Socket Preservation Using Atorvastatin Loaded in Growth Factor Plasma Rich Fibrin Scaffold: A Randomized Clinical Trial

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Abstract*:* **Background:** Plasma rich in growth factors (PRGF) technology develops a fibrin network to promote tissue regeneration through growth factor delivery from the platelets, while statins stimulate the differentiation of stem cells to osteoblasts and bone formation. The purpose of this study is to evaluate clinically and histologically the use of PRGF derived fibrin scaffold as a carrier for atorvastatin for alveolar socket preservation. **Methods:** Thirty patients having premolars scheduled for extraction were randomly assigned into three equal groups; **Group I** received PRGF fibrin scaffold loaded with Atorvastatin (PRGF/ATV), **Group II** received (PRGF) alone and **Group III** had their sockets left to heal spontaneously (Control). Clinical parameters included ridge width, average buccal and palatal crest heights measured at baseline and 8 weeks' post-extraction. Core biopsies stained with Masson's Trichrome were examined. **Results:** Group I had the lowest mean percentage reduction in ridge width, Group II had the lowest reduction in buccal and palatal heights. The mean ridge width and height of Group I and II was higher and statistically different from that of the control Group III at 8 weeks' post-extraction but differences between groups were not significant in any of the clinical parameter. The total collagen surface area and the average trabecular size were significantly higher in socket augmented with PRGF than from those augmented with (PRGF/ATV) and both were significantly higher from that of control group. **Conclusions:** Sites augmented with PRGF and PRGF/ATV showed significantly higher newly formed osteoid tissue and mineralized bone trabeculae compared to spontaneously healed socket after 8 weeks. This finding suggested that PRGF fibrin scaffold was osteoconductive and acted as a natural scaffold for new bone formation and proved bone stimulatory effect of Atorvastatin. However, none of the interventions was completely successful in preserving alveolar dimensions in a full manner.

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1. Introduction

Alveolar ridge reduction following extraction can reach approximately 50% over 6–12 months [1]. It is usually estimated that the loss in alveolar bone height is much less than that encountered with the bone width which is usually noted at the buccal side [2]. This bone loss not only makes some obstacles to the clinicians during fabrication of an implant-supported or traditional prosthesis but also make implant placement unachievable [3].

According to the Osteology Consensus Group 2012 "ridge preservation is a general term for interventions that aim to preserve the ridge volume within the envelope existing at the time of extraction" [4]. Many approaches are tried in order to minimize the risk for alveolar bone loss [5,6]. These approaches are focusing in utilization of autogenous materials inside the extraction socket aiming for enhancement of bone regeneration [5,6].

Statins are group of lipid-lowering medicines used for prevention of cardiovascular problems since they are potent inhibitors of co-enzyme A reductase and accordingly are used orally to treat hypercholesterolaemia and hyperlipidemia [7,8]. In addition, statins have also been reported to over-express bone anabolic factors, such as bone morphogenic proteins-2 (BMP-2) and vascular endothelial growth factor (VEGF) hence promoting the ability of osteoblasts to differentiate also to assist in bone calcification [9]. Again, it has been reported that statins can reduce osteoclastic activity probably via bisphosphonates-similar mechanisms [10,11]. Also,

statins can modulate the inflammatory response by affecting the profile of inflammatory mediators, leukocyte–endothelial cell interaction as well as the major histocompatibility complex- II (MHC-II) expression [12].

Due to its known promoting effect on osteogenesis and inhibitory action on bone resorption, Atrovastatin (ATV), one of statins, is widely used to overcome problems encountered after bone fracture and during bone remodeling process [13].

Aiming for usage of ATV in vivo for promotion of bone regeneration, many challenges are encountered. One important challenge is to find out a suitable delivery system that ensures continuous release of ATV in a sufficient concentration that optimize the bone healing properties of it [14]. Another challenge is the retention of ATV molecules for a period sufficient for cell growth and differentiation [15]. In addition, the availability of a suitable matrix that allows osteoblasts to infiltrate the area in a sufficient number without being altered by formation of a fibrous capsule of the carrier is also considered as a limitation [15]. The bulk of the delivery system as well as the degradation products that might alter the process of tissue regeneration are also challenging [16].

One promising autologous delivery system for tissue regeneration is the plasma rich in growth factors (PRGF) vehicles that are widely investigated nowadays for its ability to activate release of growth factors from platelets and also to promote formation of a suitable fibrin network [17]. This system is now widely accepted as a successful system that improves the post-surgical clinical outcomes [18]. Although the prognostic benefits of PRGF due to its stimulatory effect on already differentiated cells of the bone are proven [19], the lack of stem cells differentiation by PRGF highlights the importance of delivery of a successful combination that ensures maximum regenerative effect [20]. On the other hand, the benefits described by statins in bone regeneration [21,22], and the lack of clinical investigation of the combined use with PRGF in socket preservation should be investigated.

