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SOCKET PRESERVATION USING ATORVASTATIN VERSUS PLASMA RICH FIBRIN: A RANDOMIZED CLINICAL AND HISTOMORPHOMETRIC CONTROLLED TRIAL

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ABSTRACT

Introduction: Statins such as atorvastatin (ATV), aside from their lipid lowering effects, are capable of modulating the process of bone turnover and regeneration owing to their effects on certain cells including osteoblasts, osteoclasts and stem cells of mesenchymal origin. Platelet rich fibrin (PRF) on the other hand is an autogenous platelet concentrate with a fibrin matrix having biomechanical properties that allows it to be used in regeneration. The purpose of this study is to evaluate the use of ATV gel and PRF in alveolar socket preservation.

Patients and Methods: Thirty patients having upper premolars and canines scheduled for extraction were randomly assigned into three equal groups. Group I received ATV gel, Group II received PRF while Group III had their sockets left to heal spontaneously (control). Clinical parameters included ridge width, average crest heights measured at baseline and 8 weeks' post-extraction. Core biopsies stained with Masson's Trichrome were examined.

Results: Group II had the lowest mean percentage reduction in ridge width however, the differences were non-significant regarding both ridge width and height. The total collagen surface area and the average trabecular size were significantly higher in sockets augmented with PRF. This finding suggested that PRF fibrin scaffold was osteoconductive and acted as a natural scaffold for new bone formation.

Conclusion: This study showed no significant difference between ATV and PRF compared to the control as regards to clinical measurements. Sites augmented with ATV, PRF showed higher newly formed osteoid tissue and mineralized bone trabeculae than spontaneously healed sockets.

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INTRODUCTION

Successful oral rehabilitation with dental implants requires the maintenance of hard and soft tissue volume for best esthetic and functional outcomes. [1] However, significant dimensional changes are known to occur during the first year after tooth extraction with major changes occurring in the first 8 weeks due to high osteoclastic activity prevalent during this period. Generally, the modelling and remodelling processes taking place result in qualitative and quantitative changes in the extraction socket with a reduction in the bone crest dimension. [2]

Aside from reducing the loss of alveolar bone volume, socket preservation additionally has the advantages of reducing the need for further bone grafting procedures, improving stability of implants as well as allowing earlier implant restoration. [2] In addition to several biomaterials such as autogenous, allogenic, xenogenic and alloplastic bone grafts, autogenous platelet concentrates (APC)have been reported in literature to be used in socket preservation with the aim of osteoid tissue formation via the release of cytokines and growth factors. [3] Among these are platelet rich fibrin (PRF) with the advantages of a natural polymerization taking place during the centrifugation process without the need for any anticoagulant or additive making it simpler and less expensive to prepare. [4] It also has the advantages of incorporation of leukocytes which may stimulate stem cells proliferation and differentiation as well as its natural fibrin network which could result in slow release of growth factors for more than seven days.[5] A recent review article,[6] investigating the effect of platelet concentrates in alveolar ridge preservation, has concluded that Leukocyte Rich Fibrin and PRF may have beneficial effects on the reduction in both vertical and horizontal alveolar ridge dimensions, however, it did not draw any conclusions regarding the percentage of new vital bone. Statins or inhibitors of 3-hydroxy-3-glutarylcoenzyme A reductase; a rate limiting enzyme in the mevalonate pathway, like simvastatin (SMV) and atorvastatin (ATV), are widely used to treat patients with hyperlipidemia. [7] However, due to the diversity of biomolecules produced by this pathway, statins have multiple effects which are beyond their being cardioprotective. Among these are, anti-inflammatory, immunomodulatory, as well as anabolic effect on bone metabolism as first reported by Mundy et al. in 1999. [8,9] The effect on bone metabolism is mediated through the stimulation of the expression of vascular endothelial growth factor (VEGF) and bone morphogenic protein-2 (BMP-2), [10] as well as the promotion of osteoblast differentiation and mineralization in MC3T3-E1 cell line in tissue culture which are widely used to study the maturation of pre-osteoblastic cells into a matrix mineralizing osteoblast. [11] Moreover, the local delivery of statins has gained interest due to several factors including reduction of systemic side effects including liver toxicity and myositis [12,13], as well as the ability to deliver the desired dosage to the targeted areas. [14] With ATV showing better anti-inflammatory and antioxidant properties as well as superior pharmacokinetic properties,[15, 16] this study compared the use of direct application of ATV gel compared to PRF in socket augmentation both clinically and histomorphometrically in contrast with single extraction sockets left to heal without augmentation.

