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Effect of Injectable platelet-rich fibrin (i-PRF) on new bone formation in surgical expansion with mini-screw assisted rapid palatal expander: A dog model study

Khawla M. Awni[1], Zaid Dewachi[1] and Osama Hazim Al-Hyani[2]

Abstract

OBJECTIVES: The objective of the study is to test whether the local injection of i-PRF may affect osteoblast, blood vessels and new bone formation in surgically expanded mid palatal suture using maxillary skeletal expander.

MATERIALS AND METHODS: Eighteen adult male local breed dogs were divided randomly into three groups: group I (control), includes the expansion with maxillary skeletal expander that was done without corticotomy and i-PRF. Group II, involves the expansion that was done with mid palatal corticotomy but without i-PRF injection. Group III, has the expansion that was done with mid palatal corticotomy and has injection with 2 ml of i-PRF. Each group consisted of 6 dogs which were subdivided into 3 dogs. Three dogs were sacrificed after 15 days and the other 3 dogs were sacrificed after 45 days. The number of osteoblast, blood vessels and new bone formation percentage were statistically analyzed using Sigma plot platform. Mean and standard error, ANOVA and Duncan were performed among the different groups. Values of $P \leq 0.05$ were considered significant.

RESULTS: After expansion, the i-PRF group demonstrated a considerable increase in the amount of new bone in the mid-palatal suture at 15 and 45 days compared with other 2 groups which were indicated by highest percentages of new bone formation (29.30% of 15 days and 76.55% at 45 days) if compared to control group which were (7.72% at 15 days and 22.30% at 45 days). The corticotomy groups were in between, 15.33% and 46.84% respectively. Moreover the number of osteoblasts was higher in corticotomy with i-PRF group on 15 days and decreased on 45 days than the other two groups, while the blood vessels were highest in this group than the other two groups both on 15 and 45 days.

CONCLUSION: I-PRF enhanced the production of osteoblast, blood vessels, and new bone in the surgically expanded mid palatal suture.

Keywords:

Bone formation, injectable platelet rich fibrin (i-PRF), mid palatal expansion, MSE2

[1]Departments of Orthodontics, College of Dentistry and [2]Surgery and Obstetrics, College of Veterinary Medicine, University of Mosul, Iraq

Address for correspondence:
Dr. Khawla M. Awni, Department of Orthodontics, Collage of Dentistry, University of Mosul, Iraq.
E-mail: Khawlaawni76@uomosul.edu.iq

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Introduction

One of the most prevalent orthodontic problems in both children and adults is the transverse maxillary insufficiency. This problem hints to an unpleasant smile as well as improper occlusion, crowding, stenosis, and buccal segment crossbite.[1,2]

In mixed and deciduous dentitions, the prevalence of maxillary transverse deficit is currently estimated to be between 8% and 23%, with less than 10% prevalence in adults.[3]

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The difficulties of transverse maxillary inadequacy and loss of space are extremely difficult to solve.[4] Treatment of the narrow maxillary arch requires the application of orthopedic forces or a surgical intervention for expansion.[5] Rapid palatal expansion type with tooth-born expander has been used for the treatment of maxillary transverse deficiency.[6]

The side effects of rapid palatal expander are common, which include dehiscence of bone or fenestration, root resorption, gingival recession, buccal tipping of posterior teeth, in addition to relapse.[7]

To decrease the unwanted effects of tooth-borne RPE and increase skeletal expansion, several forms of bone-borne RPE have been produced.[8]

Patients who have minimal or no growth remaining often refuse to undergo surgery, leading to attempts to correct these deficiencies without surgery, which formed the basis for the development of micro-implant-assisted rapid palatal expander (MARPE).[5]

MARPE appliance, developed by Dr. Won Moon, is considered to be a type of the RPE appliance and has been developed for correction of transverse malocclusion. It has proved to be a viable and efficient nonsurgical option for young adults.[8]

To obtain skeletal changes with MARPE, the force should be sufficient to stunned zones of resistance present in the midface region like the mid-palatal suture (the first that needs to be disrupted), pterygoid junctions, zygomatic buttresses, and piriform aperture pillars. Force is functional directly into the center of resistance of the maxilla by micro-implants and not the tooth as in familiar tooth palatal expanders. As a result of this force, buccal tipping of the tooth is reduced, and a more parallel suture opening is obtained.[9] Once more, clinicians have announced various minimal offensive surgical procedures used with MARPE, called cortico-punctures or micro-osteoperforations, to improve the ratio of tooth movement and decrease iatrogenic harm produced by the long period wear of fixed appliances.[10,11]

Cortico-puncture was introduced in clinical practice as a surgical procedure to reduce the period of orthodontic treatment. It eliminates the cortical bone that struggles orthodontic force and keeps the blood circulation and continuity of bone tissues to decrease the threat of necrosis and enable tooth movement.[12]

