Effect of osteogenic periosteal distraction by a modified Hyrax device with and without platelet-rich fibrin on bone formation in a rabbit model: A pilot study

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Abstract. This study evaluated the effect of a modified Hyrax device and platelet-rich fibrin (PRF) on osteogenic periosteal distraction (OPD). Twelve adult male New Zealand white rabbits were separated into two main groups (six in each) according to the duration of the consolidation period (4 or 8 weeks). In each main group, the animals underwent OPD of the left and right sides of the mandible and were divided into four subgroups (three animals per group): device vs. device + PRF, and PRF vs. sham. Radiographic, histological, histomorphometric, and micro-computed tomography (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 + PRF group presented the highest percentages of bone volume and bone area at 4 weeks (56.67 ± 12.67%, 41.37 ± 7.57%) and at 8 weeks (49.67 ± 8.33%, 55.46 ± 10.67%; significantly higher than the other groups, P < 0.001), followed by the device group at 4 weeks (33.00 ± 1.73%, 33.21 ± 11.00%) and at 8 weeks (30.00 ± 3.00%, 23.25 ± 5.46%). In conclusion, the modified Hyrax device was used successfully for OPD in a rabbit model to gain vertical ridge augmentation, and greater bone maturation was achieved with the addition of PRF.

Keywords: Distraction osteogenesis; Histomorphometry; Hyrax device; Osteogenic periosteal distraction; Platelet-rich fibrin; Micro-CT; Rabbit.

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Introduction

Reconstruction of the atrophic edentulous ridge always requires adequate bone height and width for ideal dental restoration of the edentulous area. Several methods have been used for alveolar ridge augmentation such as bone grafting, guided bone regeneration (GBR), and alveolar distraction osteogenesis (DO)1-7. Bone grafts have some disadvantages such as morbidity of the donor site and surface resorption of the grafted bone, while the GBR technique is of use in a limited area of the alveolar ridge defect7,8,9-11. DO is another technique to gain new bone and works by separating one portion of bone and gradually changing its position with...
respect to the bone direction, allowing for new bone to fill the space between the portion and the overall bone\textsuperscript{1,2,11}. DO also has some drawbacks, as it is technically complicated and may cause trauma to the patient\textsuperscript{13}. Osteogenic periosteal distraction (OPD) comprises a combination of guided bone regeneration, tissue expansion, and DO. It is proposed to produce new bone formation by osteogenic periosteal distraction without corticotomy\textsuperscript{14}. Newly formed bone is obtained from mesenchymal stem cells which are under tension and capable of differentiating into osteoblasts\textsuperscript{5}. In a previous study, OPD was performed on the lateral surface of the mandible in rabbits, either with or without cortical bone perforation; it was shown that more bone formation was induced in the group with decortication of the mandibular cortex than in the non-decorticated group\textsuperscript{16}.

The Hyrax, an orthodontic device, is normally used in children for widening of the upper jaw by separating the mid-palatal suture to treat crowding caused by a narrow arch form\textsuperscript{17}. Platelet-rich fibrin (PRF), introduced by Dohan et al.\textsuperscript{18}, is a source of autologous growth factors that improves the healing of soft and hard tissues\textsuperscript{19-21}. In a previous study\textsuperscript{22}, it was shown that PRF enhances bone formation when used in combination with grafting material or when used alone.

It is hypothesized that by gradual periosteal lifting, in which the space can be maintained, osteogenesis would be induced and bone regeneration would be fostered by growth factors from PRF. The aim of this study was to evaluate the effect of a modified Hyrax device and PRF on supraosseous osteogenesis underneath the periosteal pouch in a rabbit mandible model, using micro-computed tomography (micro-CT) and histomorphometric analysis.

**Materials and methods**

This study was conducted with the approval of the university animal care centre research committee. The estimated sample size for a two-sample comparison of means was computed by using the assumption that the OPD group would gain at least 30 ± 5% of bone and the sham group around 15 ± 5% of bone; \( \alpha \) was set at 0.05 and \( \beta \) was set at 0.1. The sample size for each group was three, with an estimated power of 0.96.

