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Comparing efficacy of the platelet rich plasma and advanced platelet rich fibrin on tibial bone defect regeneration in dogs

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Abstract

This study aimed to evaluate the efficacy of platelet rich plasma (PRP) and advanced platelet rich fibrin (A-PRF) on regeneration of induced tibial bone defects in dogs. A 7mm circular and 10mm deep tibial defect was made at upper end of the right and left tibiae of 12 adult clinically-proven healthy male mongrel dogs. The animals were randomly divided into three groups: a control group, PRP group and A-PRF group. Regeneration of the tibial defect was evaluated by radiography, computed tomography (CT), and histopathological examination at 6 and 12 weeks postoperatively (PO). At 6 weeks PO, the tibial defect, in the control group, was partially filled with fibrous tissue and appeared radiolucent under radiography. While in PRP group, the defect was shown to be partially filled with newly formed bone and appeared more radiopaque than it did with the control group. As for A-PRF tibial defect, it was completely closed with newly formed bone and appeared more radiopaque than the PRP group did. At 12 weeks PO, the tibial defect was partially filled with newly formed bone and looked more radiopaque in control group and completely closed with newly formed bone and seemed radiopaque in PRP group. Interestingly, the tibial defect of the A-PRF group was completely closed with newly formed bone and couldn't have been differentiated from the neighboring normal bone tissue. In conclusion, using of PRP and A-PRF improved bone healing. However, using A-PRF is more likely to heal tibial defect in the early weeks of injury than PRP would.

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Introduction

Massive bone losses, caused by fractures, inflammation, tumors, implants loosening and corrective osteotomies, are a clinical challenge facing orthopedic, dental and plastic surgeons, which need surgical interference as they are not able to regenerate spontaneously (1-3). Autogenous bone graft is the gold standard for treatment of such condition as it has all of the ideal characters of graft material (4), but many side effects interfere with its application in all pathological cases such as: fracture, low availability, and donor-site morbidity (5,6). Allografts are the alternative but preparation and processing costs, infection and immune reaction limit their use (7,8) Therefore, the use of other bone substitutes, allowing for a good bone regeneration without host morbidity, is important (9,10). Platelet concentrates are a biological source of growth factors obtained from the blood of a patient in the form of: insulin-like growth factor (IGF), platelets derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) which are essential for bone and soft tissues regeneration (11,12). Platelet rich plasma (PRP), obtained by double centrifugation of patient's blood with addition of anticoagulant, has been used in regeneration of soft and bone tissues (13-17). In spite of their use for many years, the results are controversial (18). In the past twenty years, a new generation of platelet concentrates, known as

platelet rich fibrin (PRF), was produced by single centrifugation of whole blood without addition of anticoagulant (19). PRF obtained as a fibrin clot that is suitable for wound and bone healing (20,21). More recently, it was figured out that lowering the centrifugation speed helps the cells to migrate upwardly inside the fibrin clot and to release more of growth factors. Through this process a new form of PRF was obtained, called advanced-PRF (A-PRF) (22).

In spite of these *in vitro* studies, *in vivo* experimental studies on animals to evaluate their efficacy on regeneration of bone are lacked. Therefore, the present study aimed to compare the efficacy of PRP and A-PRF on regeneration of induced tibial bone defects in dogs.

Materials and methods

Ethical approve

Animal handling and surgical protocols were conducted according to the guidelines of animal care and use committee of Zagazig University, Egypt (Reference No: ZU-IACUC/2/F/111/2021).

Animals

The present study was carried out on a total of 12 adult healthy male mongrel dogs with age 3.42±0.99 years and body weight of 20.58±3.26 Kg. All animals were kept under constant temperature and were allowed sufficient food and water. The animals were subjected to induced 7mm circular and10mm deep bone defect at the proximal extremity of the right and left tibiae. The animals were divided into three groups: Control group, PRP group and A-PRF group.