In the mid-palatal suture, stimulating mineralization and new bone formation after RME will be essential to counterattack the retractive force created by surrounding tissue and to maintain the stability of the maxillary structure which contract the retention time following expansion, and to prevent setback of the arch width.[13]

Platelets have become more popular in recent years. Platelets, which contain growth factors, are essential for cell migration, proliferation, differentiation, and angiogenesis, and are used in tissue regeneration procedure. Blood is centrifuged at varying speeds, and thrombin and anticoagulant are used or not used to make autologous platelet concentrates. A fibrin clot is formed as a result of these handling protocols, which contain platelets and leukocytes.[14]

Activation factors and external chemicals may increase the contamination threats that scratch the use of platelet rich plasma in clinical routine and elegant procedure. Advances in the development of different platelet concentrates led to the creation of plateletrich fibrin (PRF), which considered a fully autologous system. PRF is achieved by onestep centrifugation and without any anticoagulants. PRF comprises a high number of leukocytes, fibrin, platelets, and plasma proteins. It is likely to create either a liquid or a solid PRF dependent on the centrifugation protocol and the blood collected tube.[15]

Contemporary research deliveres new protocol for liquid type of PRF named injectable PRF (i-PRF). This type of PRF is obtained by consuming blood in plastic tube and centrifuged at 700 rpm (60 g) for 3 minutes without anticoagulant.Plastic tubes used in this type of protocol do not trigger the coagulation process because they possess hydrophobic surface. So, this technique permits the split-up of blood components in a few minutes of centrifugation, leaving the orange layer (plasma, platelets and clotting factors) at the top of the tube, which is simply extracted and used as an injectable form.[16]

Until now, no study was launched in concern the effect of local application of i-PRF on bone regeneration of the surgically expanded mid-palatal suture, so this study will be another headlong step in orthodontic field and with our respect to all important studies done before.

Materials and Methods

Animals and groups

Eighteen skeletally mature male local breed dogs (using CBCT to each dog before the experiment to confirm the maturity of mid palatal suture), 1.5-2 years and weighing 15-20 kg were used. The dogs were clinically well as determined by physical investigation, normal haemogram, and clinical chemical profiles all dogs housed discretely in a controlled environment in animal house in a 12-hour light/dark atmosphere at similar

conditions of humidity, temperature, and aeration. They were fed with a home-made diet containing chicken and rice twice a day with sufficient tap of water all the period of study. The animal upkeep was done with the aid of appropriately skilled veterinarian. The experimental segment of the study started after an adaptation period of 2 weeks.

The sample was randomly divided into 3 main groups. Group I (control group), includes expansion that was done without corticotomy and i-PRF injection. Group II, involves the expansion that was done with mid palatal corticotomy but without i-PRF injection. Group III, has the expansion that was done with mid palatal corticotomy and i-PRF injection.

Each group consists of 6 dogs which are subdivided randomly into 2 subgroups, (3 dogs) were sacrificed after 15 and 45 days.

MARPE appliance

MSE2 screw expander that used was designed by Dr. Won Moon, UCLA School of Dentistry,(Biomaterials Korea, INK) it is a version of the Micro-implant Assisted Rapid Palatal Expander (MARPE) and was introduced to the market in 2010. It uses 4 titanium alloy TADs for fixation to the palate and a wrench for activation. It attaches to 2 molar bands (Dentaurum, Germany) via 4 legs for stabilization.The screw expander of 10 mm, 4 mini-implant 1.8 mm width, 11 mm length was used.

Anesthesia was inducted by I.M injection in the thigh muscle of a mixture of 10% Ketamine hydrochloride (10 mg/kg) (Nederhorst den Berg, Holland), and 2% Xylazine (3 mg/kg) (Venray, Holland).

Perforated special tray that was previously fabricated of cold cure acrylic (New Stetic S.A., Colombia) was used. The impression was taken by rubber base impression material (Dentplus, E.E.C.), and powered with stone [Figure 1a, and b]. Stainless steel bands (Dentaurum, Germany) were prepared on the upper right and left 1st molars after mesial and distal disking of the teeth from the cast, MSE2 placed on the cast leaving 1 mm clearance between the screw and the cast, the holding arms were soldered to the bands with 3-4 mm clearance from the cast. After soldering, the appliance was finished and polished by polishing machine to be ready for insertion [Figure 1c].

In the day of insertion of appliance, the dog was anesthetize, disking of the mesial and distal sides of upper 1st molars by disking bur to occupy the bands and the MSE2 was placed on the palate with bands cemented on the 1st molars using glass ionomer cement (Medifil, Germany).The 4 mini-screws were placed in the miniscrew holes of the expander and screwed with manual screw driver diagonally, one miniscrew at each time [Figure 2a].

