group: consisted of 20 extraction sockets as a control. Patient will be followed at 3,7 and 14 days to evaluate the healing index. Bone density and dimensional changes will be evaluated by (CBCT) after 3 and 6 months. **Results:** The vertical bone and horizontal bone resorption showed a statistically significant decrease in study group than control by (study: 6,1 %, control 10 %) at 3 months. The bone density was increased in both groups after 3 and 6 months, however it was a statistically significant increase in study group than control the healing is improved with a statistically significant increase in the second week than the first week in both groups. **Conclusion:** using of leukocyte with platelet rich fibrin (L-PRF) together with collagen membrane accelerates the formation of bone filling in the extraction bony socket, increasing its density and minimizing its dimensional change.

KEYWORDS: Extraction socket, alveolar ridge preservation, Platelet-Rich Fibrin, collagen membrane.

INTRODUCTION

Post extraction, the alveolar ridge is prone to major resorption in a vertical and horizontal dimension after tooth extraction which as normal physiological response that reduced vertical height about 40% and width 60%. These changes of hard and soft tissues complicates further treatment^(1,2).

Alveolar ridge preservation (ARP) is a relatively new surgical procedure aimed at retaining maximum bone and soft tissue after a tooth has been removed. By maintaining the original ridge morphology, there will be a minimal need for aug-

mentation procedures thereby allowing the resultant restoration to be placed in an aesthetically and functionally ideal position⁽³⁾.

Although different ARP techniques have been developed, most of which include the use of a graft material that is placed into the extraction socket, many of them have disadvantage as increased overall cost as well as increases the risk of disease transmission. On the other hand autogenous bone is considered the gold standard among all graft material. However, it requires a second surgical donor site. Studies also indicate that in many cases, the graft material is not totally incorporated into

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the newly formed bone and when compared to sites without graft material, they show less vital bone formation^(3,4).

Recently, great attention has been directed towards leukocyte and platelet rich fibrin (L-PRF).⁽⁵⁾ leukocyte and platelet rich fibrin (L-PRF) is the second generation platelet hydrogel obtained through simple and rapid centrifugation of whole blood sample in absence of anticoagulant and bovine thrombine. L-PRF is easy and inexpensive to prepare for frequent use in private practice, and it exists in the form of L-PRF a plug or membranes. It releases a significant quantity of autologous growth factors particularly platelet derived growth factor (PDGF), transforming growth factor (TGF) and vascular endothelial growth factor (VEGF), cytokines, and healing proteins for up to 28 days^(6,8).

L-PRF has osteoinductive effect over osteoblasts and mesenchymal cells enhanced the bone formation and overcome crestal bone resorption, it has anti-inflammatory and anti-infective properties that improve the healing⁽⁹⁾. The aim of this study was to evaluate the effectiveness of LPRF to improve the soft and bone tissues healing after tooth extractions.

SUBJECTS AND METHODS

Forty patients have one of maxillary premolars indicated for extraction were selected randomized from those who attended the outpatient clinic of Oral and Maxillofacial Surgery Department at the Faculty of Dental Medicine, Boys, Cairo, Al Azhar University. They have non-restorable badly decayed teeth and seeking treatment. All patients were subjected to thorough medical history, and clinical and radiographic dental examination.

Inclusion criteria:

Select age range between (20-40) years old. In addition the indicated tooth should be one of maxillary premolars, present of adjacent teeth and possibility for follow up for 6 months.

Exclusion criteria:

Exclude all of them Uncontrolled diabetes mellitus, history of chemotherapy or radiotherapy, acute infection at the extraction site, soft tissue recession at the extraction site, periodontal disease on adjacent teeth, and smoker person.

Each patient received an informed consent to be signed by him\her.

The study included 40 teeth (extracted sockets) which are divided into two groups:

- **Study group:** consisted of 20 extraction sockets that received L-PRF as a grafting material covered by a membrane, Fig. 1.
- **Control group:** consisted of 20 extraction sockets allowed to heal spontaneously and not grafted, to serve as a control.

Preparation of the leukocyte platelet rich fibrin:

A plastic sterile syringe was used to draw 10ml of the patients own blood and transferred in to sterile plain tube, which was then immediately placed in a centrifugal machine. The machin was adjusted to 2700 rpm for 12 minutes. A fibrin clot was then obtained in the middle of the tube, just below the red corpuscles at the bottom and acellular plasma at the top. The upper colored layer was then removed and middle fraction collected, 2 mm below lower dividing line, which is the PRF. Thus it is necessary to preserve a small red layer at the PRF clot end to collect as many platelets and leukocytes as possible. This part was done using a scissor. The clot was placed into the cylinder in the PRF box and slowly compressed with a piston into the extraction site, resulting in a "plug".

