

LEUKOCYTE-PLATELET RICH FIBRIN (L-PRF) AND L-PRF MIXED WITH PARTICULATE XENOGRAFT AS ALVEOLAR RIDGE PRESERVATION MATERIALS AFTER EXTRACTION OF TEETH WITH CHRONIC PERIAPICAL INFECTION (A RANDOMIZED CONTROLLED CLINICAL STUDY WITH RADIOGRAPHIC AND HISTOMORPHOMETRICAL ASSESSMENT)

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ABSTRACT

Aim: The present study was performed to evaluate the use of L-PRF and L-PRF mixed with particulate xenograft as alveolar ridge preservation materials after extraction of a tooth with chronic infection. Evaluation of the buccolingual dimensional changes of alveolar ridge was the primary objective while the secondary objectives were evaluation of the quality of regenerated bone histomorphometrically and assessment of implant stability.

Subjects and methods: This is a randomized controlled clinical trial in which thirty patients were randomly distributed into three groups. Group 1: Ten extraction sockets where alveolar ridge preservation was performed using L-PRF plug. Group 2: Ten extraction sockets where alveolar ridge preservation was performed using L-PRF mixed with particulate xenograft. Group 3: Ten extraction sockets where alveolar ridge preservation was performed using xenograft and collagen membrane. Evaluation of the alveolar ridge dimensional changes was performed radiographically. Histological and histomorphometrical assessment of the regenerated bone was also performed.

Results: Regarding alveolar ridge buccolingual changes radiographically, The highest mean percent decrease was recorded in L-PRF group with the least value recorded in L-PRF mixed with xenograft group. Regarding the Area fraction of newly formed bone and osteocytes count, The highest mean value was recorded in L-PRF mixed with xenograft group with the least value recorded in L-PRF group.

Conclusion: L-PRF mixed with xenograft group showed better dimensional and histological outcomes in alveolar ridge preservation in comparison to L-PRF alone or xenograft and collagen membrane.

KEYWORDS: L-PRF, Alveolar ridge preservation, xenografts, platelets concentrates

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INTRODUCTION

Alveolar ridge resorption follows tooth extraction and many approaches have been introduced to overcome these dimensional changes (*Schropp et al., 2003; Araujo et al., 2005*). Among these approaches is the immediate dental implant. The intact labial plate, absence of infection, sufficiency of bone apical to the extracted tooth and many other prerequisites should be taken in consideration to ensure safe and correct placement of immediate implants with high success rate. (*Vera et al., 2012; Morton et al., 2014*).

The term “Alveolar ridge preservation” denotes any procedure performed at the time of tooth extraction to minimize alveolar bone loss and enhance regeneration in the extraction site (*Darby et al., 2008; Choi et al., 2015*). Atraumatic extraction and selection of the proper graft material according to the socket condition and the remaining walls are mandatory to ensure maximum bone gain (*Kassim, 2014; Choi et al., 2015*). Alveolar ridge preservation is commonly performed using different biomaterials as bone grafts, collagen sponges, barrier membranes, or platelets concentrates (*Horvath et al., 2013; Annunziata et al., 2018, Stumbras et al., 2019*).

Autogenous bone graft is the gold standard for osteogenesis but the second site surgery, postoperative pain, hematoma formation, blood loss, nerve injury and infection are its most common limitations. (*Conrad et al., 1995; Reynolds et al., 2003; Tomlin et al., 2014*). Xenografts yield successful results in ridge preservation. They stimulate the bone by load transmission during normal jaw function. Forces on the bone-graft interface may contribute to bone preservation if they are within the physiologic range (*Kalk et al., 1993; Barone et al., 2008; Barone et al., 2013*). Xenografts alone have neither osteogenic potential nor osteoinductive potential but when combined with autogenous matrices or platelet concentrates natural bone formation is enhanced (*Lovelace et al.,*

1998; Laurell et al., 1998; Monteiro et al., 2003, Annunziata et al., 2018).

Cell-mediated bone graft resorption is an active process that involves both osteoclastic and osteoblastic activity while solution-mediated resorption is a passive process depending on tissues PH. Rapid graft resorption by chemical dissolution in infected sites has been documented due to the acidic PH. This type of chemical dissolution is rapid and sometimes the graft disappear before the new bone formation. (*Schilling et al., 2004; Thompson et al., 2006*)

Platelets concentrates can be classified depending on the leukocyte content and fibrin architecture into Pure Platelet-Rich Plasma (P-PRP) or Leukocyte-Poor Platelet-Rich Plasma, Leukocyte-and Platelet-Rich Plasma (L-PRP), Pure Platelet-Rich Fibrin (P-PRF) or Leukocyte-Poor Platelet-Rich Fibrin, Leukocyte and Platelet-Rich Fibrin (L-PRF). (*Ehrenfest et al., 2014*);

According to the polymerization technique of the fibrin network in each type of the platelet concentrates, the growth factors release rate is determined. PRP delivers all their content within 1 hour after administration; however growth factor release is prolonged for 1 to 4 weeks in case of Platelet-Rich Fibrin (PRF) (*Anitua et al., 2012; Borsani et al., 2015*). The bovine thrombin and calcium chloride used in PRP preparation results in bilateral junction fibrin network that is not ideal for cytokine entrapment and cellular migration. On the other hand, the natural and slow polymerization during PRF preparation results in fibrin network formed of an equilateral junction that allows cytokines incorporation in the network and increases their life span (*Dohan et al., 2006; Dohan et al., 2012*).