This study investigates the utilization of a combined system of PRGF as fibrin scaffold and statin as progenitor stem cells differentiation agent in socket preservation. Therefore, the objective of this study was to compare the dimensional changes and bone quality of 8 weeks' post-extraction augmented sockets with Atorvastatin loaded in PRGF derived fibrin scaffold versus PRGF alone.

2. Methods

Study design

This study was a prospective, examiner-blinded, controlled, randomized clinical trial with histomorphometric analysis conducted in accordance with the Helsinki Guidelines. The study protocol was approved by the research ethics committee in faculty of dentistry Ain Shams University (approval No.FDASU-RECM041515) and registered as a clinical trial at https://www.clinicaltrials.gov (registration number NCT03231137). The procedures were fully explained to the patients and they all signed the informed consent before enrollment.

Sample size

The power analysis revealed that a sample size of 27 was needed to detect a 2mm difference in the primary outcomes (ridge width or height) after 8 weeks, assuming a maximum standard deviation of 1.5 mm, 80% power, a 0.05 cutoff for significance. 30 patients were recruited for each group to compensate for potential dropouts.

Eligibility criteria

Patients were recruited between June 2013 and March 2017 and a total of 58 patients admitted for tooth extraction were examined in the outpatient clinic of Oral Medicine, Periodontology, and Oral Diagnosis department, Faculty of Dentistry, Ain Shams University. Information including age, gender, disease process, medical history, drug history and family history, were documented.

A total of 28 patients leaved during screening, and 30 patients were enrolled in the study. The following criteria of selection are followed according to guidelines of (clinicaltrials.gov); males or females between the age of 20 to 50 years and without any systemic illnesses having premolars indicated for extraction (badly decayed non restorable tooth, tooth contraindicated for crown preparation such as tooth with subgingival caries, broken roots, teeth with advanced periodontal disease or having grade 3 mobility and remaining roots), with 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 as determined by clinical examination and periapical radiographs (Socket type I) [23].

According to exclusion criteria of (clinicaltrials.gov), patients were excluded if they fall in the following categories: 1) smokers; 2) pregnant and breast feeding females; 3) previous radiation, chemotherapy, or immunosuppressive treatments; 4) known hypersensitivity to statin drugs; 5) teeth with periapical infections; 6) type II or III sockets or with dehiscence or fenestrations. 2 patients were lost to follow up and replaced by others.

Randomization, blinding and grouping

The three treatment protocols were randomly allocated to patients in order of inclusion according to a predetermined randomization-list using free online randomization generator (https://www.randomizer.org).

Individuals blinded to the study examined all participants and each participant was given a number. The outcome assessors were also blinded to patient allocation. Patients undergoing single tooth extraction were assigned to either Group I (PRGF/ATV) (n=10) having a fibrin scaffold of platelet rich in growth factors loaded with Atorvastatin powder placed to fill the extraction socket or Group II (PRGF) (n=10) in which platelet rich in growth factors fibrin scaffold placed to fill the extraction socket as a positive control group or Group III (Control) (n=10) where sockets are left to heal spontaneously as a negative control group.

Pre-surgical acrylic stent preparation

At baseline before extraction procedure a master cast of each patient was made with dental stone utilizing alginate impressions (Acrolgine & Acrostone, Acrostone Co. Ltd. Cairo, Egypt). For standardization of clinical measurements, a clear rigid acrylic stent of 1 mm thickness was fabricated over the cast after removal of the tooth planned for extraction the cemento-enamel junction of the adjacent tooth as reference point using vacuum forming sheets (ProForm. Keystone Industries. New Jersey. USA). This stent was extended mid way from ridge crest to vestibular depth both buccaly and palataly and hollowed at 6 points (mesiobuccal, midbuccal, distobuccal, mesiopalatal, midpalatal and distopalatal) guided by the line angles of the tooth (Figure 1A).