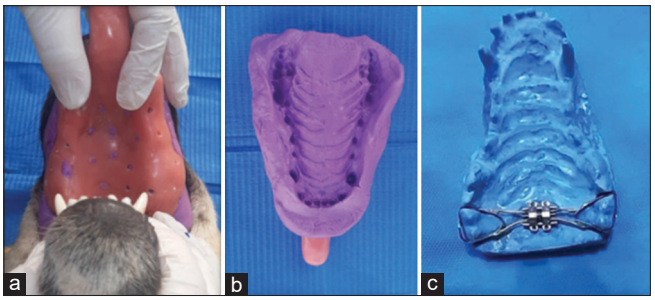

Figure 1: (a) The impression was taken to the dog with special tray made from cold cure acrylic; (b) rubber base impression material after setting; (c) MSE2 soldered and finished on the cast

The activation protocol was done according to Dr. Won Moon, in which expander was rotated by ratchet 6 times/daily (3 at one time in the morning and 3 in one time at the evening), so the anesthesia was decreased to a minimal amount which is enough to anesthetize the animal during activation that usually takes only few minutes. The activation continued until the screw opened nearly 10 mm (which is the amount of opening that can be obtained from the expander) [Figure 2 b, and c].

Corticotomy

The surgical procedures were carried out at the Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Mosul.

The dog was sedated with an intramuscular injection of 10% ketamine (10 mg/kg) mixed with 2% xylazine (3 mg/kg intramuscularly). The soft tissue of the palate was cut from the canines area backward to the 4th premolars in the mid palatal suture region.

Another 2 horizontal cutting also done anteriorly and posteriorly, reflection of mucoperiosteal flap was done bilaterally. Puncting of hard palate in the mid palatal suture was done using round bur (2 mm in diameter) and micromotor hand piece (Strong 204, 35000 rpm, 280N/cm, Korea). The speed decreased during surgical procedures according to the density of the palatal bone and it was done with copious saline irrigation to prevent necrosis of the tissues. Five perforations were prepared alongside the mid palatal suture with 5 mm space between each 2 perforations (the depth of each perforation was 2 mm). Another cuttings were done using fissure bur between each 2 perforations laterally and diagonally to the mid palatal suture.

Finally, suturing with 0.1 black silk sutures using simple interrupted suture technique; [Figure 3 a, b, c, and, d].

The dogs were injected with 1 ml/10 kg body weight per day of penicillin (PEN/STREP, Nederhorst den Berg, Holland) for up to 5 days and 1 ml of Metalgin (SPI, Saudi) twice daily up to 3 days. All operated dogs resumed their consciousness and activities normally nearly 4-5 hours postoperatively. After 1 week, the suture was removed and the MSE2 was placed and activated as mentioned previously.

Preparation and injection of i-PRF

Collection of the blood was done by using 10-ml plastic tubes (i-PRF, Choukron plastic, pan vacuum without any additive, USA) from the jugular vein. The tube placed quickly (less than 1 minute) in the centrifuge without storage (PC-02 Centrifuge, Hettich Universal 320 Zentrifugen, France) using 700 rotation/minutes for 3 minutes at room temperature to isolate the i-PRF. The upper plasma layer was collected and designated as i-PRF then drawn from the tube and delivered by disposable 5 ml syringe.[16]

The injection was done along the mid palatal suture from anterior (canine areas) to posterior region (4th premolars), 2 ml injected only in one time to each animal. The injection of i-PRF was done after opening the suture, and mid diastema was appeared (nearly after 1 week of expansion) [Figure 4 a, b and c]

Specimen preparation

The animals were euthanized by injecting an overdose of a combination of Xylazine and ketamine. The procedure was done without causing any suffering to the animals.

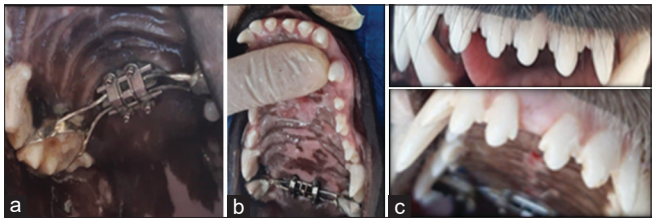

Figure 2: (a) Insertion of MSE2 in dog's mouth; (b) finishing of expansion and the diastema appear anteriorly; (c) pre and post expansion

The MSE2 was removed from the mouth by unscrewing the mini-screws and remove of bands from the molars by band removal plier, maxilla separated from the mouth, and the other region of the head by using a cutting machine, manual scalpel and surgical scissor to remove the soft tissues. After rinsing with running water, the pieces fixed in containers were filled with 10% buffered formalin solution to be ready for histological processing which include the following steps.[17]

