Efficacy of Nano Biphasic Calcium Phosphate Compared with Freeze Dried Bone Allograft in Socket Preservation Sealed with PRF Membrane

Nattaphol Boonsri

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Author Mr. Nattaphol Boonsri

Major Program Oral and Maxillofacial Surgery

Major Advisor :

……..

(Assoc. Prof. Prisana Pripatnanont)

Co-advisor

……..

(Dr. Naruporn Monmaturapoj)

Examining Committee :

……..Chairperson

(Assoc. Prof. *Dr. Somchai Sessirisombat)*

……..

(Assoc. Prof. Prisana Pripatnanont)

……..

(Dr. Naruporn Monmaturapoj)

……..

(Asst. Prof. Sompid Kintarak)

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

……..

(Assoc.Prof.Dr.Teerapol Srichana)

Dean of Graduate School

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

……...

(Assoc. Prof. Prisana Pripatnanont)

Major advisor

……...

(Mr. Nattaphol Boonsri)

Candidate

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

……...

(Mr. Nattaphol Boonsri)

Candidate

ชื่อวิทยานิพนธ์ การศึกษาประสิทธิภาพของวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต เปรียบเทียบกับกระดูกเอกพันธ์แห้งแบบระเหิดในการคงสภาพเบ้าฟันที่ปิดด้วยเยื่อพีอาร์เอฟ

ชื่อผู้เขียน นายณัฐพล บุญศรี

สาขาวิชา ศัลยศาสตร์ช่องปากและแมกซิลโลเฟเชียล

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บทคัดย่อ

หลังจากการถอนฟัน กระดูกเบ้าฟันเกิดการปรับรูปของกระดูกรอบๆกระดูกเบ้าฟันนำไปสู่การลดลงของปริมาณกระดูกของสันกระดูกขากรรไกรและส่งผลให้เกิดความยากลำบากในการวางแผนฝังรากฟันเทียม การคงสภาพกระดูกเบ้าฟันมีจุดมุ่งหมายเพื่อลดปัญหาเหล่านี้ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบประสิทธิภาพของวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต เปรียบเทียบกับ กระดูกเอกพันธ์แห้งแบบระเหิดในการคงสภาพเบ้าฟันที่ปิดด้วยเยื่อพีอาร์เอฟ

การศึกษาได้แบ่งออกเป็น 2 ส่วน ส่วนแรกทำการศึกษาในห้องปฏิบัติการ เยื่อพีอาร์เอฟถูกเตรียมเป็น 2 แบบ แบบปกติ กับแบบให้ความร้อน เพื่อศึกษาดูลักษณะทางสัณฐานวิทยาของเยื่อพีอาร์เอฟ โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด และการสลายของเยื่อพีอาร์เอฟ โดยการชั่งน้ำหนักดูปริมาณที่หายไป

ส่วนที่สอง ทำการศึกษาทางคลินิกแบบสุ่ม ในผู้ป่วย 16 คนที่ถอนฟันรากฟันเดียว และทำการคงสภาพกระดูกเบ้าฟันเพื่อทำการฝังรากเทียมในเวลาต่อมา ผู้ป่วยแบ่งออกเป็น 2 กลุ่ม กลุ่มละ 10 ซี่ เพื่อทำการปลูกถ่ายกระดูกเบ้าฟันโดยใช้กระดูกเอกพันธ์แห้งแบบระเหิดหรือวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟตที่ปิดด้วยเยื่อพีอาร์เอฟ การหายของเนื้อเยื่ออ่อนได้รับการประเมินโดยการวัดโดยตรงของบริเวณปากกระดูกเบ้าฟัน การเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรได้รับการวัดโดยชิ้นหล่อศึกษาและโคนบีมคอมพิวเตอร์โทโมกราฟ หลังจากนั้นผู้ป่วยจะถูกติดตามและเก็บข้อมูลที่ 2, 6, 8 และ 12 สัปดาห์หลังจากถอนฟันไป

ภายหลัง 12 สัปดาห์ ทำการตรวจชิ้นเนื้อกระดูกและประเมินปริมาณของกระดูกและวัสดุปลูกโดยการถ่ายภาพระดับไมโครเมตรและตรวจสัณฐานวิทยาโดยวิธีจุลพยาธิวิทยา