**Animal preparations**

Twelve adult male New Zealand white rabbits with a mean weight of 3.5 kg were used in this study. These rabbits were separated into two main groups of six rabbits each according to the duration of the consolidation period—either 4 or 8 weeks. The rabbits in each group were divided into four subgroups of three animals according to the experiment performed on the left and right mandibles: sham (right) vs. device + PRF (left), and device (right) vs. PRF (left). A device was used only on one side of the mandible in each animal and not used on both sides in order to avoid interference between devices. The device was activated to achieve 7 mm distraction, being moved 0.5 mm twice a day for 7 days.

**Distractors**

Hyrax devices (Leone S.p.A., Firenze, Italy) were modified for use as osteogenic periosteal distractors by welding a micro-titanium plate (Medicon eG, Tuttingen, Germany) to one end (two rod arms) of the device, while the other end was bent into L-shaped rod arms (Fig. 1). The device was designed to be fixed to the body of the rabbit mandible with the welded micro-titanium plate and microscrews (3 mm in length). The other end (L-shaped rod arms), being 3 cm in total length including the 7-mm length of the bottom part, was designed for placement underneath the periosteum over the alveolar crest, for lifting of the periosteum during device activation (Fig. 1).

**PRF preparation**

Autologous PRF was prepared according to the PRF protocol developed by Dohan et al.\textsuperscript{18}. Ten milliliters of autologous whole blood was collected from the central ear artery of the rabbit before anaesthesia and placed into a 10-ml glass tube without anticoagulant. This was centrifuged immediately at 3000 rpm for 10 min using a table centrifuge (EBRA20; Andreas Hetlich GmbH & Co. KG, Tuttingen, Germany), separating the sample into three layers: a red blood cell layer at the bottom, PRF in the middle, and platelet-poor plasma at the top of the tube (Fig. 2). The PRF in the middle part was removed from the tube using straight tissue forceps and cut from the red blood cells. This was then used underneath the periosteum in the PRF subgroup and the device + PRF subgroup.

**Surgical procedure**

General anaesthesia was induced by intramuscular injection of ketamine HCl 25 mg/kg (Calypsol 50 mg/ml; Gedeon Richter Ltd, Budapest, Hungary) and diazepam 5 mg/kg (10 mg/2 ml/ampoule), approximately 30 min prior to the administration of intravenous thiopental sodium 15 mg/kg (Anesthyl 1 g/vial; Jagsonpal Pharmaceuticals Ltd, Haryana, India). Thiopental sodium was titrated at a rate of 2 mg/kg every 15 min with a maximum dose of 30 mg/kg to maintain unconsciousness.

The surgical field in the area of the ramus and body of the mandible was shaved and disinfected with 10% povidone–iodine (Polidine solution 10%, New Life Pharma Co., Ltd, Bangkok, Thailand). Local anaesthesia infiltration of 1% lidocaine HCl with 1:100,000

![Fig. 1](image1.png)  
Fig. 1. (A) Lateral view of the modified Hyrax device. (B) Image showing rigid attachment of the device to the lateral aspect of a rabbit mandible.

![Fig. 2](image2.png)  
Fig. 2. (A) Blood sample after centrifugation showing the three layers: platelet-poor plasma (PPP) at the top, platelet-rich fibrin (PRF) in the middle, and red blood cells at the bottom. (B) Platelet-rich fibrin gel (PRF).
adrenalin 1.8 ml (Drocanil-A 1%; M&H Manufacturing Co., Ltd, Samutprakarn, Thailand) was administered to the surgical site for hemostasis and as an adjunct to control pain during and post surgery. A 5-cm sub-mandibular incision was made through the skin, the muscle, and the periosteum. A periosteal flap was then raised to expose the lateral aspect of the ramus. In all groups, defects were created at a site free of dentition, mesial to the mandibular molar tooth (7 mm in length and 2 mm in depth measured by periodontal probe); the mental nerve was avoided by identifying the nerve location. The reason for creating the defect in the area free of dentition was to gain enough room for vertical distraction so as not to interfere with the maxillary teeth during mastication. Reference points were established by creating a small hollow at the anterior and posterior corners of the defect and filling these with gutta percha (Fig. 3). The device was fixed to the mandible with 3-mm titanium screws. The active arms of the device were placed on the defect underneath the periosteum.