Postoperative evaluation:

Clinical evaluation:

Patient were followed at 3,7 and 14 days to evaluate the healing index.

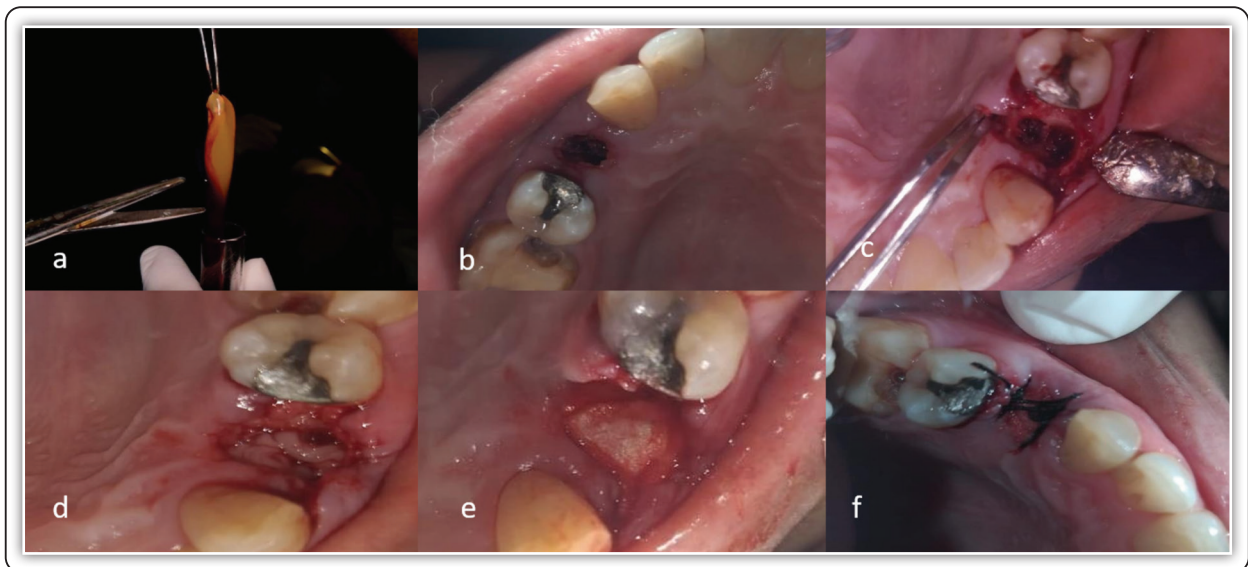


FIG (1) a), showing L-PRF separation, b), Clinical photograph of the extraction socket after debridement, c), showing buccal and palatal gingival freeing, d), showing L-PRF packing into fresh extraction socket, e), covering L-PRF with collagen membrane, and f), suturing with 3/0 silk.

Radiographic evaluation:

Cone beam computed tomography (CBCT) will be taken at the time of extraction, as a base line. Then bone density and dimensional changes will be evaluated by (CBCT) after 3 and 6 months.

Statistical analysis:

Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), therefore independent t test were used for intergroup comparison. Repeated measures analysis of variance (ANOVA) and paired t test were used to compare different observation times. ANOVA test was followed by Tukey's post hoc test if ANOVA revealed a significant difference. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 19.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

In regards to buccal resorption, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months ($p=0.00$).

Palatal resorption: a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was not statistically significant at 3 and 6 months ($p=0.062$, $p=0.063$).

Horizontal Dimensional bone change (buccopalatal width):

Buccal plate width resorption. At 1mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months ($p=0.00$). At 3mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months ($p=0.00$, $p=0.0036$ respectively). At 5mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups

was not statistically significant at 3 and 6 months (p=0.403, p=0.0689 respectively).

Palatal plate width resorption:

At 1mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months (p=0.00). At 3mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was not statistically significant at 3 and 6 months (p=0.1154, p=0.0067 respectively). At 5mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was not statistically significant at 3 and 6 months (p=0.06, p=0.467 respectively).

Total ridge width change:

At 1mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months (p=0.00). At 3mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months (p=0.00, p=0.00125 respectively). At 5mm, a higher mean value was recorded in control group. Independent t test revealed that the difference between groups was statistically significant at 3 and 6 months (p=0.029, p=0.0037 respectively).

Bone density by CBCT :

A higher mean value was recorded in study (LPRF+ collagen membrane) group. However, independent t test revealed that the difference between groups was not statistically significant at baseline (p=0.245), but was statistically significant at 3 and 6 months (p=0.000, p=0.0004 respectively).

Soft tissue healing index:

A higher mean value was recorded in study (LPRF+ collagen membrane) group. However, independent t test revealed that the difference between groups was not statistically significant at 3 days, 1 week and 2 weeks (p=0.74, p=0.89, p=0.6 respectively).

