รายงานวิจัยฉบับสมบูรณ์

ประสิทธิภาพของไบเฟสิกแคลเซียมฟอสเฟตร่วมกับเพลตเลตริชไฟบรินในการคงสภาพกระดูกเบ้าฟัน

Efficacy of Biphasic Calcium Phosphate and Platelet Rich Fibrin in socket preservation

รองศาสตราจารย์ ทพญ. ปริศนา ปริพัฒนานนท์

โครงการวิจัยนี้ได้รับทุนสนับสนุนจาก...งบประมาณแผ่นดิน มหาวิทยาลัยสงขลานครินทร์
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ส่วนที่ 2 **เนื้อหา** ประกอบด้วย

1. ชื่อชุดโครงการ (ระบุกรณีเป็นโครงการย่อยภายใต้ชุดโครงการ)

   (ภาษาไทย) วัสดุทดแทนกระดูกและสารโครงร่างสำหรับการสร้างกระดูกใหม่

   (ภาษาอังกฤษ) Bone substitute material and osteoconductive scaffold for bone regeneration

2. ชื่อโครงการเดี่ยว หรือโครงการย่อยทุกโครงการ

   ชื่อโครงการวิจัย (ภาษาไทย) ประสิทธิภาพของไบเฟสิกแคลเซียมฟอสเฟตร่วมกับเพลตเลตริชไฟบรินในการคงสภาพกระดูกเบ้าฟัน

   (ภาษาอังกฤษ) Efficacy of Biphasic Calcium Phosphate and Platelet Rich Fibrin in socket preservation

3. คณะนักวิจัย และหน่วยงานต้นสังกัด (คณะ/ภาควิชา หรือหน่วยงาน)

   รองศาสตราจารย์ ทพญ. ปริศนา ปริพัฒนานนท์

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   หน่วยงานสนับสนุน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

   โทรศัพท์/โทรสาร 074 – 429876

   E – mail prisana.p@psu.ac.th

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บทคัดย่อ

ภายหลังการถอนฟัน กระดูกเบ้าฟันเกิดการปรับรูปและสลายตัวนำไปสู่การฝ่อลีบของกระดูกขากรรไกรทำให้ไม่เพียงพอต่อการรองรับฟันเทียมและรากฟันเทียม การคงสภาพกระดูกเบ้าฟันเป็นการใส่กระดูกปลูกถ่ายในเบ้าฟันเพื่อทำหน้าที่เป็นโครงร่างสำหรับการสร้างกระดูกในเบ้าฟันเพื่อให้เบ้าฟันคงรูปร่างอยู่ได้ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบประสิทธิภาพของวัสดุทดแทนกระดูกไบเฟสิกแคลเซียมฟอสเฟต กับกระดูกเอกพันธ์แห้งแบบระเหิดในการคงสภาพเบ้าฟันที่ปิดด้วยเยื่อเพลตเลตริชไฟบริน (พีอาร์เอฟ)

การศึกษานี้เป็นการศึกษาทางคลินิกในผู้ป่วยที่รับการถอนฟันที่มีรากเดียว และได้รับการคงสภาพกระดูกเบ้าฟันสำหรับการฝังรากเทียม ผู้ป่วยแบ่งเป็น 2 กลุ่ม กลุ่มละ 10 ซี่ฟันทำการปลูกถ่ายกระดูกเบ้าฟันด้วยกระดูกเอกพันธุ์แห้งแบบระเหิด หรือวัสดุทดแทนกระดูกไบเฟสิกแคลเซียมฟอสเฟตและปิดเบ้าฟันด้วยเยื่อพีอาร์เอฟ ทำการประเมินการหายของเนื้อเยื่ออ่อนโดยการวัดปากเบ้าฟันและประเมินการเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรโดยวัดจากชิ้นหล่อศึกษาและโคนบีมคอมพิวเตอร์โทโมกราฟ ที่ 2, 6, 8 และ 12 สัปดาห์ และที่ 12 สัปดาห์ ทำการตรวจชิ้นเนื้อกระดูกและประเมินปริมาณของกระดูกและวัสดุปลูกถ่ายด้วยภาพถ่ายรังสีทางไมโครคอมพิวเตอร์โทโมกราฟและตรวจสัณฐานวิทยาโดยวิธีจุลพยาธิวิทยา

ผลการศึกษาพบว่าการหายของเนื้อเยื่ออ่อนของบริเวณปากเบ้าฟันไม่แตกต่างกันระหว่าง 2 กลุ่มในแต่ละช่วงเวลา แต่ในแต่ละกลุ่มมีการหายของเนื้อเยื่ออ่อนต่างกันโดยปากแผลลดลงในแต่ละช่วงเวลาที่ 6, 8 และ 12 สัปดาห์อย่างนัยสำคัญทางสถิติ ($P < 0.05$) การเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรโดยชิ้นหล่อศึกษาในแนวความกว้างและความสูงของทั้ง 2 กลุ่ม ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ แต่พบการลดลงความกว้างของสันกระดูกขากรรไกรด้านแก้ม ด้านเพดานหรือด้านลิ้น และความสูงในกลุ่มเดียวกันลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ทั้งสองกลุ่ม การเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรโดยโคนบีมคอมพิวเตอร์โทโมกราฟทั้งของสองกลุ่มไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ

ภาพรังสีไมโครคอมพิวเตอร์โทโมกราฟพบว่าปริมาณการสร้างกระดูกใหม่และวัสดุที่หลงเหลือของกระดูกเอกพันธ์แห้งแบบระเหิด (22.37±9.61,17.31±14.53) และไบเฟสิกแคลเซียมฟอสเฟต(16.89±7.46,15.94±7.39) ไม่มีความแตกต่างกัน แต่จากการวัดทางจุลพยาธิวิทยาพบวัสดุที่หลงเหลือของกระดูกเอกพันธ์แห้งแบบระเหิด (29.38±7.96) และไบเฟสิกแคลเซียมฟอสเฟต (22.50±2.64) มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) แต่ไม่พบความแตกต่างในการสร้างกระดูกใหม่ของกระดูกเอกพันธ์แห้งแบบระเหิด (20.17±4.59) และไบเฟสิกแคลเซียมฟอสเฟต (18.40±7.20) จึงสรุปได้ว่าไบเฟสิกแคลเซียมฟอสเฟตและกระดูกเอกพันธ์แห้งแบบระเหิดเหมาะสมสำหรับการทำการคงสภาพกระดูกเบ้าฟัน และเยื่อพีอาร์เอฟมีประสิทธิภาพในการสร้างเนื้อเยื่ออ่อนเหมาะสมสำหรับการใช้ปิดเบ้าฟัน

Abstract

Background: After tooth extraction, socket wall remodeling leads to reduction of hard and soft tissue volume of the alveolar ridge and results to difficulty in denture construction and implant placement. Socket preservation is the method to fill the socket with bone grafting material to act as a scaffold for new bone formation and maintain ridge contour. This study aimed to compare the efficacy of biphasic calcium phosphate (BCP) with freeze-dried bone allograft (FDBA) in socket preservation sealed with platelet-rich fibrin (PRF) membrane.

Material and Methods: The study was a randomized clinical controlled trial, conducted in patients whom socket preservation and later implantation were performed. Patients were allocated into 2 groups of 10 sockets which were grafted with either FDBA or BCP. All socket orifices were sealed with PRF membrane. Soft tissue healing was assessed by direct measurement of socket orifice dimension, the dimensional change of the ridge reduction was measured by using cast-based measurement and cone beam computed tomography (CBCT). Patients were followed 2, 6, 8 and 12 weeks after extraction. At 12 weeks, bone biopsy was performed; Micro-CT and histomorphometric analysis were used to evaluate newly form bone and residual grafts.

Results: Soft tissue healing of the socket orifice of both groups were not statistically significant different at each follow-up period ($P>0.05$). Each group showed statistically significant reduction of socket orifice dimension among each time frame at 6, 8 and 12 weeks ($P < 0.05$). The cast-based measurements of dimensional change in width and height reduction of both groups showed no statistically significant differences. There were statistically significant differences in width reduction at the buccal, palatal and height among each follow-up time of both groups ($P<0.05$). The CBCT measurement of the dimensional change in the both groups showed no significant difference. The percentage of the newly form bone volume fraction from Micro-CT analysis of FDBA (22.37±9.61) and BCP (16.89±7.46) and the percentage of residual graft volume fraction of FDBA (17.31±14.53) and BCP (15.94±7.39) were no significant different. However, the histomorphometry analysis indicated that the residual graft of the BCP group (29.38±7.96) was more residual than the FDBA group (22.50±2.64) significantly, but no significant different was detected for the the newly formed bone (FDBA 20.17±4.59%, BCP 18.40±7.20%).

Conclusion: BCP and FDBA sealed with PRF membrane were comparably effective in maintaining soft tissues and minimizing alveolar ridge resorption.

กิตติกรรมประกาศ

งานวิจัยฉบับนี้มีจุดมุ่งหมายเพื่อเปรียบเทียบประสิทธิภาพของวัสดุทดแทนกระดูกไบเฟสิกแคลเซียมฟอสเฟต กับกระดูกเอกพันธ์แห้งแบบระเหิดในการคงสภาพเบ้าฟันที่ปิดด้วยเยื่อเพลตเลตริชไฟบริน (พีอาร์เอฟ) ผู้วิจัยขอขอบคุณ มหาวิทยาลัยสงขลานครินทร์ที่ได้สนับสนุนทุนการวิจัยครั้งนี้ และคณะผู้บริหารและเจ้าหน้าที่คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ที่ได้ให้คำปรึกษาและข้อมูลเพื่อใช้ในการประกอบการจัดทำงานวิจัย

การดำเนินการวิจัยมิอาจสำเร็จลุล่วงไปได้หากปราศจากความร่วมมือของคณาจารย์ในคณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ที่ให้การสนับสนุนทันวิจัย อุปกรณ์ในการทำงานวิจัย รวมถึงสถานที่ในการดำเนินการจัดทำวิจัย จนโครงการนี้สำเร็จลุล่วงไปด้วยดี

รองศาสตราจารย์ ทพญ. ปริศนา ปริพัฒนานนท์

สารบัญ

บทที่ 1
บทนำ

Alveolar ridge resorption leads to soft tissue and hard tissue deficiency after a certain time of tooth extraction. Alveolar bone width reduction is usually more pronounced than alveolar bone height reduction (Lekovic, Camargo et al. 1998). In the first year after tooth extraction, the bone width reduced around 50% from original width (from 12 mm to 5.9 mm, on average), and two-thirds reduction occurred within the first 3 months (Schropp, Wenzel et al. 2003). The deformities lead to difficulty in implant placement and denture construction. Socket preservation has been a proposed method of preserving the natural tissue contours at extraction sites for later implant placement (Horowitz, Holtzclaw et al. 2012).

Socket preservation should be considered at the time of tooth extraction to alleviate the need for future ridge augmentation. Many graft materials, such as autogenous bone, allograft, xenograft, and alloplasts, have been used in an attempt to maintain the dimensions of alveolar ridge after extraction (Darby, Chen et al. 2009). Allografts are among commonly used materials and have been used safely in humans for bone regeneration without an adverse antigenic response (Becker, Urist et al. 1996). However, transmitted diseases and religion restriction flavor the use of synthetic material particularly calcium phosphate-based (Ca-P) for bone graft material. Calcium phosphate-based bioceramics are structural similarities to natural bone such as biodegradability, bioactivity, and osteoconductivity (LeGeros 2008).