SUBJECTS AND METHODS

Thirty patients [25 females (83.3%) and 5 males (16.4%)] with a mean age of 32.6 (7.9) were recruited for this study from the outpatient clinic of Oral Medicine, Periodontology, and Oral Diagnosis Department, Faculty of Dentistry, Ain Shams University, with potential 30 teeth indicated for extraction and socket preservation. The 30 sites met the following inclusion criterion of Type I socket [17]; where the facial soft tissue and buccal plate of bone at normal levels in relation to

cement-enamel junction of the pre-extracted tooth and remain intact post-extraction and showing no periapical infections. Patients were excluded from the study if they were smokers, females who were pregnant or breast feeding, those who have received irradiation, chemotherapy or immunosuppressive drugs. The sites were randomly allocated to one of the following groups using an online randomization generator (https://www.randomizer.org): [**Group I: (ATV)**, **Group II: (PRF)** and **Group III: (Control)**]. The 30 sites in the study were all in upper arch and included 13 first premolars (43.3%), 14 second premolars (46.7%), 1 canines (6.6%) and 2 first molars (10%).

Atraumatic extraction under local anesthesia using periotome and extraction forceps was performed by the same investigator followed by socket curettage using bone curettes to insure intact socket walls**.** All patients were followed to completion and committed to treatment protocol. All patients received their implant and supra structure after 2 months, the implants diameters ranged from 3.5-5 mm and lengths ranged from 10-14 mm.

ATV gel preparation: Accurately weighed methyl cellulose was added to a required amount of biocompatible solvent to prepare methyl cellulose in situ gel. The vial was heated at 50°C to 60°C and shaken well with a mechanical shaker to obtain a clear solution. Weighed amounts of ATV[**] was added to the above solutions and dissolved completely to obtain a homogeneous phase of polymer, solvent and drug. Thus, the ATV in situ gels were prepared with a concentration of ~1.2% ***(Thylin et al., 2002)*** [(18)]**.** Each 100g w/w gel contained: Atorvastatin 1.2 g Propylene glycol 34 g, Povidone K25 0.67 g, Hydroxy propyl methyl, Cellulose (medium viscosity) 4g, Hydroxy ethyl cellulose (N10) 3g, Water 57.13g

PRF preparation: 10 ml of whole venous blood was collected in plain vacutainer tubes without anticoagulant (BD Vacutainer® blood collection tubes). The tubes were then placed in a centrifugal machine at 3000 revolutions per minute (rpm) for 12 minutes. After that, it settled into the following layers: red lower fraction containing red blood cells, upper straw-coloured cellular plasma and the middle fraction containing the fibrin clot. The upper straw-coloured layer was then removed and middle fraction was collected, 2 mm below lower dividing line, which is the PRF. In this technique, the absence of anticoagulant results in activation of the majority of platelets and release of coagulation cascade factors. Fibrinogen is initially concentrated in the upper part of the tube, then the circulating thrombin changes it into fibrin. The clot is measured in the middle part of the tube. [(19)]

Stent Fabrication A study model was made from an alginate impression, and a clear rigid acrylic stent of 1 mm thickness was fabricated to be rigid enough to ensure the same path of insertion. The stent was formed from a doughy mixture of a plasticized liquid monomer and a methyl methacrylate polymer in a ratio of 1:3-3.5 parts by weight. When heated to a temperature above 120° F. (49° C.), the stent is malleable and ready to be moulded on a model of the patient to attain an approximation of the tissue surfaces. This stent was hollowed at 6 points 3 of them buccal (mesiobuccal, midbuccal, distobuccal), and 3 palatal (mesiopalatal, midpalatal, distopalatal)

Relative Ridge height and width measurements: The relative ridge height was measured from the reference point in the stent (at the level of cemento-enamel junction of neighboring teeth) to the level of crestal palatal or buccal bone All measures are taken by periodontal probe[**§] The average of the 3 buccal measurements was considered the relative buccal ridge height while the average of the 3 palatal measurements was considered the relative

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** Periodontal probe UNC 15, Standard handle # 30, University of North Carolina , USA

palatal ridge height for each patient. Alveolar ridge width was measured using bone calliper in millimetres* and guided by acrylic stent.