- **Decalcification:** It was done by 10% forming acid and hydrochloric acid 10% for 4 weeks at 37° C.
- **Dehydration:** By using ethyl alcohol, 70%, 90% and 100% primary alcohol, then 100% secondary alcohol (1.5 hour for each).
- **Clearing:** Specimens passed 3 times through xylene for 1.5 hour to complete the clearance.
- **Paraffin wax embedding:** Putting the specimens in a dish of melted paraffin, then, putting the dish in a temperature of 56-60° C until the xylene was replaced by paraffin (1 hour for each dish).Finally, the specimens were placed in a centre of the paraffin block.
- **Sectioning:** Sectioning of 5-µm thickness, putting glass slide and the wax was molten away using hot oven, then, cleared using xylene.
- **Staining with Hematoxylin and Eosin:** Firstly, dehydration was done in descending alcohol and stained with Mayer's hematoxyline (7-10) minutes, washed with water for 1-5 minutes to remove the excess stains, staining with eosin for 1-2 minutes, dehydrated in absolute alcohol for 2-3 minutes, cleared with xylene. Lastly, the cover slips fixed on the stained tissue using Dixtrene Plasticiser Xylene (D.P.X).

Histomorphometric analysis

Histomorphometric evaluation was performed in a blind analysis by experienced researcher. Three histological sections from the canines area backward to the 4th premolars in the region of mid palatal suture were analysed for each animal and the results were taken as an average of the counts (with no significant differences were seen among the 3 histological sections of each animal). These were observed under a light

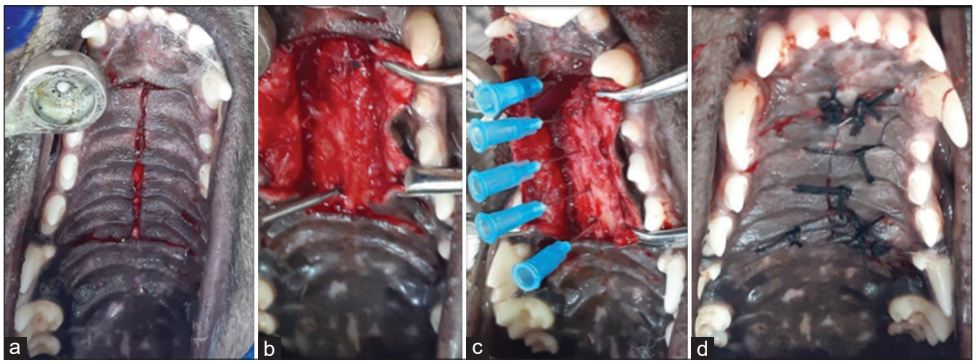

Figure 3: (a) The flap extended from canines to 4th premolars; (b) reflection of flap; (c) 5 perforations in the midpalatal suture using round bur; (d) suturing the flap

microscope (B-383PLi, OPTIKA, Italy by using digital camera, OPTIKA, Italy and a PROVIEW Program).

Each slide section was divided into four quadrants; the mean value of these quadrants was estimated to ensure approximately complete scanning of each histological section. This mean is the number of osteoblasts (obs) and blood vessels (bvs).

The numbers of obs were counted at 400 x, while the numbers of bvs were counted at 100x. The percentage of ossified to non-ossified area was calculated to obtain the amount of newly formed bone.[18]

To assess the validity of the reading of slides, inter-examiner calibration by second examiner, and intra-examiner calibration by the same operator with time laps of two weeks were done by repeating the counting of osteoblasts, blood vessels, and the measurements of percentages of newly formed bone often randomly selected slides. There were no significant differences by paired *t*-test at the level of $P \leq 0.05$.

Statistical analysis

The number of osteoblasts, newly formed bone percentage, and blood vessels were statistically analysed using Sigma plot program, mean and standard error, ANOVA and Duncan test was implemented among the different groups. Values of $P \leq 0.05$ were deliberated significant.

Results

The study showed that MSE2 appliance was effective in expanding of mid palatal suture in all groups. It was tolerated by the dogs and all animals survived till the end of the study. No adverse side effects including weight loss, inflammation, wound infection, dehiscence, or appliance failure occurred.

Histological findings

On day 15, the control group showed mature connective tissue which characterized by the presence of collagen fiber with few number of newly blood vessels in the central of the biopsy of palatal suture gap, while the corticotomy group showed dense connective tissue, disorganized congested blood vessels and irregular trabeculae bordering the suture space. In corticotomy group with i-PRF, there were mature bone lamellae formations with the connective tissue were perceived in the central of the palatal suture and formation of bone marrow within the bone tissues [Figure 5]. On day 45, irregular bone lamellae with presence of bone marrow space, small osteocytes lacunae were seen in the control group. In corticotomy group, bone marrow space, bone lamellae, osteocytes and remnant of connective tissue were present, where corticotomy group with i-PRF showed mature bone formation with large bone space also observed [Figure 6].