ผลการทดลองส่วนแรก จากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่าเยื่อพีอาร์เอฟ แบบปกติกับแบบที่ให้ความร้อนนั้นมีลักษณะรูปร่างจากการส่องด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดที่คล้ายกัน โดยส่วนล่างมีส่วนประกอบของเกล็ดเลือดและเซลล์เม็ดเลือดขาวบนพื้นผิวเยื่อพีอาร์เอฟ ส่วนกลางมีเส้นใยไฟบรินที่ปกคลุมด้วยเกล็ดเลือดและเม็ดเลือดขาวบางๆ แต่น้อยกว่าส่วนล่าง ส่วนบนมีลักษณะคล้ายกับตาข่ายไฟบรินของเยื่อพีอาร์เอฟ การสลายของเยื่อพีอาร์เอฟ แบบปกติในส่วนบนพบว่ามีการสูญเสียน้ำหนักที่น้อยกว่าส่วนล่างอย่างมีนัยสำคัญทางสถิติตั้งแต่วันที่ 10 ถึง วันที่ 14 ในส่วนของเยื่อพีอาร์เอฟ ที่ได้รับความร้อนพบว่ามีน้ำหนักลดลงมากกว่าเมื่อเทียบกับเยื่อพีอาร์เอฟ แบบปกติในทุกส่วน และเยื่อพีอาร์เอฟ ที่ได้รับความร้อนพบว่ามีการสูญเสียน้ำหนักอย่างมีนัยสำคัญทางสถิติภายในกลุ่มของวันที่ 1 และ 2

ผลการทดลองส่วนที่สอง การหายของเนื้อเยื่ออ่อนของบริเวณปากกระดูกเบ้าฟันไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ระหว่าง 2 กลุ่มในแต่ละช่วงเวลา หลังจากการถอนฟัน ($p > 0.05$) แต่ทั้งสองกลุ่มแสดงการหายของเนื้อเยื่ออ่อนโดยขนาดของปากแผลบริเวณปากกระดูกเบ้าฟันลดลงอย่างมีนัยสำคัญทางสถิติ($P < 0.05$)ในแต่ละกลุ่ม ที่ช่วงเวลาที่ 6, 8 และ 12 สัปดาห์ การเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรโดยชิ้นหล่อศึกษาในแนวความกว้างและความสูงของทั้ง 2 กลุ่ม ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ แต่พบการลดลงความกว้างของสันกระดูกขากรรไกรด้านแก้ม ด้านเพดานหรือด้านลิ้น และความสูงในกลุ่มเดียวกันลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ทั้งสองกลุ่ม การเปลี่ยนแปลงมิติของสันกระดูกขากรรไกรโดยโคนบีมคอมพิวเตอร์โทโมกราฟทั้งของสองกลุ่มไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ

การวัดโดยการถ่ายภาพรังสีระดับไมโครเมตรพบว่าปริมาณการสร้างกระดูกใหม่ของกระดูกเอกพันธ์แห้งแบบระเหิดและวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต (22.37±9.61,16.89±7.46)และวัสดุที่หลงเหลือของกระดูกเอกพันธ์แห้งแบบระเหิดและวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต(17.31±14.53,15.94±7.39) ไม่มีความแตกต่างกัน แต่อย่างไรก็ตามการวัดทางจุลพยาธิวิทยาพบมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ระหว่างกลุ่มของวัสดุที่หลงเหลือของกระดูกเอกพันธ์แห้งแบบระเหิดและวัสดุทดแทน

กระดูกนา โนไบเฟสิกแคลเซียมฟอสเฟต(29.38±7.96, 22.50±2.64) แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติการสร้างกระดูกใหม่ของกระดูกเอกพันธ์แห้งแบบระเหิดและวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต(20.17±4.59, 18.40±7.20) จึงสรุปได้ว่าวัสดุทดแทนกระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟตและกระดูกเอกพันธ์แห้งแบบระเหิดเหมาะสมสำหรับการทำการคงสภาพกระดูกเบ้าฟัน และเยื่อพีอาร์เอฟแบบปกติมีประสิทธิภาพในการสร้างเนื้อเยื่ออ่อนเหมาะสมสำหรับการใช้ปิดปากกระดูกเบ้าฟัน อย่างไรก็ตามกระดูกเอกพันธ์แห้งแบบระเหิดมีปริมาณการสร้างกระดูกใหม่ที่มากกว่ากระดูกนาโนไบเฟสิกแคลเซียมฟอสเฟต ดังนั้นอัตราส่วนของไฮดรอกซีอะปาไทต์และทีซีพีอาจจะยังไม่เหมาะสม