L-PRF contains platelets and their products, plasma proteins, leukocytes, and cytokines entrapped in fibrin matrix. Different components of L-PRF works together against bacteria and in immune regulation (*Ehrenfest et al., 2009*;

Ehrenfest et al., 2010; Drago et al., 2014). PRF clot could be considered as an immune organizing node that can be beneficial at chronic infection (*Dohan et al., 2006*). The antibacterial properties of the L-PRF against many periodontal pathogens have been documented (*Castro et al., 2019*)

Moreover, growth factors and platelet concentrates play a significant role in cellular proliferation, migration, and extracellular matrix formation and showed promising results in regenerative therapy (*Gestrelus et al., 1997; Kaigler et al., 2011; Hauserm et al 2013*). Using L-PRF mixed with bone graft enhances graft volume and quality (*Choukroun et al., 2006; Hauser et al., 2013; Jung et al., 2013*). New applications of L-PRF are introduced including alveolar ridge preservation based on its easy handling, ideal mechanical and biological properties (*Su et al., 2009; Ehrenfest et al., 2010; Sammartino et al., 2011; Annunziata et al., 2018*).

Taking in consideration the ability of L-PRF to enhance healing and regeneration beside antimicrobial capacity, this study was conducted to evaluate the use of L-PRF in ARP after extraction of a tooth with chronic infection.

Aim of the Study

The present study was performed to evaluate the use of L-PRF and L-PRF mixed with particulate xenograft as alveolar ridge preservation materials after extraction of a tooth with chronic infection.

Primary objective: Evaluation of the buccolingual dimensional changes of alveolar ridge using CBCT.

Secondary objectives: Evaluation of the quality of regenerated bone histomorphometrically and assessment of implant stability.

SUBJECTS AND METHODS

This is a randomized controlled clinical trial in which thirty patients were selected from the outpatient clinic of the department of Oral Medicine,

Periodontology, and Oral Diagnosis, Faculty of Dentistry, Ain Shams University. A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference would be found between tested groups regarding buccolingual dimensional changes of alveolar ridge using CBCT. By adopting an alpha (α) level of (0.05), a beta (β) of (0.2) (i.e., power=80%), and an effect size (f) of (0.637) calculated based on the results of a previous study (**Reda et al 2020**) the predicted sample size (n) was found to be (27) samples (i.e., 9 samples per group). Three patients were added to compensate for drop outs. Sample size calculation was performed using G*Power version 3.1.9.7 (**Faul et al 2007**)

Before starting the study the protocol of the research was revised and accepted by the Ethics Committee of the Faculty of Dentistry Ain Shams University and the ethics approval number is (FDASU-REC IM021727).

Eligibility criteria:

- All patients are 20 - 40 years old patients, male or female, systemically free according to Burket's oral medicine health history questionnaire (*Greenberg et al., 2012*).
- All patients have non restorable maxillary anterior tooth or premolar with chronic periapical infection limited to the periapical area.
- Teeth with chronic periodontitis were excluded.
- All patients have intact buccal and lingual socket walls according to preoperative CBCT and re-evaluated clinically after extraction; socket type 1 (*Elian et al., 2007*).
- Pregnant females, smokers, and vulnerable patients are excluded from the study.

Patients grouping and treatment protocol:

Thirty Patients were randomly distributed by using computer software* into three groups.

* QUICKCALCS

Group 1: Ten extraction sockets where alveolar ridge preservation was performed using L-PRF plug. **Group 2:** Ten extraction sockets where alveolar ridge preservation was performed using L-PRF mixed with particulate xenograft. **Group 3:** Ten extraction sockets where alveolar ridge preservation was performed using xenograft * and collagen membrane.**

Preoperative analysis included patient history, medical history, clinical and radiographic examination at the first visit. All the study procedures were explained to the patients and they signed an informed detailed consent form before starting any treatment. One week before the extraction (first surgery) scaling and oral hygiene instructions were given to all patients.

Surgical procedures

After local anaesthesia (Articaine 4%), atraumatic extraction using periostomes and luxators was performed. The socket was then curetted to remove any periapical infection tissue remnants then irrigation with chlorhexidine mouth rinse 0.1% was done.

L-PRF was then prepared according to the protocol developed by *Choukroun et al. (2006)*. 10ml blood sample was collected in plain plastic glass-coated without anticoagulant. The blood was then immediately centrifuged at 2700 rpm for 12 minutes. L-PRF was obtained and manipulated in the form of plug for group 1 or shredded and mixed with particulate xenograft to form one mass for group 2. (**figure 1,2,3**) The extraction sockets in both groups are fully filled up to the alveolar crest. (**figure 4,5**) In group 3, xenograft is mixed with saline and placed in extraction socket to fill the socket till the alveolar crest then covered by collagen membrane where margins of the collagen membrane are placed beneath margins of the

* Hypro oss, Bioimplon, Germany.