Number of osteoblast cells

Histological finding showed that the number of osteoblasts was higher significantly in the two experimental groups than the control group on day 15 with the highest number was in the corticotomy with i-PRF group. The number of osteoblasts was considerably different between groups. [$p \leq 0.05$; Table 1; Figure 5]. On day 45, the reverse occurred in which the number of the osteoblasts was the lowest in the corticotomy with i-PRF group and highest in the control groups, while the corticotomy group was in between.[Table 1; Figures 6 and 7].

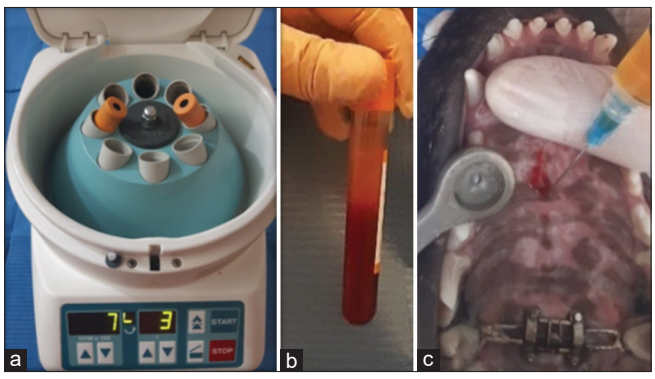

Figure 4: (a) Blood placed in centrifuge; (b) i-PRF after centrifugation; (c) injection of i-PRF into the palate

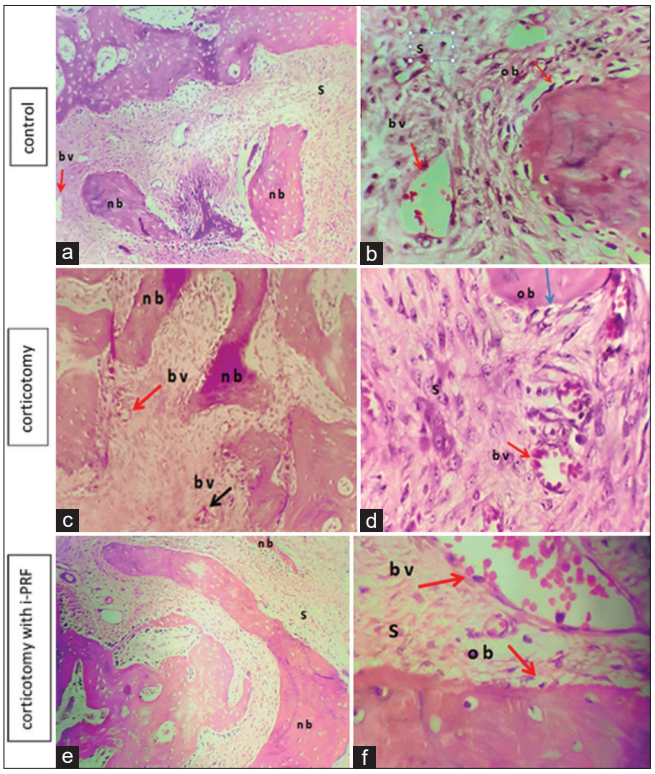

Figure 5: Photomicrograph of expanded mid-palatal suture of control and experimental groups after 15 days of expansion. s, suture; bv, blood vessel; nb, new bone; ob, osteoblast. (H&E,100x and 400x). control group (a and b), corticotomy group (c and d) and corticotomy with i-PRF group (e and f)

Number of blood vessels

On day 15, corticotomy with i-PRF group displayed a significant increase in the number of blood vessels relative to the other study groups. Likewise, numbers of blood vessels were significantly higher in the corticotomy group than control group [Table 2; Figure 5].

On day 45, the number of blood vessels was decreased in all groups with significant different between the three groups, the corticotomy with i-PRF group was the highest and the control group was the lowest value [$P \leq 0.05$; Table 2; Figures 6 and 7].

New bone formation

The groups compared were based on newly formed bone percentage ($P \leq 0.05$), and significant differences were established among the groups. The results revealed that a substantial increase in new bone formation in the corticotomy with i-PRF group than the other two groups. The corticotomy group was significantly higher than that in control group [Table 3; Figures 5,6,8].