Thesis Title	Efficacy of Nano Biphasic Calcium Phosphate Compared with Freeze Dried Bone Allograft in Socket Preservation Sealed with PRF Membrane
Author	Mr. Nattaphol Boonsri
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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: The study was divided into 2 parts. Part I in vitro, PRF membrane were prepared into 2 types; Standard PRF and Warm PRF (warm in water bath at 80°C for 15 seconds). The characteristic and morphology were assessed by SEM observation. The degradation rate of PRF membrane was assessed by using weight loss. Part II, the randomized clinical controlled trial was conducted in 16 patients whom socket preservation and later implantation were performed. Patients were allocated into 2 groups of 10 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, the 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. At 12 weeks, bone

biopsy was performed, then Micro-CT and Histomorphometric were used to analyze newly form bone and residual grafts.

Results: In SEM observations, the standard and warm PRF membrane shared similar SEM image features. The lower part showed activated platelets aggregated and some white blood cells on the PRF membrane surface. The middle part showed fibrin fibers with covered by some platelets and some white blood cells but less than the lower part. The upper part presented resemble fibrin meshwork. The degradation rate of the standard PRF membrane, the weight reduction of the upper part were significant different than the lower part from day 10 to day 14. The warm PRF membrane lose weight more than the standard PRF membrane in all three parts and lose 60% to 80% in the second weeks and the weight reduction of the warm PRF membrane was significant different from day 1 to day 2. Soft tissue healing at socket orifice, there were 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 of between groups showed no statistically significant differences. But 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 different. The Micro-CT analysis demonstrated that the percentage of the newly form bone volume fraction 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 detected between both groups. However, the histomorphometry analysis indicated that the residual graft of the BCP group (29.38±7.96) showed significant different more particles than the FDBA group (22.50±2.64), but no significant

different were detected between groups 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. However, the ratio of HA to TCP should be revised and long-term evaluation is needed.

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List of Abbreviations and Symbols

A°	=	Angstrom
ANOVA	=	One-way analysis of variance
ABM	=	Anorganic bovine bone matrix
BCP	=	Biphasic calcium phosphate
B-L	=	Buccal-Lingual
BMP	=	Bone morphogenetic protein
°C	=	*Celsius*
Ca-P	=	Calcium phosphate-based
CBCT	=	Cone beam computer tomography
CDA	=	Calcium-deficient apatite
CEJ	=	Cement-enamel junction
CO_2	=	Carbon dioxide
CS	=	Calcium sulfate
DFDBA	=	Demineralized freeze-dried bone allograft
DMEM	=	Dulbecco's Modified Eagle's Medium
FDBA	=	Freeze Dried Bone Allograft
FGF	=	Fibroblast growth factor
FOV	=	*Field of View*
et al	=	And others
Fig.	=	Figure
HA	=	Hydroxyapatite
H&E	=	Hematoxylin-eosin
IL-1	=	Interleukin-1
g	=	Gram
kVp	=	Kilovoltage peak
mA	=	Milliampere

List of Abbreviations and Symbols (Continued)

M-D	=	Mesial-Distal
mg	=	Milligram
micro-CT	=	Micro computed tomography
ml	=	*Milliliter*
mm	=	Millimetre
MGCSH	=	*Medical Grade Calcium Sulfate Hemihydrate*
MTEC	=	The National Metal and Materials Technology Center of Thailand
NB	=	Newly formed bone
no.	=	Number
OB	=	Osteoblast
OC	=	Osteoclast
OT	=	Osteoid
PAF-4	=	Platelet activating factor-4
PDGF	=	Platelet-derived growth factor
PLGA	=	*Poly-lactic-co-glycolic acid*
PRF	=	Platelet-rich fibrin
PRP	=	Platelet-rich plasma
PSU	=	Prince of Songkla University
RBCs	=	Red blood cell
RG	=	Residual graft
ROI	=	Region of interest
RPM	=	Round per minute
s	=	Second
SEM	=	Scanning electron microscope
TCP	=	Tricalcium phosphate
TGF-β	=	Transforming growth factor-beta

