Effect of Periosteal Distraction by A Modified Hyrax Device with and without Platelet Rich Fibrin on Bone Formation in Rabbit's Model

Faisal Omar Abdullah Balabid

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Oral and Maxillofacial Surgery

Prince of Songkla University

2014

Copyright of Prince of Songkla University

Thesis Title	Effect of Periosteal Distraction by A Modified Hyrax Device with and without Platelet Rich Fibrin on Bone Formation in Rabbit's Model
Author	Mr.Faisal Omar Abdullah Balabid
Major Program	Oral and Maxillofacial Surgery

Major Advisor :

……..……

(Assoc.Prof.Prisana Pripatnanont)

Co-advisors :

……..……

(Asst.Prof.Settakorn Pongpanich)

……..……

(Asst.Prof.Surapong Vongvatcharanon)

Examining Committee :

…….……..…Chairperson

(Assoc.Prof.Dr.Theeralaksna Suddhasthira)

……..……

(Assoc.Prof.Prisana Pripatnanont)

……..……

(Asst.Prof.Settakorn Pongpanich)

……..……

(Assoc.Prof.Panyarak Ngamsritragul)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Oral and Maxillofacial Surgery

……

(Assoc.Prof.Dr.Teerapol Srichana)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……Signature

(Assoc.Prof.Prisana Pripatnanont)

Major Advisor

……Signature

(Mr.Faisal Omar Abdullah Balabid)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……Signature

(Mr.Faisal Omar Abdullah Balabid)

Candidate

Thesis Title	Effect of Periosteal Distraction by A Modified Hyrax Device with and without Platelet Rich Fibrin on Bone Formation in Rabbit's Model
Author	Mr.Faisal Omar Abdullah Balabid
Major Program	Oral and Maxillofacial Surgery
Academic Year	2013

Abstract

Objective: This study aimed to evaluate new bone formation by distraction of alveolar periosteum using modified design of hyrax device and platelet rich fibrin.

Material and Methods: Twelves rabbits were divided into 2 groups of 6 each according to the duration of consolidation period of 4 and 8 weeks. Both groups were divided into 4 subgroups: sham, PRF, device and device with PRF group to achieve 7mm distraction, 0.5mm twice a day.

Result: Histomorphometric evaluation, new bone formation was observed on the lateral and vertical side of the mandible of all groups. The device with PRF group showed highest bone formation followed by the device group.

Conclusion: Periosteal distraction osteogenesis can be an option for vertical ridge augmentation.

Acknowledgements

This research project would not have been possible without the support of many people. I am expressing my gratitude to Associate Professor **Prisana Pripatnanont,** Assistant Professor **Settakorn Pongpanich** and Assistant Professor **Surapong Vongvatcharanon** who were abundantly helpful and offered invaluable assistance, support and guidance. Deepest gratitude is also to the members of the supervisory committee Associate Professor Theeralaksna Suddhasthira and Associate Professor Panyarak Ngamsritragul, without whose knowledge and assistance this study would not have been successful. Special thanks also to all friends for invaluable assistance. I would like also to convey my thanks to the Faculty of Dentistry for providing the financial means and laboratory facilities as well as the entire staff in Prince of Songkla University.

My sincere thanks also to Assistant Professor Komgrid Charngkaew, Siriraj hospital, Mahidol University, for facilitating scanning of histological slides.

I am expressing my gratitude to my parents and family members; for their understanding & endless support, through the duration of my studies.

"Lord, enable me to appreciate the blessings You have bestowed upon me and upon my parents, and to act with righteousness, pleasing You. And improve my children for me. I have sincerely repented to You, and I am of those who have surrendered."

Faisal Omar Abdullah Balabid

Contents

List of Tables

List of Figures

List of Figures (Continued)

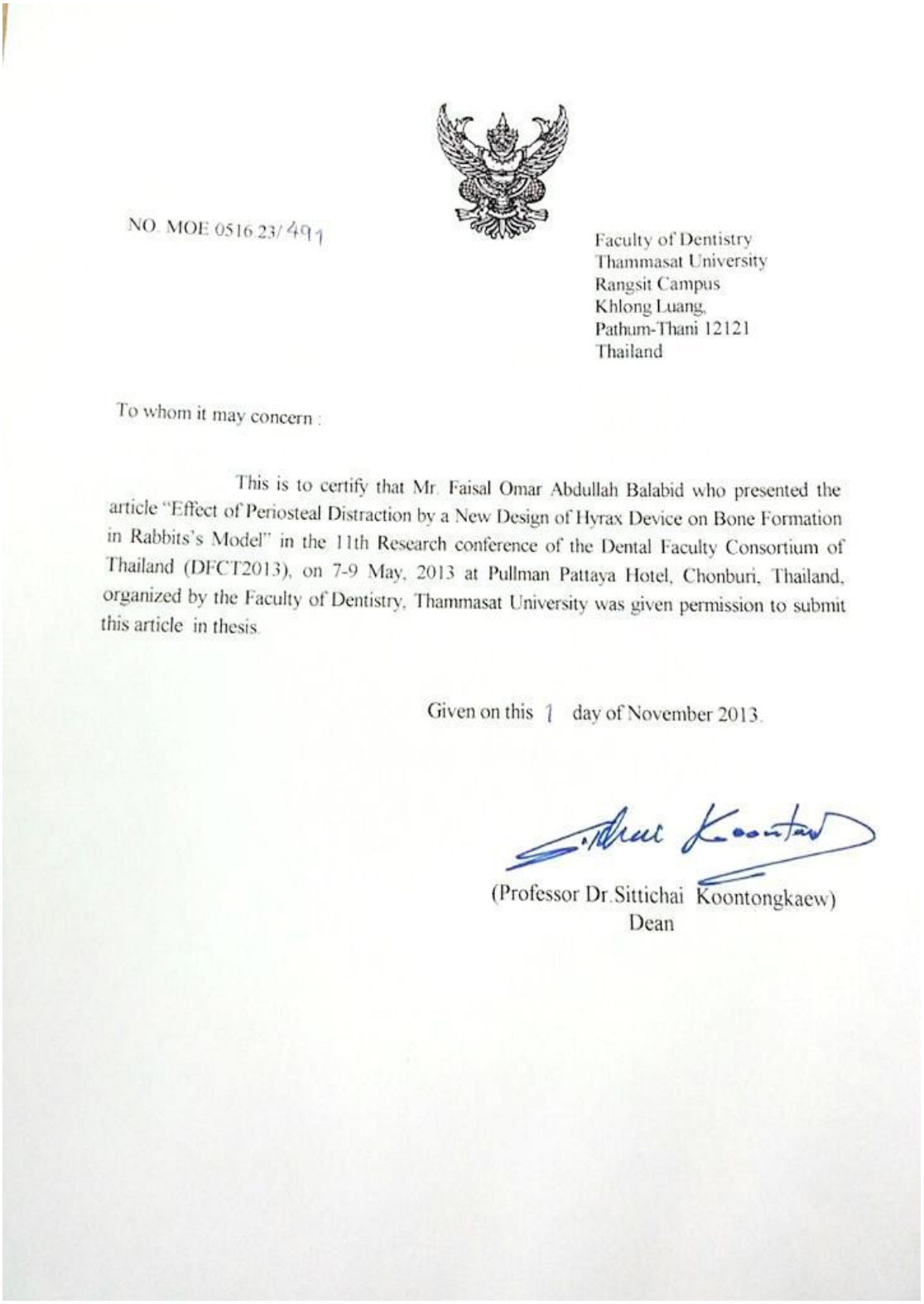
List of Abbreviations and Symbols

ANOVA	=	One-way analysis of Variance
BV/TV	=	Bone volume fraction
DL	=	Defect Line
2D	=	Two Dimension
et al	=	and others
H&E	=	Haematoxylin-eosin
Kg	=	Kilogram
kVp	=	Kilo voltage peak
Micro-CT	=	Micro Computed Tomography
Mm	=	Millimetre
NB	=	New Bone
PRF	=	Platelet Rich Fibrin
TR	=	Tooth Root
TRS	=	Tooth Root Space

List of Papers and Proceedings

1. Pripatnanont P, Balabid F, Pongpanich S**,** Vongvatcharanon S. Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model. ***Int J Oral Maxillofac Surg.***
2. Balabid F, Pripatnanont P**,** Pongpanich S**,** Vongvatcharanon **S**. EFFECT OF PERIOSTEAL DISTRACTION BY A NEW DESIGN OF HYRAX DEVICE ON BONE FORMATION IN RABBIT'S MODEL. ***The 11th Dental Faculty Consortium of Thailand Academic Meeting and Research Presentation (DFCT2013)*** May; 169-173.

Reprint were made with permission from the publisher



NO. MOE 0516.23/ 491

Faculty of Dentistry
Thammasat University
Rangsit Campus
Khlong Luang,
Pathum-Thani 12121
Thailand

To whom it may concern :

This is to certify that Mr. Faisal Omar Abdullah Balabid who presented the article "Effect of Periosteal Distraction by a New Design of Hyrax Device on Bone Formation in Rabbits's Model" in the 11th Research conference of the Dental Faculty Consortium of Thailand (DFCT2013), on 7-9 May, 2013 at Pullman Pattaya Hotel, Chonburi, Thailand, organized by the Faculty of Dentistry, Thammasat University was given permission to submit this article in thesis.

Given on this 1 day of November 2013.

(Professor Dr.Sittichai Koontongkaew)
Dean

Reprint were made with permission from the Proceeding

28-July-2014

Ms. Ref. No.: IJOMS-D-14-00601

Title: Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model International Journal of Oral & Maxillofacial Surgery

Dear Prof. Pripatnanont,

Your submission entitled "Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model" has been assigned the following manuscript number: IJOMS-D-14-00601.

You may check on the progress of your paper by logging on to the Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/ijoms/

Thank you for submitting your work to this journal.

Kind regards,

Jacqui Merrison

Editorial Office

International Journal of Oral & Maxillofacial Surgery

Introduction

Alveolar ridge height may be lost due to periodontitis or trauma or after dental extraction. If socket preservation is not performed immediately after extraction, the alveolus narrows and its vertical dimension may often be reduced and the defect may be difficult to manage[1]. In some cases alveolar bone loss can be severe. Severe bone loss may cause difficulty for the construction with a conventional prosthesis or being restored with dental implants. Vertical ridge augmentation remains a challenge in the reconstruction of the atrophic maxilla and mandible. Since there is the need to expand the soft-tissue envelope to achieve the contour of bony architecture [2].

Alveolar distraction osteogenesis (DO) is a technically challenging operation used to increase the bone height and width to gain both the bony contour and soft tissue. The most common complications of DO were insufficient bone formation following the consolidation period, regression of distraction distance and problems related to the distractor device. The type of device used and an augmentation rate of more than 0.5 mm/24 h were significantly related to insufficient bone formation and evidence of complications [3]. To avoid this complication and difficulties of device application, modified hyrax device to distract the periosteum without the need to distract the bone, it has some advantage's more than other technique, preservation the periosteum with need to diminishes the blood supply in periosteum while incision it during the operation, gain bone by activating the periosteum and maintain the gap gained between the alveolar ridge and periosteum from collapse. As from literature review, the *Periosteum* is a specialized connective tissue that forms the fibro-vascular membrane covering the entire surface of bone except for its articular cartilage, ligament or tendon insertions and the surface of sesamoid bones[4]. It consists of two discrete layers: the outer fibrous layer containing fibroblasts, vessels and fibers of Sharpey and the inner cambium layer containing nerves, capillaries, osteoblasts and undifferentiated mesenchymal stem cells (MSCs)[5]. In children the cambium layer contributes to increasing the diameter of the bone during growth. In adults, the cambium cells may be activated after mechanical stimulation (trauma), infection and by some tumors[4], also this layer serves as a reservoir of undifferentiated pluripotential mesenchymal cells, able to differentiate into chondrogenic and osteoblastic lineages and as a source of growth factors playing important role in the healing and remodelling process at the outer surface of the cortical bone[5].

Platelet-rich fibrin (PRF) is denfined as an autologous leukocyte- and platelet-rich fibrin (L-PRF) biomaterial [6]. PRF is a fibrin clot rich in platelets without addition of thrombin during preparation [7]. PRF derives from a natural and progressive polymerization occurring during centrifugation. A progressive or relatively slow polymerization mode may increase incorporation of the circulating cytokines in the fibrin meshes. Blood sample after centrifugation was divided into three layers: a base of red blood cells at the bottom, acellular plasma on the top, and a clot of PRF in the middle [6].Platelet-derived growth factor and TGF-β have been identified in PRF. It is hypothesized that these soluble molecules are trapped in the fibrin meshes of the PRF and can be released in a controllable, and relatively long-term effect[8].
A blood sample is taken without anticoagulant in 10-mL tubes which are immediately centrifuged at 3000 rpm for 10 minutes prior to anaesthesia.[9].The idea behind PRF uses as to serve as a growth factor and cytokines in surgical site and promoting the healing process.

