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Clinical, histological and radiographic evaluation of bone healing after tooth extraction using autologous platelet rich fibrin and 1% alendronate gel before dental implant

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***Abstract*---**Purpose: The present study was performed to evaluate clinically, radiographically and histologically the effect of autologous platelet rich fibrin (PRF) and 1% alendronate (ALN) gel in alveolar ridge preservation after tooth extraction. Materials and method: In this study, thirty patients with teeth indicated for extraction were divided randomly into three groups: Group A: the patients received 1% ALN gel mixed with PRF as a grafting material for the extraction socket. Group B: the patients received PRF only and in Group C the socket left without any a grafting material. All patients were evaluated clinically and radiographically at; base line and after 3 months. Also, histological evaluation using bone core biopsy harvested at the re-entry surgery before implant placement. Results of the present study were recorded, tabulated and statistically analyzed. Results: Histological, clinical and radiographic findings showed a statistically significant difference in group A when compared to both group B and group C. Conclusions: The addition of ALN to PRF leaded to a noticeable socket preservation and reduction in the amount of bone resorption during the observation period of the study.

***Keywords*---**clinical, histological, radiographic evaluation, bone healing, tooth extraction, dental implant.

Introduction

Extraction of teeth with an inflammatory state may result in extensive bone resorption which influence the optimal placement of dental implants [(1)]. Four stages of socket healing occurred, the last stage (bone-reorganization stage); occurred after six weeks and continued for six months[(2)]. It was reported that [(3)] height loss [(4)] and resorption patterns following single-tooth extraction was within

the first 3 months[4].Most changes occur in bone width but slight changes in bone height were reported [5]. Although atraumatic tooth extraction helps [6], ridge preservation techniques needed [7] to prevent alveolar changes [8].Ridge preservation by adding graft material into the socket leads to better repair and healing of the socket [9]. An ideal grafting material should be easly resorbable [10] and produce a strong foundation for osteointegration with dental implant [11]. Graft materials either natural transplants (autografts, allografts and xenografts) or synthetic materials (alloplasts) [12, 13]. Non-absorbable graft particles delay the socket healing process [14] and might reduce the bone to implant contact [15, 16].

Platelet rich fibrin (PRF) is platelet concentrate of second generation [17] consists of an assembly of cytokines, glycanic chains and structural glycoprotein's enmeshed between a slowly polymerized fibrin networks. pro-inflammatory cytokines and anti-inflammatory cytokine, angiogenesis promoter, main angiogenesis soluble factors, Angiopoietin included in the fibrin gel [18] not provided by another platelet concentrate [19]. Valuable effects of PRF have been demonstrated in different soft tissue and bone regenerative techniques [20, 21] as regeneration of periodontal tissue [22, 23].

Alendronate (ALN) used to prevents bone resorption without affecting mineralization or bone quality [23] and has antimicrobial properties[24]. Alendronate has been administered systematically in animals, but it showed some systemic side effects [25] so it has been administered locally to prevent bone loss due to periodontitis [26]. Local application effect of bisphosphonates on ridge preservation in humans remains poorly investigated[26].Previously, studies demonstrated that locally delivered 1% ALN gel was effective as an adjuvant to mechanotherapy in treatment of periodontitis [27, 28]. The present study tried to evaluate the efficacy of alendronate with platelet rich fibrin in the alveolar ridge preservation post tooth extraction.

Patients and Method

The present study is randomized, controlled clinical trial study carried out on 30 patients of both sex (15 males, 15 females ranged in age from 20-45 years) with un-restorable maxillary anteriors or premolars. All patients were selected from outpatient of Oral Medicine and Periodontology Department clinic, Faculty of Dentistry, Al-Azhar University, Assiut Branch. Approval to conduct this work was sought and granted by the ethical committee, Faculties of Dentistry Al-Azhar University. Written consent was obtained from the patients included in the current study.
Inclusion criteria: Adult patients with isolated hopeless tooth indicated for extraction and planned for delayed implant placement
Exclusion criteria: Systemic or local disease/condition that would compromise post-operative healing Patients grouping: Patients were classified randomly into the following equal three groups using on line software (http://www.randomizer.org). Group (A) contains 10 patients received autologous platelet rich fibrin (PRF) mixed with 1% Alendronate (ALN) gel as a grafting material for the extraction socket. Group (B) contains 10 patients received autologous platelet rich fibrin (PRF) as a grafting material for the extraction

socket. Group (C) contains 10 patients without any grafting materials for the extraction sockets.