Most common Ca-P materials are hydroxyapatite (HA), tricalcium phosphate (TCP) and biphasic calcium phosphate (BCP). Biphasic calcium phosphate, a two-phase of hydroxyapatite and tricalcium phosphate, provokes bone regeneration by osteoconductive properties and controls solubility by varying the ratio of TCP. Hydroxyapatite is a good matrix scaffold for new bone formation and slowly resorbed, while β-tricalcium phosphate is in rapid resorption phase, stimulates new bone formation by dissolving into calcium and phosphate ions (LeGeros, Lin et al. 2003). This grafting material has a greater capacity for enhancing new bone regeneration and can be resorbed and subsequently replaced by host bone in a shorter time. Previous work in the Department of Oral and Maxillofacial Surgery PSU, nano biphasic calcium phosphate has been fabricated by using polymeric sponge method and showed high biocompatibility with osteoblastic cell proliferation. BCP at the ratio of HA:β-TCP,50:50 showed good cellular affinity and biocompatibility with the highest osteocalcin activity (Ebrahimi, Pripatnanont et al. 2012; Ebrahimi, Pripatnanont et al. 2014). When BCP particles (ratio of HA:beta-TCP,9:1 and 8:2) had been tested in the rabbit model, both ratios enhanced bone formation and presented good

osteoconductive properties, biocompatibility with the living tissue and excellent space maintaining capacity with slow degradation rates (Pripatnanont, Praserttham et al. 2016).

Socket preservation procedure consists of the filling of bone substitute into tooth socket and the sealing of socket orifice with sealing material such as a free gingival graft or other material. Platelet-rich fibrin (PRF), a rich source of autogenous cytokines and growth factors can be considered as a biological membrane when pressed. They are angiogenesis, immunogenicity and can enhance epithelialization (Dohan, Choukroun et al. 2006; Dohan Ehrenfest, De Peppo et al. 2009; Dohan Ehrenfest, Del Corso et al. 2010). In this study, PRF would be warm with hot water and then compressed into a membrane for socket seal in socket preservation.

The use of bone substitute allows new bone formation in extraction sockets. However, different grafting materials and differing healing periods make different bone formation and graft degradation. Biphasic Calcium Phosphate is the material that can control the degradation rate by varying the ratio of HA/TCP. BCP seems to be an ideal bioceramic material that can be used in each situation depending on degradation rate needed.

The study aimed to use BCP as a grafting material for socket preservation and to evaluate whether BCP can preserve hard and soft tissue volume of the tooth socket after tooth extraction.

บทที่ 2
วัตถุประสงค์

General objective

1. To evaluate the efficacy of BCP combined with PRF membrane in socket preservation compared with FDBA combined with PRF membrane

Special objective

1. To compare soft tissue maturation after socket preservation with BCP or FDBA sealed with PRF.
2. To compare dimensional change after socket preservation with BCP or FDBA sealed with PRF by using cast-based measurement, and cone beam computed tomogram.
3. To compare bone formation after socket preservation with BCP or FDBA sealed with PRF by using Micro-CT, Histology.
4. To compare degradation rate of standard PRF membrane and warm PRF membrane.

บทที่ 3
วิธีการทดลอง

Study groups

The study protocol was approved by the Ethic Committee of the Faculty of Dentistry, Prince of Songkla University, Songkla, Thailand (Ethic no. EC5609-21-P-HR). All patients were required to read, understand, and sign the consent form, which included a thorough explanation of expected benefits and possible risks. Patients who underwent extraction of the single-root tooth and subsequent single-tooth implant placement were included in this study. The reasons for extraction were included periodontal disease, endodontic failure, advanced unrestorable caries, or tooth fracture. Patients were recruited and based on following inclusion criteria: patients must be above 20 years of age ; required single tooth extraction and later dental implant placement and the extracted teeth were bounded by adjacent teeth and exclusion criteria: patients who were smokers ; metabolic bone disease, pregnancy, history of malignancy or radiotherapy or chemotherapy for malignancy in the past 5 years ; history of autoimmune disease and long-term steroidal or antibiotic and sign of any active infection and not able to follow instructions related to the study procedures.

Then patients agreed to participate the study were randomized using sealed envelopes prepared by an independent party from the study into the following two groups shown in Table 1. Group 1 -- tooth extraction, socket grafted with Freeze-dried bone allograft particles size 100-1000 μm sealed with PRF membrane; Group 2 -- tooth extraction, socket grafted with BCP (HA/TCP 50/50) particles size 200-500 μm sealed with PRF membrane. Following initial screening procedures each patient underwent a site-specific intraoral and radiographic examination (Figure 1).

Table 1 Study group

Study Groups	Detail	Socket
FDBA	Freeze dried bone allograft seal with PRF membrane	n = 10
BCP	BCP (HA/TCP 50/50) particles seal with PRF membrane	n = 10

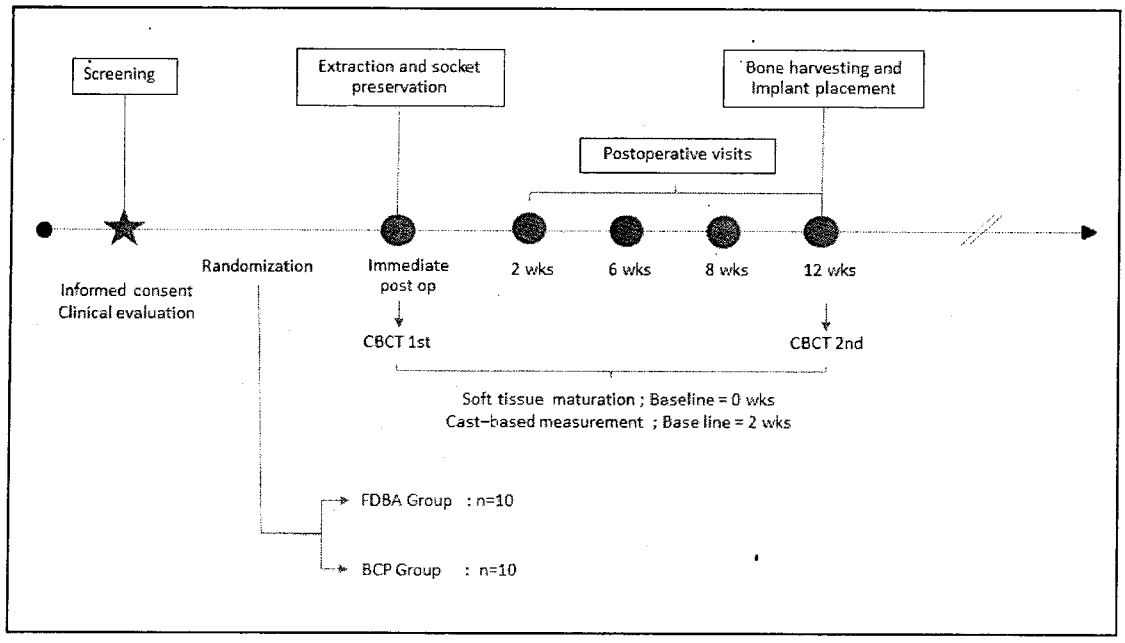

Figure 1 Timeline of the study.

Sample size

Estimate sample size for two-sample comparison of means was computed by using data from Toloue and coworker study (Toloue, Chesnoiu-Matei et al. 2012) in Figure 2

	n	Mean	SD	SE	Minimum	Maximum
New bone percentage						
$CaSO_4$	13	30.92	8.87	2.46	17.64	44.62
FDBA	15	16.40	11.20	2.89	0.15	41.23

Figure 2 Histomorphometric Analysis of Calcium sulfate and FDBA

Sample size calculation:

$$n_{/gr} = (Z_{(1-\alpha)} + Z_{(1-\beta)})^2 \, 2\sigma^2 / \text{Mean different}^2$$

σ^2 = Pooled variance

$$= (n_1-1)sd_1^2 + (n_2-1)sd_2^2 / n_1+n_2-2$$

Assumption :

$\alpha = 0.05$ (two-sided) $\rightarrow Z_{(1-\alpha)} = 1.96$

$\beta = 0.2 \rightarrow Z_{(1-\beta)} = 0.84$

σ^2= 103.85

Mean different = 14.52

$$n_{/gr} = (1.96+0.84)^2 \times 2 \times (103.85)/(14.52)^2$$

$$n_{/gr} = 7.72\ n_{/gr}$$

Sample size for each group: 10 patients (Estimated power: Power=0.8744)

Pre-operative preparation

At screening procedure visit, intraoral photographs of each patient were taken and then the impression of upper and lower arch were registered with irreversible hydrocolloid (Jeltrate® Alginate, Dentsply, Toronto, Canada) for making a surgical guide and a study model. Dental study models were casted in dental stone (GC Fujirock type 4; GC Corp, Tokyo, Japan). Before tooth extraction, intraoral radiographs of the indicated teeth were obtained using a parallel technique.

Surgical procedures

Prophylaxis with amoxicillin 1 g was prescribed 1 hour before the operation. Clindamycin 600 mg were prescribed to a patient who was an allergy to amoxicillin. The extraction sites were anesthetized with a local anesthesia (4% Articaine hydrochloride, Ubistesin 1:100,000; 3M ESPE, Platz, Seefeld, Germany). The teeth were extracted by atraumatic technique, root section in bucco-lingual direction was done and teeth segment were elevated. The teeth were removed by extraction forceps, attempted to minimize the trauma to the bone circumscribing the alveolus. The sockets were thoroughly debrided to remove granulation tissue and copiously irrigated with normal saline solution. While extraction sites were prepared and 10 mL of autologous whole blood were collected from the median cubital vein (forearm) by needle gauge no. 21 connected with a 10-ml sterile syringe without anticoagulant. Then the whole blood was transferred into a 10-mL glass tube, which will be immediately centrifuged using Hettich Zentrifugen centrifuge EBA 20 (Andreas Hettich GmbH & Co, KG, Tuttlingen, Germany) for 10 minutes at 3000 revolutions/min. A fibrin clot was obtained in the middle of the tube just between the red corpuscles at the bottom and acellular plasma at the top. The fibrin clot was collected with straight non-toothed forceps. Then the PRF gels were compressed by sterile spoons to get the membrane. The releasate or the fluid leaked from PRF was mixed with the grafting material. Then socket orifice was de-epithelized and the materials were inserted into the socket without pressure until the materials were filled up to the marginal bone level. The socket orifice was sealed by PRF membrane and retained by criss-cross suture technique with Vicryl 4-0 (ETHICON, Johnson & Johnson Medical Limited, Livingston, Scotland) suture material

Group 1 (FDBA Group), FDBA particles were mixed with releasate from PRF and were filled in the socket and the surgical wound was closed by using PRF membrane and retained by criss-cross suture technique.

Group 2 (BCP Group), The BCP particles were mixed with releasate from PRF and were filled in the socket and the surgical wound was closed by using PRF membrane and retained by criss-cross suture technique.

Postoperative Follow-up

All patients were advised to rinse their mouth with 0.12% chlorhexidine gluconate mouthwash, 1 minute, twice daily for one week. Paracetamol 500 mg (Cemol, Central Poly Trading Co., Ltd., Nonthaburi, Thailand) was prescribed postoperative every 4–6 hours until no pain and the appropriate antibiotics either amoxicillin 500 mg or clindamycin 600 mg was prescribed postoperatively 3 times daily for one week.

Reentry procedure

Patients were be reviewed at 2 weeks, 6 weeks, 8 weeks, 12 weeks for clinical measurement and then a digital x-ray were taken. Irreversible hydrocolloid impressions were registered, and dental casts (GC Fujirock type 4, GC Corp., Tokyo, Japan) were fabricated.