Periosteal distraction osteogenesis elevates the periosteum alone without an osteotomy and is a more conservative approach [10]. In recent study, the distraction of periosteum done on the lateral surface of the mandibular rabbits model with cortical bone perforation, it shows that the decortication of the mandibular cortex may induced more bone formation than the non-decorticated one, they claim that there will be influx of growth factors and increased vascularization in decorticated group and the bone regenerated in this group shows more calcified bone because the newly formed bone was more radiopaque in radiographic analysis [11].

The amount of bone formed by dynamic elevation seems to be unlimited, being dependent on the height of the mesh [12], design of the device and the area were the periosteum distracted.

Combination of PDO with autologous growth factor in PRF could enhance vertical bone augmentation and there is no study evaluates the effects of PRF in PDO.

This study aimed to utilize a new technique to distract the periosteum and foster bone formation by using PRF.

Objective of the Study

General objective:

Analysis of new bone formation in the gab created by modified design of hyrax device in mandibular rabbit.

Specific objective:

1. To evaluate the modified design of hyrax device in periosteal distraction in superior and lateral aspect of mandible in rabbit.
2. To evaluate the platelet rich fibrin PRF in promoting newly formation of bone in the created space in the mandible.

Statement of the problem

Vertical and horizontal bone augmentation due to bone loss is a challenging in dentistry.

Significance of problem

Obtaining optimum aesthetic result for implant restoration required to gain bony architecture and soft tissue contour in defected area after bone loss.

Hypothesis

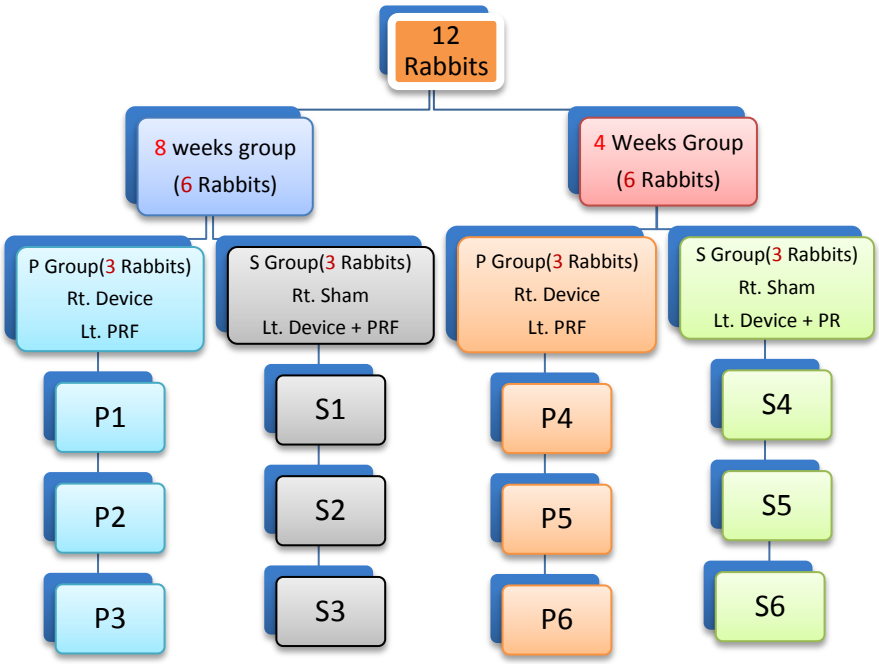
New bone formation could be gained in the gap created by distraction of the periosteum and may enhanced by PRF application.

Materials and Methods

Scope of the study

Twelves adult male New Zealand white rabbits have been used in this study with the mean weight of 3.5kg and approved by the Research committee of the animal care centre in Prince of Songkla University, Hatyai, Thailand. Twelves rabbits were divided into 2 groups of 6 each according to the duration of consolidation period of 4 and 8 weeks. Both groups were divided into 4 subgroups: Sham, PRF, Device and Device with PRF group to achieve 7mm distraction, 0.5mm twice a day.

Digital radiograph, micro computed tomography and histomorphometric analysis were performed for both consolidation periods of 4 and 8 weeks respectively. The result data were evaluated by using One-way ANOVA and Post Hoc test using Tukey HSD test. Significant was set at $P < 0.05$.



Materials

Hyrax device

Hyrax device were modified to a modified design by welding a titanium plate in one end of the device. The device was rigidly fixed on the lateral aspect of the mandible by using a 3 mini titanium screws with 3mm in length. While the other ends, the arms were modified in L shape to facilitate distraction of the periosteum.

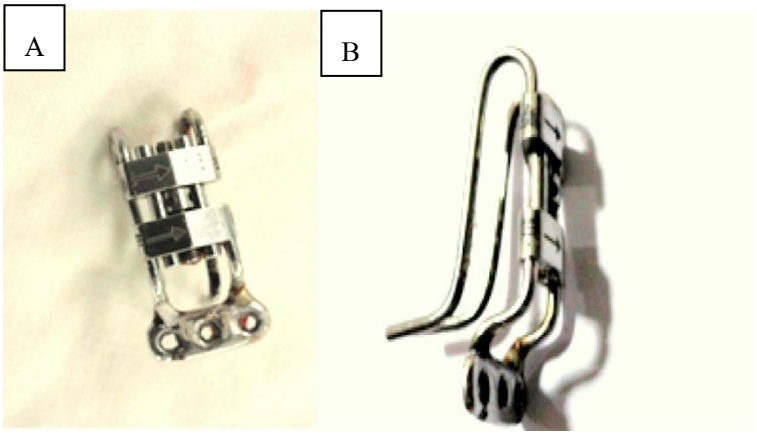


Figure 1. Modified design of Hyrax device

A) Frontal view

B) Lateral view

Platelet rich-fibrin (PRF) Preparation:

Before anaesthesia, 10ml of blood was drawn from central artery of the ear and placed into a glass tube, centrifuged immediately at 3000 revolutions/minute for 10 minutes using a table centrifuge (PC-02, Process Ltd., Nice, France). The result is the red blood cell layer at the bottom and PRF in the middle and platelet poor plasma on the top of the tube and the PRF were separated from unwanted blood.

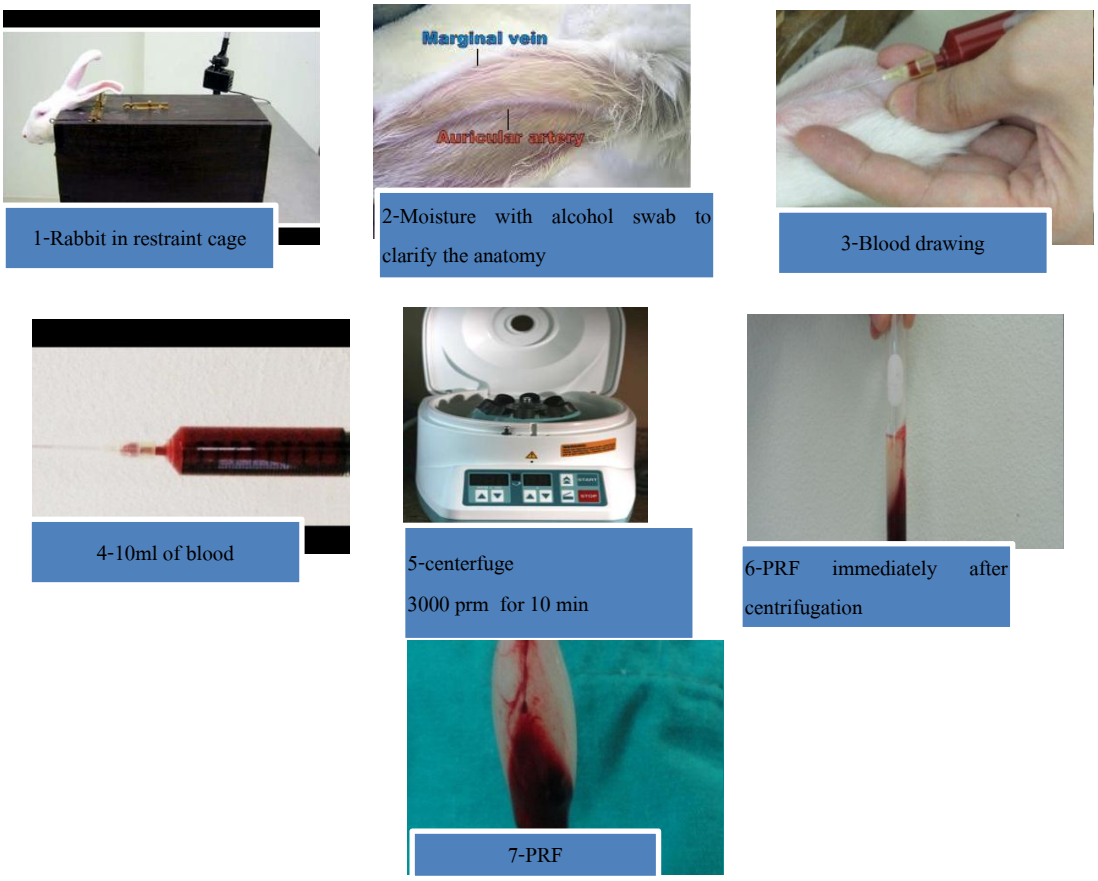


Figure 2. PRF preparation Procedure

Surgical procedure

Under general anaesthesia (25 mg/kg Ketamine and Diazepam 5mg/kg I.V of ear's vein) and local infiltrative anaesthesia (lignocaine 2%).

The surgical site was shaved and prepared with 10% iodine solution to maintain aseptic conditions. A 2-cm incision in the skin along the inferior border of the mandible done. Dissection Perform through the subcutaneous and muscle layers. The periosteum was elevated & the lateral aspect of the mandibular body was exposed. The mandibular height was measured from the lower border of the mandible to the most superior surface height of the mandible; a 2mm defect was created from the height of alveolar process with 3mm in width.

A modified palatal expansion screw was used as periosteal distraction device and connected with chrome-cobalt plate with 3 holes to fix it in the buccal cortex with mini-screw, and the length of each screw is 3mm.

The long arms of the distraction device were placed over the ridge of alveolar process and inserted by making 2 stab incisions over the skin. The wound was closed in layers, using 4-0 Vicryl sutures (Ethicon, Coated VICRYL).

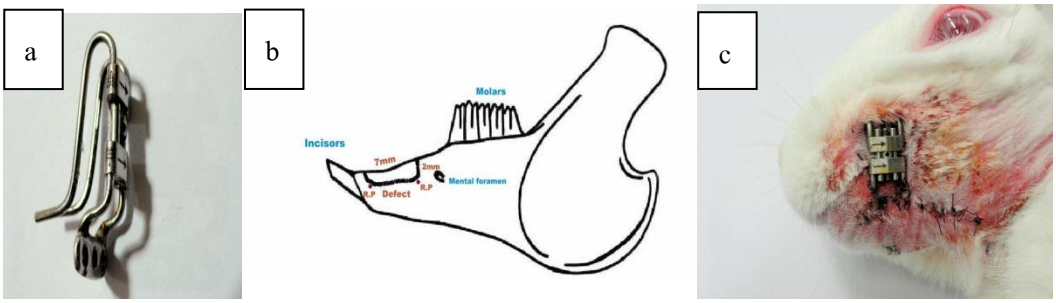


Figure 3. (a) Modified design of Hyrax device

(b) Scheme for planned surgical defected site

(c) Rigid Fixatin of the device on the lateral sapect of the mandible

1-*Povidone-Iodine Scrubto surgical site.*

2-Local anesthetic injection to surgical site

3-Flap raising until reach to lateral aspect of the mandible

4-Gutta percha application on both end site of surgical defect

5-Application of the device

6-Device fixation

7-Suturing of the surgical site.

Figure 4. Surgical procedure

Digital radiography

Digital radiograph were taken by portable x-ray device (NOMAD, Aribex Inc, USA) (Figure 5-A.B) using digital sensor size 0 attached to digital sensor holder (XCP-DS, Rinn, Densply, IL, USA) (Figure 5-C) for controlling the vertical distance of 8cm. the exposure were set at 60kVp, 2.3mA and 0.3 sec.

Images were captured on receptor (Sopix CMOS, Instrumentarium Dental, Tuusula, Finland).

The mean of distracted area in pixels was calculated and analysed by using Image Pro Plus 7.0 software (Media Cybernetics Inc., Silver Spring, MD, USA).

Figure 5. A.B-portable x-ray device, C-digital sensor

Specimen processing

The total width of the defect created in the free alveolus of the rabbits mandible was 7mm, however , the total defected area was divided into two half's and underwent for cutting procedure 3.5mm each.

After the cutting procedure, one half (distal half) underwent for histomorphometric analysis while the other half (mesial half) underwent for micro-CT analysis.