Discussion

Rapid maxillary expansion is a method usually used to correct transverse maxillary deficiency which is convoyed by posterior cross bite in order to increase the arch perimeter and release crowding in children and adolescents.[19]

Table 1: Comparisons of the number of osteoblast cells among different groups in 15 and 45 days

Group	Days	Mean	SE*	Duncan**	*P***
Control	15 days	23.25	0.62	A	0.001
Corticotomy		35.25	0.25	B	
Corticoromy with i-PRF		50.75	0.62	C	
Control	45 days	34.75	0.62	A	0.001
Corticotomy		27.00	0.40	B	
Corticotomy with i-PRF		20.50	0.28	C	

*Standard error **Different letters vertically mean a significant difference ***A significant difference existed at $P \leq 0.05$

Table 2: Comparisons of the number of blood vessels among different groups in 15 and 45 days

Group	Days	Mean	SE*	Duncan**	*P***
Control	15 days	4.00	0.40	A	0.001
Corticotomy		8.25	0.25	B	
Corticotomy with i-PRF		14.5	0.40	C	
Control	45 days	2.00	0.40	A	0.001
Corticotomy		5.00	0.40	B	
Corticotomy with i-PRF		10.50	0.28	C	

*Standard error **Different letters vertically mean a significant difference ***A significant difference existed at $P \leq 0.05$

Table 3: Comparisons of the percentage % of new bone formation among different groups in 15 and 45 days

Group	Days	Mean	SE*	Duncan**	*P***
Control	15 days	7.72	1.67	A	0.001
Corticotomy		15.33	2.97	B	
Corticotomy with i-PRF		29.30	5.76	C	
Control	45 days	22.3	1.90	A	0.001
Corticotomy		46.84	2.61	B	
Corticotomy with i-PRF		76.55	2.17	C	

*Standard error **Different letters vertically mean a significant difference ***A significant difference existed at $P \leq 0.05$

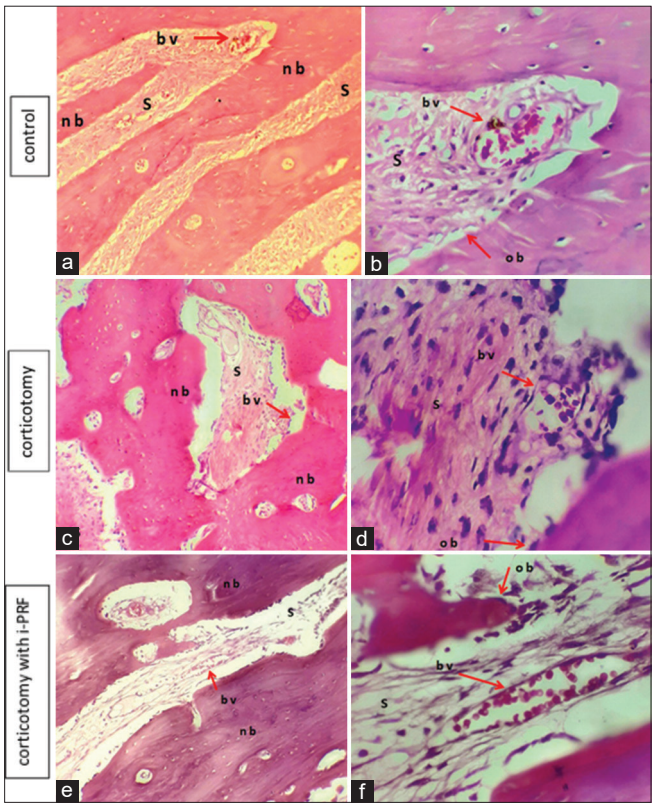

Figure 6: Photomicrograph of expanded mid-palatal suture of control and experimental groups after 45 days of expansion. s, suture; bv, blood vessel; nb, new bone; ob, osteoblast. (H&E, 100x and 400x). control group (a and b), corticotomy group (c and d) and corticotomy with i-PRF group (e and f)

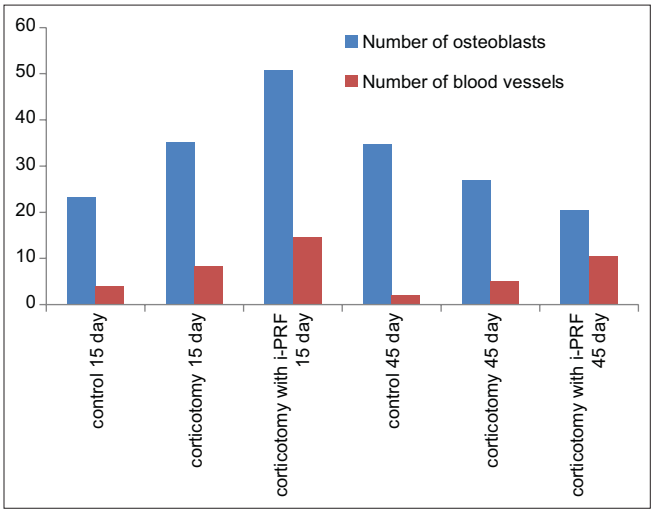

Figure 7: Comparison of the number of osteoblasts and blood vessels among the groups after 15 and 45 days of expansion