Clinical Evaluation and Data Collection

A: Direct measurement of socket orifice dimension for soft tissue healing

The dimensions of the socket orifice (mesial-distal [M-D] and buccal-lingual [B-L]) width were measured directly from the midpoint of the socket orifice of the extraction site (Figure 3). The measurements were carried out by 1 investigator using a UNC-15 periodontal probe (Hu-Friedy, *Hu-Friedy* Mfg. Co., Chicago, IL, US). Data were collected at immediate post operation (T0), follow-up time of 2 weeks (T2), 6 weeks (T6), 8 weeks (T8), and 12 weeks (T12). Socket orifice reduction was calculated as the mean percentage of reduction from the baseline (T0) to each time point.

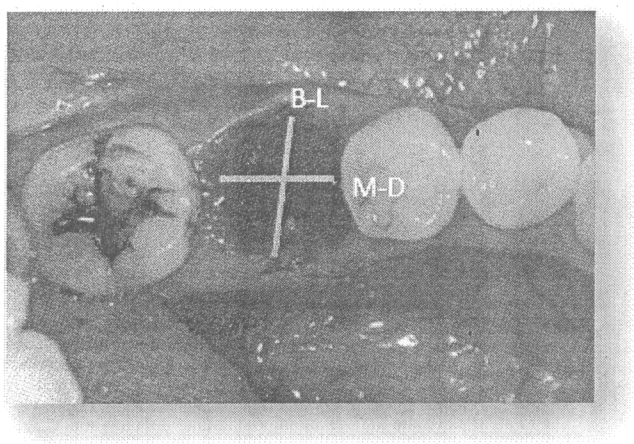

Figure 3 Direct measurement of socket orifice

B: Cast-based measurements for dimension change

Changes in the residual ridge dimensions were assessed on the dental casts obtained at the baseline of 2 weeks (T2), 6 weeks (T6), 8 weeks (T8) and 12 weeks (T12) post-operatively. Then the cast of each time point was scanned with Scanners (3Shape D700, Copenhagen, Denmark) and the casts at 6, 8, 12 weeks were superimposed with the cast at the baseline (within 2 weeks postoperatively) by using Ortho Analyzer™ software (3Shape, Copenhagen, Denmark) as in Figure 4. Then each edentulous site with the superimposed cast was measured for the dimension change of ridge width and height. The ridge width (horizontal dimension) was measured from the horizontal reference line located 3 mm below the cement-enamel junction (CEJ) of the adjacent teeth by using Ortho Analyzer™ software. Similarly, the dimension change of ridge height (vertical dimension) was measured from the mid-crestal vertical reference line of the edentulous site (Figure 5). Ridge reduction was calculated as the mean dimensional change between baseline (T2) and each time point.

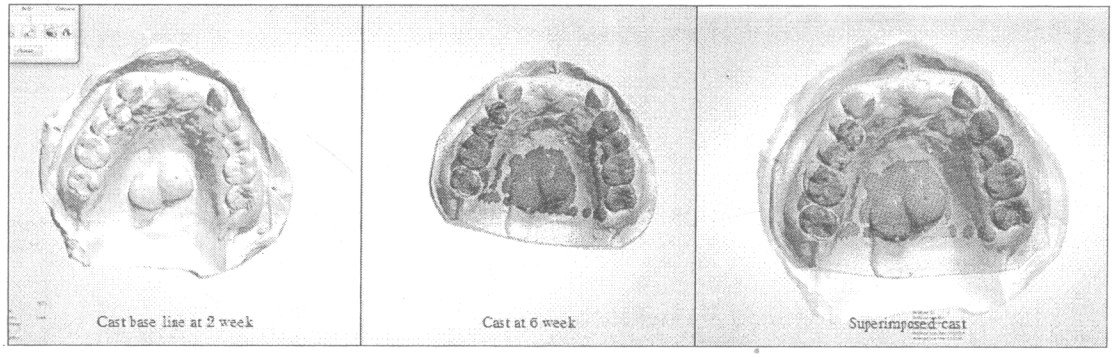

Figure 4 Superimposed cast at the baseline 2 weeks(T2) with the cast at 6 weeks

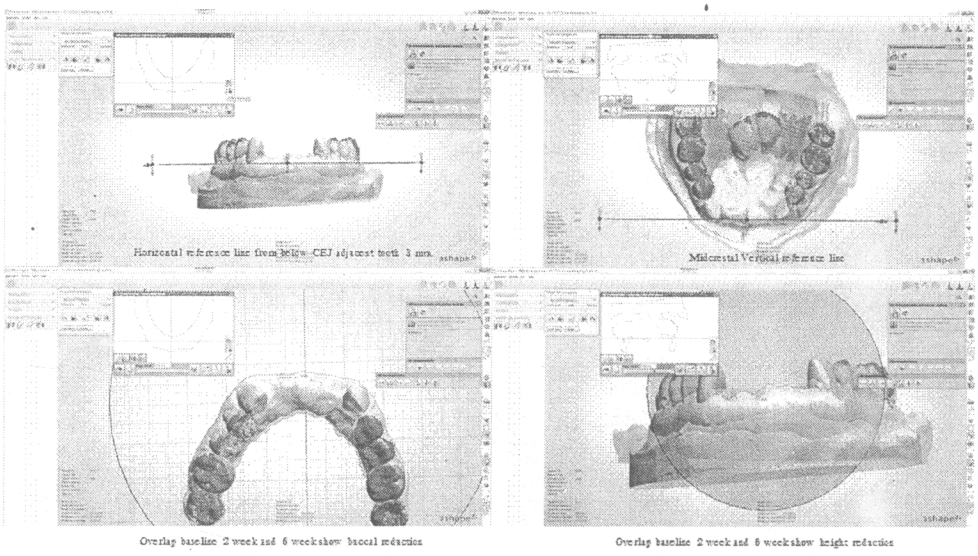

Figure 5 Measurement of the dimensional change of width and height from the reference line by using superimposed casts

C: Cone beam CT measurements for the dimension change

Cone beam CT (3D Accuitomo 170, J Morita, Kyoto, Japan) with 90 kvp, 5 mA, 30.8 s, 4x4 cm FOV, 0.08 mm isotropic voxel size at the socket preservation site were taken immediately post operation and 12 weeks postoperatively and used for measuring the dimensional change. The ridge width, buccal ridge height, palatal or lingual ridge height (Figure 6) were measured using the reference line at the bottom of the socket as the horizontal line with reference point and the lines from the peak of the buccal and the palatal crests perpendicular to the horizontal line and parallel to the mid socket line as the vertical lines. Ridge reduction was calculated as the mean dimensional change between immediately post operation and 12 weeks postoperatively.

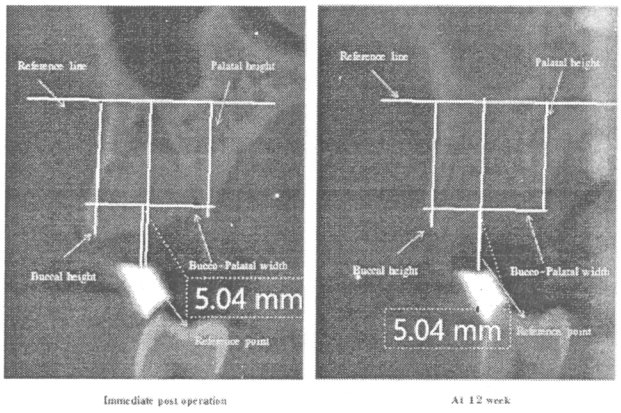

Figure 6 Dimension measurement in Cone beam CT

Histological and Histomorphometric analysis of the bone specimen

At 12 weeks, local anesthesia was administered and full mucoperiosteal buccal and lingual flaps were reflected. The clinical evaluation of grafting material were assessment by visual with grad in score on 3 – point scores: 1) Grade A, graft particles blended to the healing socket; 2) Grade B, particles were predominate in the socket; 3) Grade C, soft tissue healing filled in socket (Figure 7).

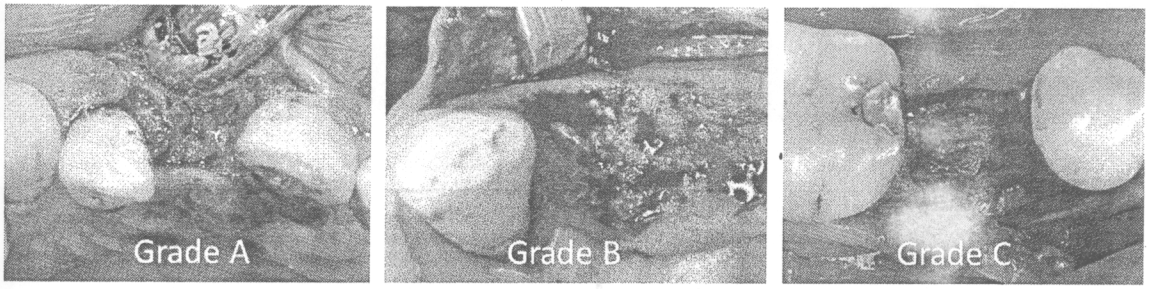

Figure 7 Grading of socket healing

After that, as part of the implant site preparation, a surgical trephine bur (3i Implant Innovations Inc.; USA) with a 2-mm inner diameter and 6-mm length were used to harvest a 6x2 mm. cylindrical bone core from the central part of the former sockets. The ridge bone were prepared to receive an appropriate-sized endosseous implant having a minimum length of 10 mm. The primary stability of the implant was assured by using a torque control ratchet. The bone specimens were fixed immediately in 10% neutral buffered formalin. And then the specimens were sent for micro-computed tomography analysis and histological analysis.

D: Micro-computed tomography analysis

To obtain a high-resolution, quantitative measure of bone formation, micro-computed tomography (micro-CT) imaging were performed. Trephined and formalin-fixed bone cores were used for micro-CT analysis (Micro-CT35, SCANCO Medical AG, Brüttisellen, *Switzerland*). After calibration, the specimens were scanned at 55 kVp, 72 μA and 4W in high-resolution mode (18.5μm^3/voxel). Scanned data were reconstructed by built-in software.

Before analysis, the grayscale threshold values were determined to discriminate new bone and materials (Humber, Sandor et al. 2010). The threshold was selected by identifying the specific threshold of new bone and materials within the bone core. The threshold value for bone mineralized was determined by tracing and clearly set at 200-1000. The threshold value for the BCP and FDBA were determined by tracing and clearly identified BCP particles were set at 500-1000 and FDBA particles were set at 320-1000. Then the new bone formation threshold was set at 200-320. After determination of the threshold values, the margins were traced to specify ROI of bone core. Then the percent of residual graft (RG) and new bone formation volume fraction (NB) were determined (Figure 8).