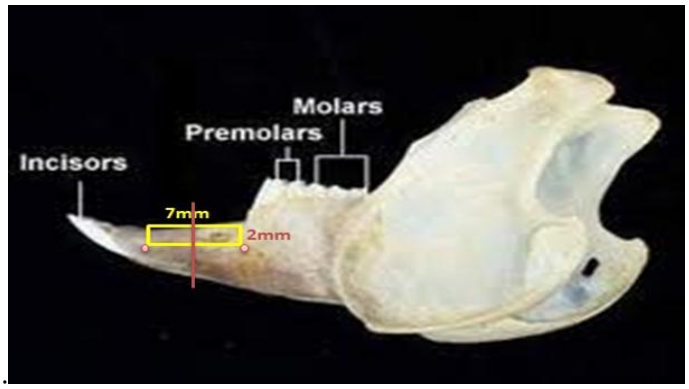


Figure 6. Mid-coronal cutting of the defected surgical site

Histology Processing

Distal halves were decalcified (by 10% formic acid) and stained with haematoxylin and eosin.

Hematoxylin and eosin (H&E) stains have been used for this study as this stains are still essential for recognizing various tissue types (figure 7). Hematoxylin has a deep blue-purple colour and stains nucleic acids. Eosin is pink and stains proteins non-specifically. In a typical tissue, nuclei are stained blue, whereas the cytoplasm and extracellular matrix have varying degrees of pink staining [13].

Figure 7. Microscopic slide of the distal half of mandibular rabbit's stained with Haematoxylin and eosin stain.

Histomorphometric analysis

All prepared histological slides from the distal half of the mandibles underwent for histological slide scanning using Aperios ScanScope System in Siriraj hospital, Mahidol University, Bangkok.

Computerized Measurements were achieved for all scanned slides using image scope program (Aperio ImageScope 2006, Inc.UK.).

The new bone formed were been recognized from the adjacent bone by identifying the defected line (DL) that has been done during the surgery.

The new formed bone (NB) outlined and the mean percentage of new bone estimated by dividing the percentage of newly formed bone over the total surface area.

Figure 8. Aperios ScanScope

Micro-CT analysis

Each specimen underwent for scanning using the Micor-CT (Micro-CT80, Scanco, Medica, AG, Basseerdorf, Swistzerland).

After the scanning completed, 10 slides images (represented the best view of the defected region with a new bone formation) was selected.

The new bone (BV) was calculated dividing the mean percentage of the new bone formed and the total bone volume for each slide selected.

Figure 9. Micro-CT80

Results

Clinical observations

All animals tolerated surgery and recovered well but two animals encountered dislodgement of the devices due to scratching to the device. Neck collar was later applied 3 days postoperatively to secure the device from animal scratching.

Gross specimen observation

Specimens were harvested at 4 and 8 weeks respectively. At 4 and 8 weeks group, PRF and Sham group showed normal contour at surgical defected side with firm consistency while the Device and Device with PRF group showed bulging of the distracted surgical defected side in lateral and superior side with firm consistency.

In all specimens there was no sign of laceration or dehiscence.

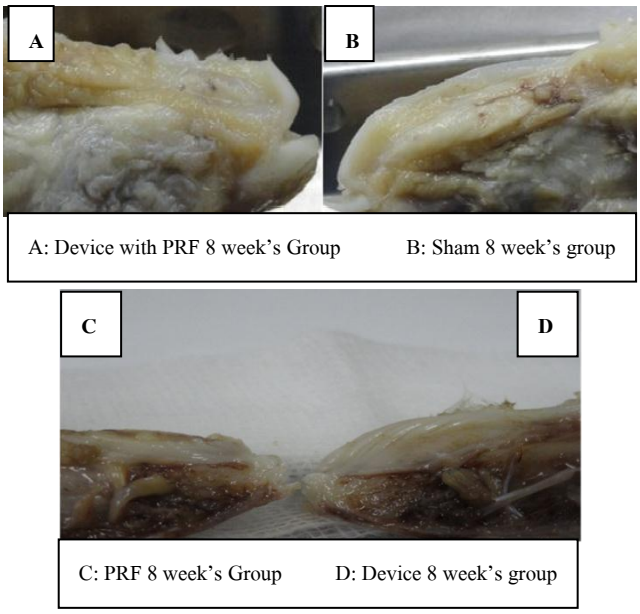


Figure 1. Gross evaluation of the 8 weeks group specimen

A) Device with PRF group, bulging of the distracted surgical defected site

B) Sham group, Normal contour of the surgical defected site

C) PRF group, Normal contour of the surgical defected site

D) Device group, bulging of the distracted surgical defected site

Digital Radiographic Evaluation

In all groups, Device with PRF, Device, PRF, and sham, digital image was done from tow views, Lateral-oblique view and lateral view.

In lateral view, all groups showed bone formation at the surgical defected side. Reference points (gutta percha) obvious noticed.

In Lateral-oblique view, Device with PRF and Device group showed lateral bone formation and has been analysed in term of pixels while the PRF and Sham group there was no bone can be noticed on the lateral aspect.

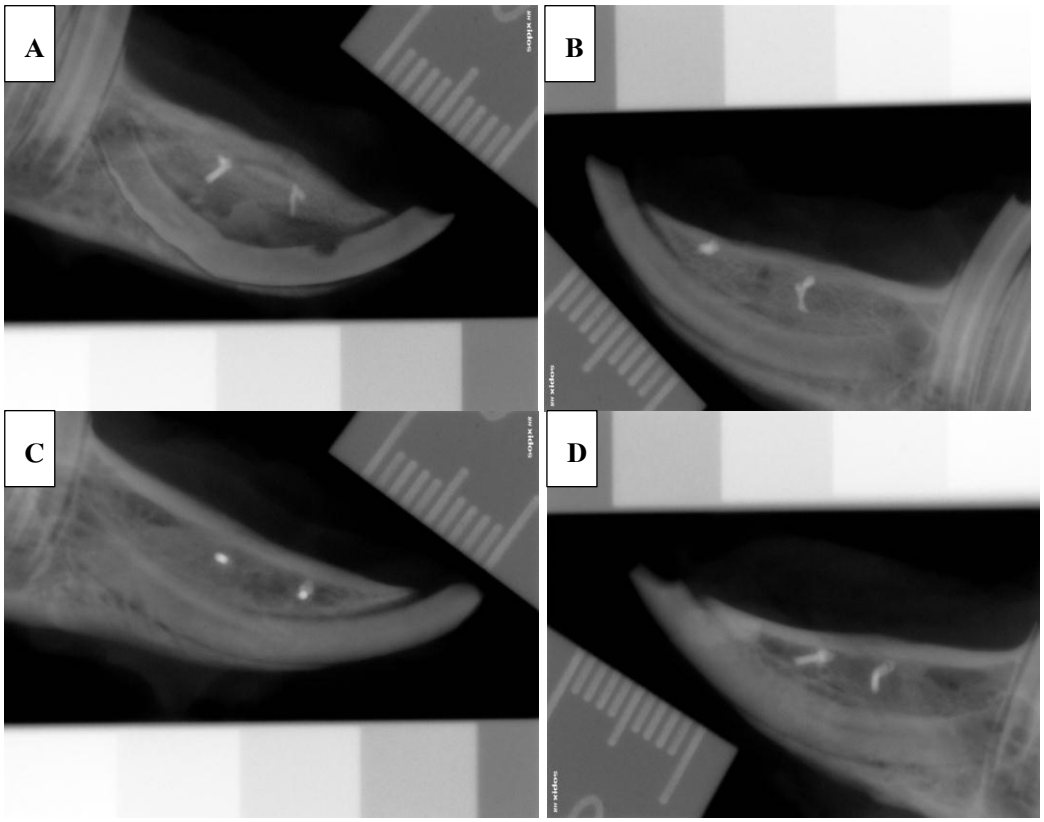


Figure 2. Lateral view of mandibular radiograph in 4 weeks group

A) Device group

B) PRF group

C) Sham group

D) Device with PRF group

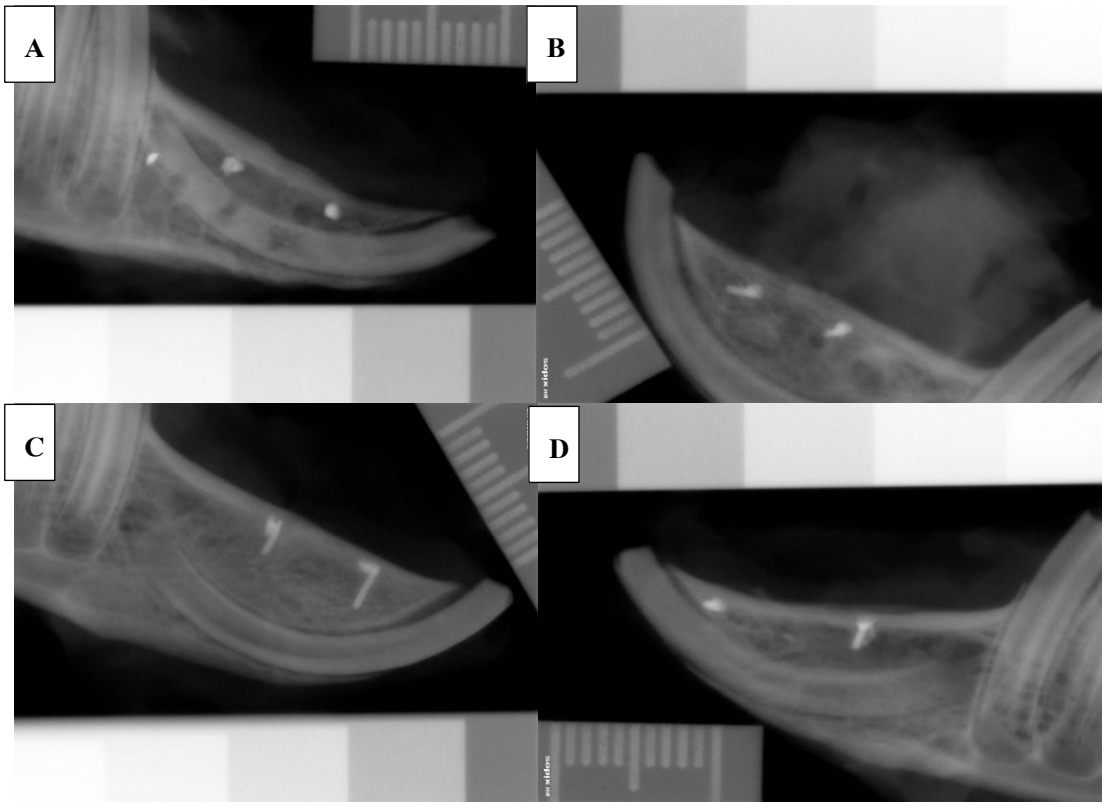


Figure 3. Lateral view of mandibular radiograph in 8 weeks group

A) Device group

B) PRF group

C) Sham group

D) Device with PRF group

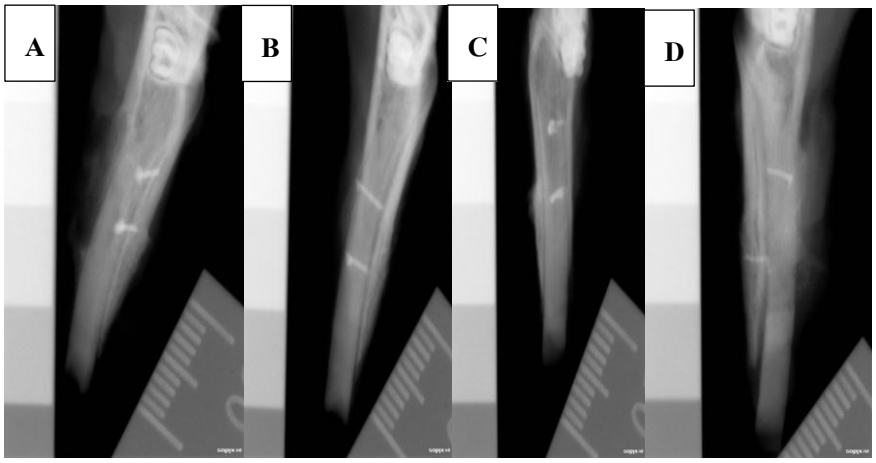


Figure 4. Lateral-oblique view of mandibular radiograph in 4 weeks group

A) Device group

B) PRF group

C) Sham group

D) Device with PRF group

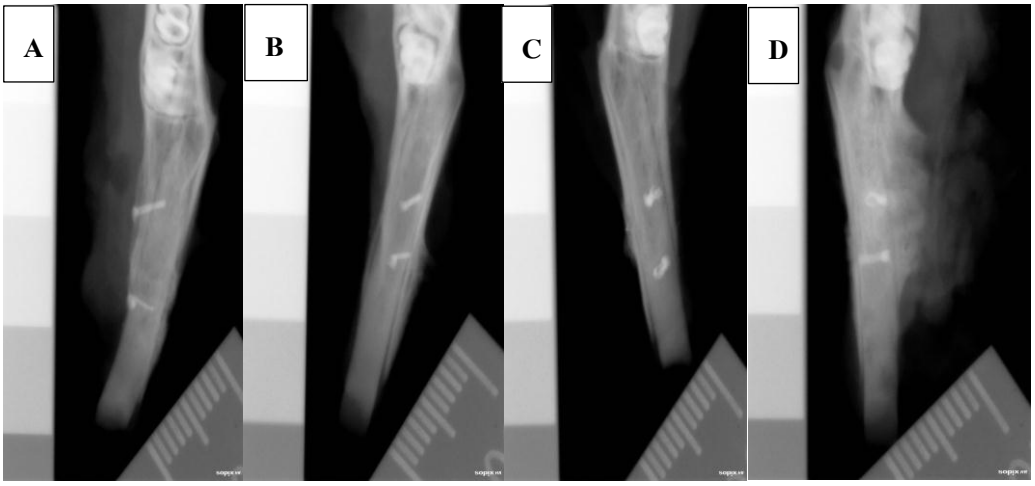


Figure 5. Lateral-oblique view of mandibular radiograph in 8 weeks group

A) Device group

B) PRF group

C) Sham group

D) Device with PRF group

Radiographic analysis

The mean of distracted area in radiographic analysis were been evaluated in the Device with PRF and Device group only while the PRF and Sham groups there was no bone detected from Lateral-oblique view in x-ray evaluation.