Atraumatic extractions were performed using a periotome, then the socket was curetted with a bone curette, alveolar ridge dimensions were measured. Eight weeks later, the second surgical procedure was performed, where new clinical measurements of the alveolar were performed then a flap was reflected, core biopsies (length 6-8 mm) were obtained using a 3mm trephine bur** followed by implant placement and flap closure. Bone biopsies were fixed in 10% formalin for 2 days and initially decalcified in 5% nitric acid for 12 hours then in EDTA solution till complete decalcification. Tissues were then embedded in paraffin wax to be sectioned longitudinally into multiple 4-µm-thick sections using innermost section of each biopsy whenever possible.

Histomorphometric examination: Sections were stained with Masson's Trichrome (HT15 - Masson Trichrome Stain Kit*** for quantitative and qualitative measurements of bone trabeculae and osteoid tissues. The principle is that the tissue is stained first with the acid dye (Biebrich Scarlet), which binds with the acidophilic tissue components. The (Aniline blue) stain the collagen which is the main component of osteoid tissue will appear blue. The less permeable components retain red so that any other tissue than collagen will be stained red.

Photomicrography: Four microscopic fields for each slide were photomicrographed at the power of 20X by digital camera**** mounted on research light microscope***** Image J software****** was used for automatic standardized measuring for mineralized bone and osteoid tissue

* Bone calliper 31.691.13 Helmut Zepf Medizintechnik GmbH. Germany
** TRE020M. Hu-Friedy Mfg. Co., Chicago, USA
*** Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany
**** C5060, Olympus, Japan
***** BX60, Olympus, Japan
****** Image J, 1.8-112, NIH, USA

Image Analysis

The histomorphometric analysis was carried out by a single calibrated examiner, masked to the treatment codes. Images were automatically corrected for brightness and contrast. Corrected images were then converted into 8-bit grey scale type. Automatic color-code threshold of gray scale images was performed to assign a red color code to the *mineralized bone trabeculae*. In an attempt to standardize the method for all analyzed images at a scale of 0-66. Automatic color-code threshold of grey scale images was performed to assign a blue color code to the *osteoid tissue* in attempt to standardize the method of all analyzed images, efforts were made to minimize the operator guided errors in favor of the automatic thresholding throughout this step at a scale of 87-242. The average size of bone trabeculae and the total surface area and area percent of mineralized bone and osteoid tissue were automatically measured. Data were then collected and tabulated, and the mean values were used for statistical analysis.

Statistical analysis

The collected data was analyzed using Statistical Package for Social Science *(SPSS 15.0 for windows; SPSS Inc, Chicago, IL, 2001)*. Data were expressed as mean and standard deviation (SD). One-Way ANOVA test was used for comparison between more than two study group means. dependent t test was used to compare changes within same group. For all tests, $P < 0.05$ was considered significant.

RESULTS

All recruited patients committed to study completion and received their implants and supra structures after 2 months with implants diameters ranging from 3.5-5 mm and length ranging from 10-14 mm.

Alveolar ridge dimensional changes

Comparison between groups regarding the mean ridge width and buccal and palatal relative ridge heights are shown in Table (1) and Figure (1). On comparing the mean relative buccal ridge height after 2 months the difference was a statistically significant difference both in Groups I and Group III, while the difference was not statistically significant in Group II, however Group II had the lowest mean percentage change after 2 months (-3.5± 4.7). As regards the relative palatal ridge height, there was a statistically significant difference in all groups after 2 months. On comparing ridge width, Group I had the highest mean ridge width at 2 months (8.8 ±1.1), while Group II showed the least mean dimensional loss (-0.35± 0.4) with mean percentage reduction (-5.17± 6.7) Generally, the percent of reduction was higher in width than in height although no significant differences were observed between all three groups regarding relative buccal height (P value 0.367), relative palatal height (P value 0.34) or ridge width (P value 0.082) respectively.