** Hypro-sorp, Bioimplon, Germany.

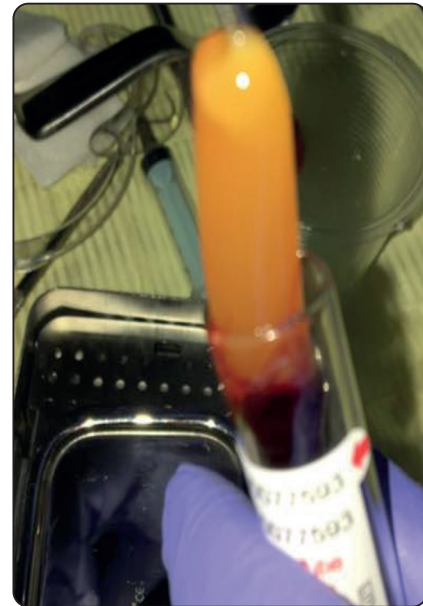


Fig. (1) (L-PRF preparation)

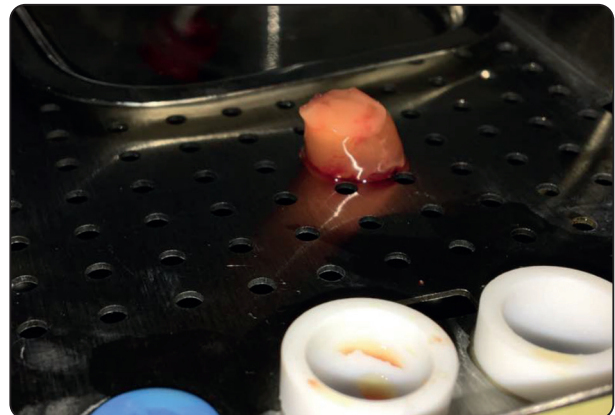


Fig. (2) (L-PRF plug used in group 1)

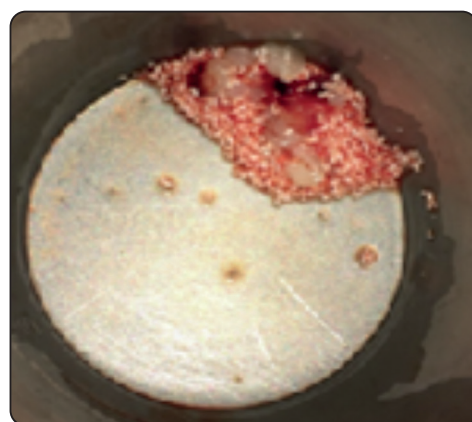


Fig. (3) (L-PRF mixed with xenograft in group 2)



Fig. (4) (Non-restorable tooth , extraction socket)



Fig. (5) (L-PRF mixed with xenograft in extraction socket)

buccal and palatal mucoperiosteum. Cross over 4-0 polypropylene sutures was then performed in the three groups.

Postoperative medications were prescribed as follows: Chlorhexidine* 0.1% mouth wash two times daily for one week (*Drago et al., 2017*). Antibiotic** (Amoxicillin - Clavulanic acid): 1gm orally tablets every 12 hours for 7 days, Metronidazole*** 500 mg orally tablets every 12 hours for 7 days, anti-inflammatory diclofenac potassium**** 50 mg orally tablets every 8 hours for 3 days, and anti-edematous***** (chymotrypsin – trypsin) 1 tablet 3

* Hexitol, arab drug company, Egypt.

** Hibiotic, Amount Pharmaceutical, Egypt.

*** Flagyl, Sanofi-Aventis, Egypt.

**** Cataflam,, Novartis, Egypt.

***** Alphintern, Amoun pharmaceutical, Egypt.

times daily for 3 days (*Ludwig et al., 1993; Dionne et al., 2001*).

Removal of suture was performed 10 days later; patient follow up visits were scheduled every month until the re-entry surgery (5 months later) for harvesting bone core biopsy***** and implant***** placement in routine fashion.

Measurements and evaluation of the alveolar ridge

Radiographic measurements

Cone beam computed tomography (CBCT) was used to evaluate the bucco-lingual width of the alveolar ridges. The base line CBCT was performed before extraction to determine alveolar socket width and height before extraction. The final CBCT was taken 5 months after alveolar ridge preservation and one week before implant placement. Cone beam computed tomography images were taken with ICAT cone beam 3D dental imaging system at 120kvp, 5Ma, exposure time 34 sec, slice thickness 0.3mm and field of view 60-70 mm.

Histological and histomorphometric assessment:

Evaluation of the grafted bone was performed by using bone core biopsy harvested at the re-entry surgery before implant placement.

***** Hu-Friedy, USA.

***** Neobiotech, Korea

Specimen staining procedure:

Bone biopsy specimens obtained were fixed in 4% formalin and then decalcified in 17% nitric acid for 12 hours. Tissues were then embedded in paraffin wax and sectioned longitudinally into multiple 5-mm thick sections. Sections then stained with Masson's Trichrome for evaluation of bone trabeculae and osteoid tissue. Other sections stained with hematoxylin and eosin (H&E) to show general layout, distribution of cells and provide general overview of tissue sample's structure (Chan, 2014).