The preparation of PRGF and the PRGF fibrin scaffold loaded with atorvastatin

- The PRGF was freshly prepared immediately before surgery [24]; 10 ml venous blood was collected from the same patient then deposited in 5 mL tubes with 3.8% w\v (1:9v\v) sodium citrate. The tube was centrifuged at 580 G (2270 rpm) for 8 minutes at room temperature (Centrifuge device- DT5, Yigtai, Japan) (Figure 2A).
- After centrifugation, the blood sample was layered into the following four distinctive layers (Figure 2B): 1) 0.5 mL Plasma poor in growth factors (PPGFs) =F1 in the uppermost part of the tube, 2) 0.5 mL Plasma with growth factors (PGFs)= F2, 3) 0.5 mL (PRGF)= F3 located immediately above the red blood cell portion in the tube, 4) Red blood cell concentrate layer.
- The 500 µL PPGF was eliminated and the PRGF was separated with 500 µL pipettes and transported to an independent tube then activated using 50 µL of 10% calcium chloride and incubated for 20 minutes in 37ͦc to produce easy to handle gelatinous layer (PRGF) fibrin scaffold for Group II patients.
- A carefully weighted 2.5 mg of raw active Atorvastatin powder (Medical Union Pharmaceuticals co. Abo Sultan-Ismailia-Suez Road, Ismailia. Egypt) using digital scale were mixed gently and regularly with the 500 µL PPGF in 10% calcium chloride to confirm equal distribution of the drug into the entire carrier, then incubated for 20 minutes in 37ͦc to produce easy to handle gelatinous layer of PRGF fibrin scaffold loaded with 1.2% Atorvastatin (PRGF/ATV) (Figure 2C) [25] for socket augmentation in Group I patients (Figure 2D).

Surgical procedure and clinical assessment

Atraumatic extraction under local anesthesia using periotome and extraction forceps was performed by the same investigator followed by socket curettage and probing using bone curettes to insure intact socket walls.

Immediately after tooth extraction; alveolar ridge width was measured using bone caliper in millimeters (Bone caliper 31.691.13 Helmut Zepf Medizintechnik GmbH. Germany) and guided by acrylic stent. The relative ridge height was measured using graduated periodontal probe (Periodontal probe UNC 15, Standard handle # 30, University of North Carolina , USA) from the reference point in the stent to the level of crestal buccal or palatal bone through the holes previously made (Figure 1B). The average of the 3 buccal and the 3 palatal measurements considered the relative buccal and palatal ridge height respectively for each patient.

Extracted sockets were grafted with assigned interventions for treatment groups or left to heal spontaneously for the control group then socket approximation using 5/0 vicryl suture material and figure of 8 suturing technique was performed. Patients were instructed to avoid chewing on sticky/hard food or using toothbrush/inter-dental aids near the treated areas and not to wear any prosthetic appliances for 1 week. Then sutures were removed and patients evaluated for any adverse reactions or inflammation at the site.

Core biopsy procedure and implant placement

A core biopsy (length 6-8 mm) using 2 mm trephine bur (TRE020M. Hu-Friedy Mfg. Co., Chicago, USA) was taken after eight weeks at the center of the socket following full thickness flap reflection and all the measurements were repeated guided by the stent. Then submerged implant (INNO Internal Implant System. Cowellmedi Co., Ltd. New Delhi. India) was placed followed by flap suturing.

Histologic analysis

- Trephine cores 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.
- Section were then stained with Masson's Trichrome (HT15 - Masson Trichrome Stain Kit. Sigma-Aldrich Chemie GmbH, Taufkirchen,

Germany); the tissue is stained first with the acid dye (Biebrich Scarlet), which binds with the acidophilic tissue components. The (Aniline blue) stain the collagen so the main component of osteoid tissue to appear blue. While the less permeable any other tissues than collagen retain the red. Slides of stained sections were then covered by glass slips and examined under biological research microscope.

- Four microscopic fields for each slide were photomicrographed at the power of 20X by digital camera (C5060, Olympus, Tokyo, Japan) mounted on research light microscope (BX60, Olympus, Tokyo, Japan) and ImageJ software (Image J, 1.8-112, NIH, USA) was used for automatic histomorphometric analysis by a single calibrated examiner, masked to the treatment codes. Automatic color-code threshold of gray scale images was performed to assign a red color code at a scale of 0-66 to the mineralized bone trabeculae and a blue color code at a scale of 87-242 to the osteoid tissue. The average size of bone trabeculae and the total surface area and area percent of mineralized bone and osteoid tissue were automatically measured. Mean values of the four fields for each slide were then tabulated for statistical analysis.