Patient preparation

Full mouth scaling and root planning (SRP) befor extraction. Clinical photographs were taken for all patients before extraction and after 3 months.

Materials preparation

A. Formulation of 1% Alendronate (ALN) gel: ALN (Alfa Aesar, Thermo Fisher Scientific, Germany) used as described by Reddy et al (2005) [29] .
B. Preparation of autologous platelet rich fibrin (PRF) as described by Choukroun et al (2006) [17].

Surgical procedures & application of graft materials:

a- Befor extraction, flap was done to expose the crestal bone
b- Curettage of the socket was followed by irrigation with 0.9 saline concentration.
c- Socket was filled with graft materials according to group types, examination daily for the first 3 days were performed. After three months dental implants were placed.

Patient assessment: Patient evaluation was done in two stages:

Healing of the extraction socket stage

All clinical and radiographic parameters were recorded at the baseline and 3 months after extraction prior to implant placement as the following:

Clinical parameters

preoperative acrylic stent for the extraction site was fabricated with two grooves on the mid-buccal and mid-palatal corresponding to the respective cortical plates.

Alveolar ridge height

a. Mid-buccal crestal height: measured using graduated periodontal prob as the distance in millimeters from a fixed reference point (FRP) on the acrylic stent to the most coronal mid-point on the buccal cortical plate.
b. Mid-palatal crestal height: measured as the distance in millimeters from a fixed reference point (FRP) on the acrylic stent to the most coronal mid-point on the palatal cortical plate using graduated periodontal prob.

Bucco-lingual width

measured at the line 2 mm apical to the most coronal point on the buccal / palatal bone plate using Ridge Mapping Caliper.

Radiographic assessment

- Cone beam computed tomography (CBCT) were performed for all patients with New Tom cone beam 3D system (110F, Teheran-ro87-gil, Gangnam-gu, Seol, Korea).
- After importing data as DICOM files into the software, All images were marked and traced in cross-sectional and axial view, and same reference points and lines were used both at baseline and at 3 months. The sagittal and coronal reference lines where intersected at each other at the center of the socket. The axial reference line was adjusted at the exact view to be parallel to the lingual cortex of bone at crestal level.

1. Radiographic alveolar ridge height: Alveolar ridge height was measured by selecting 3 sagittal sections at base line (fig1e: left figure) and measuring ridge height from fixed tangent at the base of anatomic structure (maxillary sinus or nasal floor) to the most coronal point of alveolar bone buccally (E) and palataly (F) and the mean was calculated. The same was then repeated after 3 months (fig1e: right figure).
2. Radiographic Alveolar ridge width: Alveolar ridge width was measured by selecting 3 sagittal sections at base line (fig1e: left figure) and measuring bucco-lingual ridge width at 3 points 10, 12, 14 mm from a fixed reference line at each section.
3. Bone density measurements: At the generated cross-sectional view, three density readings were taken for each site (crestal, middle and apical) in Hounsfield Unit (HU) then the mean of the three readings was acquired.

Implant placement stage

Drilling protocol

After 3 months from the grafting, mucoperiosteal flaps were elevated to allow access to the alveolar ridges of grafted sockets using crestal horizontal incision without vertical incisions. A bone core biopsy was taken by trephine bur (2mm) smaller than the initial implant drill. Then implants were inserted.