Image analysis:**Area Fraction Analysis for Assessment of Quantity of New Bone:**

For each MTC (Masson's Trichrome) stained section, three microscopic fields showing the most abundant blue/purple staining (characteristic of the newly formed osteoid) were selected and photomicrographs were captured at original magnification of 20X.

All images were captured using digital camera* which was mounted on a light microscope**. Images were then transferred to the computer system for analysis. This was performed in the Precision Measurement Unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. All the steps of immunohistochemical assessment were carried out using Image J, 1.41a*** image analysis software. Images were first corrected for brightness and contrast. Corrected images were then converted into 8-bit type grayscale. Color thresholding was then adjusted. The area fraction (AF) of the blue/purple MTC-stained osteoid was measured automatically. The area fraction represented the percentage of the newly formed osteoid to the total area of the microscopic field. The mean area fraction (MAF) for each case was calculated.

* (EOS 650D, Cannon, Japan)

** (BX60, Olympus, Japan)

*** (NIH, USA)

Osteocyte Count Analysis for Assessment of Quality of New Bone

For each H&E-stained section, three microscopic fields showing the most abundant formation of newly formed bone were selected and photomicrographs were captured at an original magnification of x40. For the estimation of osteocytes count in each microscopic field of the digital images taken the same way as those of MTC-stain, only viable osteocytes (i.e. lacunae with visible nuclei inside) in the newly formed bone were manually marked with a different color using Image J software. Thresholding was again tuned to only extract the marks of the different color to be automatically counted.

Clinical measurements:

Implant primary stability was measured in ISQ units using the Ostell Mentor**** buccally, palatally, mesially and distally, then average was calculated.



Fig. (6) Smart peg and ostell used to measure the implant primary stability)

Statistical analysis:

Statistical analysis was then performed using a commercially available software program (SPSS 18; SPSS, Chicago, IL, USA). Data were explored for normality using Kolmogorov-Smirnov test of normality. Values were normally distributed and were presented as mean, standard deviation (SD)

**** MEGA ISQ, Megagen Implants UK Ltd.

and confidence intervals. ANOVA test was used for comparison between groups and was followed by Tukey's post hoc test if a significant difference was detected between groups. Comparison between pre-post values of CT was performed using paired t test.

The percentage of change in CT was calculated by the following formula:

$$\frac{\text{Value after}-\text{value before}}{\text{Value before}} \times 100$$

The level of significance was set at $P \leq 0.05$.

RESULTS

The study included thirty patients distributed among three groups, each group consists of ten patients for ARP procedure. In L-PRF group two patients refused to continue to the end of the study after extraction. In L-PRF mixed with xenograft group, one patient did not show after ARP. In xenograft and collagen membrane group, one patient also did not show after ARP. No complications were reported after ARP in three groups. Thus the actual number of patients that completed the study is 26 patients.

TABLE (1): Mean and standard deviation (SD) of age (years) in the studied groups

		L-PRF	L-PRF & bone graft	Bone graft & membrane	F value	P value
Age (years)	Mean \pm SD	27.63 \pm 6.19	27 \pm 5.02	26.3 \pm 5.91	0.122	0.886 ns
	Min- Max	22-37	20-34	20-36		

ns= Non-Significant

TABLE (2): Gender distribution in the studied groups

Gender n (%)	L-PRF	L-PRF & bone graft	Bone graft & membrane	X ² value	P value
Males	4 (50%)	4 (44.4%)	4 (44.4%)	0.074	0.96 ns
Females	4 (50%)	5 (55.6%)	5 (55.6%)		

ns= Non-Significant

Demographic data:

There was no significant difference between the mean age values in different groups ($p=0.886$), (Table 1) In addition, no significant difference regarding gender distribution in different groups ($p=0.96$), (Table 2).

Radiographic assessment:

Bone width

Comparison between groups

Pre-operative: There was no significant difference in bone width in the different groups ($p=0.275$) (Table 3).

Post-operative: The highest mean value was recorded in L-PRF mixed with xenograft group (7.65 ± 1.34), followed by xenograft and collagen membrane group (7.17 ± 0.66), with the least value recorded in L-PRF group (6.46 ± 0.68). ANOVA test revealed a statistically significant difference between groups ($p=0.04$) (Table 3).

Percent change:

The highest mean percent decrease was recorded in L-PRF group (-23.76 ± 5.95), followed by xenograft and collagen membrane group (-13.58 ± 2.3), with the least value recorded in L-PRF mixed with xenograft group (-13.05 ± 8.71). ANOVA test revealed a statistically significant difference between groups ($p=0.014$) (**Table 3**).

Tukey's post hoc test revealed statistically no significant difference between any of the three groups in bone width preoperatively. Postoperatively, there was significant difference between L-PRF group and L-PRF mixed with xenograft group. On the other hand, there was no significant difference between the other two groups. Regarding percent change, L-PRF groups showed significant difference between the other two groups, while there was no significant difference between the other two groups (**Table 4**).

Comparison between pre-post bone width value within each group

The three groups showed a statistically significant decrease in bone width postoperatively compared to

preoperative values (**Table 5**).

Histological Assessment:

Histological evaluation of group 1 (LPRF) (**fig.7**) showed poorly formed bone trabeculae without osteoblastic rimming together with little number of osteocytes in their lacunae.