Data analysis

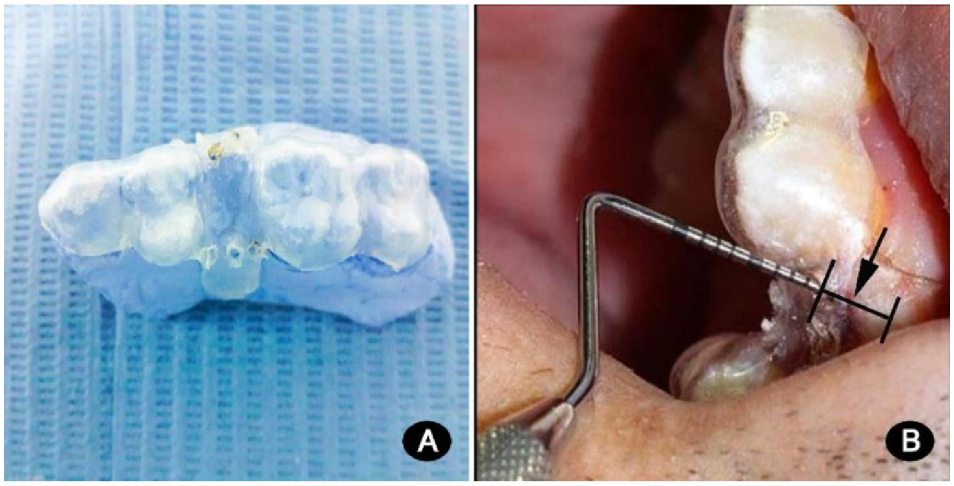

Figure 1. (A) Clear rigid acrylic stent used for standardization of clinical measurements (B) Measuring ridge height (arrow) from reference point on stent to the level of crestal bone.

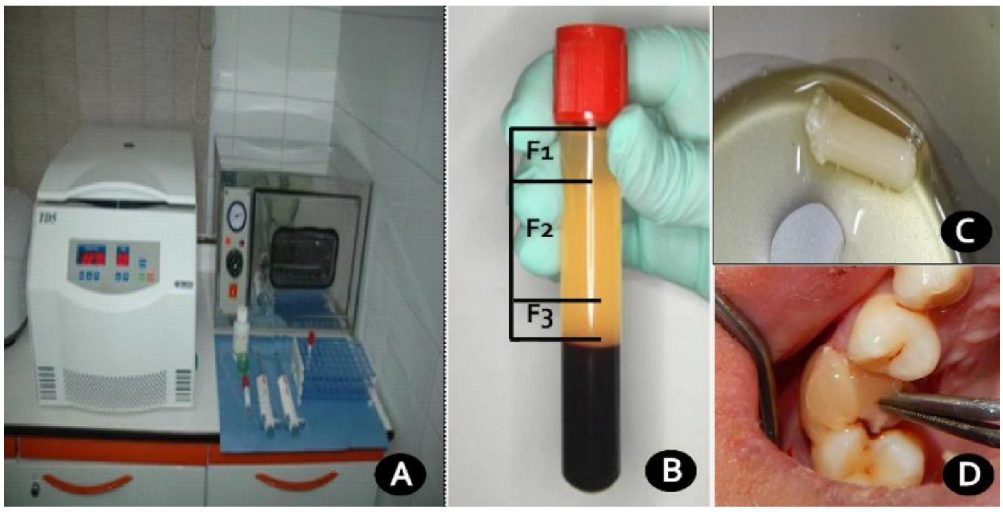

Figure 2. Preparation of plasma rich in growth factor fibrin scaffold loaded with atorvastatin (A) The centrifugation and incubator devices used in the study (B) Plasma is separated into 3 fractions: F1, F2 & F3 after centrifugation (C) Plasma rich in growth factor loaded with Atorvastatin (PRGF/ATV) (D) (PRGF/ATV) in Group I sockets.

The primary outcome variables were changes in width and height in millimeters. On the other hand, secondary outcome variables were total collagen surface area and average trabecular size of mineralized tissues in pixles. The collected data was tabulated and analyzed using Statistical Package for Social Science (SPSS 15.0 for windows; SPSS Inc, Chicago, IL, 2001). All outcomes conformed to a normal distribution (Shapiro-Wilk test, P>0.050). The two tailed, dependent student *t*-tests were used to determine the significant difference between baseline and 2 months of study parameters within each group. One-Way ANOVA test was performed for comparison between the three study groups followed by two tailed, independent student *t*-tests to investigate the significance of study parameters between each study group and the control group. Comparison between groups in gender and teeth position proportions was calculated using the Pearson $\chi 2$ test. Categorical measurements were presented as percentage, while continuous variables were expressed as mean ± standard deviation (SD) with a significance level of $P < 0.05$.