Measuring primary implant stability

Following the final seating of the implant fixtures the smart peg is screwed to the fixture then stability of each implant was measured in ISQ units using the Ostell Mentor at four points; buccal, palatal, mesial and distal. Then were averaged for each implant. Periapical post-operative x-ray was taken. Four months later, prosthetic procedures were completed.

Histological assessment

A. Specimen staining procedure: Bone biopsy specimens obtained were fixed in 10% formalin and then decalcified in 17% nitric acid for 12 hours. Tissues were then embedded in paraffin wax and sectioned into multiple 5-µm thick sections. Sections stained with hematoxylin and eosin (H&E) to show general layout, distribution of cells and provide general overview of tissue

samples structure [30]. Then stained with Masson's Trichrome (MTC) for qualitative and quantitative measurements of bone trabeculae and osteoid tissue.

B. Image analysis: For each MTC 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 (EOS 650D, Cannon, Japan) which was mounted on a light microscope (BX60, Olympus, Japan). 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 histochemical stain assessment were carried out using Image J, 1.41a, (NIH, USA) image analysis software. Images were first corrected for brightness and contrast 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.

Statistical analysis

Data were collected tabulated and statistically analyzed with IBM® SPSS Statistics Version 20 for Windows.

Results

Results of clinical parameters, radiographic assessment, histological assessment, bone density and implant stability were summarized in table1 and table 2.

Implant placement stage

Histological parameter

a- By examination of H&E stained slides under light microscop, group (A) slides showed large sized trabeculae of osteoid tissue through highly cellular stroma which contain active plump of osteocyte in lacunae. The trabeculae were surrounded by active osteoblastic rimming. Interconnection between bone trabeculae was noticed, Fig2a. Slides of group (B) showed not well formed trabeculae of osteoid tissue which contain plump of osteocyte in lacunae but they were not interconnected with each other Fig2c. However, slides of group (C) showed poorly formed bone trabeculae without obvious osteoblastic rimming Fig2e.

b- By examination of Masson's trichrome stained slides under light microscop, slides of group (A) showed large amount of newly formed bone trabeculae (blue) with many osteocytes inside lacunae and surrounded by fibro cellular matrix with few blood vessels. Union between bone trabeculae and transition from newly formed to mature bone were noticed Fig2b. Slides of group (B) showed newly formed bone trabeculae (blue) less than fully mineralized tissue appeared (red) in contrast to group (A), they are

surrounded by fewer fibrous matrix Fig2d. However, slides of group (C) showed least formed fully mineralized tissue appeared (red) Fig2f. Using percentage of newly formed bone osteoid as the mean area fraction measured by image analysis software, there was a statistically significant difference between Group (A), Group (B) and Group (C) where ($p<0.001$). A statistically significant difference was found between Group (C) and each of Group (A) and Group (B) where ($p<0.001$) and ($p=0.002$) respectively. A statistically significant difference was found between Group (A) and Group (B) where ($p=0.047$).

Implant Stability

There was no statistically significant difference between Group (A), Group (B) and Group (C) where ($p=0.420$).