The mean of distracted area in pixel for the Device with PRF group in 4 weeks consolidation period is (2.24×10^2) while in Device group is (2.63×10^2). Device with PRF significantly different from the Device group only, (p-value =0.039).

In 8 weeks consolidation period, the mean of distracted area in pixel for the Device with PRF is (2.84×10^2) and in Device group is (8.49×10^2). Device with PRF significantly different from Device group only, (p-value= 0.030).

Table 1. Radiographic analysis data of the distracted surface in Device and Device with PRF group

Group	**4 weeks-Bone Surface -Pixels**	**8 weeks-Bone surface- Pixels**
Device with PRF	6.63×10^2	8.49×10^2
Device	2.24×10^2	2.84×10^2
P Value	0.039	0.030

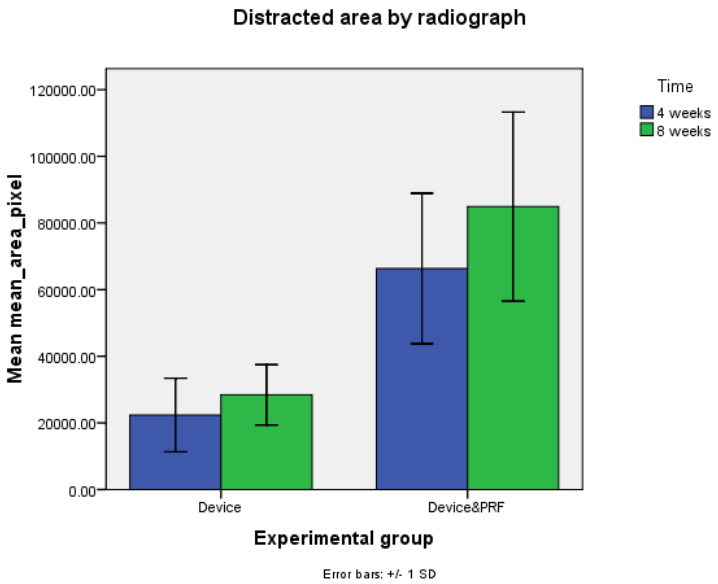


Figure 6. Distracted area by radiograph in the Device with PRF and Device groups

Histological evaluation

All groups showed an increase of new bone over the created defect particularly in the groups of device either with or without PRF.

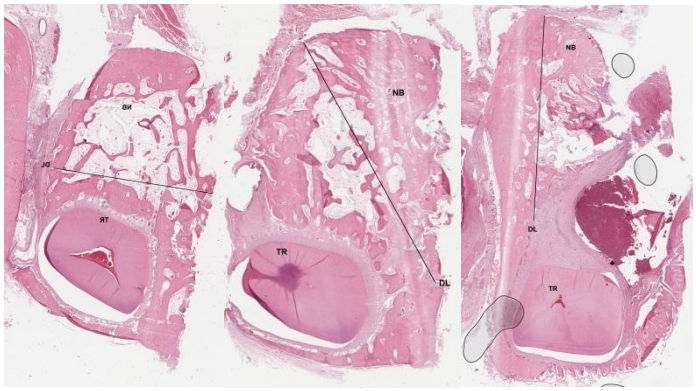
The Device group both with and without PRF showed more bone formation when compared with PRF or Sham group in both consolidation periods of 4 and 8 weeks. Some collapse was observed in 8-week group.

Device group

Increase in the width of the new bone formed in the defected area as well as the space gained from distraction of the periosteum.

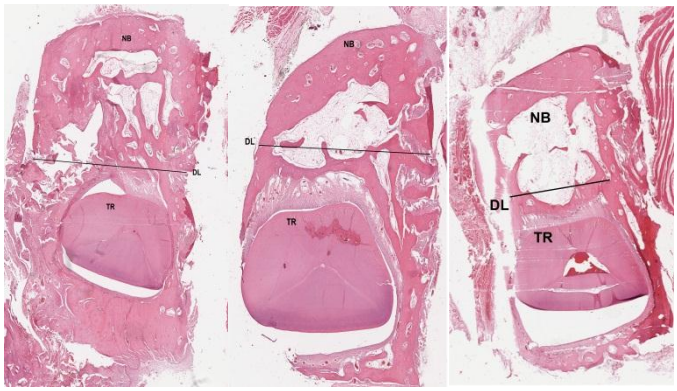
In 4 week's consolidation period group; mature bone observed and thick cortical bone and more fatty tissue between the tooth root and the cortical bone when compared to the 8 weeks group.

In 8 week's consolidation periods group; less fatty tissue observed between the tooth root and the cortical bone, denser and thicker cortical bone when compared to 4 weeks consolidation period.



(I) (II) (III)

Figure 7. Histological slide of Device group in 4 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root



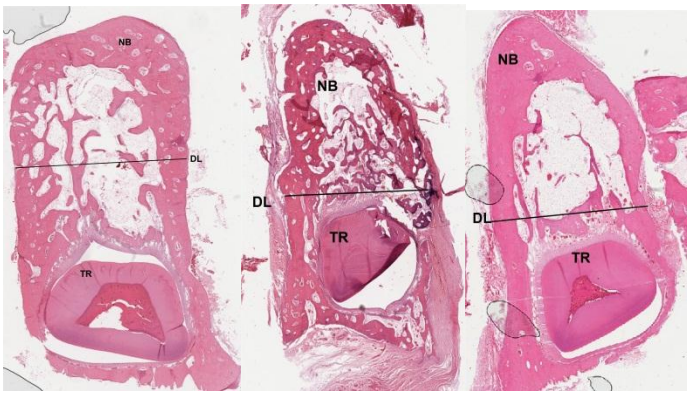
(IV) (V) (VI)

Figure 8. Histological slide of Device group in 8 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root

Device with PRF group

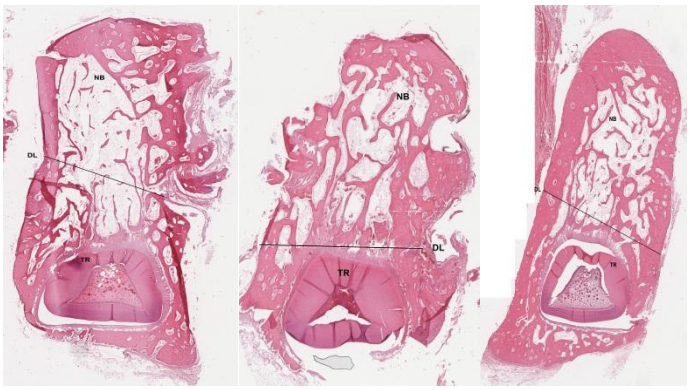
In 4 week's consolidation period group, thick cortical bone observed and more fatty tissue in-between the tooth root and the cortical bone.

In 8 week's consolidation period group, a thick cortical bone formed same as 4 weeks consolidation period, more trabecular bone observed in the fatty space in-between the tooth root and the cortical bone when compared to the 4 weeks consolidation period.



(VII) (VIII) (IX)

Figure 9. Histological slide of Device with PRF group in 4 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root



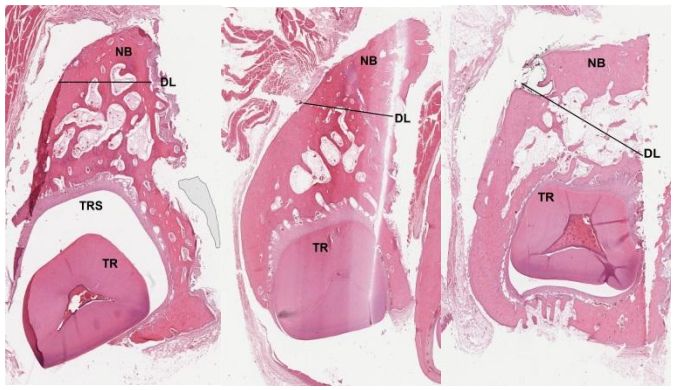
(X) (XI) (XII)

Figure 10. Histological slide of Device with PRF group in 8 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root

PRF group

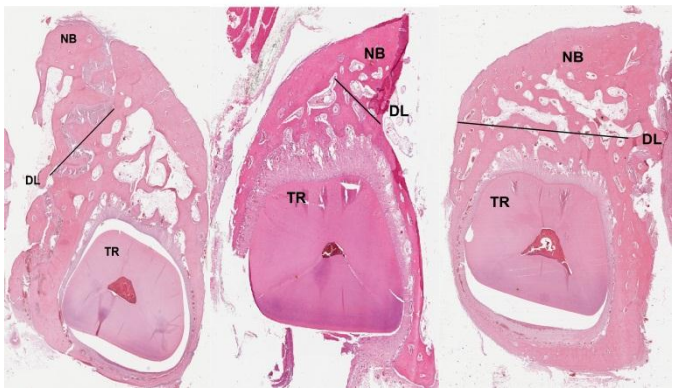
In 4 week's consolidation period group; a bony contour formed in the defect created with a thick cortical bone.

In 8 week's consolidation period group; new bone formed in the defect space created and gained normal contour but thicker and denser cortical bone when compared to 4 weeks consolidation period group.



(XIII) (XIV) (XV)

Figure 11. Histological slide of PRF group in 4 weeks consolidation period, NB New Bone; DL Defect Line; TR Tooth Root



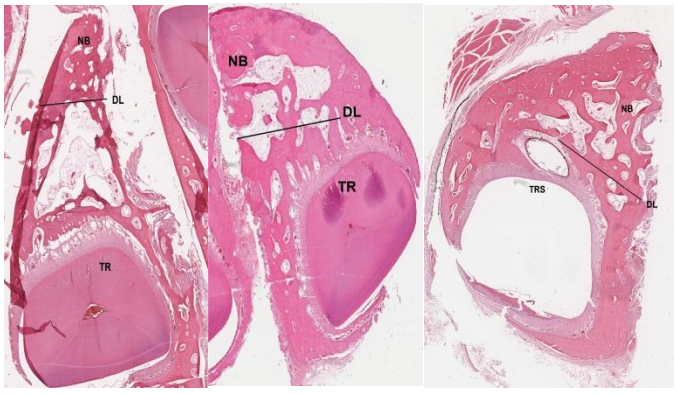
(XVI) (XVII) (XVIII)

Figure 12. Histological slide of PRF group in 8 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root

Sham group

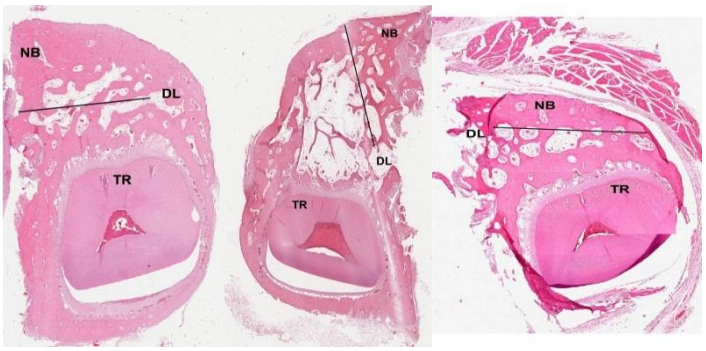
In 4 week's consolidation period; a new bone formed in the defected space and gained normal contour of the alveolus.

In 8 week's consolidation period group; a denser cortical bone formed when compared to 4 weeks group



(XIX) (XX) (XXI)

Figure 13. Histological slide of Sham group in 4 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root



(XXII) (XXIII) (XXIV)

Figure 14. Histological slide of Sham group in 8 weeks consolidation period; NB New Bone; DL Defect Line; TR Tooth Root

Histomorphometric analysis

Histomorphometric measurements of the mean percentage of the new bone (NB) formed and total surface areas, separated by the defect line (DL), were done. All groups gained new bone formation. Significant bone formation ($p<.01$) were seen in the device with PRF and device alone when compared with PRF or Shame group.