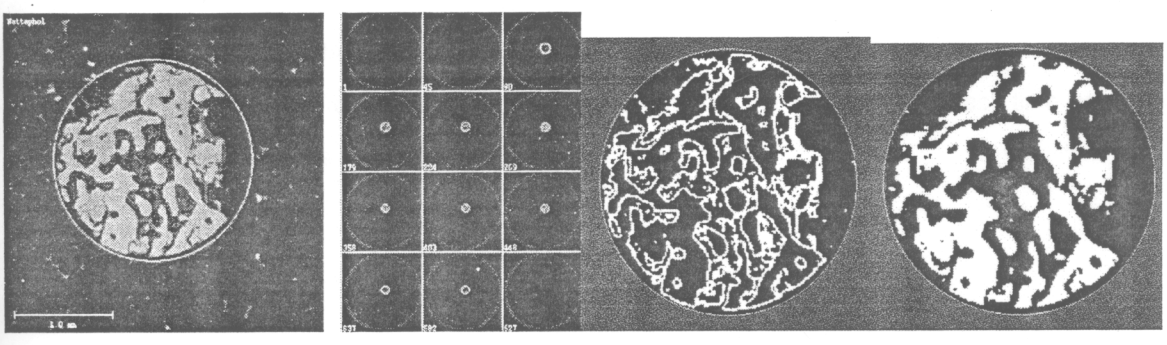

Figure 8 Micro CT analysis of Region interest (ROI)

E: Histological and Histomorphometric analysis

After completing the image analysis, the amount of bone tissue formation were analyzed by standard histology. The specimens were processed to obtain thin ground sections, according to the

technique of Donath and Breune (Donath and Breuner 1982). Briefly, the specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disc at approximately 150μm and ground down to approximately 15-25 μm with a specially designed grinding machine (EXAKT® cutting and grinding system, EXAKT® Apparatebau, Norderstedt, Hamburg, Germany). The bone blocks stained for visualization of cells and extracellular matrix with Goldner's Masson trichrome and Hematoxylin and eosin stain and slides labeled with the patient number. All slides were loaded into VS120 Virtual Slide Microscope (Olympus, Tokyo, Japan) and scanned at 40x magnifications. Digital histologic images were captured with the special software from the same company (OlyVIA 2.8, Olympus software, Tokyo, Japan). The undecalcified sections were selected for histomorphometric analysis. The quantity of new bone formation was calculated as the percentage of the newly formed bone area to the total area and the amount of residual graft area that were calculated as the percentage of each residual graft area to the total area using Image Pro Plus 7.0 (Media Cybernetics, MD, USA).

Percentage of new bone area = new bone area x 100 / total area

Percentage of residual graft area = residual graft area x 100 / total area

Statistical analysis

Statistical analysis was performed using statistical analysis software (SPSS version 15, SPSS Inc., Chicago, USA). The characteristic and morphology of PRF membrane were assessed descriptively. The microscopic features of the bone biopsy and the surrounding tissue were assessed descriptively. Data were tested for normality and presented as means±SD. Repeated measures ANOVA analysis was used to compare the dimensional change of each time point in the same group. The independent t-test was applied to compare the differences in those parameters between the two groups at each time point. The level of statistical significance was set at a $P < 0.05$.

บทที่ 4
ผลการทดลองและวิจารณ์ผลการทดลอง

Sixteen patients aged 52.87±15.61 years with 20 socket preservation participated in the study with 10 each per material group. There were 15 socket preservation in the maxilla and 5 socket preservation in the mandible. The FDBA group consisted of five incisors and five premolars. In the BCP group consisted of four incisors and six premolars. There was no any infection and 11 implant sites needed additional grafting due to buccal plate resorption at 3 months after socket preservation. At stage I implant surgery, the grafting sites were reentry, the FDBA group showed grafted material blended to the surrounding bone whereas the BCP particles were clearly seen in all cases of the BCP group.

A: Direct measurement of socket orifice dimension for soft tissue healing

PRF membrane was still presented in the socket orifice until 2 weeks postoperatively. Soft tissue healing was nearly complete at 6 weeks in both groups and completely healed within 8 weeks in both groups (Figure 9). No statistically significant differences were detected between the groups at each time point of 0, 2, 6, 8 and 12 weeks after extraction ($P > 0.05$). The mean percentage of socket orifice reduction from immediate post-extraction to 6, 8 and 12 weeks group was statistically significant different from immediate post-extraction to 2 weeks post extraction significantly ($P < 0.05$) as in Figure 10.

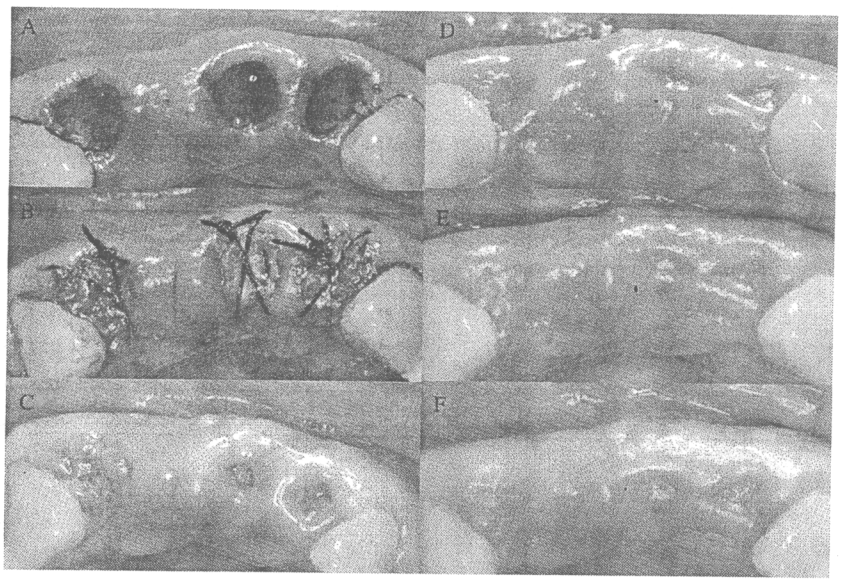

Figure 9 Progression of socket healing in socket preservation. Tooth 12 was grafted with FDBA and sealed with PRF membrane, tooth 11 was filled with PRF gel and tooth 22 was grafted with BCP and sealed with PRF membrane A) Immediate extraction B) Socket preservation C) Follow-up 2 weeks D) Follow-up 6 weeks E) Follow-up 8 weeks F) Follow-up 12 weeks

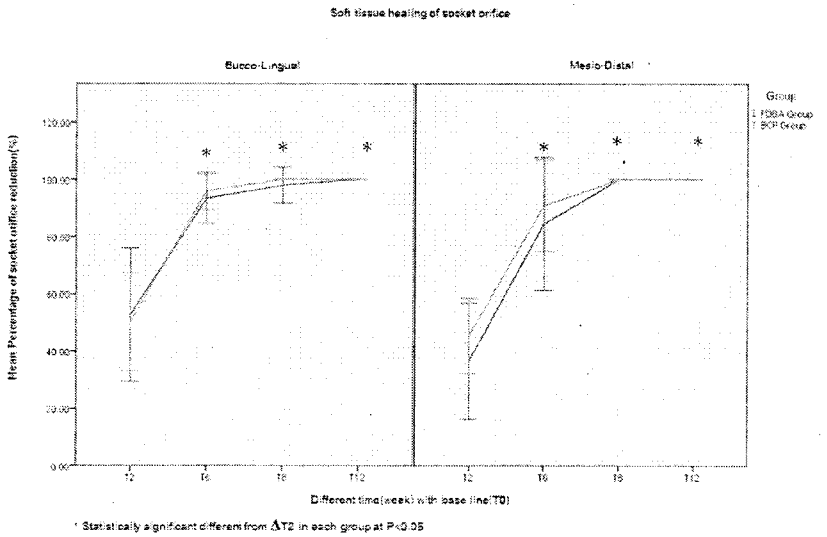

Figure 10 Mean different percentage of socket orifice reduction from immediate post extraction (T0) to each time point (T2, T6, T8, T12) in the bucco-lingual direction (left) and the mesio-distal direction (right)

B: Cast-based measurements

The ridge width and height reduction progressed with time and there were statistically significant differences among each time point of 6, 8 and 12 weeks in both groups. At the buccal side, ridge width reduction of FDBA group was more than the BCP group at all follow-up times and most pronounced at 12 weeks postoperatively (1.26±0.48 mm, 1.02±0.68 mm). The lingual side or palatal side (FDBA 0.71±0.38 mm, BCP 0.68±0.44 mm) and the height reduction (FDBA 0.66±0.21 mm, BCP 0.72±0.21 mm) were comparable and limited, and less than 1 mm at 12 weeks (Figure 11).

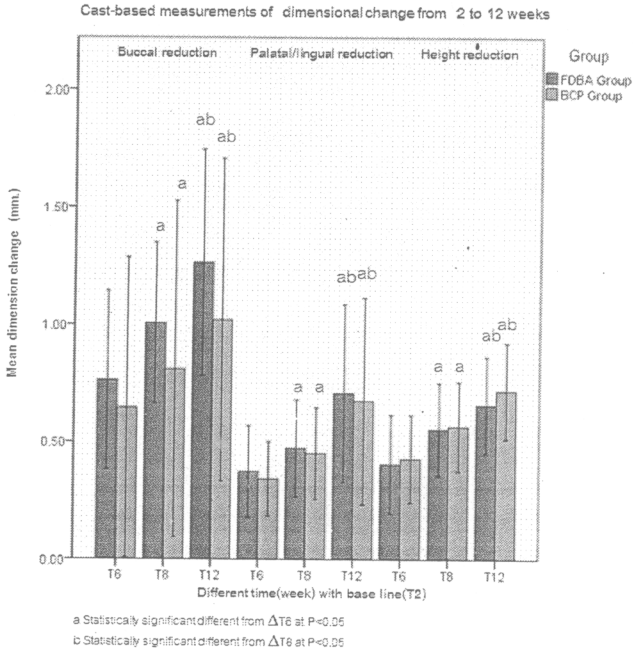

Figure 11 Dimensional change in ridge reduction at buccal, lingual and the height of the extraction site from cast-based measurements.

C: Cone beam CT measurements

At the level of 5 mm from the reference point, the FDBA and BCP groups showed similar results of ridge width reduction (0.98±0.15 mm, 0.92±0.34 mm), but FDBA showed less height reduction at buccal side (0.34±0.25 mm) and height reduction at palatal/lingual side (0.35±0.12 mm) than the BCP group (0.49±0.25 mm, 0.42±0.13 mm). There was no statistically significant difference between the groups in ridge reduction at any direction.

D: Micro-CT Analysis

At 3 months, the percentage of new bone volume fraction and residual graft volume fraction of FDBA (22.37±9.61, 17.31±14.53) was higher than BCP (16.89±7.46, 15.94±7.39), but no significant difference was detected (Figure 12,13).

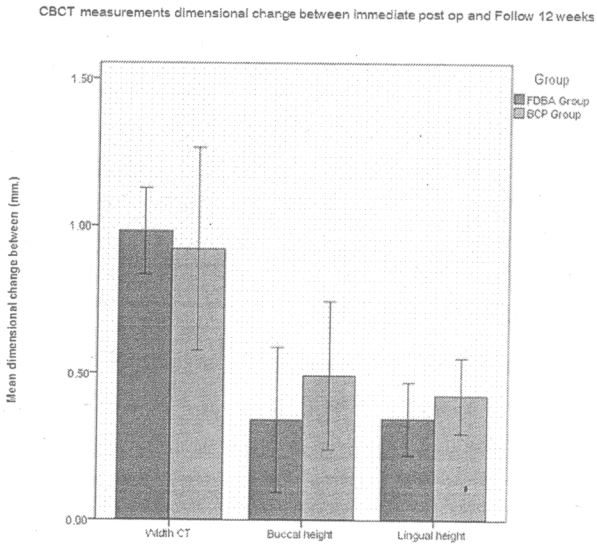

Figure 12 The dimensional change in the CBCT of FDBA and BCP from immediate post-op to follow-up period of 12 weeks.

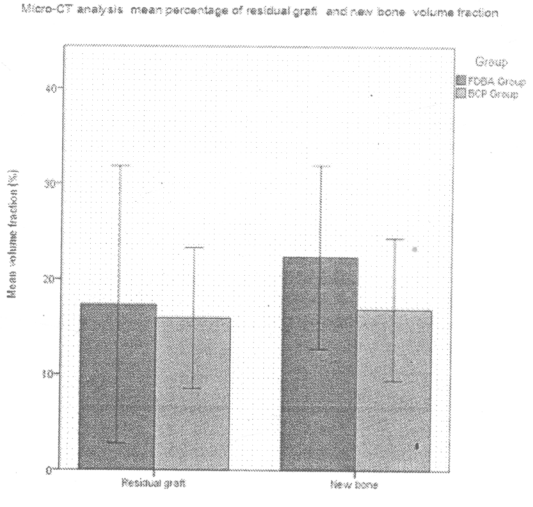

Figure 13 Micro-CT analysis; Residual graft and new bone volume fraction at 12-week postoperative period.