Preparation of PRP and A-PRF

A 30ml whole blood sample was obtained from the jugular vein of each dog for preparation of autologous PRP (Figure 1) (22) and autologous A-PRF (Figure 2) as previously described (23). For preparation of PRP, a 15ml of whole blood was collected in 3 centrifuge tubes (5 ml tubes) containing 3.8% sodium citrate as anticoagulant, then they were centrifuged twice at room temperature. The first centrifugation was performed at 1000 rpm for 7 minutes to obtain 3 layers: the top plasma layer, the middle buffy coat layer, and the lower RBCs layer. The upper two layers (Plasma and buffy coat) were collected and transferred to another new centrifuge tubes for the second centrifugation which was performed at 3000 rpm for 10 minutes to obtain two layers of plasma: the upper platelet poor plasma (PPP) and the lower PRP. The upper layer was discarded and the remnant was considered PRP. For PRP activation and gel production, a 0.1ml of 10% calcium chloride was added prior to its implantation at the defect sites. In preparation of A-PRF, a 15ml of whole blood was collected in 3 plain centrifuge tubes (5 ml tubes) without anticoagulant, then they were centrifuged at 1300 rpm for 8 minutes at room temperature to obtain fibrin clot at the upper most of the tubes. The fibrin clots were left for 15 minutes before their implantation at the defect sites.

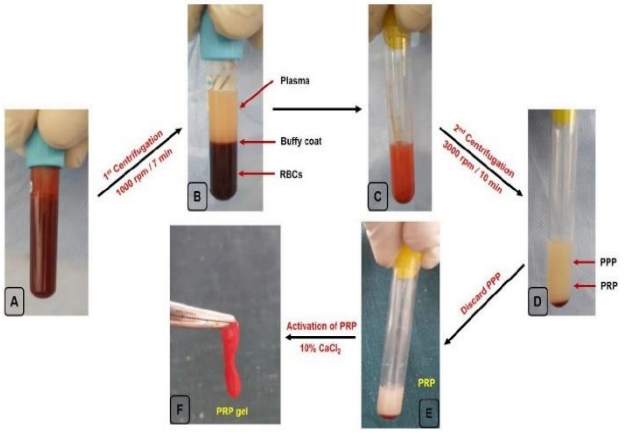

Figure 1. Preparation of PRP. A) 5 ml tube containing whole blood with anticoagulant; B) After the first centrifugation at 1000 rpm for 7 min, the blood sample was divided into three layers, plasma, buffy coat and RBCs; C) Plasma and buffy coat layers were collected into new centrifuge tube; D) After the second centrifugation at 3000 rpm for 10 min, the sample were fractioned into PPP supernatant layer and PRP sediment; E) The PPP layer was discarded and remnant PRP was obtained; F) Activation of PRP using 0.1 ml of $CaCl_2$ and PRP gel was obtained.

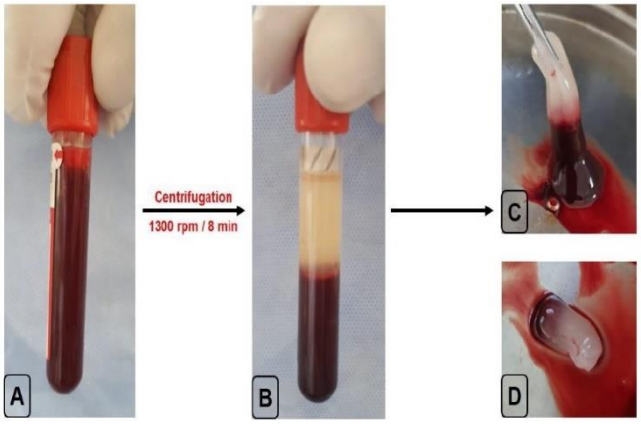

Figure 2. Preparation of A-PRF. A) 5 ml tube containing whole blood without anticoagulant; B) After centrifugation at 1300 rpm for 8 min, the blood sample was divided into upper fibrin clot and lower RBCs; C) After 15 min at room temperature, the fibrin clot was picked up using artery forceps; D) The RBCs layer was cut and the fibrin clot represented A-PRF.