3. Results

Patients included were 6 men and 24 women who had a mean age of 32.6±7.9 years. Their general baseline characteristics are listed in (Table 1) demonstrating no statistical differences by age, sex or distribution of teeth included ($P > 0.05$) among groups. None of the patients had reported any side effect or adverse reaction and they all were followed to completion and committed to treatment protocol then received their implant after 2 months (Inserted implants diameter ranged from 3.5-5 mm and length ranged from 10-14 mm).

Table 1. General characteristics of the subjects at baseline

Characteristics	Group I (PRGF/ATV)	Group II (PRGF)	Group III (Control)	*P*-value
Age (yr) [a)]	30.1±7.5	29.2±4.4	30.1±7.5	0.635
Gender [b)] **Male** **Female**	 2 (20%) 8 (80%)	 3 (30%) 7 (70%)	 1 (10%) 9 (90%)	0.535
Teeth & Position [b)] **Upper 4** **Upper 5**	 5 (50%) 5 (50%)	 4 (40%) 6 (60%)	 5 (50%) 5 (50%)	0.874
Right **Left**	5 (50%) 5 (50%)	6 (60%) 4 (40%)	6 (60%) 4 (40%)	0.873

Values are presented as mean±standard deviation or number (%).
[a)] Using (ANOVA), [b)] Using the Pearson $\chi 2$ test.

Evaluation of alveolar ridge dimensional changes

Comparisons between groups in mean of ridge width and relative buccal & palatal ridge heights are shown in (Table 2); At baseline, no significant differences ($P > 0.05$) between the groups were found among any of these clinical parameters while differences between baseline and 2 months mean width and height were significant in each group.

Table 2. Comparison between groups regarding mean of ridge width and relative buccal & palatal ridge heights at base line and after 2 months

Parameter	Baseline	2m. post-extraction	*P*-value	m Diff. (mm)	mCh. (%)
RW (mm)					
Group I (PRGF/ATV)	9.7±0.48	8.6±0.45 [b)]	0.0031 [a)]	-1.1±0.61	-11.1±6.07
Group II (PRGF)	8.0±0.94	6.6±0.96 [b)]	0.0001 [a)]	-1.4±0.51	-17.5±6.3
Group III (Control)	8.1±0.99	6.6±1.07	0.0034 [a)]	-1.5±0.84	-18.4±9.7
Relative BRH (mm)					
Group I (PRGF/ATV)	6.6±0.96	7.2±1.1 [b)]	0.009 [a)]	-0.6±0.58	-9.1±8.3
Group II (PRGF)	5.7±0.67	6.0±0.9 [b)]	0.0081 [a)]	-0.3±0.48	-5.0±8.05
Group III (Control)	7.6±0.96	8.3±0.8	0.001 [a)]	-0.7±0.89	-9.6±6.6
Relative PRH (mm)					
Group I (PRGF/ATV)	6.9±0.62	6.3±0.49 [b)]	0.0001 [a)]	-0.4±0.2	-6.7±3.5
Group II (PRGF)	6.4±0.6	6.0±0.7 [b)]	0.014 [a)]	-0.2±0.26 [b)]	-3.6±3.8
Group III (Control)	7.4±0.36	8.1±0.23	0.0004 [a)]	-0.6±0.36	-7.7±4.3

Values are presented as mean±standard deviation.
RW (mm): Ridge width in millimetre, BRH (mm): Buccal ridge height in millimetre, PRH (mm): Palatal ridge height in millimetre, PRGF/ATV: Plasma rich in growth factor loaded with Atorvastatin, PRGF: Plasma rich in growth factor. 2m: Two months, m Diff (mm). Mean difference in millimetre, m Ch. (%): Mean percentage change.
[a)] Statistically significant difference $P<0.05$ compared to baseline (Dependent *t*-test). [b)] Statistical significant difference $P<0.05$ compared to the control group (Independent *t*-test).

Ridge width

Group I had the highest mean ridge width (8.6±0.45) mm followed by Group II and was significantly different from the control Group III (6.6±1.07) mm after 2 months. Alveolar ridge in Group I had also the lowest mean dimensional loss in ridge width (-1.1±0.61) mm with mean percentage reduction (-11.1±6.07) % but was not significantly different from that of the other groups.

Relative buccal and palatal Ridge height

On comparing the mean relative buccal and palatal ridge height between groups after 2 months the different was statistical insignificant between Group I and II, however the buccal and palatal crest level was the highest in Group II as observed by the lowest mean relative buccal (6.0±0.9) mm and palatal height (6.0±0.7) mm and significantly different from that of the control Group III which had the highest mean percentage reduction in buccal (-9.6±6.6) % and palatal levels (-7.7±4.3) %. No statistical significant differences were observed between the groups regarding mean dimensional loss.