List of Abbreviations and Symbols (Continued)

VEGF	=	Vascular endothelial growth factor
VF	=	Volume fraction
W	=	Watt
UNC	=	Universal curette
μA	=	Microampere
μm^3	=	Cubic micrometer
μm	=	Micrometer

Chapter 1

Introduction

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 (1). 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(2). The deformities lead to difficulty in implant placement. However, it can be minimized such problems by an easy procedure of socket preservation in extraction sockets. Socket preservation has been a proposed method of preserving the natural tissue contours at extraction sites for later implant placement (3).

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 (4). 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 (4). Allografts are among commonly used materials and have been used safely in humans for bone regeneration without an adverse antigenic response (5). Their use has been found to predictably reconstruct missing bone and preserve the ridge after tooth extraction (6). However transmitted diseases and religion restriction flavors the use of synthetic material particularly calcium phosphate-based (Ca-P) for bone graft

material. Calcium phosphate-based bioceramics are structural similarities in some properties of bone such as biodegradability, bioactivity, and osteoconductivity (7).

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 (8). 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(9, 10). 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 (11).

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. The free gingival graft is usually used for socket seal but it has to be harvested from the palatal tissue. For general dentist, a practical and easy technique are necessary. Therefore, Platelet-rich fibrin is introduced to serve this purpose. 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 (12-14). Compressed PRF has been used as a substitute for barrier membranes(15). Kawase and coworker suggested that heat-compression technique reduced the rate of biodegradation of the PRF membrane without sacrificing its biocompatibility(15). 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. Even though autogenous bone graft is the gold standard for bone grafting but it is invasive and not practical for socket preservation. Freeze dried bone allograft (FDBA) is widely used for socket preservation and shows success in socket preservation. However, it also has a downside such as immune response or disease transmission. Synthetic material is free of disease but mostly is slow 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.

Research questions

Whether a nano BCP can preserve hard and soft tissue volume of the tooth socket after tooth extraction.

Review of Literature

After tooth extraction, healing of a socket begins with an inflammatory reaction activated, formation of a blood clot and ends with fully maturation of tissue structures. It can be divided into five different stages as in Table1(16).

Table 1 Healing Stages of Extraction Sockets

STAGE	EVENTS	DURATION
First	A coagulum of red and white blood cells forms an initial blood clot or coagulum from the severed blood vessels.	Immediately extraction
Second	Within 2-5 days, the blood clot begins to lysis (fibrinolysis) and transforms into granulation tissue with cords of epithelial cells together with new capillaries.	4 to 5 days
Third	Connective tissue that being rich in vessels and inflammatory cells replaces the granulation tissue.	14 to16 days
Fourth	Starting of the calcification process. Osteoid is formed at the base and periphery of the socket wall. The sockets are filled with woven bone and the soft tissue turns to be keratinized tissue and completely close the socket.	3 to 6 weeks
Fifth	Lamellar bone gradually replaces the woven bone within the socket. Complete bony fill, with little evidence of osteogenic activity by the 16^{th} week.	5 to10 weeks

Loss of tooth leads to loss of ridge width and ridge height. When left undisturbed, extraction sockets heal uneventfully with bone tissue in 1 to 2 months following tooth extraction (17, 18). Naturally, the normal post-extraction healing response of an alveolar socket is resorptive and healing process usually occurs with the reduction of bone width and bone height(19). The greatest amount of bone loss is in the horizontal dimension and occurs on the facial aspect of the ridge. There is also the loss of vertical ridge height, which is most pronounced on the buccal (1, 20). The loss of ridge width and height may preclude optimum implant placement and restorative aesthetics.