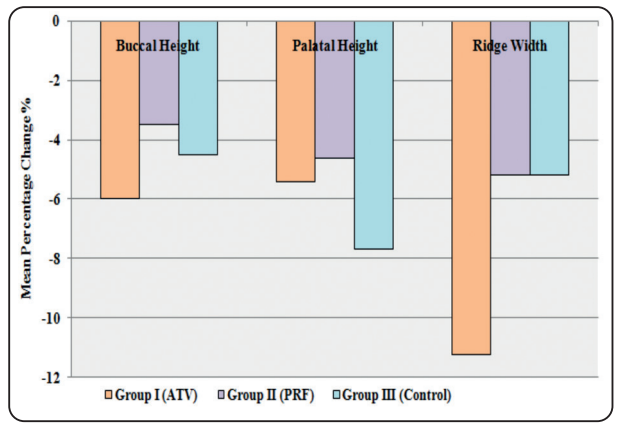

Fig. (1): Bar chart representing comparison between groups regarding mean percentage change in ridge height and width

TABLE (1)

Relative Buccal Ridge Height (mm)	Baseline Mean (SD)	After 2 m Mean (SD)	P Value	Mean difference (mm)	mCh. (%)
Group I(ATV)	7.6 (1.2)	8.1 (1.1)	0.0005*	0.47 (0.28)	-5.98 (3.7)
Group II(PRF)	6.5 (0.8)	6.7 (0.95)	0.052 NS	0.36 (0.19)	-3.5 (4.7)
Group III(control)	7.8 (0.98)	8.1 (0.87)	0.0001*	0.25 (0.35)	-4.5 (2.3)
P value	0.016*	0.004*		0.23 NS	0.367NS
Relative Palatal Ridge Height (mm)	Baseline Mean (SD)	After 2 m Mean (SD)	P Value	Mean difference in (mm)	mCh. (%)
Group I(ATV)	7.7 (1.2)	8.1 (1.1)	0.0008*	1.6 (0.69)	-5.41 (3.7)
Group II(PRF)	6.25 (0.92)	6.5 (0.83)	0.023*	0.3 (0.08)	-4.6 (5.9)
Group III(control)	7.4 (0.36)	8.1 (0.23)	0.0004*	0.6 (0.02)	-7.7 (4.3)
P value	0.0021*	0.00016*		0.097 NS	0.34NS

Ridge Width (mm)	Baseline Mean (SD)	After 2 m Mean (SD)	P value	Mean difference in (mm)	mCh. (%)
Group I(ATV)	9.8 (1.5)	8.8 (1.1)	0.003*	-1 (0.8)	-11.25 (9.17)
Group II(PRF)	8.35 (1.1)	8.1 (0.8)	0.044*	-0.35 (0.4)	-5.17 (6.7)
Group III(control)	7.7 (1.25)	8.1 (0.99)	0.036*	-0.4 (0.5)	-5.2 (6.7)
P value	0.0043*	0.163NS		0.048*	0.082NS

One-Way ANOVA test, (SD) = Standard deviation , dependent sample t- test, independent sample t- test (SD) = Standard deviation. P = Probability level. NS= Not significantly different *= Significant at (p≤0.05).

Histochemical examination of stained core biopsies

Histological examination at 20X magnification under light microscope of the Masson's Trichrome stained sections of core biopsies taken from the center of the socket 2 months post extraction in **Group I and Group II** showed newly formed osteoid tissue stained blue in color. This osteoid tissue showed many entrapped osteocytes that were observed at 40X magnification. These osteoid trabeculae were separated by fibrocellular marrow spaces that showed variable number of blood vessels. Variable degrees of mineralized bone were also observed and these mature areas of bone showed many united trabeculae with minimal marrow spaces. Beginning of formation of bony osteons was also observed denoting the start of bone remodeling. While, in **control Group III**, histological examination at 20X magnification showed newly formed osteoid tissue stained blue in color. This osteoid tissue showed some of entrapped osteocytes that were observed at 40X magnification. These osteoid trabeculae were separated by fibrous marrow spaces that showed minimal number of blood vessels. **Figure (2) (A, B, C, D, E, F)**

Histomorphometric analysis of stained core biopsies

Results of the histomorphometric analysis are shown in table (2, 3) and figure (3) The total collagen surface area and the average trabecular size were significantly higher in socket augmented with PRF in Group II than from those augmented with in Group I. However, both groups showed significantly higher values compared to Group III. Moreover, the area percent of mineralized tissue was greatest in Group II(25.34 ±10.57) compared to Groups I (21.27 ± 8.39)and III (17.21 ± 2.49) however, the difference was not statistically significant. Also the area percent of osteoid tissue was highest in Group I