Activation of the device was tested prior to wound closure. The periosteum, muscle, and skin were repositioned at the lower border of the mandible and sutured layer by layer using Vicryl 4.0. Local wound dressing was performed by applying chloramphenicol antibiotic ointment to the surgical site for 7 days (Coethine ophthalmic ointment 1%; General Drugs House Co., Ltd, Bangkok, Thailand). Postoperatively, the animals were injected with penicillin G 100,000 IU/kg (Penicillin G 500,000 units/vial; General Drugs House Co., Ltd, Bangkok, Thailand) and acetaminophen analgesic 200 mg/kg (Acetaphen injection 300 mg/2 ml/ampoule; Nida Pharma Incorporation Co., Ltd, Bangkok, Thailand) in the lateral thigh muscle for 3 days to prevent infection and pain during recovery. The rabbits were fed a standard rabbit diet, given water ad libitum, and kept in separate cages in the animal facility of the university. The animals were monitored in accordance with the guidelines of the institutional ethics committee. The clinical condition, wound condition, physical activity, food and water consumption, and weight of the animals were monitored closely.

In the sham group, the defect was created and used as a control. In the PRF group, the defect created was filled with PRF underneath the periosteum. In the device group, after the defect was created, the device was fixed rigidly on the lateral aspect of the mandible. In the device + PRF group, after the defect was created and the device was fixed, PRF was added under the active arms beneath the periosteum.

In the device and the device + PRF groups, the distraction period started after a latency period of 3 days. Distraction was performed by activating the distractor by 0.5 mm twice a day. A periosteal distraction of 7.0 mm was achieved after a distraction period of 7 days. After a consolidation period of 4 or 8 weeks, the animals were sacrificed with an overdose of 50 mg/ml thipental sodium 5 ml (Anesthal 1 g/vial) administered intravenously via the marginal ear vein. The mandibles were harvested and fixed in 10% neutral-buffered formalin for 1 week. After decalcification with 10% formic acid, the distraction regions were sectioned and stained with hematoxylin and eosin.

Digital radiography

The mandibular specimens were radiographed using a hand-held portable X-ray device (NOMAD; Aribex Inc., Utah, USA) with digital sensor size 0 attached to a digital sensor holder (XCP-DS; Rinn, Dentsply, IL, USA). The setting for all exposures was 60 kVp, 2.3 mA, and 0.3 s at a distance of 10 cm. The digital image was taken in two views, a lateral–oblique view and a lateral view. The mean optical density (OD) of the defect was calculated and analyzed using Image-Pro Plus 7.0 software (Media Cybernetics Inc., Rockville, MD, USA).

Specimen processing

The specimen of the free alveolar containing the 7-mm defect was divided into two halves (each 3.5 mm in length); one half (distal half) underwent histology and histomorphometric analysis and the other (mesial half) underwent micro-CT analysis.

Micro-CT analysis

A high-resolution micro-CT system (Micro-CT80; Scanco Medical AG, Brütisellen, Switzerland) was calibrated and the specimens were scanned perpendicular to the cranium vault at 55 kVp, 72 μA, and 4 W in high-resolution mode (18.5 μm3/voxel). Scanned data were reconstructed by built-in software.

After determination of the threshold values, the region of interest (ROI) was traced to specify the newly formed bone (BV). The percentage of new bone volume was calculated as the percentage of radiopaque voxels in a bone threshold range divided by the total bone volume (TV): percentage of new bone volume = (new bone volume/total bone volume) x 100.