E: Histology

In the FDBA group, the newly formed bone showed good continuity and well incorporated with FDBA particles. In the BCP group, BCP particles were presented with newly formed bone fused with BCP particles together with surface resorption of BCP particles and active osteoclasts (Figure 14,15).

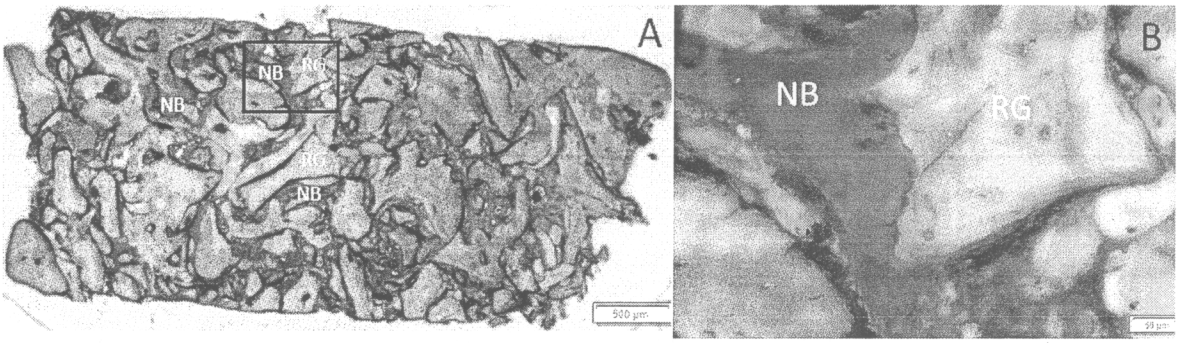

Figure 14 FDBA specimens at 3 months; (A) FDBA, original magnification. (B) FDBA shows that newly formed bone with FDBA particles on the bone core; RG = residual graft, NB = newly formed bone; Hematoxylin and eosin, original magnification x 10

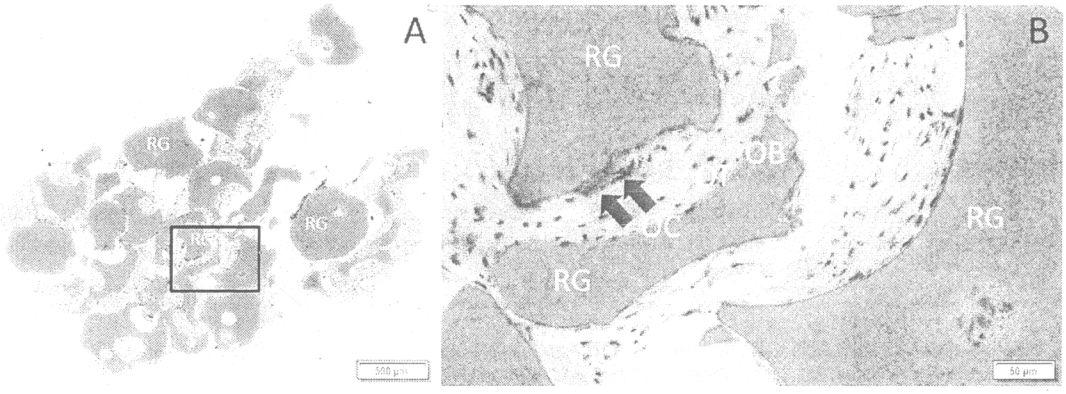

Figure 15 BCP specimens at 3 months; (A) BCP,original magnification.
(B)BCP shows surface resorption of BCP particles with active osteoclast and bone formation is also evident with the appearance of osteoid; RG = residual graft, OC = osteoclast, OT = osteoid, OB = osteoblast; Hematoxylin and eosin, original magnification x 10

F: Histomorphometric Analysis

The percentage of new bone area for FDBA (20.17±4.59) and BCP groups (18.40±7.20) were comparable but the percentage of residual graft area of FDBA (22.50±2.64) was less than the BCP group (29.38±7.96) significantly ($p<0.05$) (Figure 16).

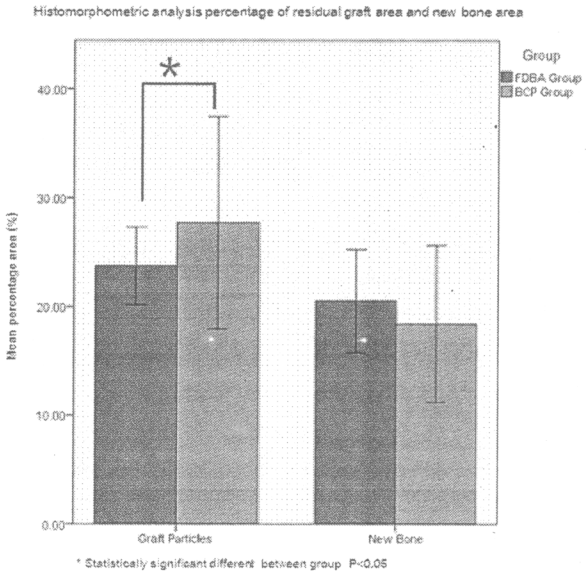

Figure 16 Histomorphometric analysis; the percentage of residual graft area and the percentage of new bone area at 12-week postoperative period.

Regarding clinical study, Ridge reduction was quantitatively evaluated through cast-based and CBCT measurement. The results from cast-based and CBCT measurement were in the same direction that the dimensional change at the buccal, lingual aspects and the height occurred immediately after tooth extraction and progressed with time. Both FDBA and BCP groups showed limited loss of ridge height and width at both buccal and lingual side without statistically significant differences. Both FDBA and BCP groups showed ridge width reduction at the buccal side more than 1.00 mm after 3 months. Although grafting material in socket preservation can maintain ridge dimension and bone volume but only the internal volume of the socket because the buccal wall still remodeled and resorbed. Furthermore, the result of CBCT measurement on the dimensional change of ridge width and height were in the same direction with the cast-based measurement. The ridge width reduction was approximately 1.00 mm in both groups (FDBA group 0.98±0.15, BCP group 0.92±0.34). However, the ridge height reduction was limited in both groups and less than 0.5 mm. Therefore, the use of biomaterial both FDBA and BCP could maintain ridge dimension and bone volume post extraction.

In this study, the histological findings showed that the FDBA and BCP could be used to promote socket preservation. No signs of inflammation surrounding the graft particles were detected, and these particles contacted both newly deposited woven bone. Most of the newly formed bone in this study was woven bone, and lack of newly formed lamellar bone with residual graft particle.

For the evaluation of new bone formation and residual graft material, most studies had been performed using histomorphometric analysis, which is limited to 2-dimension information from a representative slide. Results from Micro-CT revealed that newly form bone of the FDBA group (22.37±9.61)

was more than the BCP group (16.89±7.46). However, residual graft particle of the FDBA group (17.31±14.53) was also more than the BCP group (16.89±7.46). The histomorphometry results supported the micro-CT analysis that the FDBA group had higher newly bone formed than the BCP group. In contrast, histomorphometry results of residual grafts was opposite to the micro-CT. However, the micro-CT and histomorphometry results showed that both FDBA and BCP had effective space maintaining in socket preservation.

Regarding residual material, at 3 months, the FDBA particles were blended with the bone in the sockets and showed new bone formation higher than the BCP group (22.37±9.61 vs 16.89±7.46) in micro-CT analysis. The particles size used in this study of FDBA (100-1000 μm) and BCP (200-500 μm) were not in the same range because FDBA had been crushed manually with bone mill while the BCP had been fabricated and sieved. Therefore, the particle size of the BCP was more homogeneous than the FDBA. Moreover, the residual particles of the BCP group (15.94±7.39) was lower than the FDBA group (17.31±14.53) in micro-CT analysis but no statistical significance. It is noted that the BCP particles may be resorbed faster than the FDBA and then was replaced with connective tissue instead of bone due to the size of the particles. In addition, the result from of this study was slightly better than the previous study that used FDBA in socket preservation and found new bone formation was only 16.7% while the residual graft in the previous study was 21%(Toloue,Chesnoiu-Matei et al 2012).

In summary, it is evidence that PRF membrane was an option for socket sealing because it could be used instead of free gingival graft or other collagen material for sealing the socket. FDBA and BCP were favorable for socket preservation and both materials could maintain the ridge dimension and enhanced bone formation. However, the FDBA showed better bone formation than the BCP

บทที่ 5
สรุปผลการทดลอง

Efficacy of BCP and FDBA on preserving ridge dimension and socket preservation for Type II implant placement showed good outcome. Using a PRF membrane for socket seal is proved to be a reliable method that enhanced soft tissue healing at socket orifice in 4 weeks. FDBA demonstrated more bone regeneration when compared to BCP (HA:β-TCP = 50:50) at the 3-month time frame. Long-term evaluation is needed and the ratio of HA to TCP should be revised

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ข้อคิดเห็นและข้อเสนอแนะสาหรับการวิจัยต่อไป

BCP at the ratio of HA:BCP 50:50 could be used for socket preservation but the outcome was not greater than the FDBA. It is speculated that the TCP ratio is too high that resorb too early and enhance soft tissue infiltration into the socket orifice. Hence the ratio of BCP should be revised and use higher ratio of HA and less TCP in further study.

ภาคผนวก

Efficacy of nano biphasic calcium phosphate compared to freeze dried bone allograft in socket preservation sealed with PRF membrane

Nattapon Boonsri[1*], Prisana Pripatnanont[1] and Naruporn Monmaturapoj[2]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Prince of Songkla University, Hatyai, Songkhla, Thailand, 90112

[2]National Metal and Materials Technology Center, Thailand Science Park, Pathumthani, Thailand, 12120

*Corresponding author. E-mail address: prisana.p@psu.ac.th, nattanook.n@gmail.com

Abstract

Background: After tooth extraction, socket wall remodeling leads to reduction of hard and soft tissue volume of the alveolar ridge and results to difficulty in implant placement. Socket preservation aims to minimize these problems. This study aimed to compare the efficacy of biphasic calcium phosphate (BCP) to freeze-dried bone allograft (FDBA) in socket preservation sealed with platelet-rich fibrin (PRF) membrane. Material and Methods: This randomized clinical controlled trial was conducted in 13 patients whom socket preservation and later implantation were performed. Patients were allocated into 2 groups of 8 sockets for socket grafted with either freeze-dried bone allograft (FDBA) or biphasic calcium phosphate (BCP). All socket orifices were sealed with PRF membrane. Soft tissue healing was assessed by direct measurement of socket orifice dimension, dimensional change of the ridge reduction was measured by using cast-based measurement and cone beam computed tomography (CBCT). Patients were followed at 2, 6, 8 and 12 weeks after extraction. Results: Soft tissue healing at socket orifice was nearly complete at 6 weeks and healed completely within 8 weeks in both groups. There was no statistically significant differences in dimension of socket orifice between 2 groups at each follow-up period after extraction ($P > 0.05$). Both groups showed statistically significant reduction of socket orifice dimension among each time frame at 6, 8 and 12 weeks ($P < 0.05$). The cast-based measurements of dimensional change in width and height reduction at either buccal sides or lingual or palatal sides of both groups showed no statistically significant differences. There were statistically significant differences in width reduction at the buccal side among each follow-up time of both groups ($P<0.05$). The CBCT measurement of the dimensional change in the FDBA group and the BCP group showed comparable dimensional change in each parameters such as ridge width reduction (1.03±0.11 mm vs 0.92±0.39 mm), ridge height reduction at buccal side(0.36±0.29 mm vs 0.47±0.25 mm) , and ridge height reduction at palatal/lingual side (0.33±0.14 mm vs 0.43±0.14 mm) which were favorable outcome. Conclusion: BCP and FDBA sealed with PRF membrane were comparably effective in maintaining soft tissues and minimizing alveolar ridge resorption.