Surgical procedure

All animals were pre-medicated with atropine sulphate (Atropine sulphate, Memphis Company for pharmaceutical and chemical industries –Egypt) at a dose of 0.04 mg / Kg B W, I/M, then sedated later for 10 minutes by I/M injection of Xylazine hydrochloride 2% at a dose of 1 mg / Kg B W. General anesthesia was conducted by an injection of 2.5% thiopental sodium at a dose rate of 20 mg / Kg B W, I/V. The surgery on the tibia was performed at the medial aspect (Figure 3) of the right and left tibiae after aseptic preparation. A 3cm linear incision through skin, subcutaneous tissue, and muscles was done at the proximal extremity of the right and left tibiae of each dog. The periosteum was stripped and removed from the defect site. A 7mm circular and 10mm deep tibial bone defect was created using a drill bur with continuous cooling using sterile 0.9% saline solution (Sodium chloride 0.9%, FIPCO, Egypt). The defect was irrigated with sterile 0.9% saline solution for removal of any remaining bone dust. The defects were treated according to the group allocation as follows: control group; the defects of the right and left tibiae were left empty, n=4, PRP group; the defects of the left tibiae were treated with activated PRP, n=8 and A-PRF group; the defects of the right tibiae were treated with A-PRF, n=8. The periosteum was sutured with Vicryl No.0 (EGYSORB, Cairo, Egypt) in an interrupted pattern. The muscles, SC and skin were closed routinely. All animals were injected with I/M Cefotaxime at a dose of 30 mg /Kg B W twice daily for five successive days postoperatively (PO) and with Meloxicam at a dose of 0.2 mg / Kg B W for three successive days PO. The hind limbs were wrapped with supportive bandage for two weeks after surgery. The animals were euthanized at 6- and 12-weeks PO with an over dose of anesthesia (thiopental sodium, 35mg/Kg B W, I/V) for gross, computed tomography (CT) and histopathological examinations. The number of the samples was four for each group at each time.

Radiographic examination

Standardized ML radiography of the right and left tibiae of each dog was performed immediately after surgery, 6- and 12-weeks PO using X-ray machine (POX-300 BT, TOSHIBA, ROTANODE^{TM}, Japan) with 50-55KV and 6.3 MAs exposure factors to evaluate bone healing at the defect site. The radiographs were evaluated by two blinded independent observers for the treatment strategy using a scoring system described previously (24). The scoring ranged from -3 to +3 by comparing the opacity of the defect with that of the bone.

Computed Tomography scanning

After gross examination, the harvested tibiae of dogs from each group were scanned at 6- and 12-weeks PO for evaluation of new bone formation using helical CT device (revolution CT). Scanning was performed at 80 KV and 50 MA with a thickness of 0.024 mm per slice in medium resolution mode, 1,024 reconstruction matrix, and 200-ms integration time.

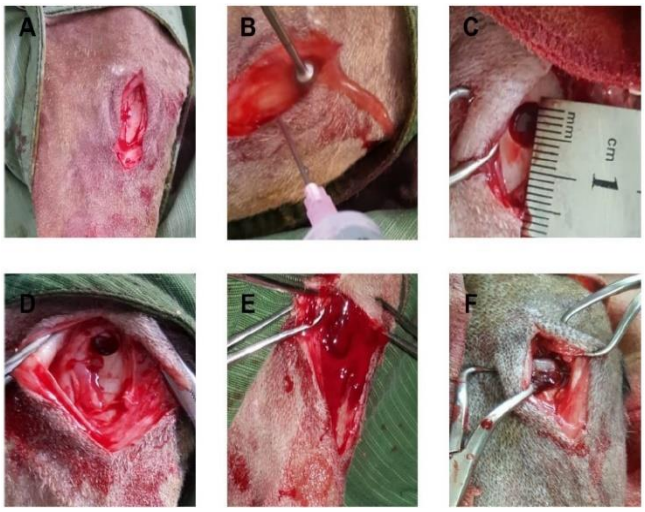

Figure 3: Creation of the tibial bone defect. A) Incision at the proximal extremity of the tibia; B & C) Creation of the 7mm circular tibial defect using drill burr under continuous cooling with sterile saline solution; D) The defect was left empty (Control group); E) The defect was treated with activated PRP (PRP group); F) The defect was treated with A-PRF (A-PRF group)**.**

Histopathological examination:

Following gross examination, the tibial defects and both host bone-defect interfaces were excised and fixed in 10% neutral buffered formalin at room temperature. After fixation, decalcification was performed with 10% Ethylene diamine tetra acetic acid (EDTA). The samples were dehydrated in ascending grades of alcohol from 70% to 100%, cleared in xylene then embedded in paraffin. Then the samples were sectioned at 4 µm, stained with hematoxylin and eosin (H&E) then examined microscopically according to previous description (25). The amount of new bone formation at the level of the defect site was evaluated using *Emery scoring system* (Table 1) (26). The histological sections were examined under the light microscope and scored by two blinded independent observers.