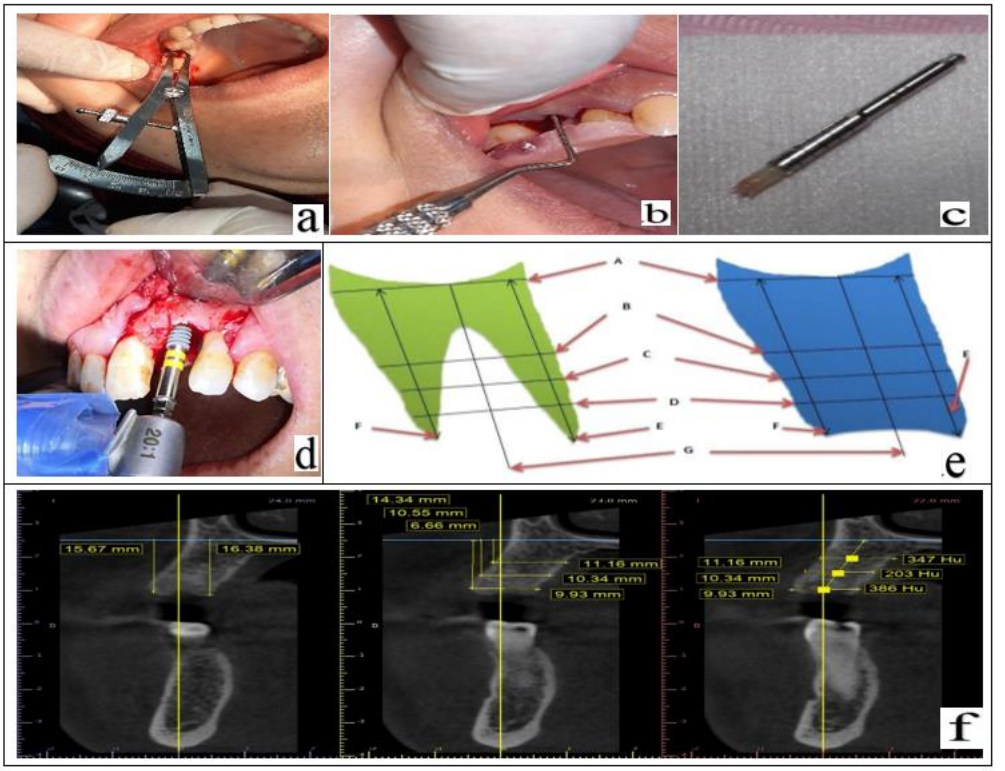

Fig 1: Clinical and radiographical photographs showing a) Core biopsy taking using trephine bur and Extracted bone. b) Fabricated acrylic stent and mid-buccal crestal height measurement.c) Measuring socket width with caliper. d) Implant placement. e) Reference lines for measuring alveolar height and width A: horizontal reference line (tangent) through the most inferior border of maxillary sinus or nasal floor. B: first horizontal line at 10 mm from the tangent. C: second horizontal line at 12 mm from the tangent. D: third horizontal line at 14 mm from the tangent.E: line extending from the tangent till the most coronal point in

buccal wall. F: line extending from the tangent tills the most coronal point in palatal wall. G: vertical reference line. left figure: socket after extraction. Right figure: ridge after 3 months. f) Pre and post-operative CBCT assessment showing intersecting reference lines tracing socket height, width and bone density measurements.

Table 1: The mean ± standard deviation (SD) and *p*-values of Clinical/radiographic parameters in mm for different groups

Clinical &radiographic parameters	Variables	Baseline		After 3m		p-value[3]
		p-value[1]	Mean ± SD	p-value[2]	Mean±SD	
buccolingual width	Group A	0.345ns	10.92±1.12	0.006*	8.10±1.38	<0.001*
	Group B		10.23±0.88		6.53±0.76	<0.001*
	Group C		10.56±1.10		6.86±0.93	<0.001*
Mid-buccal height	Group A	0.919ns	13.80±1.63	0.802ns	15.53±1.85	<0.001*
	Group B		13.74±1.36		15.98±1.65	0.019*
	Group C		13.55±1.23		15.90±1.26	<0.001*
Mid-palatal height	Group A	0.847ns	13.62±1.53	0.301ns	14.50±1.50	<0.001*
	Group B		13.30±1.78		15.05±1.73	0.035*
	Group C		13.75±2.03		15.74±1.99	<0.001*
Coronal width	Group A	0.393ns	10.07±1.02	0.004*	7.72±1.32	<0.001*
	Group B		9.53±1.00		6.21±0.72	<0.001*
	Group C		9.49±1.10		6.38±0.83	<0.001*
Middle width	Group A	0.445ns	9.95±1.52	<0.001*	9.27±1.65	<0.001*
	Group B		9.56±0.76		8.05±0.78	<0.001*
	Group C		9.28±1.09		6.75±0.92	<0.001*
Apical width	Group A	0.529ns	9.90±2.11	0.026*	9.46±2.13	<0.001*
	Group B		9.88±1.20		8.71±1.18	<0.001*
	Group C		9.20±1.19		7.49±1.10	<0.001*
Total ridge width	Group A	0.454ns	9.97±1.45	0.002*	8.82±1.51	<0.001*
	Group B		9.66±0.83		7.66±0.74	<0.001*
	Group C		9.33±1.04		6.87±0.88	<0.001*
Buccal height	Group A	0.837ns	15.68±1.63	0.086ns	14.58±1.39	<0.001*
	Group B		15.85±2.33		14.65±2.10	0.292ns
	Group C		15.25±2.81		12.67±2.78	0.002*
Palatal height	Group A	0.814ns	17.35±3.16	0.543ns	16.53±2.94	<0.001*
	Group B		17.98±2.34		16.77±2.38	0.336ns
	Group C		17.21±2.99		15.44±3.14	<0.001*