Keywords: socket preservation, platelet-rich fibrin, freeze dried bone allograft, biphasic calcium phosphate

Introduction

Alveolar ridge resorption leads to soft tissue and hard tissue deficiency after a certain time of tooth extraction. Alveolar bone width reduction are usually more pronounced than alveolar bone height reduction (Lekovic, et al., 1998). The alveolar ridge width decreased 50% in 1 year (from 12 mm to 5.9 mm, on average), and two-thirds of the reduction occurred within the first 3 months (Schropp, Wenzel, Kostopoulos, & Karring, 2003). The deformities lead to difficulty in implant placement. However, it is possible to minimize such problems by simply carrying out socket preservation procedures in extraction sockets using grafting materials with or without barrier membranes. Socket preservation has been a proposed method of

preserving the natural tissue contours at extraction sites for later implant reconstruction (Horowitz, Holtzclaw, & Rosen, 2012).

Socket preservation should be considered at the time of tooth extraction to alleviate the need for future ridge augmentation. Degrees of bone formation and residual graft materials in socket preservation depends on the materials and techniques used (Darby, Chen, & Buser, 2009). Many graft materials, such as autogenous bone, allograft, xenograft, and alloplasts, have been used in an attempt to maintain the dimensions of alveolar ridge after tooth extraction (Darby, et al., 2009). Allografts are among commonly used materials and have been used safely in humans for bone regeneration without an adverse antigenic response (Becker, et al., 1996). Their use has been found to predictably reconstruct missing bone and preserve the ridge after tooth extraction (Wang, & Tsao, 2008). However, transmitted diseases and religion restriction flavor the use of synthetic material particularly calcium phosphate-based (Ca-P) for bone graft material. Calcium phosphate-based bioceramics are structurally similarities in some properties of bone such as biodegradability, bioactivity, and osteoconductivity (LeGeros, 2008).

Most common Ca-P materials are hydroxyapatite (HA), tricalcium phosphate (TCP) and biphasic calcium phosphate (BCP). Biphasic calcium phosphate, a two-phase of hydroxyapatite and tricalcium phosphate, provokes bone regeneration by osteoconductive properties and controls solubility by varying the ratio of TCP. Hydroxyapatite is a good matrix scaffold for new bone formation and slowly resorbed. Beta-tricalcium phosphate is in rapid resorption phase, stimulates new bone formation by dissolving into calcium and phosphate ions (LeGeros, Lin, Rohanizadeh, Mijares, & LeGeros, 2003). This grafting material has a capacity in enhancing new bone regeneration and can be resorbed and subsequently replaced by host bone. Previous work in the Department of Oral and Maxillofacial Surgery Prince of Songkla University, nano biphasic calcium phosphate has been fabricated by using polymeric sponge method and showed high biocompatibility with osteoblastic cell proliferation. BCP at the ratio of HA:beta-TCP, 50:50 showed good cellular affinity and biocompatibility with the highest osteocalcin activity (Ebrahimi, Pripatnanont, Monmaturapoj, & Suttapreyasri, 2012; Ebrahimi, Pripatnanont, Suttapreyasri, & Monmaturapoj, 2013). When BCP particles (ratio of HA:beta-TCP, 9:1 and 8:2) had been tested in the rabbit model, both ratios enhanced bone formation and presented good osteoconductive properties, biocompatibility with the living tissue and excellent space maintaining capacity with slow biodegradation rates (Pripatnanont, Suttapreyasri, Leepong, Monmaturapoj, & Praserttham, 2013).

Socket preservation procedure consists of the filling of bone substitute into tooth socket and the sealing of socket orifice with sealing material such as free gingival graft or other material. Platelet-rich fibrin (PRF) membrane was introduced to be used as a socket seal in this study. PRF has been known as a rich source of autogenous cytokines and growth factors which involve with angiogenesis, immunogenicity and hard and soft tissue healing process. (Dohan, et al., 2006; Dohan Ehrenfest, De Peppo, Doglioli, & Sammartino, 2009; Dohan Ehrenfest, Del Corso, Inchingolo, & Charrier, 2010).

The use of bone substitute allows new bone formation in extraction sockets. However, different grafting materials with different degradation rate affect quality of bone formation and residual graft retention. Degradation rate of BCP can be controlled by varying the ratio of HA to beta-TCP. BCP seems to be an ideal bioceramic material that can be used in various situations depending on degradation rate needed. This study hypothesized that BCP with PRF membrane could preserve hard and soft tissue volume of alveolar socket after

tooth extraction similar to FDBA with PRF membrane. The aim of this study was to evaluate the efficacy of BCP compare to FDBA in socket preservation sealed with PRF membrane.

Materials and Methods

Study design

This study was a prospective randomized clinical study, conducted at the Oral & Maxillofacial Surgery Clinic, Prince of Songkla University, Hatyai, Songkhla, Thailand. The experimental protocol was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Prince of Songkla University (No: EC5609-21-P-HR). Sample size per group was calculated based on previous study by Toloue, Chesnoiu-Matei and Blanchard (Toloue, Chesnoiu-Matei, & Blanchard, 2012). Mean difference was 14.52, Pooled variance was 103.85 and the sample per group was 8 at α=0.05 and β=0.2. The patients who participated the study were randomized by using sealed envelopes prepared by an independent party from the study

and allocated into two study groups. FDBA Group; tooth extraction socket was grafted with freeze-dried bone allograft particles sized 200-1000 μm and sealed with PRF membrane. BCP Group; tooth extraction socket was grafted with BCP (HA/TCP:50/50) particles sized 200-500 μm, sealed with PRF membrane (Table 1).

Table 1 Study groups

Study Groups	Detail	Socket
FDBA Group	Freeze dried bone allograft seal with PRF membrane	n = 8
BCP Group	BCP (HA/TCP:50/50) particles seal with PRF membrane	n = 8

Patients and Methods

Sixteen socket and thirteen patients which required extraction of single root tooth and subsequent single-tooth implant treatment were invited to participate the study. All patients were informed about the purposes and required to read, understand, and sign the consent form. The reasons for extraction included periodontal disease, endodontic failure, advanced unrestorable caries, or tooth fracture. The recruitment of patients based on patient's age that must be above 20 years of age, require single tooth extraction with following dental implant therapy, and the extracted tooth was in the dentate area. Patients were excluded if they were in these conditions: patients who were smokers, metabolic bone disease, pregnancy, history of malignancy or radiotherapy or chemotherapy for malignancy in the past 5 years, history of autoimmune disease and long-term steroidal or antibiotic therapy, sign of any active infection and not able or not willing to follow instructions related to the study procedures.

Surgical procedures

Amoxicillin 1 g (COAMOX®500, Community Pharmacy Public Co. Ltd., Bangkok, Thailand) was prescribed 1 hour before the operation as a prophylactic regimen for wound infection, otherwise Clindamycin 600 mg was prescribed to patient who was allergy to amoxicillin. The extraction site was anesthetized with a local anesthesia, 4% articaine hydrochloride 1.8 ml (Ubistesin 1:100,000; 3M ESPE,

Platz, Seefeld, Germany). The tooth was extracted by an atraumatic technique by root sectioned in bucco-lingual direction and each tooth segment was elevated gently, attempted to minimize trauma to the bone circumscribing the alveolus. The tooth segment was removed by extraction forceps. The socket was thoroughly debrided to remove granulation tissue and copiously irrigated with normal saline solution. While extraction site was prepared, 10 mL of autologous whole blood was collected from the median cubital vein (forearm) by a needle gauge no. 21 connected with a 10-ml sterile syringe without anticoagulant. Then the whole blood was transferred into a 10-mL glass tube, which was immediately centrifuged using Hettich Zentrifugen centrifuge EBA 20 (Andreas Hettich GmbH & Co, KG, Tuttlingen, Germany) for 10 minutes at 3000 revolutions/min. A fibrin clot was obtained in the middle of the tube just between the red corpuscles at the bottom and acellular plasma at the top. The fibrin clot was collected with straight non-toothed forceps. Then the PRF gel was compressed by sterile spoons to get the PRF membrane. The releasate or the fluid leaked from PRF membrane was mixed with the grafting material. After that the socket orifice was de-epithelized and the grafting material was inserted into the socket without pressure until the material was filled up to 2 mm below the marginal bone level. The socket orifice was sealed by PRF membrane and retained by criss-cross suture technique with Vicryl 4-0 (ETHICON, Johnson & Johnson Medical Limited, Livingston, Scotland) suture material (Figure 1).

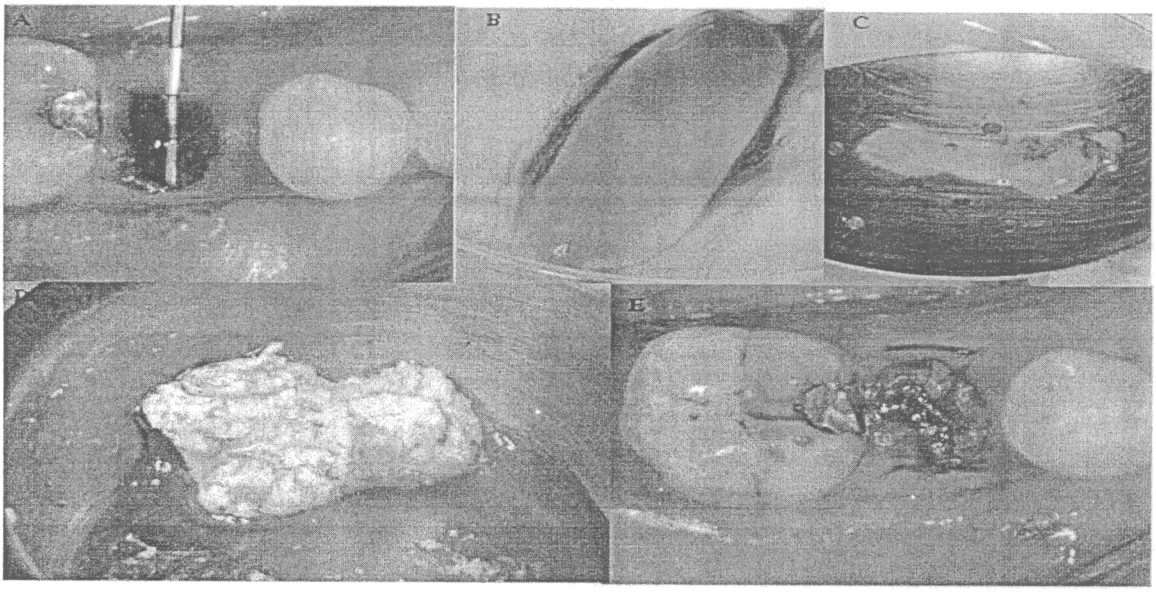

Figure 1 – Operation procedure A) Atraumatic extraction and de-epithelized socket orifice B) PRF C) PRF membrane D) Grafting material mixed with fluid leaked from PRF E) Placement of graft material and sealed with PRF membrane and sutured with criss-cross suture technique

Post-operative follow-up

All patients were advised to rinse their mouth with 0.12% chlorhexidine gluconate mouthwash, 1 minute, twice daily for one week. Paracetamol 500 mg (Cemol, Central Poly Trading Co., Ltd., Nonthaburi, Thailand) was prescribed postoperative every 4-6 hours until no pain and the appropriate antibiotics either amoxicillin 500 mg or clindamycin 600 mg was prescribed postoperatively 3 times daily for one week.