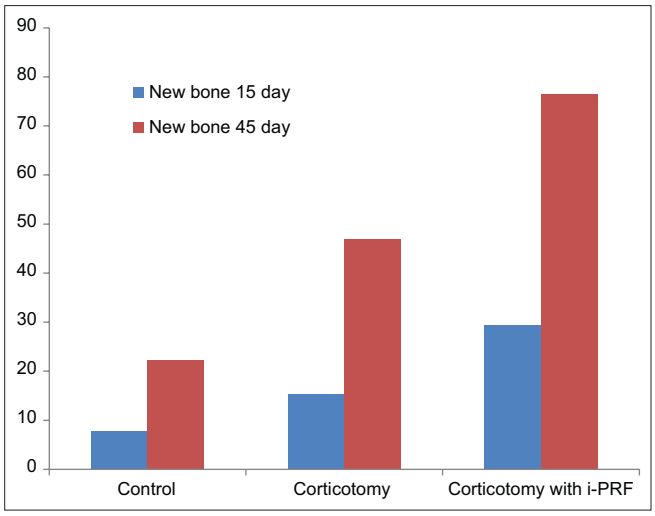

Figure 8: Comparison of the percentage of new bone formation among the groups after 15 and 45 days of expansion

Recently, an effective maxillary skeletal expansion type with a tooth bone-borne RPE appliance found on mini-screw (MARPE) was introduced which has greater orthopedic effects than dento-alveolar effects compared to RPE in adults.[20]

The dog in some ways is similar to human in bone composition and this makes it an appropriate animal for research.[21] Also, the dog is relatively small in size, ease in manipulation, with acceptable cost, ease in housing, available and tolerate to surgery. It has a large size of maxilla compared to rodents; the palate and the dental arch are wider and appropriate for the procedure. The shape of the molar teeth allowed in fabrication of the expansion appliance.

Dogs are used in many medical branches, which include surgery, physiology, toxicology, hereditary, and dental health, which is likely to increase in future.[22] The number of experimental animals in this study was reduced for ethical reasons, so it decreased to the minimal number required to achieve a scientific protocol. The age of the animals was 1.5-2 years old which resemble a skeletally mature dog. All the dogs were males to avoid the possible hormonal changes which could compromise the results. The appliances were tolerated by the animals, and appliance stability was maintained through- out the period of the study.

In this study, the local effect of i-PRF on bone formation in the mid-palatal suture after expansion was investigated by using histomorphometric method. The best of our knowledge, this is the first study that used i-PRF with expanded mid-palatal suture.

Bone histomorphometry is a reliable and dependable method which can be used to estimate bone formation in experiment, both *in vitro* and *in vivo* studies.[23] The histomorphometric parameters used in this study were the number of osteoblasts, number of blood vessels, and the percentage of new bone formation to all groups.

Mechanical force considered as an important signal which activate growth and regeneration in orthodontic and orthopedic treatments. So, expansion itself can stimulate new bone formation in mid palatal suture.[24]

Thus, our study showed that all groups have new bone in the mid palatal suture after expansion, but with different significant values. More positive results were seen in corticotomy with i-PRF group than the other two groups. The histological observations of the expanded mid palatal suture were studied in two times interval on 15 and 45 days to observe the early and later effects of i-PRF on bone regeneration in response to maxillary expansion and compared to other groups.

On 15 days, the corticotomy group showed denser collagen fibers than the control group. The corticotomy group with i-PRF showed increased number of osteoblasts, blood vessels, and new bone formation than the other two groups.

Regarding the corticotomy group, the surgery initiates the normal healing process which is called Regional Acceleratory Phenomena (RAP), which is a local response to stimulus. In this phenomenon, the tissues formed faster than the normal physiological regional regeneration process, so more bone has been formed than the control group.[25]

Zhou *et al*.,[26] 2019, explain that corticotomy stimulates osteoblast activity and maturation, modulate bone metabolism in early stage, and increased osteogenesis stability after expansion.

Some authors recommended the use of cortico-punctures in the cortex of palatine bone in the level of mid-palatal suture and facilitate the process of bone regeneration.[27, 28]

Micro-osteoperforation procedure can shorten the length of orthodontic treatment and can be safely used in routine orthodontics.[29]

The study didn't show an obvious inflammatory cells or osteoclastic activity, because the osteoclast increased initially and then began to decrease nearly at 7 days. This comes in agreement with others.[30,31] Inside the suture, there were newly formed bone after rapid maxillary expansion in all the groups which weren't in contact with the prevailing bone, that was similar to another study.[30]

The corticotomy group with i-PRF showed significant increase in the number of osteoblast, blood vessels, and

accordingly, the percentage of new bone formation than the other two groups.