Histological examination of group 2 (LPRF+ Xenograft) (**fig.8**) revealed newly formed bone (woven bone) in all sites with prominent osteoblastic rimming indicating the active bone formation process. Also, prominent abundant osteocytes were observed entrapped in this newly formed bone. The medullary spaces were seen in continuity with the newly formed bone and filled with well vascularized connective tissue with prominent capillaries and abundant fibroblasts.

On histological examination of group 3 (Xenograft + barrier membrane) (**fig.9**): newly formed bone was observed with osteoblastic rimming. Few osteocytes in their lacunae were

TABLE (3): Descriptive statistics and comparison of pre, post and percent change of bone width in different groups (ANOVA test)

	Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
					Lower Bound	Upper Bound				
Pre	L-PRF	8.47	.51	.17	8.07	8.86	8.00	9.40	1.359	.275ns
	L-PRF+ Graft	8.80	.78	.26	8.20	9.40	7.45	9.70		
	Graft+membrane	8.30	.70	.22	7.80	8.80	7.30	9.40		
Post	L-PRF	6.46 ^b	.68	.23	5.94	6.98	5.70	7.40	3.682	.040*
	L-PRF+ Graft	7.65 ^a	1.34	.45	6.62	8.69	5.00	8.80		
	Graft+membrane	7.17 ^{a,b}	.66	.21	6.70	7.64	6.40	8.20		
Percent change	L-PRF	-23.76 ^a	5.95	1.98	-28.34	-19.19	-28.75	-11.87	5.13	.014*
	L-PRF+ Graft	-13.05 ^b	8.71	4.24	-22.82	-3.28	-45.65	-5.24		
	Graft+membrane	-13.58 ^b	2.30	.73	-15.22	-11.93	-17.53	-10.00		

Significance level $p \leq 0.05$, *=significant, ns=non-significant

Tukey's post hoc test: within the same comparison, means sharing the same superscript letters are not significantly different

TABLE (4): Details of Tukey's post hoc test for bone width in different groups

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig. (p value)	95% Confidence Interval	
						Lower Bound	Upper Bound
Pre	L-PRF	L-PRF+ Graft	-.33	.32	.55	-1.12	.46
		Graft+membrane	.17	.31	.85	-.60	.94
	L-PRF+ Graft	L-PRF	.33	.32	.55	-.46	1.12
		Graft+membrane	.50	.31	.25	-.27	1.27
	Graft+membrane	L-PRF	-.17	.31	.85	-.94	.60
		L-PRF+ Graft	-.50	.31	.25	-1.27	.27
Post	L-PRF	L-PRF+ Graft	-1.19	.44	.03*	-2.29	-.09
		Graft+membrane	-.71	.43	.24	-1.79	.36
	L-PRF+ Graft	L-PRF	1.19	.44	.03*	.09	2.29
		Graft+membrane	.48	.43	.51	-.59	1.55
	Graft+membrane	L-PRF	.71	.43	.24	-.36	1.79
		L-PRF+ Graft	-.48	.43	.51	-1.55	.59
Percent change	L-PRF	L-PRF+ Graft	-10.72	3.798	.024*	-20.18	-1.26
		Graft+membrane	-10.19	3.702	.028*	-19.41	-.964
	L-PRF+ Graft	L-PRF	10.72*	3.798	.024*	1.256	20.18
		Graft+membrane	.53	3.702	.989	-8.69	9.752
	Graft+membrane	L-PRF	10.19*	3.702	.028*	.964	19.41
		L-PRF+ Graft	-.53	3.702	.989	-9.75	8.689

Significance level $p \leq 0.05$, *significant

TABLE (5): Comparison of pre and post bone width value within each group (Paired t test)

		Mean	Std. Dev	Paired difference				t	P
				Mean	Std Dev	95% C.I. lower	95% C.I. upper		
L-PRF	Pre	8.47	.51	2.01	.48	1.637	2.379	12.474	.00*
	Post	6.46	.68	1.15	1.17				
L-PRF+ Graft	Pre	8.80	.78	1.13	.20	.250	2.048	2.946	.019*
	Post	7.65	1.34	2.01	.48				
Graft+ membrane	Pre	8.30	.70	1.15	1.17	.983	1.267	17.972	.00*
	Post	7.17	.66	1.13	.20				

Significance level $p \leq 0.05$, *significant

C.I.= confidence interval

seen. Reversal lines indicating bone remodeling were observed. The medullary spaces were not in continuity with the newly formed bone. Few inflammatory cell infiltrate was noticed in the medullary spaces. Deeply stained areas were noticed representing remnants of graft.

Histomorphometrical assessment

Area fraction of newly formed bone

The highest mean value was recorded in L-PRF mixed with xenograft group (65.63±16.41), followed by xenograft and collagen membrane group (37.53±2.26), with the least value recorded in L-PRF group (28.17±9.34). ANOVA test revealed a statistically significant difference between groups (p=0.00) (Table 6).