Reentry procedure

Patients were reviewed postoperatively at 0 week (T0), 2 weeks (T2), 6 weeks (T6), 8 weeks (T8), 12 weeks (T12). Irreversible hydrocolloid impressions were registered, and dental casts (GC Fujirock type 4, GC Corp., Tokyo, Japan) were fabricated.

Clinical Evaluation and Data Collection

A : Direct measurement of socket orifice dimension for soft tissue healing

The dimensions of the socket orifice (mesial-distal [M-D] and buccal-lingual [B-L]) width were measured directly from the midpoint of inner socket orifice of the extraction site (Figure 2). The measurements were carried out by 1 investigator using a UNC-15 periodontal probe (Hu-Friedy, Hu-Friedy Mfg. Co., Chicago, IL, US). Data were collected at immediate postoperation (T0), follow-up time of 2 weeks (T2), 6 weeks (T6), 8 weeks (T8), and 12 weeks (T12). Socket orifice reduction was calculated as the mean percentage of reduction between baseline (T0) and each time point.

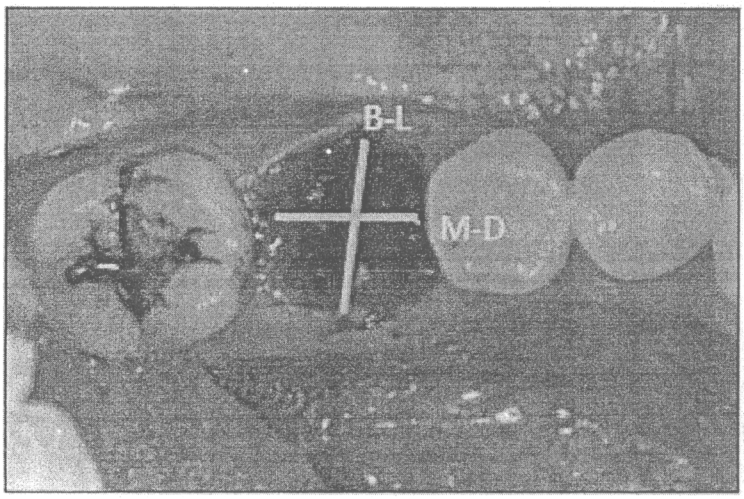

Figure 2 Measurements of socket orifice

B : Cast-based measurements for dimension change

Changes in the residual ridge dimensions were assessed on the dental casts obtained at baseline 2 week (T2), 6 week (T6), 8 week (T8) and 12 weeks (T12) post-operatively. Then the cast of each time point was scanned with Scanners (3Shape D700, Copenhagen, Denmark) and the cast at 6, 8, 12 weeks were superimposed with the cast at the baseline (within 2 weeks post operatively) by using Ortho Analzer™ software (3Shape, Copenhagen, Denmark) as in Figure 3. Then each edentulous site with superimposed cast was measured for the dimension change of ridge width and height. The ridge width (horizontal dimension) was

measured from the horizontal reference line located 3 mm below the cement-enamel junction (CEJ) of the adjacent teeth by using Ortho Analyzer™ software. Similarly, the dimension change of ridge height (vertical dimension) was measured from the midcrestal vertical reference line of the edentulous site (Figure 4). Ridge reduction was calculated as the mean dimensional change between baseline (T2) and each time point.

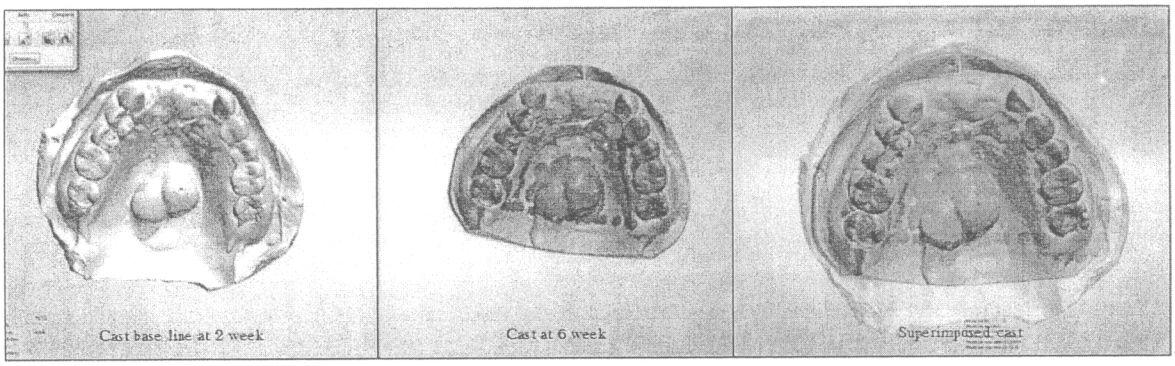

Figure 3 Superimposed cast at the baseline 2 week(T2) with the cast at 6 weeks(T6)

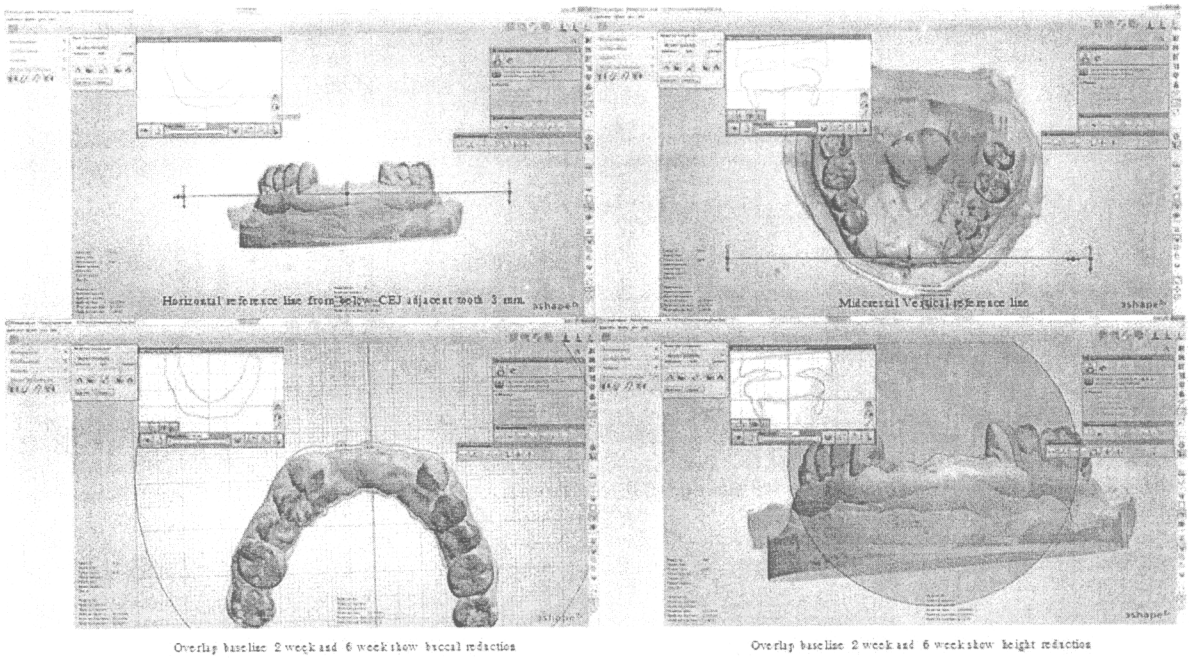

Figure 4 Measurement of the dimensional change of width and height from the reference line by using superimposed casts

C : Cone beam CT measurements for dimension change

Cone beam CT (3D Accuitomo 170, J Morita, Kyoto, Japan) with 90 kvp, 5 mA, 30.8 s, 4x4 cm FOV, 0.08 mm isotropic voxel size at the socket preservation site were taken immediately post operation and 12 weeks postoperatively and used for measuring the dimensional change. The ridge width, buccal ridge

height, palatal or lingual ridge height (Figure 5) were measured using the reference line at the bottom of the socket as the horizontal line with reference point and the lines from the peak of the buccal and the palatal crests perpendicular to the horizontal line and parallel to the mid socket line as the vertical lines. Ridge reduction was calculated as the mean dimensional change between immediately post operation and 12 weeks postoperatively

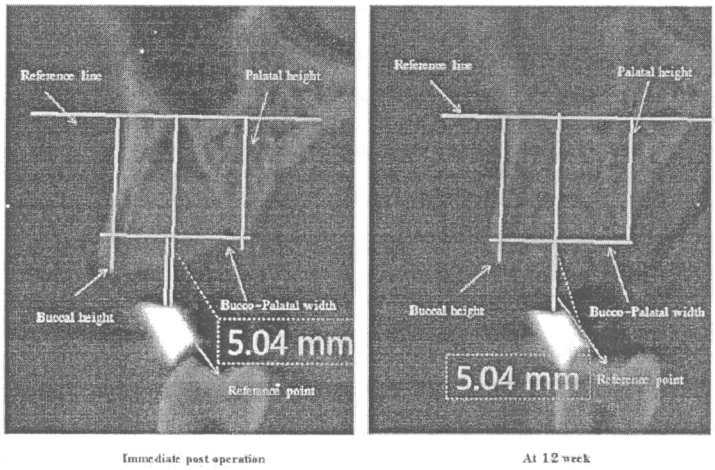

Figure 5 Dimensional measurement in CBCT

Statistical analysis

Statistical analysis was performed using statistical analysis software (SPSS version 22, SPSS Inc., Chicago, USA). Data were tested for normality and presented as means±SD. Repeated measures ANOVA analysis was used to compare the dimensional change of each time point in the same group. The independent t-test was applied to compare the differences of those parameters between the two groups at each time point. The level of statistical significance was set at a $P < 0.05$.

Result

Thirteen patients aged 51.39±15.26 years with 16 socket preservation participated in the study. There were 13 socket preservation in the maxilla and 3 socket preservation in the mandible. The FDBA group included four incisors and four premolars. In the BCP group included three incisors and five premolars. There was no any infection and 11 implant sites needed additional grafting due to buccal plate resorption. At stage I implant surgery the grafting sites were reentry and FDBA group showed blended grafted material to the surrounding bone whereas the BCP particles had been clearly seen in all cases of BCP group. Demographic data were presented in Table 2.

Table 2 Demographic data

	Number of socket	Age	Gender		Maxilla	Mandible	Incisor	Premolar	Additional graft
			Male	Female					
FDBA	8	53.29±16.07	-	-	5	3	4	4	6
BCP	8	49.49±15.24	-	-	8	0	3	5	5
Total	16	51.39±15.26	7	6	13	3	7	9	11

Clinical examination

A: Direct measurement of socket orifice dimension for soft tissue healing

Platelet-rich fibrin membrane couldn't be seen in the socket orifice after 2 weeks. Soft tissue healing was nearly complete at 6 weeks in both groups and completely healed within 8 weeks in both groups (Figure 6). The mean different percentage of socket orifice reduction between baseline (TO) and the follow-up time in M-D and B-L directions were presented in Figure 7. No statistically significant differences were detected between the groups at each time point of 0, 2, 6, 8 and 12 weeks after extraction ($P > 0.05$). Mean percentage of socket orifice reduction from immediate post extraction to 6, 8 and 12 weeks group was statistically significant different from immediate post extraction to 2 weeks post extraction significantly ($P < 0.05$).