It is probable to obtain dense or liquid form of PRF according to the blood collection tube and centrifugation procedure. Glass tube was used to obtain solid form of PRF because the platelet interacts with glass surface led to activation of their coagulation by centrifugation process. While the i-PRF collected with a plastic tube preserved it in a liquid condition for about 15-30 minutes to produce fibrin clot subsequently.[32]

In the current study, we used i-PRF to obtain a liquid formulation taking the advantages of slow and short centrifugation velocity (700 rpm for 3 minutes), so it is possible to observe an increase in the number of regenerative cells, increase concentration of growth factors compared to other PRF types which were produced at a higher centrifugation velocity. In addition, the i-PRF is easily manipulated in the mid-palatal suture. I-PRF is an easy, repeatable, minimally invasive, low cost, and totally autogenous procedure.[33]

I-PRF demonstrated sophisticated discharge of growth factors at the end of 10 days like PDGF-AB, PDGF-AA, epidermal growth factors (EGF) and insulin –like growth factor -1 (IGF-1), cellular migration, induced significantly m-RNA expression of transforming growth factor (TGF-β), and collagen -1 on 7th day, presence of leukocytes in i-PRF act as antimicrobial activity.[16,34]

These growth factors involved in cell mitosis, neo-angiogenesis and neo-collagenogenesis promoting osteoblast activity which lead to osteogenesis and new bone formation.[35]

I-PRF is able to glue all biomolecules in the fibrin scaffold leading to slight release to the adjacent media till resorption of fibrin was happened, which confirm direct administration of biomaterials to the needed environment.[36]

The literature demonstrated the scientific and the safe therapeutic approach by using PRF. You *et al*.,[37] concluded that PRF can promote the bone regeneration and it is valuable in clinical use. PRF can enhance alveolar cleft reconstruction.[38] PRF is considered as a simple, effective, and low cost therapeutic support for the treatment of extraction socket in dogs.[39]

Other studies showed that PRF is completely an autologous products without biochemical handling, in expensive, contain extended growth factors leukocytes and cytokines which contribute in the healing process.[40]

I-PRF containing platelet, Type I collagen, leukocytes, growth factors and osteocalcin can induce soft and mineralized tissue healing, it improved the effect of osteogenesis.[41,42]

Injecting i-PRF sub mucosally reduced relapse after orthodontic tooth movement and increase the bone density. So, it is a viable option to improve the stability.[43]

On 45 days, all groups shows increase in the amount of bone formation with significant amount was seen in corticotomy with i-PRF group which insures the sustained release of i-PRF in this group.

The physiological polymerization of i-PRF permits the cytokines and growth factors to be stored and slowly released which ensuring a bioactive levels for a long period up to 28 day.[44]

Study by To *et al*.,[45] shows that in control group, the new bone trabeculae was observed compared to dense bone formation in the socket after 30 days of extraction of tooth in A-PRF group, by enhancing the osteoblastic activity.

In the early stage of bone reorganization following expansion, there were many fibroblasts and blood vessels present in the suture, while in the stage of maturation, few osteoblasts and fibroblasts were present with small blood vessels and more regular trabeculae were seen, this come in agreement with other.[46]

Conclusions

I-PRF enhanced osteoblast, blood vessels and new bone formation in the expanded midpalatal suture, so this will have an impact to decrease the relapse of the expanded suture and reduce the retention period of orthodontic treatment.

Limitations of the study

The number of the animals in all groups was reduced due to the ethical reason. This may masked important findings; differences in bone density, in addition to anatomical variations (skeletal and dental) found between dogs and human like length and depth of the palate, location and connections of zygomatic apparatus with adjacent structures, location of maxillary and palatine sutures, muscles distribution, and actions beside the type dental occlusion when compared to human which may effect on expansion mechanism and resistance.

Clinical implication of the research

Our experimental results shows that local injection of i-PRF can induce a favorable effect on bone regeneration in the expanded mid-palatal suture using MSE2 type of MARPE. Accordingly, this will decrease relapse in orthodontic treatment and reduce retention time following the expansion procedure.

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Ethical approval

Experimental protocol was approved by the guidelines of the Institutional Animal Care Unit of the College of Veterinary Medicine at University of Mosul in accordance with the ethical approval of Research of Ethics Committee of the faculty of Dentistry, University of Mosul, Ministry of higher Education and Scientific Research, Iraq which permitted all protocol, trials and guidelines of the study. The approved REC reference number was U o M. Dent/A. DM.L.1/22 on January 18, 2022.

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Conflicts of interest

There are no conflicts of interest.

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