Histology processing

The distal half of the mandibular specimen was decalcified in 10% formic acid, trimmed, cut transversally, and then.
embedded in paraffin. Serial 5-μm sections were cut and stained with hematoxylin and eosin, and used for histomorphometric analysis.

**Histomorphometric analysis**

All slides were scanned via an Aperio ScanScope XT (Aperio ePathology Solutions, CA, USA) at 40× magnification. Digital histology images were captured with computer software (Aperio ImageScope 9.0; Aperio ePathology Solutions). The new bone formed was differentiated from the adjacent bone by identifying the defect line that was created during surgery. The area of newly formed bone (NB) was outlined and calculated as the percentage of newly formed bone area to the total defect area using Image-Pro Plus 7.0 (Media Cybernetics); percentage of new bone area = (new bone area/total defect area) × 100.

**Statistical analysis**

The statistical analysis was performed using SPSS version 15 statistical software (SPSS Inc., Chicago, IL, USA). Data were evaluated by one-way analysis of variance (ANOVA) and the post hoc Tukey HSD test. Significance was set at P < 0.05. Differences between the two times points for each group were analyzed using the paired t-test.

**Results**

**Animals**

All animals tolerated the surgery and recovered well. Two animals in the device 8-week consolidation period group had displacement of the device due to scratching during the consolidation period, but no infection was found. Neck collars were later applied at 3 days postoperatively to protect the device from animal scratching. The wound in each animal healed well without any infection or wound dehiscence.

**Gross specimen observation**

Specimens were harvested at 4 or 8 weeks. The PRF and the sham groups showed normal contours at the surgical site, with a firm consistency. The device and the device + PRF groups showed bulging at the distraction site on the lateral and the superior aspects of the mandible, with a firm consistency.

**Digital radiographic evaluation**

On lateral view (Figs. 4 and 5), all groups showed complete bone healing at the defect site after the 4-week consolidation period. Reference points (gutta percha markers) indicated the borders of the defect. Only the device groups with and without PRF showed slight bulging of the superior border of the defect, while the PRF and the sham groups showed normal contours of the edentulous ridge.

**Radiomorphometric analysis**

The means of the distracted area obtained from radiographic analysis were evaluated only for the device + PRF and device groups (Fig. 6) since there was no bone detected on the lateral-oblique view for the PRF and sham groups. The mean distracted area was calculated as the pixel area. The device + PRF group showed a significantly more distracted radio-opaque area at 4 weeks of consolidation (6.63 × 10^3 ± 2.26 × 10^3) and at 8 weeks of consolidation (8.49 × 10^3 ± 2.83 × 10^3) than the device group (4 weeks: 2.24 × 10^3 ± 1.10 × 10^3; 8 weeks: 2.84 × 10^5 ± 9.07 × 10^3) (t = −3.03, df = 4, P = 0.039, and t = −3.287, df = 4, P = 0.030, respectively).

**Micro-CT analysis**

The mean percentage of bone volume (% BV/TV, mean ± standard deviation) at the distracted sites is presented in Fig. 7. At 4 weeks of consolidation, the % BV/TV in the device group (33.00 ± 1.73%) was significantly (ANOVA, F = 27.69, df = 3, P < 0.001) greater than that in the PRF group (12.67 ± 0.58%; Dunnett T3, MD = 20.33, P = 0.003) and the sham group (15.00 ± 4.00%; Dunnett T3, MD = 18.00, P = 0.027). The device + PRF group showed the highest bone volume (56.67 ± 12.67%), but this was not significantly different from the other groups.