Table 2. Histomorphometric value analysis of the bone surface area in both consolidation periods

Group	**4 weeks-Bone Surface -mm^2**	**8 weeks-Bone surface-mm^2**
Device	33.22a	23.24b
Device with PRF	41.36b	55.46d
PRF	12.26	13.77
Sham	14.72	13.16

a Device group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

b Device group significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

c Device with PRF group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

d Device with PRF significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

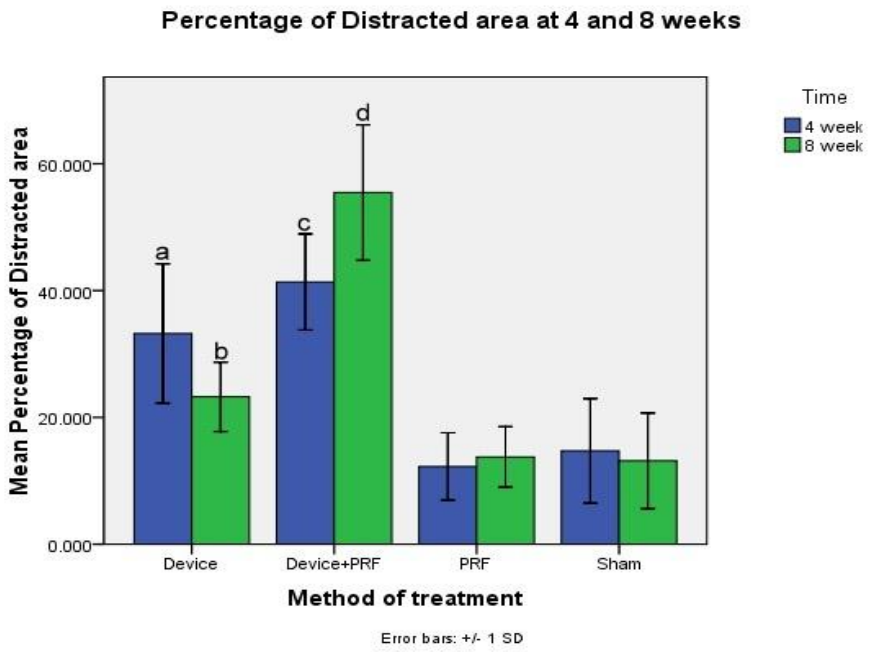


Figure 15. Histogram of the Mean percentage of distracted area in hypnological section

a Device group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

b Device group significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

c Device with PRF group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

d Device with PRF significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

Micro-CT analysis

The bone surface area in squared millimetre evaluated by calculating the new bone formed from the total surface area (BV/TV).

In 4 weeks consolidation period, significant bone volume showed in Device and Device with PRF group when compared to PRF or Sham group.

There is no significant difference between Device and Device with PRF group. In 8 weeks group, significant bone volume occurred in Device and Device with PRF when

compared to PRF or Sham group. No significant difference between the Device and Device with PRF.

Table 3. Micro-CT value analysis of the bone surface area in both consolidation periods

Group	**4 weeks-Bone Surface –mm^3**	**8 weeks-Bone surface-mm^3**
Device	33.00a	30.00b
Device with PRF	56.67c	49.00d
PRF	12.67	10.33
Sham	15.00	14.50

a Device group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

b Device group significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

c Device with PRF group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

d Device with PRF significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

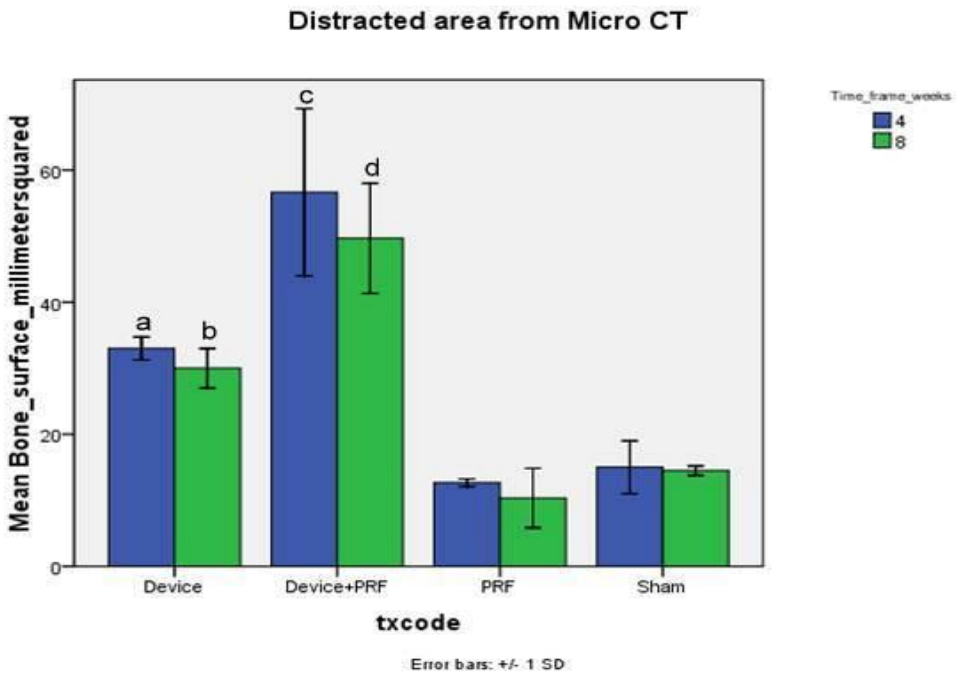


Figure 16. Histogram of the mean bone surface area in micro-CT analysis

a Device group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

b Device group significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

c Device with PRF group significantly different $p<0.05$ than PRF and Sham group in 4 weeks consolidation period

d Device with PRF significantly different $p<0.05$ than PRF and Sham group in 8 weeks consolidation period

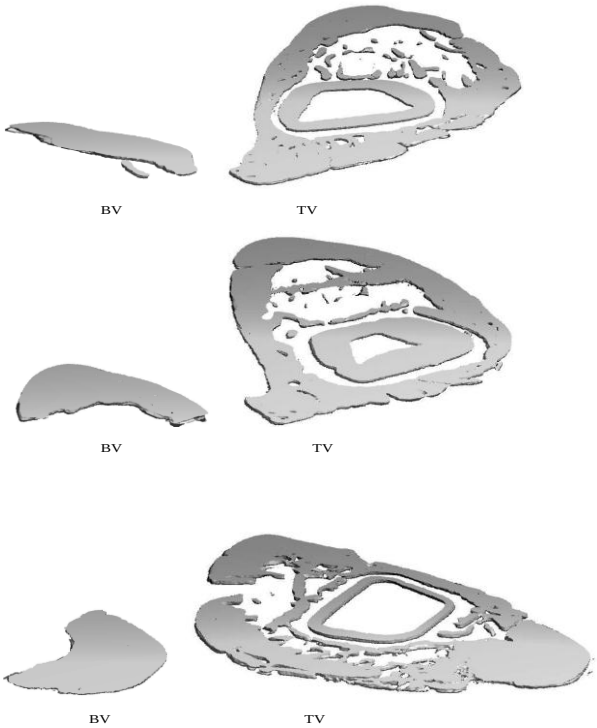


Figure 17. Micro-CT images of PRF group in 4 weeks consolidation period; BV Bone Volume; TV Total Volume

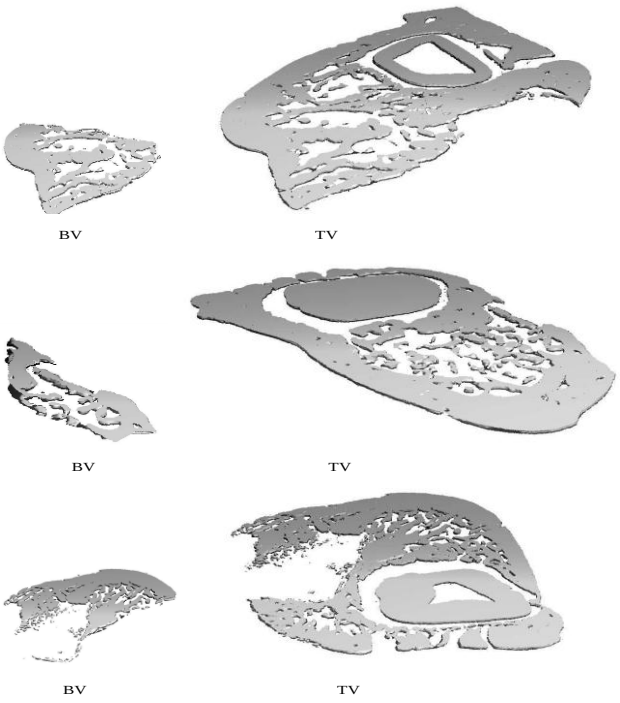


Figure 18. Micro-CT images of Device group in 4 weeks consolidation period; BV Bone Volume; TV Total Volume

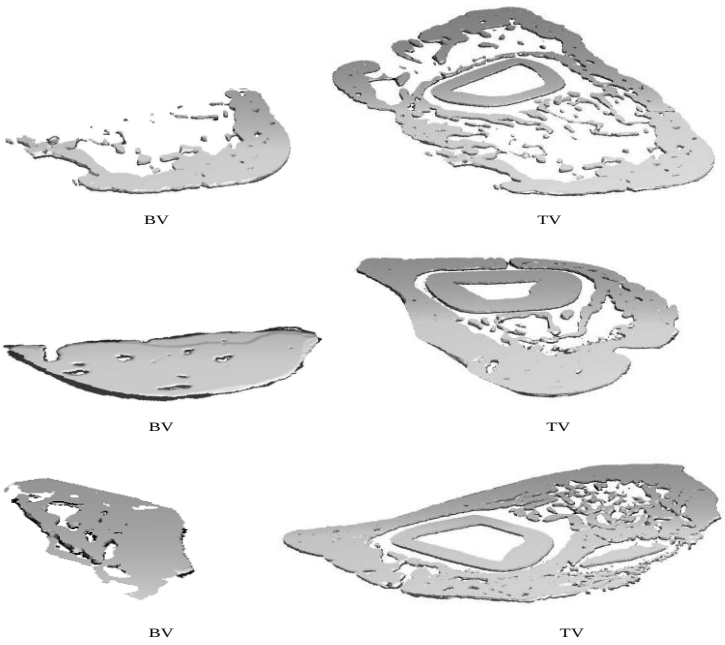


Figure 19. Micro-CT images of Device with PRF group in 4 weeks consolidation period; BV Bone Volume; TV Total Volume

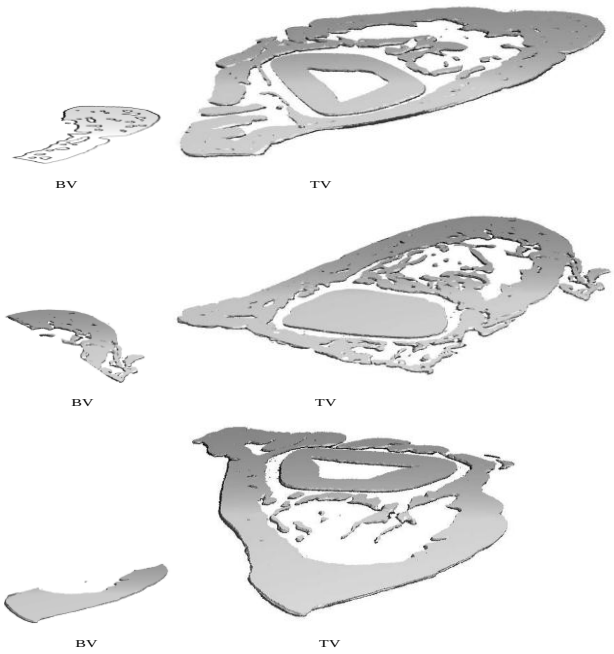


Figure 20. Micro-CT images of Sham group in 4 weeks consolidation period; BV Bone Volume; TV Total Volume

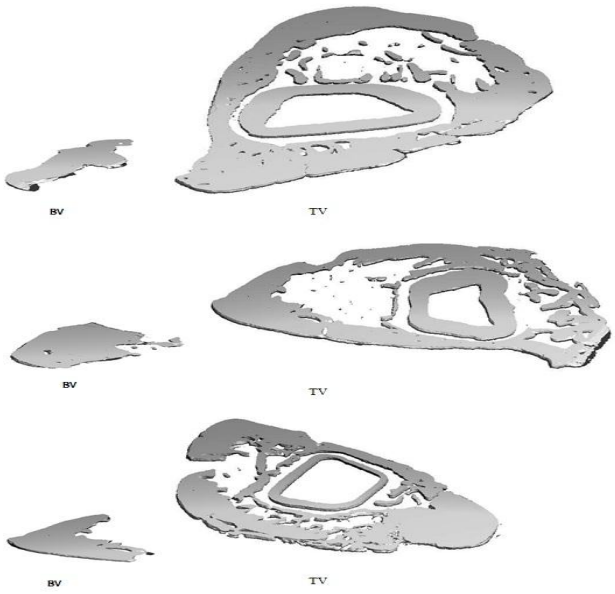


Figure 21. Micro-CT images of PRF group in 8 weeks consolidation period; BV Bone Volume; TV Total Volume