*; significant (p<0.05) ns; non-significant (p>0.05) p-value[1], between groups at base line, p-value2,between groups after 3 months; p-value[3].between the same group at base line and after 3 months

Table 2: The mean ± standard deviation (SD) and *p*-values of bone density, percentage of newly formed bone and implant stability for different groups

parameters	variables	Mean	SD	p-value
bone density in HU	Group A	658.03	107.32	0.002*
	Group B	603.18	134.06	

	Group C	458.44	95.02	
Percentage of newly formed osteoid	Group A	61.93	10.46	<0.001*
	Group B	56.13	10.44	
	Group C	38.64	9.75	
Implant stability in ISQ	Group A	61.50	8.11	0.420ns
	Group B	60.70	11.41	
	Group C	55.30	13.58	

*; significant (p<0.05) ns; non-significant (p>0.05)

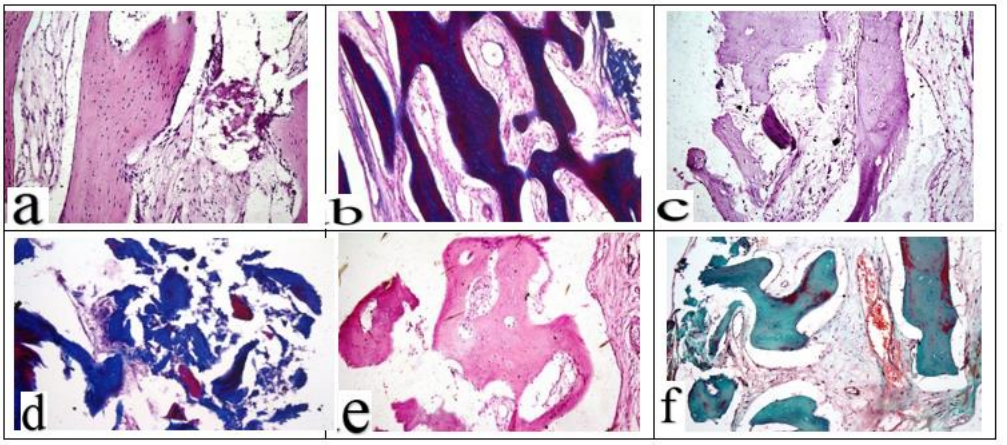

Fig2: Histological slides for bone of three groups. a- Photomicrography of group (A) showing large sized trabeculae of osteoid tissue through highly cellular stroma which contain active plump of osteocyte in lacunae. The trabeculae were surrounded by active osteoblastic rimming. Note interconnection between bone trabeculae. (H&E x10). b- Photomicrography of group (A) showing large amount of newly formed bone trabeculae (blue) with many osteocytes inside lacunae and surrounded by fibro cellular matrix with few blood vessels. Note union between bone trabeculae and transition from newly formed to mature bone. (Masson's trichrome x20). c- Photomicrography of group (B) showing not well formed trabeculae of osteoid tissue which contain plump of osteocyte in lacunae but they were not interconnecting with each other. (H&E x10) d- Photomicrography of group (B) showing newly formed bone trabeculae (blue) less than fully mineralized tissue appeared (red) in contrast to group (A), they are surrounded by few fibrous matrix (Masson's trichrome x20). e- Photomicrography of group (C) showing poorly formed bone trabeculae without osteoblastic rimming. (H&E x10). f- Photomicrography of group (C) showing least formed fully mineralized tissue (M) appeared (red). (Masson's trichrome x20)