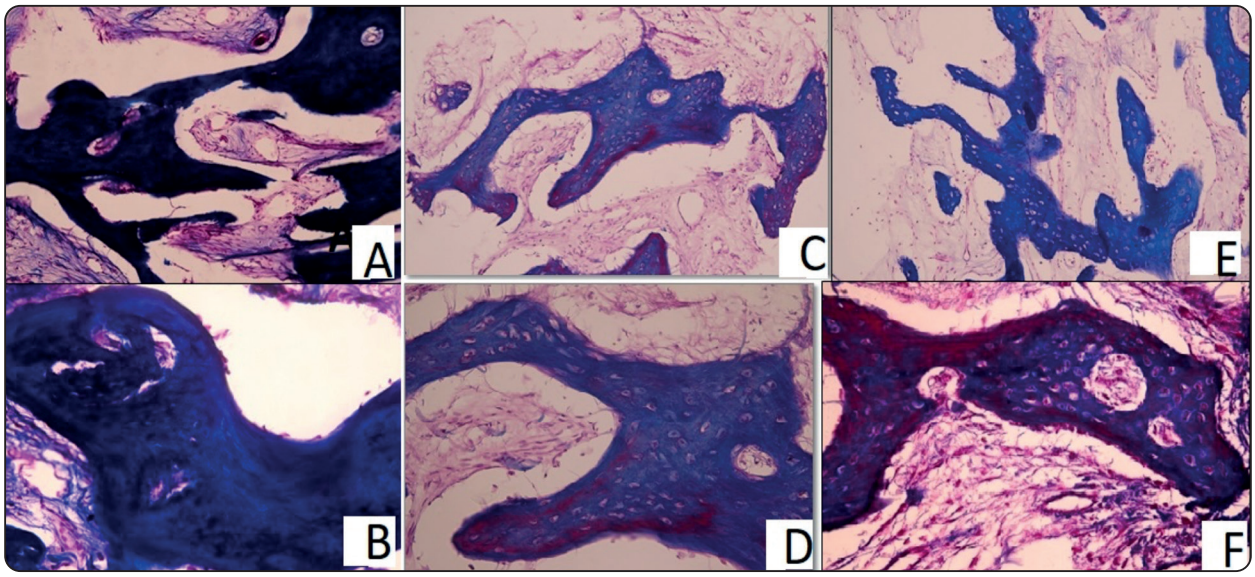

Fig (2) A: Photomicrograph of group I showing thin trabeculae of osteoid tissue (blue) separated from each other by fibrocellular tissue with numerous variable-sized blood vessels (Masson's Trichrome 20x), B: Photomicrograph of group I showing formation of bony osteons (Masson's Trichrome 40x). C: Photomicrograph of group II showing thin trabeculae of osteoid tissue (blue) rimmed with mineralized areas of woven bone (red). Note the vascular fibrocellular tissue surrounding the bone trabeculae. (Masson's Trichrome 20x). D: Photomicrograph of group II showing trabeculae of woven bone. Many osteocytes are seen inside lacunae. Beginning of formation of bone osteons is also observed (Masson's Trichrome 40x). E: Photomicrograph of group III showing few thin bone trabeculae of osteoid tissue (blue) surrounded by fibrocellular matrix with few blood vessels. (Masson's Trichrome 20x). F: Photomicrograph of group III showing woven bone trabeculae with many osteocytes inside lacunae. The surrounding matrix is fibrous with few blood vessels. (Masson's Trichrome 40x)

(28.65 ±13.21) but it was not statistically significant as well.

TABLE (2) Comparison between groups regarding the mean of total collagen surface area and average trabecular size of mineralized tissues after 2 months

Histomorphometric outcomes		Mean (SD)	P Value
Total Collagen Surface area ($Pixel^2$)	Group I(ATV)	1945.24[b] (1456.1)	<0.0001*
	Group II(PRF)	6762.12[a] (2605.77)	
	Group III(Control)	530.35[c] (483.31)	
Trabecular size (Pixel)	Group I(ATV)	3013.54[b] (986.22)	<0.0001*
	Group II(PRF)	6956.41[a] (2444.64)	
	Group III(Control)	1121.3[c] (446.38)	

One way-ANOVA- The independent-Samples T Test

TABLE (3): Comparison between area % of mineralized and osteoid tissue among the study groups

	Area%	Mean (SD)	P Value
Group I	Mineralized tissue	21.27 (8.39)	0.25[NS]
	Osteoid tissue	28.65 (13.21)	
Group II	Mineralized tissue	25.34 (10.57)	0.37[NS]
	Osteoid tissue	22.15 (10.46)	
Group III	Mineralized tissue	17.21 (2.49)	0.36[NS]
	Osteoid tissue	19.79 (5.39)	