Statistical analysis

All the results were represented in mean ± SD and were statistically analyzed using one-way ANOVA and LSD post hoc test using SPSS 17.0 software (SPSS, Chicago, USA). $P \leq 0.05$.

Table 1: Emery scoring system for tibial defect gap filling

Score	Description of the bone gap
0	Empty
1	Filled with fibrous connective tissue only
2	Filled with more fibrous tissue than cartilage
3	Filled with more cartilage than fibrous tissue
4	Filled with cartilage only
5	Filled with more cartilage than bone
6	Filled with more bone than cartilage
7	Filled only with bone

Results

Radiographic findings

At day 0 (Immediately after surgery), the created defects in the right and left tibiae of dogs in all groups appeared as clear radiolucent circular holes at the level of the proximal extremity. While at 6 weeks PO, the defects of the control group appeared radiolucent with roughened edges. In PRP group the defects were partially filled with newly formed bone and appeared more radiopaque than the control group did. The defects of the A-PRF group were completely filled with newly formed bone and appeared more radiopaque than PRP group did. At 12 weeks PO, the defects of the control group were partially filled with newly formed bone and appeared more radiopaque. The defects of PRP group were completely filled with newly formed bone and appeared radiopaque. The defects of the A-PRF group were completely closed with new bone formation and were difficult to be distinguished from the host bone (Figure 4).

Statistically, the radiographic score of the tibial defects at 6 weeks PO was significantly higher in PRP and A-PRF groups than the control group one. The radiographic score in A-PRF group was non-significantly higher than that of the PRP group (Figure 5). At 12 weeks PO, the radiographic score of the tibial defects treated with A-PRF was significantly higher than that of the control group. The radiographic score in the PRP group was non-significantly lower than that of A-PRF group and non-significantly higher than that of the control group (Figure 5).

Computed tomographic findings

The defect holes at 6 weeks PO appeared with partial filling with less radiopaque tissue in the control group, while in the PRP group the defect holes appeared partially filled with radiopaque tissue. Interestingly, the defect holes in the A-PRF group appeared partially (approximately 2/3rd of the defect area) filled with radiopaque tissue; similar to that of normal bone tissue representing newly bone formation. At 12 weeks PO, the defect holes in the control group appeared partially (approximately half of the defect area) closed with newly bone formation, while in the PRP group the defect holes appeared partially (approximately 2/3rd of the defect area) filled with radiopaque tissue similar to that of normal bone tissue representing newly-accumulated bone formation. It's to be noted that the defect holes in the A-PRF group appeared completely closed with newly formed bone similar to that of normal bone (Figure 6).

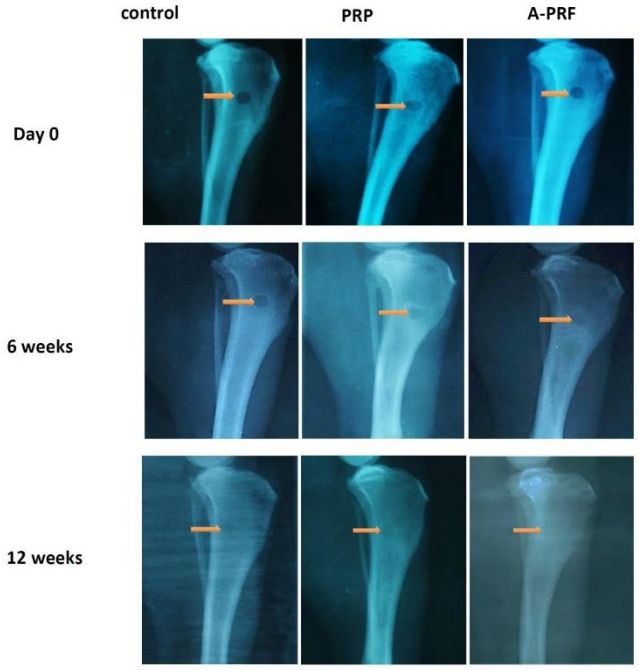

Figure 4: Medio-Lateral radiographs of the tibial bone defects of the control group, PRP group and A-PRF group immediately PO (Day 0), 6 weeks and 12 weeks PO. Note the defect site (arrows).