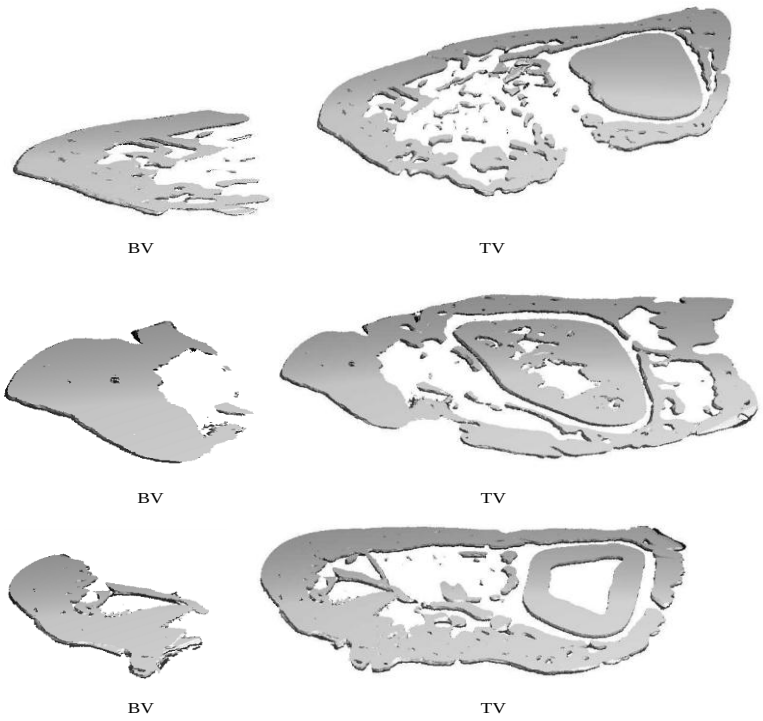


Figure 22. Micro-CT images of Device group in 8 weeks consolidation period; BV Bone Volume; TV Total Volume

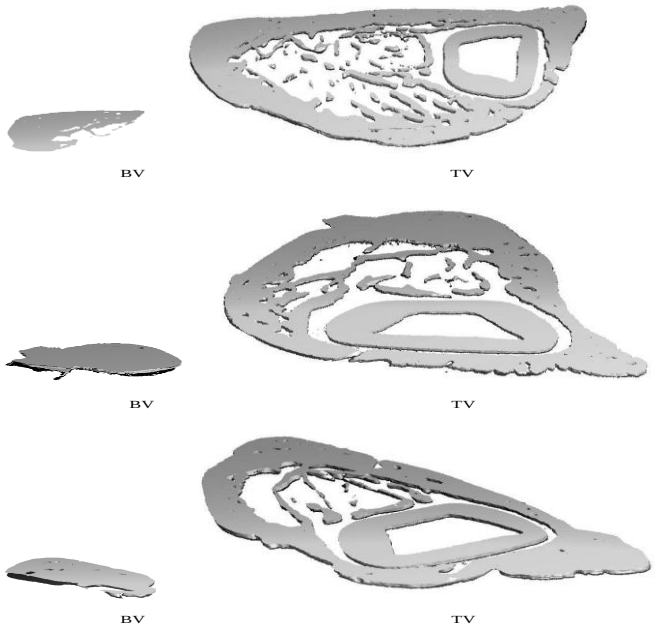


Figure 23. Micro-CT images of Sham group in 8 weeks consolidation period; BV Bone Volume; TV Total Volume

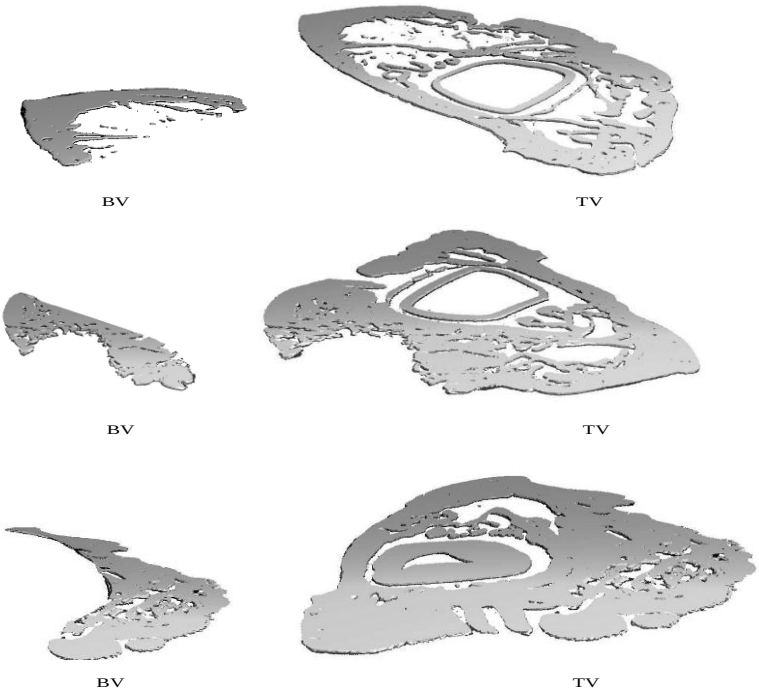


Figure 24. Micro-CT images of Device and PRF group in 8 weeks consolidation period; BV Bone Volume, TV Total Volume

Discussions

Severe bone loss in alveolar bone may cause difficulty to reconstruct with a conventional prosthesis or restored with dental implants. Ridge augmentation remains a challenge in the reconstruction of the atrophic maxilla and mandible. Since there is the need to expand the soft-tissue envelope to achieve the contour of bony architecture [2] either with block or particulate graft with guided bone regeneration. Alveolar distraction osteogenesis (DO) is a technically challenging operation used to increase bone height and width to gain both the bony contour and soft tissue. The most common complications of DO were insufficient bone formation following the consolidation period, regression of distraction distance and problems related to the distractor device.

Periosteal distraction osteogenesis (PDO) is a combination method of tissue expansion, distraction osteogenesis (DO) and guided tissue regeneration and when compared with other techniques it represents advantages over the other techniques. Unlike distraction osteogenesis where it need cut of bone in segments, therefore, no risk of teeth injuries, nerves damage, bone segment fractures and no donor site morbidity [14]. The amount of bone formation is seems directly proportion with space created by the elevation of the periosteum, therefor, the amount of bone is unlimited [15]. In addition, no bone resorption as seen in autologous bone grafting technique [16, 17] and the bone formed after distraction of the periosteum results in three dimensional bone formations.

Periosteal distraction osteogenesis (PDO) is widely accepted procedure for maxillofacial skeleton [18-20].

To avoid this complication and difficulties of device application, the hyrax device was modified to distract the periosteum without bone distraction and gain bone by activating the periosteum and maintain the gap gained with the device.

Lack of bone marrow cells may play a role in the occurrence of fatty tissue [21]. Periosteal distraction with decortication might be effective in promoting bone formation [11]; in this study cortical bone defect was performed to facilitate access of the bone marrow cells into the distracted site. Stimulatory forces may affect the maturity of the newly formed bone [22-24]; however, the mandibular bone receives masticatory forces, and this may affect the quality of new bone.

Periosteal Distraction Osteogenesis PDO can be applied in the maxillofacial area to treat small bone defects with minimal trauma compared to Distraction Osteogenesis DO [25]. On the other study, an Osteogenesis done by gradually expanding the interface between bone surface and periosteum in rabbit models. Mesenchymal stem cells were administered into the gap, the bone volume, height, bone mineral density, and bone mineral content increased significantly in newly formed bone tissue [26].

Animal model selection

Several advantages for choosing the rabbits model in this study such as, the size of Hyrax device commercially available and the rabbit's mandible size that can be easy to attach the device in the lateral aspect of the mandible and on other hand, handling of the animal is relatively easy pre and post-operative. The bone maturity of the rabbits is quite similar to those in humans. Cost and availability of the animal relatively low when compared to other animals. Housing and postoperative cost considered to be relatively low. Existing knowledge and experience for this kind of animal.

Two times interval of consolidation period of 4 and 8 weeks respectively were selected in this study to evaluate the bone generated in different period of time. Use of platelet-rich fibrin has several advantages. Platelet-rich fibrin does not use bovine thrombin or other exogenous activators in the preparation process. It forms a liquid-like matrix that comprises high concentrations of non-activated, functional, intact platelets, contained within a fibrin matrix, that release, a concentration of growth factors that is relatively constant over a 7 days period. In membrane form, it can be employed as fibrin bandage acting as a matrix to accelerate the healing of defects created. Additionally, PRF is autologous in nature making it relatively cheap as no additional expenses for synthetic membranes are added to patients. The chair side PRF preparation is also quite easy, fast and simple.

Radiographic analysis

Digital radiographic evaluation involved two dimensions analysis, therefor; some bone on the third vector of space cannot be seen or evaluated. In this study the bone on the lateral-oblique view for Sham or PRF groups did not show bone formation.

On the lateral view, all defected mandible regenerated among all groups.

Histological analysis

The histological evaluation confirmed that the surgical defected mandible healed in all groups, Device, Device with PRF, PRF, and Sham groups for both consolidation periods.

Two animals encountered dislodgement of the devices due to scratching to the device. Neck collar was later applied 3 days postoperatively to secure the device from animal scratching. In histomorphometric analysis the in four weeks group, the device group showed more bone formation than the Device with PRF group while in the eight weeks group, the Device with PRF group showed more bone formation than the device only and this is due to neck collar applied for other animal and prevented the animal from scratching. The amount of bone formed among two groups in both consolidation period of four and eight weeks is not significantly different.

Histomorphometric analysis revealed that the quantity of newly formed bone in 8weeks group was greater than in 4 weeks group and this was in accordance with the results reported by Sencimen et al.

On a study evaluate the quantity of newly formed bone by PDO in 60-day specimens was greater than in 15 or 30 day specimens [21, 27, 28].

No differences were observed between the newly formed bone and the native bone from the 15 to 60 days of PDO experimental groups [27]. The distraction area was rich in interstitial fatty tissues [21].

Continuous stimulation must be applied to mature newly formed bone in a particular direction [29, 30].

Micro-CT analysis

In micro-CT evaluation, the amount of newly formed bone from of the total surface area in both groups of Device and Device with PRF significantly different from the PRF or Sham group in both consolidation periods. The result supported the histomorphometric analysis.

The device with PRF showed more bone formation than the Device group only in both consolidation periods and not significantly different for the Device with PRF or the Devise only.

Conclusions

Distraction of the periosteum using modified design of Hyrax device with PRF is a promising method to gain bone formation in alveolar reconstruction particularly vertical bone augmentation. The device should further developed to gain effective and more predictable result to be used clinically.

Reference List

1. Laster, Z., G. Cohen, and R. Nagler, A novel technique for vertical bone augmentation in the premaxillary region. ***J Oral Maxillofac Surg***, 2009. 67(12): p. 2669-72.
2. Louis, P.J., Vertical ridge augmentation using titanium mesh. ***Oral Maxillofac Surg Clin North Am***, 2010. 22(3): p. 353-68, v.
3. Saulacic, N., J. Zix, and T. Iizuka, Complication rates and associated factors in alveolar distraction osteogenesis: a comprehensive review. ***Int J Oral Maxillofac Surg***, 2009. 38(3): p. 210-7.
4. Simon, T.M., et al., Cambium cell stimulation from surgical release of the periosteum. ***J Orthop Res***, 2003. 21(3): p. 470-480.
5. Malizos, K.N. and L.K. Papatheodorou, The heating potential of the periosteum Molecular aspects. ***Injury Int.J.Care Injured***, 2005. 36: p. 13-19.
6. Ehrenfest, D.M.D., et al., Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. ***Arch Oral Biol***, 2010. 55(3): p. 185-194.
7. He, L., et al., A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2009. 108(5): p. 707-713.
8. Lundquist, R., M.H. Dziegiel, and M.S. Agren, Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. ***Wound Repair Regen***, 2008. 16(3): p. 356-363.
9. Choukroun, J., et al., Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2006. 101(3): p. 299-303.
10. Estrada, J.I., et al., Periosteal distraction osteogenesis: preliminary experimental evaluation in rabbits and dogs. ***Br J Oral Maxillofac Surg***, 2007. 45(5): p. 402-5.
11. Oda, T., K. Kinoshita, and M. Ueda, Effects of cortical bone perforation on periosteal distraction: an experimental study in the rabbit mandible. ***J Oral Maxillofac Surg***, 2009. 67(7): p. 1478-85.