The dependent-Samples T Test

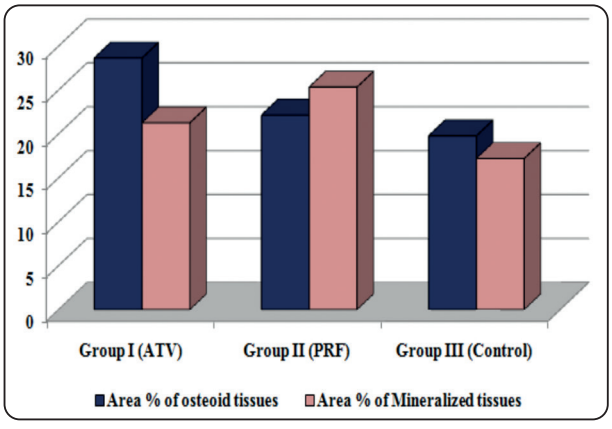

Fig 3: Bar chart representing comparison in area percent of mineralized and osteoid tissue among study groups.

DISCUSSION

The use of a prosthodontically driven implant placement protocol may necessitate the preservation of the dimensions of the alveolar ridge that was present prior to tooth extraction.[20]

This study was designed to evaluate the effect of direct application of atorvastatin gel versus platelet rich fibrin in socket preservation procedures and comparing both to spontaneously healing sockets. To our knowledge, this is the first study to attempt the use of ATV gel as a sole socket preservation material. Since it has been shown that after tooth extraction, alveolar ridge remodelling begins and continues for several months with the majority of changes occurring in the first three months,[21] therefore, the 8 weeks healing period was selected for the clinical and histologic results in order to check the quantity and quality of the bone targeting the stage of healing that may allow early placement of implants. In order to ensure accurate measurements, prefabricated stents with set measurements points were used. The selection of Masson's trichrome stain was due to its ability to differentiate between mineralized and osteoid tissue as well as the ease of interpretation of the results. This allowed a much more precise measurement of the outcome that radiographic measurements for instance.

The results showed that both ATV and PRF generally resulted in less ridge dimensional changes following tooth extraction than sockets left to heal spontaneously, however the difference between the three groups was statistically non-significant. Most systematic reviews reported a significant reduction in the alveolar ridge changes following alveolar ridge preservation, however, most of their results were based on the use of grafting materials namely autografts, allografts, xenografts and alloplasts with and without membranes with the results generally in favour of Autografts, xenografts as opposed to alloplasts. [22, 23, 24] The lack of agreement of our results to those systematic reviews may be attributed

to several factors, one of which is that no graft material was used in this study as compared to those. The lack of a grafting material may have resulted in lack of the scaffolding effect that it generally provides during socket preservation procedures where we only used ATV gel or PRF. The second factor is the variability of the period of assessment of 8 weeks period in the present study compared to a minimum of 6 months period in most systematic reviews. A third factor could be that the socket preservation material may not be on the determinant of success but rather one of several others including the local condition of the socket and atraumatic extraction techniques. However, it should be noted that although the difference between test groups regarding ridge dimension was non-significant, it was still within the range reported by Tomlin et al 2014 [25] who showed that sockets augmented with bone graft, had horizontal ridge loss ranged from 0.75 – 2.0 mm and vertical ridge loss ranged from 0.48 – 2.48 mm.

Histochemical analysis showed newly formed bone in both test groups as well as the control. This was in accordance with a study performed by Trombelli [26] who showed that the granulation tissue generally seen in the early stages of socket healing to be replaced by a matrix of osteoid tissue. On the other hand, regarding the histomorphometric analysis results, PRF showed higher total collagen surface area and average trabecular size than the ATV group. While both groups showed higher values than the control group, and the difference was statistically significant. This may indicate more favourable bone formation in the PRF group, the difference might be attributed to possible failure of even distribution of ATV in the gel which may have resulted in extra physiologic level of statin in the early phases of healing in addition to lack of scaffolding which may explain the better histomorphometric outcome shown with the PRF group.

CONCLUSION

Although the differences between the two test and the control groups were non-significant regarding alveolar dimensions , there was a quality of the newly formed bone that was better in the PRF group followed by the ATV in comparison with the control. This may indicate that the PRF fibrin scaffold was osteoconductive and acted as a natural scaffold for new bone formation..