TABLE (1) Comparison between groups regarding Vertical Dimensional bone change, Horizontal Dimensional.

	Control group	Study group (L-PRF)	p
Vertical Dimensional			
Buccal resorption			
3 months	1.4±0.3	0.7±0.13	0.00*
6 months	2.31±0.32	1.38±0.41	0.00*
Palatal resorption			
3 months	0.6±0.12	0.53±0.11	0.062NS
6 months	1.02±0.31	0.92±0.35	0.0633NS
Horizontal Dimensional			
Buccal plate width resorption			
3 months			
1mm	3.04±0.63	1.1±0.13	0.00*
3mm	1.1±0.1	0.65±0.12	0.00*
5mm	0.6±0.31	0.42±0.9	0.403NS
6 months			
1mm	4.5±1.2	2.47±0.41	0.00*
3mm	2.1±0.51	1.7±0.27	0.0036*
5mm	1.01±0.32	0.96±0.25	0.0689NS
Palatal plate width resorption			
3 months			
1mm	1.75±0.6	0.7±0.17	0.00*
3mm	0.43±0.18	0.35±0.13	0.1154NS
5mm	0.21±0.07	0.17±0.06	0.06NS
6 months			
1mm	2.9±0.83	1.64±0.24	0.00*
3mm	0.82±0.25	0.69±0.18	0.067NS
5mm	0.43±0.15	0.28±0.9	0.467NS

TABLE (2) Comparison between groups regarding Total ridge width change and Soft tissue healing index 3 days, 1 week and 2 weeks after extraction.

	Control group	Study group (L-PRF)	p
Total ridge width change			
3 months			
1mm	4.92±1.01	1.8±0.42	0.00*
3mm	1.53±0.33	1.0±0.18	0.00*
5mm	0.81±0.42	0.59±0.11	0.029*
6 months			
1mm	7.41±1.41	4.03±0.89	0.00*
3mm	2.92±0.82	2.39±0.38	0.0125*
5mm	1.44±0.36	1.12±0.29	0.0037*
Bone density			
T0 (baseline)	57.61±12.4	63.1±16.7	0.245NS
T3 (3months)	132.58±32.4	245.75±43.3	0.000*
T6 (6 months)	493.35±98.8	620.84±109.3	0.0004*
Soft tissue healing index			
3 days	2.97±0.53	3.02±0.42	0.74 NS
1 week	3.89±0.39	4.01±0.92	0.89 NS
2 weeks	4.62±0.8	4.83±1.6	0.60 NS

DISCUSSION

Regenerative medicine provided number of biomaterials used in socket preservation to minimize the changes in quantity and quality of ridge after tooth removal. One of the substances used is platelet rich fibrin with leukocytes which is a good candidate for bone substitutes due to its osteoinductive effects over osteoblasts and mesenchymal cells which enhance the bone formation and overcome crestal bone resorption as well as its anti-inflammatory and anti-infective properties that improve the healing of both soft and hard tissues⁽⁹⁾.

In the present study, socket preservation was done using L-PRF plug form, that directly applied into the empty socket immediately after single extraction of upper premolars teeth, then the graft was covered by collagen membrane. Both study and control groups showed a statistically significant increase in bone resorption post-operatively after 3 and 6 months, this coincided with result of schropp et al⁽¹⁰⁾ The causes of resorption was explained by study of Araujo et al⁽¹¹⁾ who reported that the change started after tooth removal resulted from osteoclasts activation by losing of periodontal ligament fibers, bundle bone and blood supply. On the other hand, Morjaria et al⁽¹²⁾ who analysed the outcomes of ridge preservation in a systematic review which were randomized controlled clinical trials, in few cases the using of grafting materials in socket preservation vertical bone gain may occur.

In the present study, the horizontal bone resorption was greater than the vertical and more prominent in the vestibular bone plates than oral plate, shifting the ridge palatal, as showed in result of Iasella et al⁽¹³⁾. Partially, this can be explained by the fact that the vestibular bone plates are generally thinner than palatal⁽¹⁴⁾. Tan and co-workers⁽¹⁵⁾ reported in their systematic review of 20 studies which were based on the dimensional changes in non-grafted sockets at six months after tooth extraction, found that an average horizontal bone loss was (3.8 mm) and vertical loss was (1.2 mm), this was in agreement with the present study (3.92mm and 1.6 mm).

There is a statistically significantly less mean of reduction in vertical and horizontal bone in study group than control. As showed in result of Temmerman et al⁽¹⁶⁾ and Kotsakis et al⁽¹⁷⁾ they used L-PRF plug within extracted socket covered by membrane in ridge preservation, at 2 or 3 months found that a significant less reduction at L-PRF sites. This is due to the growth factors released from activated platelets of L-PRF, those promote the differentiation of osteoblasts as well as inhibiting of the osteoclasts activity. Jang E. et al⁽¹⁸⁾.