At 8 weeks of consolidation, the device + PRF group showed the highest mean % BV/TV (49.67 ± 8.33%), which was significantly higher than that of the other groups (ANOVA, F = 32.01, df = 3, P < 0.001). The device group also showed a significantly higher bone volume (30.00 ± 3.00%) than the PRF group (10.33 ± 4.51%; Tukey HSD, MD = 19.67, P = 0.011), but the difference between the device group and the sham group was not significant (14.50 ± 0.71%; Tukey HSD, MD = 15.50, P = 0.058).

**Histology**

All groups showed an increase in the area of bone over the defect created as indicated by the defect line (Fig. 8). The device group, both with and without PRF, showed more bone formation when compared with the PRF or the sham group for both consolidation periods (4 and 8 weeks). Some collapse was observed in the device group at 8 weeks of consolidation due to displacement of the device in two animals prior to the use of neck collars as a precautionary measure.

**Device group**

At 4 weeks of consolidation, new bone formation with a large marrow space was observed between the defect line and the cortical bone for the device group. The cortical bone was not uniformly formed (Fig. 9). At 8 weeks of consolidation, the
cortical bone was denser and thicker than at 4 weeks of consolidation (Fig. 10).

Device + PRF group
At 4 weeks of consolidation, a full contoured and uniform construction of the cortical bone with a large marrow space was observed in the device + PRF group (Fig. 9). At 8 weeks of consolidation (Fig. 10), a full contour of thick cortical bone was seen, with more trabecular bone between the tooth root and the cortical bone than at 4 weeks.

PRF group
In the 4-week and 8-week consolidation period groups (Figs. 9 and 10), the defect healed with thick cortical bone and dense trabecular bone in the PRF group. However, the cortical bone was thicker and denser in the 8-week group than in the 4-week group.

Sham group
After the 4-week and 8-week consolidation periods (Figs. 9 and 10), the defect had healed and gained a normal contour of the alveolus in the sham group. The cortical bone in the 8-week group was denser than that in the 4-week group.

Histomorphometric analysis
The histomorphometric measurements of the mean percentage of new bone (NB) formed are presented in Fig. 11. All groups gained new bone formation. The mean percentage of new bone formation for the 4 weeks consolidation period in the device + PRF group (41.36 ± 7.57%) was significantly different (ANOVA, F = 8.78, df = 3, P < 0.007) from the PRF group (12.26 ± 5.31%) and the sham group (14.72 ± 8.25%), but not significantly different from the device group (33.21 ± 11.00%). At 8 weeks of consolidation, the mean percentage of new bone formation for the device + PRF group (55.46 ± 10.67%) was significantly different (ANOVA, F = 21.32, df = 3, P < 0.001) from the device (23.25 ± 5.46%), the PRF (13.77 ± 4.78%), and the sham (13.16 ± 7.52%) groups.

Discussion
Reconstruction of a severely atrophic ridge requires not only bone augmentation but also soft tissue coverage and reconstruction. The soft tissue is usually insufficient and needs extension from nearby

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**Fig. 5.** Lateral view radiographs of the mandible after the 4-week (A–D) and 8-week (E–H) consolidation periods. All groups showed a healed defect; the opaque spots indicate the reference points at the corners of the created defect. Device (A and E), device + PRF (B and F), PRF (C and G), and sham (D and H).

**Fig. 6.** Radiographic assessment of the newly formed bone as the pixel area under the distracted periosteum area in the device + PRF group and the device group. (a) Significantly different from the device group at each time point (P = 0.039 and P = 0.030, respectively).
Fig. 7. Micro-CT analysis: the mean percentage of newly formed bone volume in the distracted periosteum area of the experimental groups at 4 and 8 weeks of consolidation. (a) Significantly different from the other groups at the same time point \( P = 0.001 \). (b) Significantly different from the PRF group \( P = 0.003 \) and the sham group \( P = 0.027 \) at the same time point. (c) Significantly different from the PRF group at the same time point \( P = 0.011 \).

Fig. 8. Photomicrographs demonstrating the defect line at 4 and 8 weeks of consolidation (hematoxylin and eosin stain at 4X magnification). (A) Defect line in the device group at 4 weeks of consolidation. (B) Defect line in the device group at 8 weeks of consolidation.