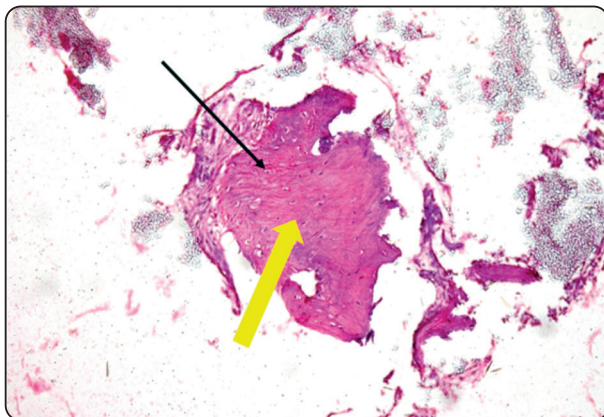


Fig. (7): Group 1 Photo micrograph stained with H&E showing poorly formed bone trabeculae (yellow arrows) with little number of osteocytes in their lacunae (black arrows) (original magnification x20).

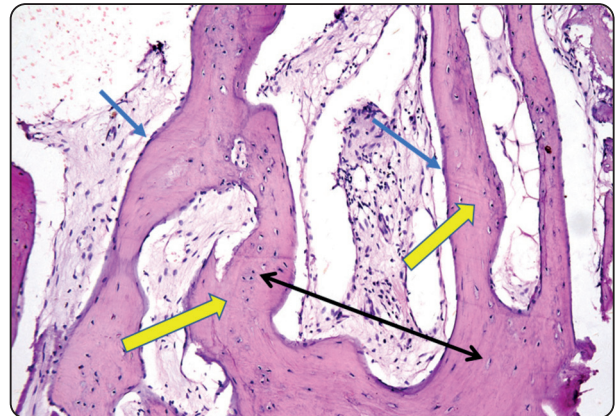


Fig. (8): Group 2 Photo micrograph stained with H&E showing well-formed bone trabeculae (yellow arrows) containing osteocytes in their lacunae (black arrows) with osteoblast rimming (blue arrows) (original magnification x20)



Fig. (9): Group 3 Photo micrograph stained with H&E showing well-formed bone trabeculae (yellow arrows) containing osteocytes in their lacunae (black arrows) with osteoblast rimming (blue arrows) (original magnification x20).

Table 6: Descriptive statistics and comparison of Area fraction of newly formed bone in different groups (ANOVA test)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
L-PRF	28.17 ^b	9.34	2.95	21.48	34.85	14.02	42.41	31.54	0.00*
L-PRF + Graft	65.63 ^a	16.41	5.19	53.89	77.36	37.08	86.13		
Graft + membrane	37.53 ^b	2.26	.72	35.92	39.15	33.80	40.00		

Significance level p≤0.05, *significant

Tukey's post hoc test : Groups sharing the same superscript letter of are not significantly different

Tukey’s post hoc test revealed statistically significant difference in the area fraction of newly formed bone between L-PRF group and L-PRF mixed with xenograft group, in addition to L-PRF mixed with xenograft group and xenograft and collagen membrane group. On the other hand, non-significant difference was detected between L-PRF group and xenograft and the membrane group (Table 6, 7).

Osteocytes count

The highest mean value was recorded in

L-PRF mixed with xenograft group (116.5±34.68), followed by xenograft and collagen membrane group (98.6±4.17), with the least value recorded in L-PRF group (87.87±8.46). ANOVA test revealed a statistically significant difference between groups (p=0.016) (Table 8).

Tukey’s post hoc test revealed statistically significant difference in the osteocyte count between L-PRF group and L-PRF mixed with xenograft group. No significant difference was detected between xenograft and collagen membrane group and each of the other two groups (Table 8, 9).

TABLE (7): Details of Tukey’s post hoc test for area fraction of newly formed bone

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
L-PRF	L-PRF+ Graft	-37.46	4.91	.00*	-49.64	-25.29
	Graft+membrane	-9.37	4.91	.16ns	-21.54	2.80
L-PRF+ Graft	L-PRF	37.46	4.91	.00*	25.29	49.64
	Graft+membrane	28.10	4.91	.00*	15.92	40.27
Graft+membrane	L-PRF	9.37	4.91	.16 ns	-2.80	21.54
	L-PRF+ Graft	-28.10	4.91	.00*	-40.27	-15.92

Significance level p≤0.05, *significant, ns=non- significant

TABLE (8): Descriptive statistics and comparison of osteocytes count in newly formed bone in different groups (ANOVA test)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
L-PRF	87.87 ^b	8.46	2.67	81.82	93.92	80.00	104.00	4.860	.016*
L-PRF+ Graft	116.50 ^a	34.68	10.97	91.69	141.31	52.00	141.00		
Graft+membrane	98.60 ^{a, b}	4.17	1.32	95.62	101.58	91.00	104.00		

Significance level p≤0.05, *significant

Tukey’s post hoc test : Groups sharing the same superscript letter of are not significantly different

Table 9: Details of Tukey’s post hoc test for osteocytes count of newly formed bone

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
L-PRF	L-PRF+ Graft	-28.63330*	9.28	.01*	-51.64	-5.63
	Graft+membrane	-10.73	9.28	.49	-33.74	12.27
L-PRF+	L-PRF	28.63330*	9.28	.01*	5.63	51.64
	Graft	17.90	9.28	.15	-5.11	40.91
Graft+	L-PRF	10.73	9.28	.49	-12.27	33.74
	L-PRF+ Graft	-17.90	9.28	.15	-40.91	5.11

Significance level $p \leq 0.05$, *significant

Clinical assessment:

Primary stability of the implant

The highest mean value was recorded in L-PRF mixed with xenograft group (74.4±5.82), followed by xenograft and collagen membrane

group (64±8.39), with the least value recorded in L-PRF group (46.4±5.32). ANOVA test revealed a statistically significant difference between groups (p=0.00) (Table 10). Tukey’s post hoc test revealed a significant difference between each 2 groups (Table 11).