Discussion

The progressive resorption of the bony ridge that follows tooth extraction is a physiological phenomenon that can lead to potential esthetic and functional challenges for clinicians and patients [31, 32]. Alveolar ridge preservation (ARP) was developed as a therapy to minimize remodeling and to preserve alveolar bone following tooth extraction [33]. Materials available for this purpose generally consist of matrix scaffolding materials and/or biologic agents which can be used

separately or together to achieve the desired surgical outcome [30,34,35]. Of the available biomaterials, platelet-rich fibrin [36]and Alendronate (ALN)[37]. Studies have observed that topical application of ALN was highly effective in reducing the bone resorption after mucoperiosteal flap surgery [38, 39].

The present study was designed to evaluate the efficacy of 1% alendronate gel mixed with PRF in alveolar ridge preservation following tooth extraction.The present study was conducted on medically free patients and excluded smokers, pregnant and lactating women and medically compromised patients, because these conditions affect the response to treatment in the form of healing term and pattern which reflects on and affect the accuracy of the study results [40]. It was concluded that, by 4-6 weeks after tooth extraction, most parts of the alveolus are filled with woven bone, while the soft tissue becomes keratinized and the healing process completed within 3 months [41], so the follow up period for the present study was 3 months. As the esthetics received more emphasis with treatment planning, resorption of the alveolar ridge following tooth extraction especially in the anterior region has become a significant problem [42]. It was also reported that the buccal wall of the alveolar bone undergoes greater vertical atrophy than the lingual wall [43]. So, the maxillary anteriors and premolars were the interested subjects in the present study. A fabricated occlusal stent in clinical measurements was used as it is stably seated on the occlusal surfaces of multiple adjacent teeth to the extraction site allowed for reproducible measurements of the alveolar ridge [44].

Cone beam computed tomography was used for radiographic analysis of the quality and quantity of preserved sockets, as CBCT scan is a highly accurate tool in the axial and cross sectional image planes at different areas of the maxillofacial region. One of the major uses of CBCT is the pre surgical implant planning for measuring alveolar bone height, width and density. Its accuracy, reproducibility, lower patient radiation dose and faster scanning time [45] all provided useful informations for the study.

Regarding the loss in buccolingual width of the alveolar ridge, the present study showed a statistically significant differences in group A (ALN + PRF) when compared to PRF only or control group. After 3 months, ALN gel mixed with PRF group showed noticeable reduction in the amount of bone loss clinically in the bucco-lingual width. A significant statistical difference was found between the three groups with 2.82 mm mean of bone loss in group A comparing to group B 3.70 mm and group C with 3.7mm. The clinical alveolar crestal height showed more amount of bone loss in buccal side. No significant statistical difference in the crestal height either buccal or palatal between the different groups. Group A showed 1.73 mm mean loss in buccal bone, while group B and C showed 2.24 and 2.35 mm bone loss respectively. The mean palatal bone loss was 0.88 mm in group A, 1.75 mm in group B and 1.99 mm in group C.

These results are in agreement with different clinical studies concluded that, the use of combined regenerative therapies rendered more favorable vertical and horizontal socket preservation[44,46,47]. Generally, the buccal bone showed more bone loss than palatal bone because less blood supply and high stress bearing area [48]. The total radiographic buccolingual width of the alveolar ridge showed a

noticeable preservation of extraction socket width in ALN+PRF group with a statistically significant difference when compared to control group. Radiographic bone height showed a statistically significant difference in group A when compared to group C only, as the means of buccal bone loss in the three groups were 1.1, 1.2 and 2.58 mm respectively and the means of palatal bone loss in the three groups were 0.82,1.21 and 1.77 mm respectively.