12. Kessler, P., et al., Dynamic periosteal elevation. ***Br J Oral Maxillofac Surg***, 2007. 45(4): p. 284-7.
13. Nagata, N., et al., Characterization of amiodarone pneumonitis as related to inflammatory cells and surfactant apoprotein. ***Chest***, 1997. 112(4): p. 1068-74.
14. Neukam, F.W., [Bone transplantation in combination with implants]. ***Niedersachs Zahnarztebl***, 1990. 25(8): p. 446-50.
15. Yamauchi, K., et al., Periosteal expansion osteogenesis using highly purified beta-tricalcium phosphate blocks: a pilot study in dogs. ***J Periodontol***, 2008. 79(6): p. 999-1005.
16. Tudor, C., et al., Bone regeneration in osseous defects-application of particulated human and bovine materials. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2008. 105(4): p. 430-6.
17. Schlegel, K.A., et al., Sinus floor elevation using autogenous bone or bone substitute combined with platelet-rich plasma. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2007. 104(3): p. e15-25.
18. Cope, J.B., M.L. Samchukov, and D.E. Muirhead, Distraction osteogenesis and histogenesis in beagle dogs: the effect of gradual mandibular osteodistraction on bone and gingiva. ***J Periodontol***, 2002. 73(3): p. 271-82.
19. Oda, T., et al., Segmental mandibular reconstruction by distraction osteogenesis under skin flaps. ***Int J Oral Maxillofac Surg***, 1998. 27(1): p. 9-13.
20. Rachmiel, A., et al., Distraction osteogenesis for tracheostomy dependent children with severe micrognathia. ***J Craniofac Surg***, 2012. 23(2): p. 459-63.
21. Altug, H.A., et al., Histomorphometric analysis of different latency periods effect on new bone obtained by periosteal distraction: an experimental study in the rabbit model. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2011. 111(5): p. 539-46.
22. Gal, J.A., et al., Analysis of human mandibular mechanics based on screw theory and in vivo data. ***J Biomech***, 2004. 37(9): p. 1405-12.
23. Gittelson, G.L., Vertical dimension of occlusion in implant dentistry: significance and approach. ***Implant Dent***, 2002. 11(1): p. 33-40.
24. Karoglan, M., et al., Development of a static and dynamic simulator for osteosyntheses of the mandible. ***Technol Health Care***, 2006. 14(4-5): p. 449-55.

25. Zakaria, O., M. Madi, and S. Kasugai, Induced osteogenesis using a new periosteal distractor. ***J Oral Maxillofac Surg***, 2012. 70(3): p. e225-34.
26. Sato, K., et al., Osteogenesis by gradually expanding the interface between bone surface and periosteum enhanced by bone marrow stem cell administration in rabbits. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2010. 110(1): p. 32-40.
27. Sencimen, M., et al., Histomorphometrical analysis of new bone obtained by distraction osteogenesis and osteogenesis by periosteal distraction in rabbits. ***Int J Oral Maxillofac Surg***, 2007. 36(3): p. 235-42.
28. Bayar, G.R., et al., Histomorphometric analysis of new bone obtained by osteogenic periosteal distraction in ovariectomized rabbits. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod***, 2012. 113(4): p. 472-9.
29. Ilizarov, G.A., The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. C***lin Orthop Relat Res***, 1989(238): p. 249-81.
30. Ilizarov, G.A., The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. ***Clin Orthop Relat Res***, 1989(239): p. 263-85.
31. Jensen, O.T., et al., Anterior maxillary alveolar distraction osteogenesis: a prospective 5-year clinical study. ***Int J Oral Maxillofac Implants***, 2002. 17(1): p. 52-68.

Appendix 1

Elsevier Editorial System(tm) for International Journal of Oral & Maxillofacial Surgery
Manuscript Draft

Manuscript Number: IJOMS-D-14-00601

Title: Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model

Article Type: Original Article

Keywords: Distraction osteogenesis, Histomorphometry, Hyrax device, Periosteal distraction, Platelet rich fibrin, Micro-CT, Rabbit

Corresponding Author: Mrs. Prisana Pripatnanont, DDS

Corresponding Author's Institution: Faculty of Dentistry, Prince of Songkla University

First Author: Faisal Balabid, DDS

Order of Authors: Faisal Balabid, DDS; Prisana Pripatnanont, DDS; Settakorn Pongpanich, DDS, MD; Surapong Vongvatcharanon, DDS, MD

Abstract: The purpose of this study was to evaluate the effect of the modified design of the Hyrax device and platelet rich fibrin (PRF) on periosteal distraction. Twelve adult male white New Zealand rabbits were grouped into two main groups of six animals according to the consolidation period of four and eight weeks. In each main group, the animals underwent periosteal distraction at the left and right sides of the mandible and divided into four subgroups (with three animals per group); device versus device with PRF and PRF versus sham. Radiographic, histologic, histomorphometric and micro-CT analyses were performed. New bone formation was observed on the lateral and vertical sides of the mandible of all groups. Micro CT and histomorphometry showed that the device with PRF group presented highest bone formation (56.67±12.67mm^3, 41.4±7.57 mm^2) at 4 weeks and (49.67±8.33, 55.5±10.67) at 8 weeks followed by the device group (33.0±1.73mm^3, 33.2±10.99mm^2) at 4 weeks and (30.0±3.0mm^3, 23.2±5.55 mm^2) at 8 weeks respectively. In conclusion, the modified Hyrax device could be used for periosteum distraction in rabbit's model to gain vertical ridge augmentation, and more bone formation could be enhanced by adding PRF.

Journal: INTERNATIONAL JOURNAL OF ORAL & MAXILLOFACIAL SURGERY	**Title of Paper**: *Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model*

Declarations

The following additional information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned to you. If you have nothing to declare in any of these categories then this should be stated.

Please state any conflict of interests. A conflict of interest exists when an author or the author's institution has financial or personal relationships with other people or organisations that inappropriately influence (bias) his or her actions. Financial relationships are easily identifiable, but conflicts can also occur because of personal relationships, academic competition, or intellectual passion. A conflict can be actual or potential, and full disclosure to The Editor is the safest course.

Competing Interests

No

Please state any sources of funding for your research

Graduate school and faculty of Dentistry, Prince of Songkla University

DOES YOUR STUDY INVOLVE HUMAN SUBJECTS? Please cross out whichever is not applicable.

~~Yes~~

No

If your study involves human subjects you MUST have obtained ethical approval.
Please state whether Ethical Approval was given, by whom and the relevant Judgement's reference number

-

Patient Consent – please state that written patient consent has been obtained to publish clinical photographs. If you have no clinical photographs, please state "not required".
The Journal may request a copy of this consent prior to acceptance

-

Please add a statement to confirm that all authors have viewed and agreed to the submission

The authors confirm that all authors have viewed and agreed to the submission to INTERNATIONAL *JOURNAL OF ORAL & MAXILLOFACIAL SURGERY.*

This information must also be inserted into your manuscript under the acknowledgements section prior to the References.

INTERNATIONAL JOURNAL OF ORAL & MAXILLOFACIAL SURGERY

CONFIRMATION OF AUTHORSHIP FORM

Title of Submission: Effect of periosteal distraction by a modified Hyrax device with and without platelet rich fibrin on bone formation in rabbit model

Contributing Authors:-

Author: (surname and initials)	**Signature (Mandatory)**
1. Pripatnanont P.	
2. Balabid F.	
3. Pongpanich S.	
4. Vongvatcharanon S.	

"I warrant that all the authors listed above have made a significant contribution to this manuscript and have agreed to its submission to the IJOMS".

Signed (corresponding author) .....

Name and Title of Corresponding Author: Associate Professor Prisana Pripatnanont

This form can be downloaded from the Guide to Authors (http://ees.elsevier.com/ijoms) and must be signed by all authors. This signed form must be submitted prior to acceptance of your manuscript.

The form can either be scanned and uploaded into your submission, or alternatively, faxed to 01865 843992, or sent by post to the following address :-

IJOMS Editorial Office
c/o Jacqui Merrison
Elsevier Limited
The Boulevard
Langford Lane
Oxford
OX5 1GB

Appendix 2


30th Anniversary
DFCT
The Dental Faculty Consortium of Thailand
The Best of Thai Dental Research
11th
Welcome to
Scientific Meeting
7-9 May, 2013
Pullman Pattaya Hotel,
Chonburi
IMPORTANT DATES
Abstract submissions deadline 22 March 2013
Acceptance notifications 12 April 2013
Registration deadline 30 April 2013
For more detail;
http://www.dentistry.tu.ac.th/Dentistry/DFCT/DFCT.htm
Email : dfct.tu@gmail.com
www.facebook.com/dfct.thailand
Tel. +662 986 9213 Ext. 7105, 7152 Fax. +662 986 9051

Welcome Message

by Conference Chair

Faculty of Dentistry, Thammasat University would like to welcome you to the 11th Dental Faculty Consortium of Thailand Academic Meeting and Research Presentation (DFCT2013), at the Pullman Pattaya Hotel, Chonburi. The theme for the meeting is "The Best of Thai Dental Research". This is an opportunity to bring together the dental leaders, educators, researchers, as well as students to exchange ideas and contribute to the latest developments, innovations in this rapidly advancing, multidisciplinary field of research in dentistry. The scope of DFCT2013 has expanded to cover dental education administration, research, as well as national policy and strategy in the dentistry profession. Our scientific program is rich and varied with 1 keynote speech, 1 special lecture from Prof. JM ten Cate (Global President of International Association for Dental Research), 9 invited talks and around 80 scientific abstracts split between 3 parallel 2 oral sessions and 1 poster session. There are 16 full papers submitted by graduate students as part of their graduation requirement. Besides, there is a Dean forum on research trend in dentistry in Thailand. This meeting will also mark a very special occasion, the 30th anniversary of DFCT (established since 1983). DFCT has members from eight universities, Chiang Mai, Chulalongkorn, Khon Kaen, Mahidol, Naresuan, Prince of Songkla, Srinakarinwirot, and Thammasat.

As a conference chair of DFCT2013, I know that the success of the conference depends ultimately on the many people who have worked with us in planning and organizing both the scientific program and supporting social arrangements. In particular, we thank the Scientific Program Committee Chair for an international arrangement of the abstract/paper submission and reviewing system; the Award Judging Committee Chair for advice and brilliant suggestion on organizing junior and senior research competitions; the Scientific Program Committee for their thorough and timely reviewing of the abstracts/papers, and our sponsors who have helped us to keep down the costs of DFCT2013 for all participants. Recognition should go to the Local Organizing Committee members who have all worked extremely hard for the details of important aspects of the conference programs and social activities.

On behalf of the DFCT2013 organizing committee, I am honored and delighted to welcome you to the conference and believe we have chosen a venue that guarantees a successful scientific conference amid the scenery of Pattaya beach.

Warm Regards

Prof.Dr. Sittichai Koontongkaew
Dean, Faculty of Dentistry, Thammasat University
Conference Chair

PP33

Effect of periosteal distraction by a new design of hyrax device on bone formation in rabbit's model

Faisal Balabid, Prisana Pripatnanont, Settakorn Pongpanich and Surapong Vongvatcharanon*

Vertical ridge augmentation is a challenging in the reconstruction of the atrophic mandible. This study aimed to evaluate the new bone formation by distraction of alveolar periosteum using modified design of Hyrax device and platelet rich fibrin. Twelves rabbits were divided into 2 groups of 6 each according to the duration of consolidation period of 4 and 8 weeks. Both groups were divided into 4 subgroups: Sham, PRF, device and device with PRF group to achieve 7mm distraction, 0.5mm twice a day. In the histomorphometric evaluation, new bone formation observed on the lateral and vertical side of the mandible of all groups. The device with PRF group showed significant bone formation followed by the device group. Periosteal distraction osteogenesis can be an option for vertical ridge augmentation.

EFFECT OF PERIOSTEAL DISTRACTION BY A NEW DESIGN OF HYRAX DEVICE ON BONE FORMATION IN RABBIT'S MODEL

Faisal Balabid, Prisana Pripatnanont, Settakorn Pongpanich, Surapong Vongvatcharanon

Faculty of Dentistry, Prince of Songkla University, Hatyai, Thailand

Abstract

Vertical ridge augmentation is a challenging in the reconstruction of the atrophic mandible. This study aimed to evaluate new bone formation by distraction of alveolar periosteum using modified design of hyrax device and platelet rich fibrin. Twelves rabbits were divided into 2 groups of 6 each according to the duration of consolidation period of 4 and 8 weeks. Both groups were divided into 4 subgroups: sham, PRF, device and device with PRF group to achieve 7mm distraction, 0.5mm twice a day.

Histomorphometric evaluation, new bone formation was observed on the lateral and vertical side of the mandible of all groups. The device with PRF group showed highest bone formation followed by the device group.

Periosteal distraction osteogenesis can be an option for vertical ridge augmentation.

Keywords:
Hyrax device, periosteal distraction osteogenesis, platelet rich fibrin.

Introduction

Patients with edentulous jaws usually require adequate bone height and width for ideal restoration of the edentulous area [1, 2]. Several methods have been used for alveolar ridge augmentation such as bone grafting, guided bone regeneration (GBR) and alveolar distraction osteogenesis (DO).