REFERENCES

1. Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: what can we learn? Periodontol 2000. 2015;68(1):122-34.
2. Pagni G, Pellegrini G, Giannobile WV, Rasperini G. Postextraction alveolar ridge preservation: biological basis and treatments. Int J Dent. 2012;2012:151030-43.
3. Bielecki T, Dohan Ehrenfest DM. Leukocyte- and platelet-rich Plasma (L-PRP)/fibrin (L-PRF) in medicine - past, present, future. Curr Pharm Biotechnol. 2012;13(7):i-ii.
4. Dohan DM, Choukroun J, Diss A, et al. Plateletrich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e45-e50.
5. Ehrenfest DMD, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors. 2009;27(1):63-69.
6. Niu W, Wang P, Ge S, Ji P. Effects of platelet concentrates used in alveolar ridge preservation: A systematic review. Implant Dent. 2018;27(4):498-506.
7. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation 2000; 101: 207–213.
8. Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez. Stimulation of bone formation in vitro and in rodents by statins. Science. 1999; 286:1946–1949.
9. Ruan F, Zheng Q, Wang J. Mechanisms of bone anabolism regulated by statins. Biosci Rep. 2012; 32:511–519.
10. Maeda T, Kawane T, Horiuchi N. Statins augment vascular endothelial growth factor expression in osteoblastic cells

via inhibition of protein prenylation. Endocrinology. 2003; 144:681–692.

11. Maeda T, Matsunuma A, Kurahashi I, Yanagawa T, Yoshida H, Horiuchi N. Induction of osteoblast differentiation indices by statins in MC3T3-E1 cells. Journal of Cellular Biochemistry. 2004; 92, 458–471.
12. Sinzinger H, Wolfram R, Peskar BA. Muscular side effects of statins. J Cardiovasc Pharmacol .2002; 40: 163-71.
13. Cohen DE, Anania FA, Chalasani N. An assessment of statin safety by hepatologists. Am J Cardiol. 2006; 97: S77–8.
14. Shah SR, Werlang CA, Kasper FK, Mikos AG. Novel applications of statins for bone regeneration. NATL SCI REV. 2015; 2: 85–99.
15. Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 1998;81:582-587.
16. Martande SS, Kumari M, Pradeep AR, Singh SP, Suke DK. Comparative evaluation of efficacy of subgingivally delivered 1.2% atorvastatin and 1.2% simvastatin in the treatment of intrabony defects in chronic periodontitis: a randomized controlled trial. J Dent Res Dent Clin Dent Prospect 2017; 11(1): 18-25
17. Elian N, Cho SC, Froum S, Smith RB, Tarnow DP. A simplified socket classification and repair technique. Pract. Proced. Aesthet. Dent. (2007);19:99–104
18. Pradeep AR, Kumari M, Rao NS, Martande SS, Naik SB. Clinical efficacy of subgingivally delivered 1.2% atorvastatin in chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2013;84(7):871-9.
19. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Plateletrich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):e37-44.
20. Atieh MA, Alsabeeha_NHM, Payne_AGT, Duncan_W, Faggion_CM, Esposito_M. Interventions for replacing missing teeth: alveolar ridge preservation techniques for dental implant site development. Cochrane Database Syst. Rev. 2015;5: Art. No.: CD010176
21. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. International Journal of Periodontics & Restorative Dentistry 2003;23(4):313
22. HämmerleCH, AraújoMG, SimionM, Osteology Consensus Group 2011. Evidence-based knowledge on the biology and treatment of extraction sockets. Clin Oral Implan Res 2012;23 Suppl 5:80-2.
23. Vignoletti_F, Matesanz_P, Rodrigo_D, Figuero_E, Martin_C, Sanz_M. Surgical protocols for ridge preservation aEer tooth extraction. A systematic review. Clin Oral Implan Res 2012;23 Suppl 5:22-38.
24. Avila-Ortiz G, Chambrone L, Vignoletti F. Effect of alveolar ridge preservation interventions following tooth extraction: A systematic review and meta-analysis. J Clin Periodontol, 2019;46:195-223.
25. Tomlin, E.M. Nelson. S.J. Rossmann, J.A. Ridge preservation for implant therapy: a review of the literature. Open Dent. J. 8:66–76 (2014).
26. Trombelli L. Modeling and remodeling of human extraction sockets. J. Clin. Periodontol. 2008;35:630–9