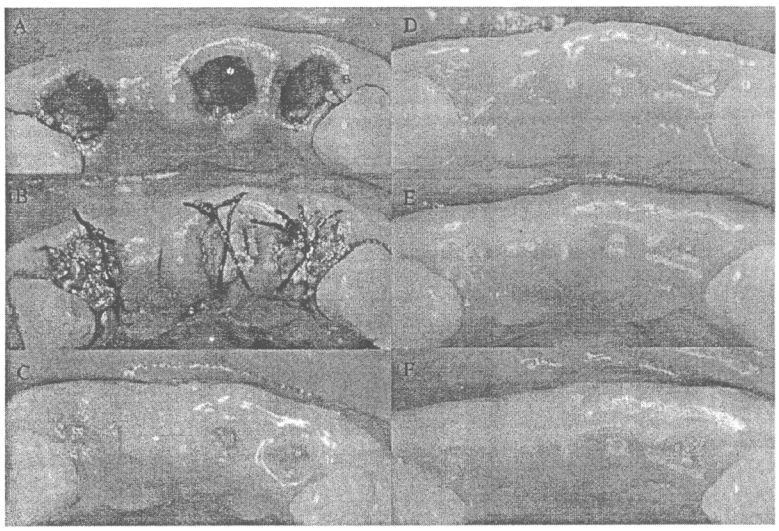

Figure 6 A case of socket preservation on the area 12 with FDBA seal with PRF membrane, the area 11 with PRF gel and the 22 with BCP seal with PRF membrane A) Immediate extraction B) Socket preservation C) Follow-up 2 weeks D) Follow-up 6 weeks E) Follow-up 8 weeks F) Follow-up 12 weeks

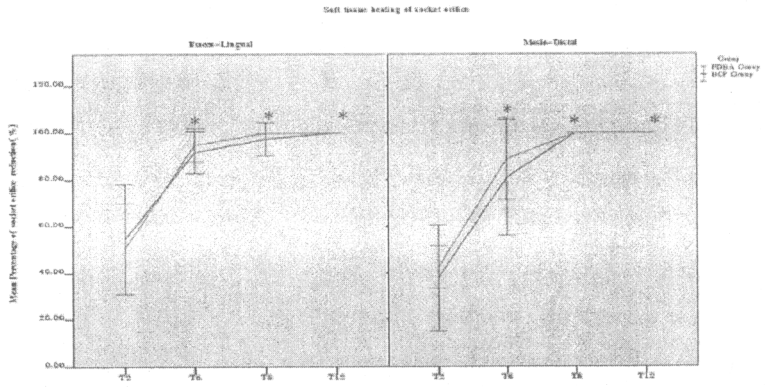

Figure 7 Mean different percentage of socket orifice reduction from immediate post extraction (T0) to each time point (T2, T6, T8, T12) in the bucco-lingual direction (left) and in the mesio-distal direction
(right)

B : Cast-based measurements

The cast-based measurements of the dimensional change at the buccal side, lingual/palatal side and height were shown in Figure 8. The ridge width and height reduction progressed with time and there were statistically significant differences at each time point of 6, 8 and 12 weeks in the buccal side of both groups. Ridge width reduction at the buccal side of FDBA group was the most pronounced at 12 weeks postoperatively (1.32±0.53 mm) and more than the BCP group (1.03±0.78 mm) and also at the other time points. There was no statistically significant difference between the group at the buccal side, the lingual or palatal side and the height reduction. The lingual side (FDBA 0.78±0.40 mm, BCP 0.73±0.48 mm) and the height reduction (FDBA 0.71±0.20 mm, BCP 0.70±0.21 mm) were very similar and limited and less than 1 mm at 12 weeks.

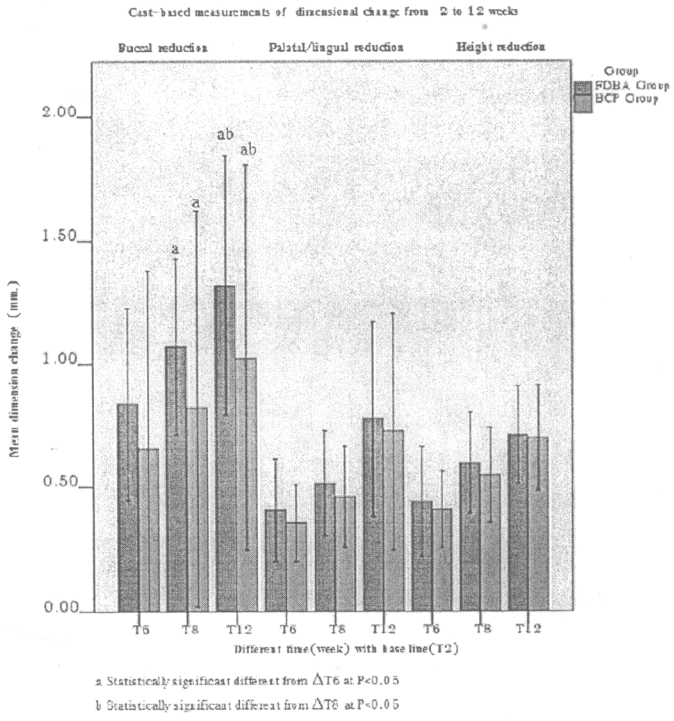

Figure 8 The buccal, lingual side and the height of the contour changes of the extraction site from cast-based measurements

C : Cone beam CT measurements

The morphological ridge width and height from the cone beam CT were presented in Figure 9. FDBA group showed 1.03±0.11 mm ridge width reduction, 0.36±0.29 mm height reduction at buccal side, and 0.33±0.14 mm height reduction at palatal/lingual side while the BCP group showed 0.92±0.39 mm ridge width reduction, 0.47±0.25 mm height reduction at buccal side, 0.43±0.14 mm 0.33±0.14 mm height reduction at palatal/lingual side respectively. No statistically significant differences were detected between the groups for bucco-palatal/lingual width and also no statistically significant differences were detected between the groups for buccal ridge height and palatal/lingual ridge height.

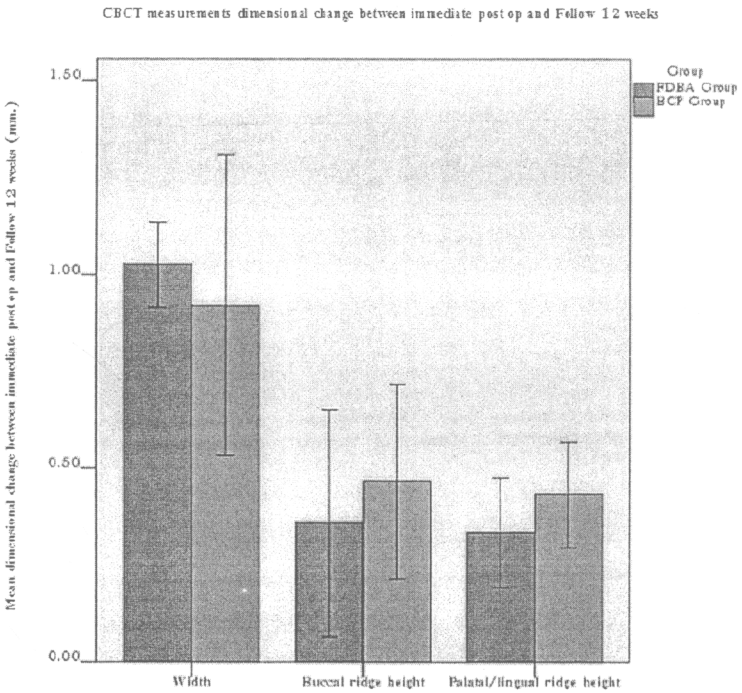

Figure 9 The dimensional change measured by CBCT of both groups. There was no statistical difference between groups

Discussion

This study evaluated the efficacy of biphasic calcium phosphate (BCP) compared to freeze-dried bone allograft (FDBA) in socket preservation which was sealed with PRF membranes. Ridge reduction was quantitatively evaluated through cast-based and CBCT measurement. The results from cast-based and CBCT measurement are in the same direction that the dimensional change at the buccal, lingual aspects and the height occurred immediately after tooth extraction and progressed with time. Both FDBA and BCP groups showed limited loss of ridge width at the buccal and the lingual side and ridge height and showed no statistically significant differences between the groups. In each group, there was no significant loss of ridge width at the lingual side and ridge height at each time point, but there was a significant loss in ridge width at the buccal side in both groups. Regarding the cast-based measurement, the results indicated that BCP performs as well as

FDBA in preserving ridge height and width and keeping the ridge contour after 3 months. Although FDBA and BCP grafting material were used for socket preservation for maintaining ridge dimension and bone volume, the buccal wall still remodeled and resorbed. Previous reports have shown maximal ridge width resorption after 6 months to 1 year after extraction (Lekovic, et al., 1998; Schropp, et al., 2003). Iasella, et al (Iasella, et al., 2003) used freeze-dried bone allograft (FDBA) concomitant with collagen membrane for preserving residual ridge contour and assessed dimensional change by using direct measurement with modified digital caliper and acrylic stent, and reported that the dimensional change were 1.3 mm in ridge height and 1.17 mm in ridge width during 4-6 months. Furthermore, Eskow and Mealey (Eskow, & Mealey, 2014) also assessed dimensional change by using direct measurement and reported the dimensional change were 2.00 mm in ridge width, 1.00 mm of buccal ridge height, 1.94 of lingual ridge height during 18 weeks with the use of cancellous freeze dried bone allograft (FDBA). In the present study, 3 months of short-term socket preservation was the time frame for investigating the capacity of FDBA and BCP to preserve socket which was shorter from other studies. The results of the study agree showed less reduction than those studies might due to shorter time frame, therefore large reduction still progress afterward.

Regarding non grafting site from previous studies Cardaropoli, Tamagnone, Roffredo, Gaveglio, & Cardaropoli (Cardaropoli, Tamagnone, Roffredo, Gaveglio, & Cardaropoli, 2012), by using cast-base measurement, it was found that non grafting site loss more ridge width (4.48 ± 0.65 mm) and ridge height (1.54 ± 0.33 mm) at 4 month post extraction compared to Bio-oss® (Geistlich Biomaterials, Wollhusen, Switzerland). Also from the study by Lekovic et al (Lekovic, et al., 1998) who compared non grafting site to bioresorbable membrane for socket preservation and found that non grafting site loss of 4.59 ± 0.23 mm for ridge width and 1.50 ± 0.21 mm for ridge height during 6 months. Therefore, it can be concluded that using biomaterial for socket preservation could reduce ridge width and height reduction and this present study showed comparable results when compared to other reports.

However, systematic reviews demonstrate that even with ridge preservation techniques, there remains some loss of the bone width and height after healing (Ten Heggeler, Slot, & Van der Weijden, 2011). Furthermore, regarding the result of the dimensional change of ridge width and height by using CBCT measurement, the dimensional changes were in the same direction with the cast-based measurement. The ridge width reduction was approximately 1 mm in both groups and the FDBA group (1.03±0.11) showed more reduction than the BCP group (0.92±0.39). However the ridge height reduction was very limited in both groups less than 0.5 mm. Regarding the results of our study, the use of biomaterial both FDBA and BCP could maintain ridge dimension and bone volume post extraction.

Regarding, PRF membrane was used for socket seal in this study and showed that the socket healed within 6 weeks and was clearly showed complete maturation of mucosa covering the socket orifice within 8 weeks. It was evidence that PRF membrane was an option for socket sealing because it could be used instead of free gingival graft or other collagen material seal the socket. In summary, FDBA and BCP were favorable for socket preservation since both groups could maintain the ridge dimension, and the dimensional change was within acceptable limit.

Conclusion

BCP and FDBA sealed with PRF membrane were effective in maintaining soft tissue and hard tissue contour of alveolar socket in 3-month-period after tooth extraction.

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