Fig. 9. Photomicrographs of the cross-sectional views of the distracted area at 4 weeks of consolidation (hematoxylin and eosin stain at 2X magnification). NB, new bone; DL, defect line; TR, tooth root. (A) The device group showed newly formed bone with large marrow and non-uniform cortex. (B) The device + PRF group showed newly formed bone with large marrow and uniform cortex. (C) The PRF group showed a healed defect site with dense cortex. (D) The sham group showed a healed defect site with dense cortex.
As well as the arms of the device, the rate of distraction may cause tearing of the periosteum during stretching and result in a small volume of bone. In this study, periosteum stretching was checked during the operation by full activation of the device prior to wound closure to ensure the function of the device and integrity of the periosteum. Regarding the activation rate of 0.5 mm twice a day, which is greater than that used in a previous study\(^{(25)}\), the histological results showed no soft tissue invasion into the periosteal pouch, indicating that the periosteum was intact and tolerated the rate of activation of 0.5 mm twice a day for 7 days. Nevertheless, in another study, bone of adequate stock and density was obtained using a dental implant distractor with whole periosteum, half periosteum, or no periosteum, although the whole periosteum gave the best result\(^{(27)}\).

Therefore the enveloping periosteum should be intact and preserved as much as possible during the distraction period.

Histomorphometrically, the quantity of newly formed bone in the device + PRF group was significantly different from all of the other groups at 8 weeks of consolidation, and differed significantly from the PRF and sham groups at 4 weeks of consolidation. These data agreed with the percentage of bone volume from the micro-CT analysis: the device group with and without PRF gained more bone formation than the groups without the device. PRF did not alter the quantity of new bone when there was no device. The device + PRF group gained more space than the device alone due to the space occupied by the PRF content. PRF enlarged the interface between the original bone surface and the periosteum thus inducing supraosseous neogenesis and gaining more bone than the device without PRF. Moreover, the device + PRF group showed dense trabecular bone, which may have been the result of the PRF fostering bone maturation by releasing various growth factors during the early period of bone formation. At 8 weeks, the devices in two animals in the device without PRF group were displaced; this resulted in less bone formation than at 4 weeks, and the cortex bone in this group presented an irregular pattern (Fig. 10A). When comparing the quantity of newly formed bone between 4 and 8 weeks, there was no statistically significant difference within any group. Nevertheless, the bone at 8 weeks was more mature than at 4 weeks. Therefore the space from the device could be maintained and allowed bone maturation during 4 to 8 weeks.

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**Fig. 10.** Photomicrographs of the cross-sectional views of the distracted area at 8 weeks of consolidation (hematoxylin and eosin stain at 2× magnification). NB, new bone; DL, defect line; TR, tooth root. (A) The device group showed newly formed bone with large marrow and irregular contours of cortex bone. (B) The device + PRF group showed uniform new bone formation with dense cortex and trabecular bone. (C) The PRF group showed dense cortex and trabecular bone. (D) The sham group showed dense cortex bone.

**Fig. 11.** Histomorphometry analysis: the mean percentage of newly formed bone area in the distracted periosteum area of the experimental groups at 4 and 8 weeks of consolidation. (a) Significantly different from the other groups at 8 weeks of consolidation \((P = 0.000)\). (b) Significantly different from the PRF group \((P = 0.011)\) and the sham group \((P = 0.018)\) at 4 weeks of consolidation.
In conclusion, the modified Hyrax device was effective for OPD but requires further modification prior to clinical application. PRF is an adjunct therapy for enhancing soft and hard tissue healing and fostering bone maturation.

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Competing interests
None.

Ethical approval
This study was approved by the Research Committee of the Animal Care Centre of Prince of Songkla University, Hatyai, Thailand (Ref. No. 32/2012, September 19, 2012).

Patient consent
Not required.

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