Generally, the percentage reduction in ridge width was higher than that of ridge height and in the buccal level than that of palatal in all groups, although no significant differences were observed between groups regarding mean percentage reduction in any of the clinical parameters (Figure 3).

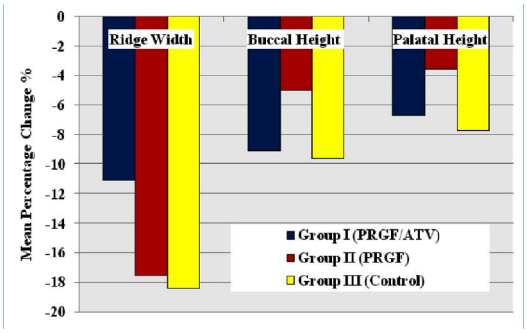

Figure 3. Mean Percentage change in ridge dimensions among study groups

Histological observations of stained core biopsies

Masson's Trichrome stained sections of core biopsies taken from the center of the socket two months' post-extraction showed newly formed osteoid tissue stained blue in color in all groups. Group I (Figure 4 A, B) and Group II (Figure 4C, D) showed entrapped osteocytes and osteoid trabeculae with observed mature areas of bone showing united trabeculae, minimal marrow spaces and mineralized areas, and the bony osteons formation denoted the start of bone remodeling. In control Group III, the osteoid trabeculae were separated by fibrous marrow spaces that showed minimal number of blood vessels with some of entrapped osteocytes (Figure 4 E, F).

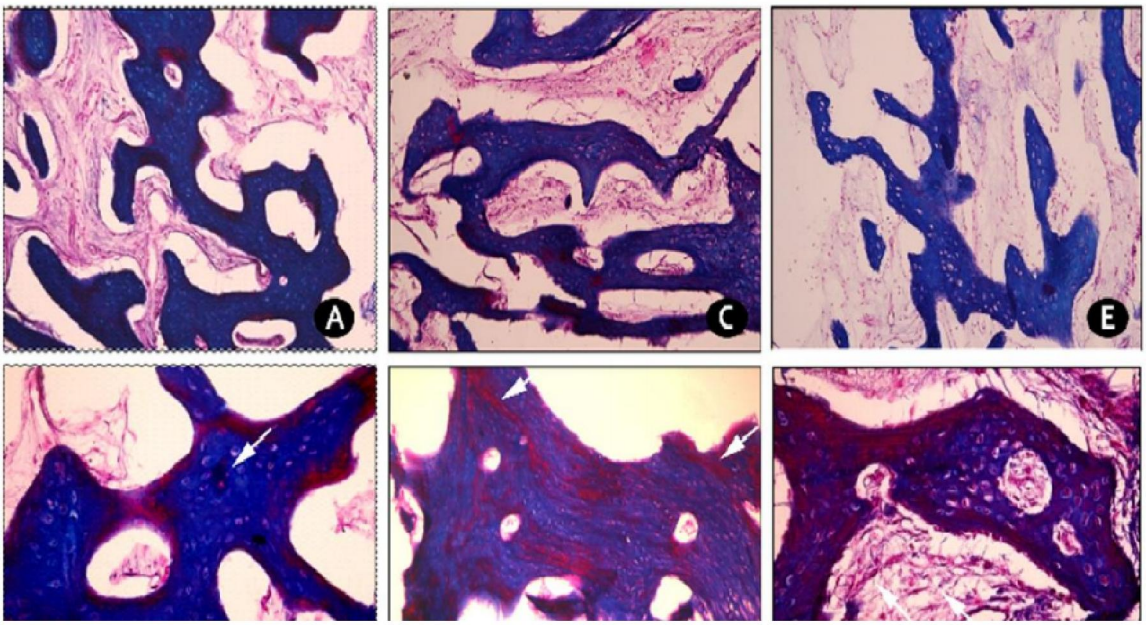

Figure 4. Representative photomicrographs of core biopsies specimens from study groups after 8 weeks of healing. Group I: (A) Thin trabeculae of osteoid tissue (blue) rimmed with mineralized areas of woven bone (red) surrounded by vascular fibrocellular tissue surrounding (B) Many osteocytes are seen inside lacunae with beginning of formation of bone osteons (arrow). Group II: (C) Thick connected trabeculae of osteoid tissue (blue) separated from each other by fibrocellular tissue with numerous variable-sized blood vessels (D) Many mineralized areas of woven bone (arrows) seen rimming the trabeculae with numerous osteones. Group III (E) Few thin bone trabeculae of osteoid tissue (blue) surrounded by fibrocellular matrix with few blood vessels (F) Woven bone trabeculae with many osteocytes inside lacunae. The surrounding matrix is fibrous with few blood vessels (arrows). Masson's Trichrome 20x (Upper panel A, C, E), Masson's Trichrome 40x (Lower panel B, D, F)