TABLE (10): Descriptive statistics and comparison of stability in different groups (ANOVA test)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
				Lower Bound	Upper Bound				
L-PRF	46.40 ^c	5.32	1.68	42.60	50.20	38.00	56.00	45.34	0.00*
L-PRF+ Graft	74.40 ^a	5.82	1.84	70.24	78.56	67.00	82.00		
Graft+membrane	64.00 ^b	8.39	2.65	58.00	70.00	50.00	76.00		

Significance level $p \leq 0.05$, *significant

Groups sharing the same superscript letter of are not significantly different

TABLE (11): Details of Tukey’s post hoc test for stability in different groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
L-PRF	L-PRF+ Graft	-28.00	2.97	.00*	-35.37	-20.63
	Graft+membrane	-17.60	2.97	.00*	-24.97	-10.23
L-PRF+ Graft	L-PRF	28.00	2.97	.00*	20.63	35.37
	Graft+membrane	10.40	2.97	.00*	3.03	17.77
Graft+	L-PRF	17.60	2.97	.00*	10.23	24.97
	L-PRF+ Graft	-10.40	2.97	.00*	-17.77	-3.03

Significance level $p \leq 0.05$, *significant

DISCUSSION

It has been documented that guided bone regeneration in the presence of infection at the grafted site may be associated with poor regenerative outcomes and this may be related to fast graft dissolution in the acidic PH. (Yuan *et al.*, 2001; Habibovic *et al.*, 2006). Thus, previous studies suggested different protocols that must be followed for ARP in infected sockets to ensure accepted regenerative results. Mixing bone graft with antibiotics, complete removal of the infected tissues, primary wound closure, maintaining adequate blood supply, and systemic antibiotic administration are among these protocols. (Winkler *et al.*, 2000, Thomas *et al.*, 2011).

The use of L-PRF in the present study was based upon its antibacterial properties which is beneficial in presence of chronic infection. These antibacterial properties are attributed to L-PRF content of leukocytes, antimicrobial peptides, plasma complement, fibrinopeptide and platelets factor 4. Moreover, L-PRF is able to stimulate proliferation and differentiation of osteoblasts, thus it is able to enhance bone regeneration (Su *et al.*, 2009; Ehrenfest *et al.*, 2009; Ehrenfest *et al.*, 2010; Drago *et al.*, 2014; Davis *et al.*, 2014; Castro *et al.*, 2019).

In the present study, L-PRF mixed with xenograft was utilized for ARP in chronic infected sockets. According to Lekovic and co-workers L-PRF mixed with xenograft in periodontal defect regeneration results in significant effect on bone regeneration (Lekovic *et al.*, 2012). Moreover, Zhang *et al.* (2012) reported positive bone formation at lateral window sinus augmentation using L-PRF mixed with xenograft.

Regarding the choice of xenogenic bone graft, this was based on its osteoconductive potential, and slow resorption rate that may allow enough time for new tissue formation (Araujo *et al.*, 2009; Titsinides *et al.*, 2019). Polypropylene sutures selection was based upon ideal criteria of

this type of suture material regarding regenerative procedures as being non-biodegradable, hygienic material and able to maintain its tensile strength for long time (Gaspar *et al.*, 1983). Chlorhexidine and systemic antibiotics were used in the study as prophylaxis against secondary infection and to enhance regenerative outcomes according to many previous studies (Bowe *et al.*, 2011; Froum *et al.*, 2015; Drago *et al.*, 2017). The same antibiotic dose and duration was prescribed to all patients in the three groups to avoid any differences between groups that may have an effect on the regenerative outcomes.

Medically free patients were selected to avoid any confounding factors. Smokers were excluded due to unfavourable suspected outcomes (Mombelli & Cionca 2006; Keenan & Veitz Keenan 2016). Relatively young age was selected to avoid age related factors that could affect results of the study (Amarya *et al.*, 2015).

Maximum preservation of tissues was performed via flapless approaches and a traumatic extraction during surgeries (Darby *et al.*, 2008; Blanco *et al.*, 2011; Dym *et al.*, 2012). Implant and core biopsy were performed 5 months following ARP to allow time for new bone formation, and for any pre-existing infection for resolution (Kotsakis *et al.*, 2014; Renzo *et al.*, 2017; Lai *et al.*, 2019).

Regarding the amount of newly formed bone per area fraction, the highest mean value was recorded in L-PRF mixed xenograft group (group 2), followed by xenograft and barrier membrane group (group 3), with the least value recorded in L-PRF group. Regarding osteocyte count; the highest mean value was recorded in L-PRF mixed xenograft group, followed by xenograft and barrier membrane group, with the least value recorded in L-PRF group. Tatullo *et al.*, 2012 showed similar results at sinus floor augmentation using L-PRF mixed with xenograft in comparison to xenograft alone. Moreover, superior results of graft vascularization, and high osteocyte count was recorded with L-PRF mixed with xenograft.