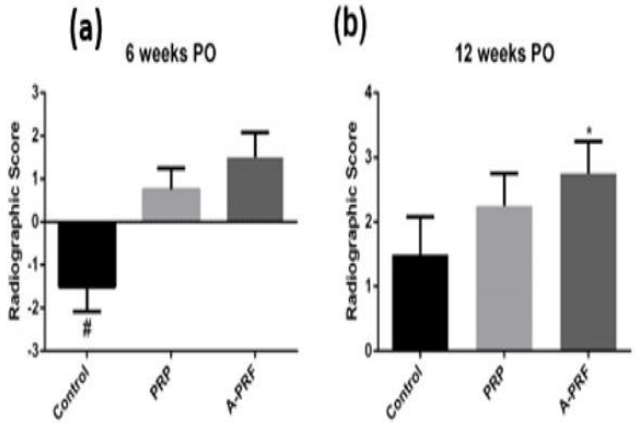

Figure 5: Radiographic score of the tibial bone defects at 6 weeks PO (a) and 12 weeks PO (b). # Significantly lower than the other groups, *significantly higher than the other groups.

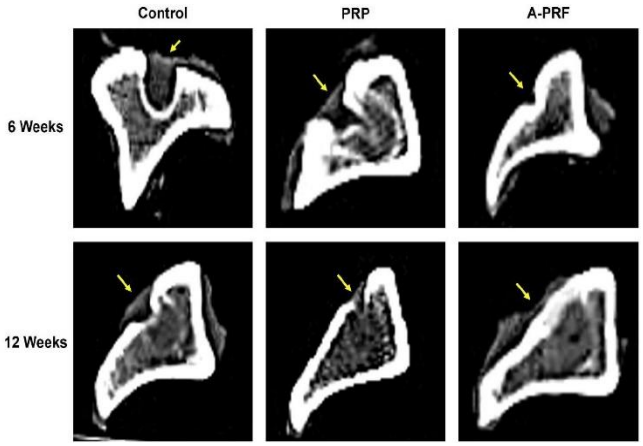

Figure 6: Computed tomography images of the tibial bone defects at 6 and 12 weeks PO of the control group, PRP group and A-PRF group. Note the defect holes (arrows).

Histopathological findings

The stained sections at 6 weeks PO in the control group appeared partially restored with granulation tissues which consisted of fibrous tissues, newly formed blood vessels and proliferating fibroblasts surrounded by normal osseous tissues. while those of the PRP group looked partially restored with granulation tissues which consisted of fibrous tissues with hyperplasia of osteoblasts surrounded by normal osseous tissues. In the A-PRF, the sections seemed completely restored with granulation tissues which consisted of fibrous tissues with hyperplasia of osteoblasts surrounded by normal osseous tissues. At 12 weeks PO, the section in the control group showed maturatin of the granulation tissues with callus formation while those of the PRP group showed maturation of granulation tissues and callus formation with ill-developed Haversian system with hyperplasia of osteoblasts and marrow formation. Interestingly, the sections in the A-PRF group showed callus formation with well-developed Haversian system and large number of osteocytes. Well-developed marrow spaces were also recorded (Figure 7).

Statistically, the histological scores of the tibial defects at 6 weeks PO were significantly higher in PRP and A-PRF groups than they were in the control group while those of the PRP group were non-significantly lower than those of the A-PRF group (Figure 8). At 12 weeks PO, the histological scores of the tibial defects were significantly higher in the A-PRF group than those of the PRP and the control groups. The histological scores of the tibial defects in the PRP group were non-significantly higher than those of the control group (Figure 8).