Histomorphometric measurements

Results of the histomorphometric analysis are shown in (Table 3). The total collagen surface area and the average trabecular size were significantly higher in socket augmented with PRGF in Group II than from those augmented with atorvastatin loaded PRGF fibrin scaffold in Group I. Moreover, the area percent of mineralized tissue was nearly 1.5-fold higher in the Group II (25.4±7.6) % than in the Group I (17.2±5.2) % and the control Group III (17.2±2.4) %, that showed a statistically significant difference (P=0.01). Although the area percent of osteoid tissue was the highest in Group I followed by Group II and Group III but the difference was statistically insignificant.

Table 3. Comparison between groups regarding the mean of total collagen surface area, average trabecular size and area % of mineralized and osteoid tissues after 2 months

Parameter	Group I (PRGF/ATV)	Group II (PRGF)	Group III (Control)	*P*-value
Total collagen surface area (Pixel^2)	3201.9±4329.5	6866.1±3267.9 [b)]	530.3±483.3	0.0058[a)]
Trabecular size (Pixel)	4243.2±4115.9	6507.08±2312.2 [b)]	1121.3±446.3	0.009 [a)]
Area % of mineralized tissues	17.2±5.2	25.4±7.6 [b)]	17.2±2.4	0.01[a)]
Area % of osteoid tissues	25.7±13.9	21.1±6.2	19.7±5.3	0.4513

Values are presented as mean±standard deviation.

[a)] Significant difference $P<0.05$ between groups (ANOVA). [b)] Statistical significant difference $P<0.05$ compared to the control group (Independent *t*-test).

4. Discussion

The present study was designed to evaluate for the first time the combined effect of PRGF as an active natural scaffold for Atorvastatin to the effect of PRGF alone and comparing that to spontaneously healed socket. The (8 weeks) post-extraction comparison was to assess the ridge dimensions and quality of newly formed bone in stage of healing which may allow placement of implant as early as possible. For accurate assessment; prefabricated stents were used to standardize all clinical measures and Masson's trichrome stain was selected due to its ability to differentiate between mineralized and osteoid tissue and easy interpretation, thus measuring our outcomes directly and more precisely than radiographic measures. We also took every effort to eliminate bias by random allocation of interventions and blinding of outcome assessors.

The results showed that both Atorvastatin loaded in PRGF fibrin scaffold (PRGF/ATV) and the (PRGF) alone resulted in less ridge dimensional loss following tooth extraction than sockets left to heal spontaneously but the difference between interventions was statistical insignificant. The favorable effect of (PRGF/ATV) was observed mainly on ridge width changes while (PRGF) preserved the ridge height more.

There was no significant additional clinical effect of Atorvastatin when loaded on PRGF over just using PRGF, Furthermore, the histomorphometric analysis revealed that the total collagen surface area, average trabecular size and area % of mineralized tissues was larger in stained sections from socket augmented with (PRGF) than with (PRGF/ATV) but both showed significantly more newly formed mature bone than the spontaneously healed socket.

The experimental study done by Rivera et al [26] in 2013 revealed that systemic simvastatin administration did not enhance the efficacy of platelet-rich plasma or plasma rich in growth factors on alveolar bone repair in rats in accordance to our results. While the clinical studies done by Pradeep and his team published in 2016 concluded that 1.2% ATV failed to augment the regenerative potential of PRF. While Rosuvastatin (1.2%) and PRF resulted in significantly greater periodontal benefits compared to PRF alone in treatment of infrabony defects, However the results can't be compared to our data due to difference in interventions and defect type [27,28].

In the present study; possible failure of even distribution of the atorvastatin in the PRGF scaffold may resulted in rapid degranulation of platelets and with the angiogenic effect of PRGF; statin burst may have occurred, conclusively, resulted in undesired localized high concentration of both platelet growth factors and statin in extra physiologic levels at early stage of healing as well as early shrinkage and resorption of the scaffold which may explain the higher mineralized tissue and better clinical effect on ridge height with PRGF alone. Therefore, further in vitro investigation for this combination is strongly recommended to consider these factors in the future clinical studies.