The changes in alveolar width in this study was also compared favorably to changes in alveolar dimensions noted in other investigations using different socket preservation techniques, however the difference in measurement methodologies employed makes it difficult to compare the results directly. Iasella et al⁽¹³⁾ evaluated the dimensional ridge change of extracted socket after using of demineralized freeze-dried bone allograft (DFDBA) in conjunction with a collagen membrane, after four months the mean ridge width change was (1.2 mm and 2.6 mm) in study and control groups respectively, this coincided with the present study in 3 months, the mean buccolingual width change was (1.13 mm and 2.41mm).

The rate of horizontal bone resorption decreased at the first weeks of grafting, then increased at the end of 6 months, which explained the degradation of collagen membrane that has a negative effect, that performed in study of Olaechea et al⁽¹⁷⁾ who evaluated the absorption rate of three different collagen membranes, noted that the rates were variable over different time periods according to the source of membrane from 15 days to 90 days.

The vertical bone resorption showed a statistically significant decrease in study group than control by (study: 6,1 %, control 10 %) at 3 months. This coincided with result of Simon et al.⁽⁶⁾ who used L-PRF within extracted socket, after four months, it was reported that the net loss of alveolar bone height was (0.67mm 7.13%). Moreover, the preservation of alveolar bone height obtained with using of L-PRF was comparable to other modalities used for socket preservation. Lekovic et al⁽¹⁴⁾ recorded an 11.59% net loss of height after 4 months of healing when a polygalactide membrane was used for ridge preservation in intact extraction sockets. In addition, Jambhekar et al⁽¹⁹⁾ who analysed the outcomes of ridge preservation in a systematic review which were randomized controlled clinical trials, found that the vertical bone loss of buccal plate decreased with xenografts by (0.57 mm) , allografts (0.58mm),

alloplasts (0.77 mm) and sockets without any graft (1.74 mm). This means that the L-PRF graft occupies a good position among the other grafting materials used in socket preservation. On the other hand, Suttapreyasri and Leepong⁽²⁰⁾ showed that the L-PRF group demonstrated faster bone healing compared with the control group. However, the overall resorption of marginal bone levels mesial and distal to the extraction site in L-PRF was comparable to that of the control with no significant difference. The difference in results between their study and our study may be related to their using of study casts, it is not a precise method to evaluate dimensional changes, as impression materials may undergo contraction or expansion.

The bone density was increased in both groups after 3 and 6 months, however it was a statistically significant increase in study group than control, as showed in result of Zhao et al⁽²¹⁾ and Doiphode et al⁽²²⁾ they used L-PRF plug within extracted socket and found that a significant increase in bone density at 2, 4 and 6 months than control group. This clarified the important role of L-PRF in activation and proliferation of osteoblasts, osteocytes and mesenchymal stem cells as well as the role of platelet factors that enhance bone formation as well as rapid filling the empty socket with new bone⁽²³⁾. On the other hand, Karan et al⁽²⁴⁾ who assessed the bone density after application of L-PRF within periapical lesions using CBCT imaging, it was found that the bone density increased in L-PRF and control groups with no significant difference. The difference in results between their study and the present one related to their utilization of flap technique versus the flapless used in the current study, they stated that by elevating the periosteum, the blood supply of the exposed bone surface was compromised leading to increase of osteoclastic activity and bone resorption. In addition their evaluation of periapical defect after one year.

In the present study, the healing is improved with a statistically significant increase in the second

week than the first week in both groups. However, the study group showed rapid as well as improved healing than control group without significant deference, as showed in result of Ercan et al⁽²⁵⁾).

On the other hand, Alissa et al⁽²⁶⁾ who evaluated the soft tissue healing one week after L-PRF graft within extracted socket, their study showed significant improve and rapid healing at L-PRF site than control. The difference in results between their study and the present one related to our using of sutures in each group that approximates the wound edge which improve healing. Moreover, postoperative antibiotic, analgesic and mouthwash prescription decrease the incidence of infection, where it had positive effect on socket healing for 3 persons in the present study who had infected socket, this was agreed with result of Epstein et al⁽²⁷⁾ who preferred using postoperative antibiotic to prevent such infection. As well as only healthy patients without systemic diseases enrolled in the current study, decrease the incidence of postoperative complications. Gerdtts et al⁽²⁸⁾.

CONCLUSION

The outcome of the present study concluded that, after a traumatic extraction of the premolar tooth using of leukocyte with platelet rich fibrin (L-PRF) together with collagen membrane, minimize a dimensional change in socket and improve soft tissue healing.

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