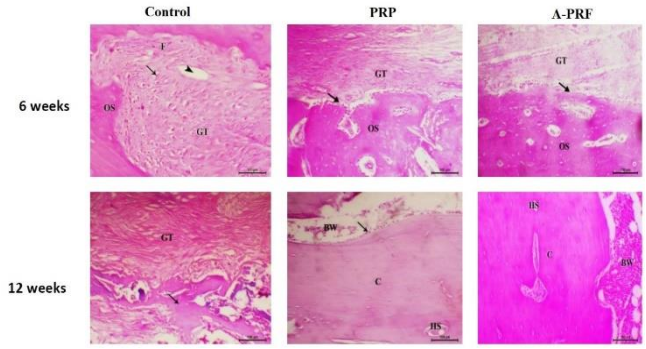

Figure 7: Photomicrograph of the tibial bone defects of the control, PRP and A-PRF groups at 6- and 12-weeks PO stained with H&E staining with power of zoom (200X and 400X). (GT) granulation tissues, (F) fibrous tissues, (arrow head) newly formed blood vessels, (arrow) proliferating fibroblasts, (OS) normal osseous tissues, (C) callus formation, (HS) haversian system, (BW) marrow spaces (bar100 µm).

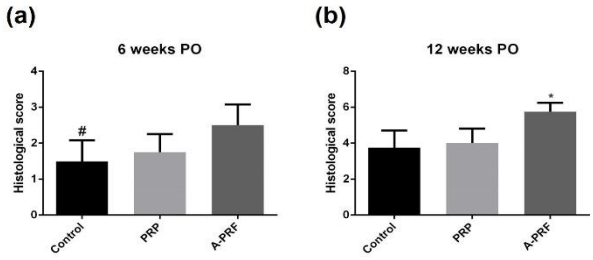

Figure 8: Histological score of the tibial bone defects at 6 weeks PO (a) and 12 weeks PO (b). # Significantly lower than the other groups, * significantly higher than the other groups.

Discussion

Platelet concentrates, obtained from the patient blood, are a biological source for several growth factors essential for repair including: IGF, PDGF and bFGF (11,12). Therefore, the aim of this study was to evaluate the efficacy of PRP and A-PRF as bone filling materials so as to improve bone regeneration of induced tibial bone defects in dogs. Using dogs, as a model in this kind of experimental studies, was due to the similarities in biological healing process and bone structures of dogs and humans (27).

In present study, radiographic and CT findings revealed early closure of the tibial bone defects after 6 weeks from surgery and complete closure of the defects after 12 weeks from surgery in the A-PRF treated group in comparison with PRP treated group and the control group. In the PRP treated group, the defects showed more bone formation than those

of the control group. Histopathological, the A-PRF treated defects showed more callus formation with well-developed Haversian system, marrow spaces and large number of osteocytes, while those treated with PRP showed moderate callus formation with ill-developed Haversian system, marrow spaces and hyperplastic osteoblasts. In the control group, the defects showed minimal callus formation in comparison with the PRP and A-PRF treated groups. These results were in line with the previous studies where they revealed that PRF is better in promoting bone and soft tissue healing in comparison with PRP (28). The improvement of healing ability of A-PRF over PRP may be attributed to the fact that A-PRF released the highest total growth factors than PRP did, which stimulate cell proliferation and regulating angiogenesis (29).

Also, our results ran parallel with a previous study which had revealed that sacroiliac joint dysfunction patients, that received PRF injection, experienced an improvement compared to those received PRP (30). In addition, another study indicated that using of A-PRF with or without combination of coral powder accelerated and improved bone healing (24). It was reported that the ability of A-PRF to mimic the immunology and physiology of wound healing is due to the high release of growth factors concentration and anti-inflammatory cytokines that stimulate tissue cicatrization, differentiation and proliferation of bone cell and vessels formation (31). It was also revealed that A-PRF is the better form of platelet concentrates in regenerative periodontal therapy as it has a sustained release of growth factors over time (32).

Our results were in agreement with the previous studies which found an increase in histological picture in bone formation with the combination of PRF to biphasic calcium phosphate in an induced bone defects in tibia of sheep (33). On the other hand, non-significant difference in histological findings of PRF were shown when used in induced tibial bone defect in rabbit but the general results of the experiment, which indicated that PRF had increased new bone tissue formation and induced early bone healing, was reported (34).