Bone grafts have some disadvantages like morbidity of the donor site and resorption of the grafted bone on the recipient side, while GBR technique is limited to a small defect of the alveolar ridge[3].

Distraction osteogenesis is another technique for bone regeneration, which is a procedure whereby the bone is stretched to increase its volume. According to processes in distraction osteogenesis, at least one portion of bone is partially separated. The portion's position is changed gradually with respect to the bone direction. Time is then availed for new bone to fill in the space between the portion and the overall bone. Distraction osteogenesis, however, has some drawbacks such as technically complicated for surgeons and trauma to patients[3].

Periosteum distraction is a combination of guided bone regeneration, tissue expansion and distraction osteogenesis.

It is possible to produce new bone formation by periosteal distraction osteogenesis (PDO) without corticotomy obtaining newly formed bone is possible by mesenchymal stem cells that are under tension and capable of differentiating into osteoblasts[1].

Platelet-rich fibrin (PRF), introduced by Choukroun, is the source of autologous growth factor that improves the healing of soft and hard tissues [4, 5].

Combination of PDO with autologous growth factor in PRF could enhance vertical bone augmentation and there is no study evaluates the effects of PRF in PDO.

This study aimed to utilize a new technique to distract the periosteum and foster bone formation by using PRF.

MATERIALS and METHODS

Animal care

Twelves adult male New Zealand white rabbits have been used in this study with the mean weight of 3.5kg and approved by the Research committee of the animal care centre in Prince of Songkla University, Hatyai, Thailand. Twelves rabbits were divided into 2 groups of 6 each according to the duration of consolidation period of 4 and 8 weeks. Both groups were divided into 4 subgroups: Sham, PRF, Device and Device with PRF group to achieve 7mm distraction, 0.5mm twice a day.

Distractors

Hyrax device were modified to a new design by welding a titanium plate in one end of the device. The device was rigidly fixed on the lateral aspect of the mandible by using a 3 mini titanium screws with 3mm in length. While the other ends, the arms were modified in L shape to facilitate distraction of the periosteum (Figure1).

Figure 1-Modified design of Hyrax device

Surgical method

Platelet rich-fibrin (PRF) Preparation:

Before anaesthesia, 10ml of blood was drawn and placed into a non-coagulate tube, centrifuged immediately at 3000 revolutions/minute for 10 minutes using a table centrifuge (PC-02, Process Ltd., Nice, France). The result is the red blood cell layer at the bottom and PRF in the middle and platelet poor plasma on the top of the tube and the PRF were separated from unwanted blood (Figure 2).

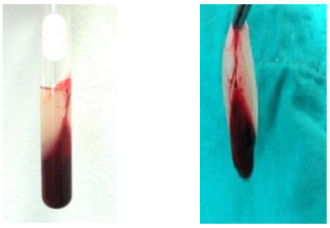


Figure 2-A) Blood separated into 3 layers after centrifugation B)-Prepared PRF

Anaesthesia of the animal

Rabbits were sedated with Ketamine Injection (25 mg/kg IM) and Diazepam (5mg/kg) intravenously and maintained by ketamine drip in normal saline solution (10–15 mg/kg).
Betadine swabbed at the surgical site and sub-cutaneous infiltration of local anaesthesia of 2% Articaine with epinephrine (1: 100,000, 1.8ml) was given in lateral aspect of the ramus and submandibular area.

Surgical method

Sub-mandibular incision of 5 cm was done and a periosteal flap was raised through the skin, muscle and periosteum on the lateral aspect of the ramus. A 7mm in length and 2mm depth defect were created on a free dentition site mesial to the mandibular molar tooth, avoiding the mental nerve by identifying the nerve location. Reference points at both side of defect were created and filled up with a gutta-percha. The device was fixed with 3mm titanium screws. The periosteal flap was repositioned and closed in layers using Vicryl suture 3/0.
Local wound dressing was done by applying antibiotic ointment to the surgical site for 7 days (Figure 3).

Figure 3- Rigid fixed device

Postoperative care

Post-operatively the animals were injected with antibiotic penicillin G (47.000 - 84.000 IU/kg) on the lateral thigh muscle and Acetaminophen analgesic (200 mg/kg) per day for 3 days respectively to ensure no sign of infection or pain until full recovery. The animals were observed in general activities and behaviour to indicate good health.

Periosteal distraction

The rabbits were divided into 2 groups of 6 each according to the consolidation period of 4 and 8 weeks. In both groups, the distraction period started after the latency period of 3 days. Distraction was performed by activating the distractor 0.5 mm twice per day. A periosteal distraction of 7.0 mm was achieved after a distraction period of 7 days.
After the time of 4 and 8 weeks the animals were sacrificed using the high dose of thiopental. The mandibles were harvested and fixed in 10% neutral-buffered formalin for 1 week. After decalcification (by 10% formic acid), the distraction regions were sectioned, and stained with haematoxylin and eosin.

Histomorphometric analysis

Histomorphometric measurements were achieved by using an image program Aperio ImageScope (2006 Aperio Technologies, Inc.UK) to evaluate the mean percentage of the new bone (NB) formed and total surface area separated by the defect line (DL).

Statistical analysis

Data were evaluated by using One-way ANOVA and Post Hoc test using Tukey HSD test. Significant was set at P (0.05).

Result

Clinical evaluation

All animals tolerated surgery and recovered well but two animals encountered dislodgement of the devices due to scratching to the device. Neck collar was later applied 3 days postoperatively to secure the device from animal scratching. All groups gained bone height over the created defect.

Histology examination

All groups showed an increase of new bone over the created defect particularly in the groups of device either with or without PRF.

The Device group both with and without PRF showed more bone formation when compared with PRF or Sham group in both consolidation periods of 4 and 8 weeks. Some collapse was observed in 8-week group (Figure 4).

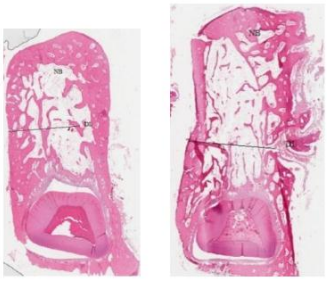


Figure 4- A) *Device group of 8 weeks consolidation period, B) Device with PRF group of 8 weeks consolidation period*

The non-device groups either PRF or sham showed limited bone formation and looked not difference (Figure5).

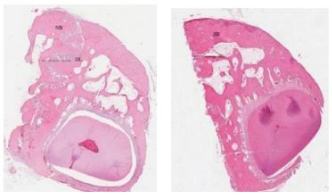


Figure 5- A) PRF group of 8 weeks consolidation period B)Sham group of 8 weeks consolidation period

Histomorphometric analysis

Histomorphometric measurements of the mean percentage of the new bone (NB) formed and total surface area, separated by the defect line (DL), were done. All groups gained new bone formation. Significant bone formation ($p<.01$) were seen in the device with PRF and device alone when compared with PRF or Shame group (Figure 6).

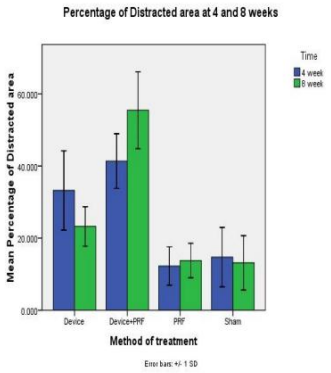


Figure 6- Histogram of new bone formation of all groups

Device with PRF group gained the highest bone formation among all 4 groups with the mean percentage of 60 precent from the total surface area at 8 weeks but not statically significant different from 4-week consolidation period.

DISCUSSION

Severe bone loss in alveolar bone may cause difficulty to reconstruct with a conventional prosthesis or restored with dental implants. Vertical ridge augmentation remains a challenge in the reconstruction of the atrophic maxilla and mandible. Since there is the need to expand the soft-tissue envelope to achieve the contour of bony architecture [7] either with block or particulate graft with guided bone regeneration. Alveolar distraction osteogenesis (DO) is a technically challenging operation used to increase bone height and width to gain both the bony contour and soft tissue. The most common complications of DO were insufficient bone formation following the consolidation period, regression of distraction distance and problems related to the distractor device. To avoid this complication and difficulties of device application, the hyrax device was modified to distract the periosteum without bone distraction and gain bone by activating the periosteum and maintain the gap gained with the device. Platelet-rich fibrin (PRF), an autologous leukocyte- and platelet-rich fibrin (L-PRF) biomaterial [9,10], was uses to as growth factor and cytokines carrier in the surgical site to promote the healing process. In the present study, bone formation was seen in all groups at 8 weeks of consolidation period buy more pronounce in the device group with and without PRF. This presentation confirmed the concept of created space and maintains that gap for bone to regenerate. The use of PRF alone gain better organized bone than the sham group but there was no difference in bone height and bone area. Therefore PRF alone is not sufficient to be used for bone regeneration but could enhance better bone formation in case that created space can be maintained. Dislodgement of the device observed in first 2 animal of this study due to scratching of the animal to the device. Dislodgement leads to collapsed in bone formation in 8 weeks consolidation period of the device group. Neck collar was applied 3 days postoperatively to secure the device from animal scratching. Space maintaining for a certain time is crucial for bone regeneration, rigid device or other methods to prevent collapse of periosteum has to be modified.

Conclusion

Distraction of the periosteum using modified Hyrax device design with PRF is a promising method to gain bone formation in alveolar reconstruction particularly vertical bone augmentation. The device should further developed to gain effective and more predictable result to be used clinically.

Acknowledgments

This work was supported by a grant from Faculty of Dentistry and Graduate school, Prince of Songkla University. My sincere thanks also to Assistant Professor Komgrid Charngkaew, Siriraj hospital, Mahidol university, for facilitating of scanning histological slides.

References:

[1] Jensen, O.T., et al., *Anterior maxillary alveolar distraction osteogenesis: a prospective 5-year clinical study.* Int J Oral Maxillofac Implants, 2002. **17**(1): p. 52-68.

[2] Jensen, O.T. and Z. Laster, *Preventing complications arising in alveolar distraction osteogenesis*. J Oral Maxillofac Surg, 2002. **60**(10): p. 1217-8.

[3] Saulacic, N., et al., *Analysis of New Bone Formation Induced by Periosteal Distraction in a Rat Calvarium Model.* Clin Implant Dent Relat Res, 2011.

[4] Chang, I.C., C.H. Tsai, and Y.C. Chang, *Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts.* J Biomed Mater Res A, 2010. **95**(1): p. 327-32.

[5] Chang, Y.C. and J.H. Zhao, *Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects.* Aust Dent J, 2011. **56**(4): p. 365-71.

[6] Laster, Z., G. Cohen, and R. Nagler, *A Novel Technique for Vertical Bone Augmentation in the Premaxillary Region.* Journal of Oral and Maxillofacial Surgery, 2009. **67**(12): p. 2669-2672.

[7] Louis, P.J., *Vertical ridge augmentation using titanium mesh.* Oral Maxillofac Surg Clin North Am, 2010. **22**(3): p. 353-68, v.

[8] Saulacic, N., J. Zix, and T. Iizuka, *Complication rates and associated factors in alveolar distraction osteogenesis: a comprehensive review.* Int J Oral Maxillofac Surg, 2009. **38**(3): p. 210-7.

[9] Ehrenfest, D.M.D., et al., *Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way.* Archives of Oral Biology, 2010. **55**(3): p. 185-194.

[10] He, L., et al., *A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro.* Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology, 2009. **108**(5): p. 707-713.

Appendix 3

PRINCE OF SONGKLA UNIVERSITY
15 Karnjanawanij Road, Hat Yai, Songkhla 90110, Thailand
Tel (66-74) 286958 Fax (66-74) 286961
Website : www.psu.ac.th

MOE 0521.11/๑๒๘ Ref.32/2012

September ๑๙ , 2012

This is to certify that the research project entitled "Analysis of new bone obtained by mandibular periosteal distraction using modified hyrax palatal expander in rabbits" which was conducted by Asst.Prof.Settakorn Pongpanich, Faculty of Dentistry, Prince of Songkla University, has been approved by The Animal Ethic Committee, Prince of Songkla University.

Kitja Sawangjaroen

Kitja Sawangjaroen, Ph.D.
Chairman,
The Animal Ethic Committee, Prince of Songkla University

Vitae

Name Mr. Faisal Omar Abdullah Balabid

Student ID 5210820009

Educational Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Ajman University	2006
Graduate Diploma OMFS	Prince of Songkla University	2009

List of Publication and Proceedings

Balabid F, Pripatnanont P, Pongpanich S, Vongvatcharanon S. EFFECT OF PERIOSTEAL DISTRACTION BY A NEW DESIGN OF HYRAX DEVICE ON BONE FORMATION IN RABBIT'S MODEL. ***The 11th Dental Faculty Consortium of Thailand Academic Meeting and Research Presentation (DFCT2013)*** May; 169-173.