Radiographic bone density represented a statistically significant difference in group A when compared to both group B and group C. in addition, there was a statistically significant difference between group B and group C. The means of bone density in the three groups were 658.03, 603.18 and 458 HU respectively. Radiographic quantity and quality of bone in the form of linear measurement of bone height, width and density exhibited noticeable preservation of the extraction socket and reduction of the degree of alveolar bone loss with statistical significance in ALN+PRF group when compared to PRF group or control group. These results are inconsistency with those using melatonin + β-TCP when compared to β-TCP after complete healing of the extraction socket, and in agreement with other radiographic studies concluded that, alveolar socket preservation by different combined grafting materials consistently rendered more favorable results [49, 50], with the exception of two studies; the non-grafted sockets exhibited slightly better results in terms of radiographic bone height as compared to the grafted sockets; however, these differences were not clinically or statistically significant [51]. In another study, the application of an alloplast (β-TCP with a polylactide–co-glycolide coating) without membrane, rendered significantly inferior results as compared to the control group [52]. In the study using A-PRF and A-PRF + FDBA, Micro-CT analysis of bone cores from healed sockets demonstrated no significant difference in bone density for treatment groups. Significantly less bone mineral density was noted using blood clot alone compared to FDBA alone [44].

In addition to hematoxylin-eosin, Masson trichrome method gives good contrast between mineralized and un-mineralized bone and aids in quantitative and qualitative measurements of bone trabecule and ostoied tissue [53]. Histological findings showed a statistically significant difference in group A when compared to both group B and group C and there was a statistically significant difference between group B and group C. Photomicrography of group (A) showing large amount of newly formed bone trabeculae with many osteocytes inside lacunae and surrounded by fibro cellular matrix with few blood vessels. Photomicrography of group (B) showing newly formed bone trabeculae less than fully mineralized tissue appeared in contrast to group (A), they are surrounded by few fibrous matrices. BPs therapy in tooth extraction model decreased osteoclasts on the bone surface and increased mononuclear and non-attached osteoclasts [54]. The non-attached osteoclasts are not able to resorb bone but may be involved in bone remodeling and activation of osteoblasts [55].

The histological examination of the present study resemble that obtained by using melatonin with β-tri-calcium phosphate samples exhibited earlier bone maturation with complete bone formation in the test group than the control group [46]. Another study reported no statistically significant difference in the ratio between the PRF and control groups in the formation of new bone [56].

Histomorphometric analysis for ridge preservation using A-PRF resulted in the most vital bone formation across all groups, and this bone formation was significantly greater than observed in the FDBA treatment group which demonstrated the least amount of vital bone formation as the presence of mineralized graft material may have contributed to the decreased vital bone formation at sites treated with FDBA [44]. ALN solution in different concentration treatment resulted in more osteoid formation within the extraction sockets in dogs compared with the control group. Higher bone volume was found in ALN groups than in the control at 2-week and 8-week healing periods[47].

The implant stability was measured for the three groups with no significant statistical difference in the present study. The means of implant stability for Group A, B and C were 61.5, 60.7 and 55.3 ISQ respectively. If implant stability quotient (ISQ) lies between 55-85 resonance frequency analysis (RFA) units is acceptable range for obtaining sufficient mechanical stability [57].While both radiographic bone density and histological parameters showed a significant difference in both Groups A and B which will affect the secondary implant stability, so increasing durability with good prognosis for implant.

In conclusion, the addition of ALN to PRF leaded to a noticeable socket preservation and reduction the amount of bone resorption on the clinical and radiographic levels during the observation period of the study. Histological evaluation of alveolar ridge socket preserved with 1% ALN gel mixed with PRF revealed highly regenerative capacity and more transition from newly formed to mature bone than other groups.While the mixture of ALN and PRF showed a significant difference in the formed bone density, it has no effect on the primary implant stability quotient.

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