Positive effects of the use of PRP in regeneration of bone (35,36) and soft tissue (37,38) were reported. It was indicated that PRP form a good bone bridging without any complications (35). As for soft tissue healing, PRP injection improved regenerative process in arthroplasty (37) and gastric ulcerations restoration (38) through enhancing macrophage migration, angiogenesis, decreasing inflammation at the surgical site and necrosis. These findings support the results of the present study where the use of PRP enhanced bone regeneration more than the control group did.

On the other hand, the negative effect of PRP in regeneration of bone was reported in previous studies (18,39). It was reported that the use of PRP alone or combination of it with autologous bone had resulted in less bone remodeling in comparison with the use of autologous bone alone (40). In addition, less bone formation and defect closure in comparison with the use of simvastatin on regeneration of critical-sized calvarial bone defects was reported (18). This negative effect might be due to the use of anticoagulant in PRP preparation that affects the growth factors and cellular content of PRP (41).

Conclusion

From the results of this study, it could be concluded that A-PRF and PRP had a good efficacy in bone generation, while the use of A-PRF showed a rapid regenerative ability in comparison with PRP.

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Conflict of Interest

The authors declare no conflict of interest.

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مقارنة تأثير البلازما الغنية بالصفائح الدموية والفيبرين الغني بالصفائح الدموية المتقدم على التئام العيب العظمي بعظمة القصبة في الكلاب

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الخلاصة

هدفت هذه الدراسة الى المقارنة بين تأثير البلازما الغنية بالصفائح الدموية والفيبرين الغني بالصفائح الدموية المتقدم على التئام العيب العظمي بعظمة القصبة في الكلاب. تم احداث عيب عظمي دائري بمساحة ٧ ملم وعمق ١٠ ملم في الجزء العلوي من عظمة القصبة اليمنى واليسرى في عدد ١٢ كلب ذكر بالغ وسليم بالفحص السريري. وقسمت الحيوانات عشوائيا الى ثلاثة مجموعات وتم علاجها كالتالي: مجموعة السيطرة (العدد=٤)، مجموعة البلازما الغنية بالصفائح الدموية (العدد=٨ في عظمة القصبة اليسرى)، مجموعة الفيبرين الغني بالصفائح الدموية المتقدم (العدد=٨ في عظمة القصبة اليمنى). تم تقييم التئام العيب العظمي من خلال الاشعة السينية، الاشعة المقطعية، الفحص العيني والفحص النسجي بعد ٦ و ١٢ اسبوع من الجراحة. اظهرت النتائج عند الاسبوع السادس من الجراحة، ان العيب العظمي في مجموعة السيطرة قد ملئت بنسيج ليفي ولم تعطي مردود بالأشعة السينية بينما في مجموعة البلازما الغنية بالصفائح الدموية، امتلئ العيب العظمي نسبيا بنسيج عظمي جديد وقد اظهرت مردودا بالأشعة السينية عن مجموعة السيطرة، اما العيب العظمي في مجموعة الفيبرين الغني بالصفائح الدموية المتقدم قد امتلئ بنسيج عظمي جديد وقد اظهرت مردودا بالأشعة السينية عن مجموعة البلازما الغنية بالصفائح الدموية. عند الاسبوع الثاني عشر من الجراحة امتلئ العيب العظمي في المجموعة الحاكمة بنسيج عظمي جديد وأظهرت مردودا بالأشعة السينية، اما العيب العظمي في مجموعة البلازما الغنية بالصفائح الدموية امتلئت بتكوين عظمي جديد وأظهرت مردودا بالأشعة السينية. ومن الجدير بالذكر ان العيب العظمي في مجموعة الفيبرين الغني بالصفائح الدموية المتقدم قد ملئت بتكوين عظمي جديد ولا يمكن تمييزها من النسيج العظمي الطبيعي المحيط بها. مما سبق، اتضح ان استخدام البلازما الغنية بالصفائح الدموية والفيبرين الغني بالصفائح الدموية المتقدم يحفز التئام العظام، ولكن استخدام الفيبرين الغني بالصفائح الدموية المتقدم يزيد من فرصة التئام العيوب العظمية في الاسابيع الاولى من حدوثها مقارنة بالبلازما الغنية بالصفائح الدموية.