The mean dimensional loss, and percentage reduction in ridge width and height were not significantly different from the control group which may indicate that the socket preservation agents are not the key factor in successful ridge preservation however other factors like the atraumatic extraction and baseline condition of the alveolus as well patient systemic and local factors which were considered in inclusion and exclusion criteria of this study may play major role.

These results were comparable to the reported dimensional ridge loss after tooth extraction in the randomized controlled trials regardless of the technique used as reviewed by De Risi V, et al [29] in 2013 which found a more post-extraction ridge width loss in control sites compared with test sites and a more range of ridge height loss in control sites versus test sites with a follow-up time after extraction of at least 3 months.

Systematic reviews also revealed that the amount of bone resorption in the alveolar ridge is generally more obvious horizontally rather than vertically and in buccal aspect more than palatal one, a finding in accordance to results of the present study [30–32], these data further confirmed that no method has completely prevented physiological bone resorption in particular buccal bone, probably because it is composed mostly of bundle bone thus more prone to resorption [1].

Based on the review made by Tomlin et al 2014; 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 [33]. This range was comparable to the loss reported with the autologeous and pharmacological interventions used in the present study. However, non-easy preparation and handling of PRGF during surgical procedure are factors that should be considered for cost effective measures.

Histological findings revealed the presence of newly formed bone in all sites regardless of the interventions used. These results were in accordance with a study done by Trombelli [34] to monitor 6-month healing period of human extraction sockets and showed that granulation tissue present in comparatively large amounts in the early phase of socket healing was replaced with provisional matrix of osteoid tissue and mineralized woven bone in the presence of osteoblasts.

Better organization of bone trabeculae indicating more favorable bone regeneration in PRGF-treated extraction sockets compared with control sites are also reported [24]. However, similar bone volume and tissue mineral content in tested group compared to control group at 8 weeks were reported [35]. These variabilities in the findings among studies may be partly due to difference in composition of combined delivery system and variability in period of assessment.

Wu et al [36] in 2008 investigated the effect of local simvastatin application carried on polylactic acid/polyglycolic acid copolymer on alveolar bone of rats following tooth extraction and concluded that larger newly formed bone island was observed as well as higher bone formation rate and quality in the Simvastatin group than in the control group of spontaneously healed socket at 4 weeks in accordance with our study. However Nishimura [22] in another experimental study although reported higher bone mineral content and thickness of cortical bone with simvastatin but observed less bone fill in extraction socket compared to control.

Conclusions

Although there was no significant difference between test and control groups regarding reduction in residual ridge dimensions after extraction, there was significantly higher newly formed mineralized bone trabeculae and osteoid tissue in sites augmented with PRGF and those augmented with Atorvastatin loaded on PRGF fibrin scaffold compared to spontaneously healed socket after 8 weeks. These findings might indicate that the PRGF fibrin scaffold was osteoconductive and acted as a natural scaffold for new bone formation and proved bone stimulatory effect of Atorvastatin. However, none of the interventions was completely successful in fully preserving alveolar dimensions.

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Declarations

Ethics approval and consent to participate

- This study was a prospective, examiner-blinded, controlled, randomized clinical trial with histomorphometric analysis conducted in accordance with the Helsinki Guidelines.
- The study protocol was approved by the research ethics committee in faculty of dentistry Ain Shams University ***(approval No.FDASU-RECM041515)*** and registered as a clinical trial at https://www.clinicaltrials.gov ***(registration number NCT03231137)***.
- The procedures were fully explained to the patients and they all signed the informed consent before enrolment.

Consent for publication

- All patients are signed the informed consent for publication of the images.

Availability of data and materials

- Research labs from which data sets that support the findings of this study are already mentioned in methods chapter of this manuscript and are available from the authors upon reasonable request.

Competing Interests

"The authors declare that they have no competing interests"

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Study design, data collection, run of the methodology of this article as well as interpreting the results all are performed without being a part of a grant body. Furthermore, the equipment and laboratory facilities utilized in this work are the local resources of the research units in the Faculty of Dentistry, Ain Shams University.

Author Contributions

Ihab S. Abd El-Hamid; contributed in study design, revising the results, writing the discussion, finalizing and editing the manuscript.

Khalid Atef Abdel Ghaffar; contributed to study design, revising the results and supervising the work.

Ola M. Ezzatt; contributed to study design, running methodology, revising the results and discussion and citation of the references.

Fatma H. El Demerdash; contributed in study design, formal analysis and revising the results and discussion.

Noha N. EL-Zalabany; contributed to conceptualisation, study investigation, reviewing, running methodology and results writing.

Marwan F. Antar; contributed to conceptualisation, study investigation, reviewing, running methodology and results writing.

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