The superior results of group 2 over group 3 can be explained as follow; in group 3 xenograft osteoconductive properties, and slow resorption rate serves as scaffold for bone ingrowth and angiogenesis which helps for new bone formation; however, xenograft do not completely get substituted by newly formed bone as remnant of graft material were seen in histology assessment remaining within the regenerated bone which affects the amount of newly formed bone per area fraction. *Nappe et al., 2016* showed similar results after using xenograft in socket preservation as histomorphometric analysis showed 45% of newly formed bone with 41% of remaining graft material in the socket (*Cordaro et al., 2008*). In group 2; L-PRF can accelerate tissue healing, wound re-epithelization by creating sealed cavity form oral bacteria to avoid open healing state after ARP, graft infection, and bone graft loss. Moreover, it accelerates angiogenesis and new vascularization, thus fasten bone graft creep substitution and new bone formation. (*Jung et al., 2013*).

In group 1 using L-PRF alone in socket preservation improved bone quality formation as the L-PRF affects proliferation and differentiation of osteoblast through increased alkaline phosphatase activity, and the slow release of growth factors, and acting simultaneously as a scaffold for cellular invasion, angiogenesis which promote the osteoconductive effect. However fast resorption rate and lack of strong physical properties did not provide enough time for bone forming cell to produce more volume. Thus, lower percent of newly formed bone has been produced in this group (*Clark et al., 2001; Kawase et al., 2003; Ehrenfest et al., 2009*)

Comparing the dimensional stability at the alveolar ridge width radiographically between the three groups revealed that group 2 showed the highest stability with least percent of ridge resorption followed by group 3, where group 1 recorded the

greater percent of ridge resorption. Results of group 1 was in accordance with studies by *Zhang et al., 2018* and *De Angelis et al., 2019* which used L-PRF solely with the aim of socket preservation and showed non-significant results to maintain alveolar ridge contour but adding it to other grafts had better effect on bone regeneration in extraction socket. Superior results of group 2 in comparison to group 1 can be related to xenograft used in group 2 which has slow resorption rate which gives enough time for new bone formation, on the other hand using L-PRF only effect lasts only for few weeks due to fast resorption rate. L-PRF used as an adjunct to a bone graft in group 2 will enhance the graft volume without interfering with bone maturation (*Wenz et al., 2001; Ehrenfest et al., 2018*). This result is similar to *De Angelis et al., 2019*.

Superior results of group 2 in comparison to group 3 can be related to the role of L-PRF in the presence of chronic infection. L-PRF has antibacterial effect due to their content of leukocytes. Others demonstrated L-PRF anti-inflammatory effect against lipopolysaccharide (LPS) induced inflammatory response. Furthermore, L-PRF benefits of the entrapped growth factors within the fibrin matrix and accelerates proliferation, differentiation of osteoblasts and BMSCs and decrease bone resorption (*Wenz et al., 2001; Dohan et al., 2006; Wang et al., 2020*). L-PRF fibrin network entraps macrophages, B&T lymphocytes, and plasma cells and their products within the fibrin matrix at different concentration for each subtype. These cells are involved in bone remodelling process and regulate bone remodelling and decrease bone loss (*Weitzmann and Ofotokun., 2016; Nogueira et al., 2020*).

Moreover, L-PRF in group 2 eliminates complication associated with barrier membrane use in group 3 associated with soft tissue closure. L-PRF enhances soft tissue healing following socket

preservation procedure, on the contrary leaving collagen membrane exposed to the oral cavity has a negative effect on the bone regeneration outcome (*Eskan et al., 2017; Garcia et al., 2018*).

Regarding implant primary stability, the highest mean value was recorded in L-PRF mixed xenograft group, followed by xenograft and barrier membrane group, with the least value recorded in L-PRF group. This may be explained by different bone quality obtained in the three groups as implant primary stability is affected by bone quality (*Atsumi et al., 2007*). Group 2 demonstrated superior results in comparison to group 3 related to the quality of newly formed bone produced at the time of implant insertion. As previously mentioned, group 2 showed higher percent of newly formed bone in comparison to group 3. According to *Dohan et al., 2006*, L-PRF entraps high amounts of TGF β and PDGF and slowly release them. TGF β and PDGF are the highest concentration of growth factors reported promoting bone formation and initial collagen production. Collagen synthesis creates extracellular matrix for calcium deposition. Also fibrinogen content of L-PRF may contribute to increase bone mineralization. Hence, platelet concentrates seem not only help a formation of better-quality bone with higher density, but also improve qualities of other biomaterials when combined together (*Compston et al., 2006; Dohan et al., 2006; Uggeri et al., 2007; Stumbras et al., 2019*).

CONCLUSION

Within the limitations of this study, it can be concluded that using L-PRF mixed with xenograft is a simple technique to decrease post-extraction alveolar ridge dimensional changes in cases with chronic infection. Moreover it showed better dimensional and histological outcomes in alveolar ridge preservation in comparison to L-PRF alone or xenograft and collagen membrane.

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