At the Third International Team for implantology (ITI) Consensus Conference, the basic protocols were defined according to the time between tooth extraction and implant installation(21). Type 1: immediate implant placement, implants are placed in fresh extraction sockets. The objective is to engage the remaining socket walls with the implant. Type 2: early implant placement, implants are placed approximately 4–8 weeks after tooth extraction. The objective is to optimize soft tissue healing and may be need lateral bone augmentation. Type 3: early-delayed/conventional implant placement, the implants are placed in the healed alveolar ridge that dimensional changes have occurred (12–16 weeks).

Ridge dimension is important for planning implant placement, socket preservation is essential to ensure preserving ridge dimensions after tooth extraction. The socket preservation techniques have been proposed by several investigators as a possible way to preserve the ridge width and ridge height for optimum implant placement(1, 20, 22, 23). This may also help to avoid additional pre-implant bone grafting procedures. The materials such as autografts, allografts, xenografts and synthetic materials can be used alone or combined with barrier membrane for grafting in socket preservation procedures(24-31).

Block and coworkers suggested that using human mineralized bone had potential to reconstruct missing bone resulting from tooth extraction and to preserve bone after tooth extraction(32). It was confirmed with the study from Froum and coworkers which reported that freeze-dried bone allograft and bioactive glass had a positive effect on socket healing but were different in the residual material(26). In addition, Toloue and coworkers reported that calcium sulfate was as effective as freeze-dried bone allograft in preserving post-extraction ridge dimensions(33). Lasella and coworkers found that using freeze-dried bone allograft and a collagen membrane improved ridge height and width dimension(22). Guarnieri and coworkers reported that medical grade calcium sulfate hemihydrate seemed to be an ideal graft material in extraction socket bone regeneration because it was almost completely resorbable, and it allowed a new trabecular bone arrangement at 3 months(34) and seemed to be effective in accelerating the bone healing process, reduce alveolar ridge resorption following tooth extraction and to positively influence the bone volume over a 3-month period(35).

While there are many ridge preservation studies using various bone substitutes, there are few studies demonstrating the use of synthetic BCP in ridge preservation (Table 2). Kesmas and coworkers found that ridge preservation with BCP (HA/β-TCP:60/40) and a collagen membrane could be an alternative treatment for maintaining ridge dimension before implant placement(36). The quality and quantity of newly formed bone after the use of BCP/PLGA for ridge preservation could be adequate for successful implant therapy after tooth extraction(37).

Table 2 Summary of socket preservation in human

Author	Material & Method	Time	Dimension change	New bone formation Histomorphometry	Residual graft Histomorphometry
Block MS et al. , 2002 (32)	human mineralized bone graft	4 months	-	The human mineralized cancellous bone grafts appeared similar to native bone with small remnants of the original grafts visible.	
Froum et al. , 2002 (26)	DFDBA vs. Bioactive glass	6-8 months	-	DFDBA = 34.7% Bioactive glass = 59.5%	DFDBA = 13.5% Bioactive glass = 5.5%
Iasella JM et al. , 2003 (22)	DFDBA with collagen membrane	4-6 months	Width changes of Ridge preservation group = 1.2±0.9 mm Height changes of Ridge preservation group = 1.3±2.0 mm	DFDBA = 28%	DFDBA = 37%

Table 2 (Continued)

Author	Material & Method	Time	Dimension change	New bone formation Histomorphometry	Residual graft Histomorphometry
Guarnieri et al. , 2004 (34)	Calcium sulfate (Surgiplaster®)	3 months	-	MGCSH = 58%	-
Aimetti et al. , 2009 (35)	Calcium sulfate (Surgiplaster®)	3 months	Vertical resorption of the buccal socket walls = 0.5 mm Reduction of the width =2.0 mm	MGCSH = 58.8 %	-
Kesmas S et al. , 2010 (36)	BCP (HA/β:TCP) (60/40) (BoneCeramic®) with collagen membrane	4 months	Horizontal ridge changes = 2 mm Height ridge changes = 1.5 mm	BCP = 28.00±36.75%,	BCP = 15.83 ±8.70%,

Table 2 (Continued)

Author	Material & Method	Time	Dimension change	New bone formation Histomorphometry	Residual graft Histomorphometry
Cardaropoli D et al., 2012 (38)	Bio-Oss collagen with collagen membrane	4 months	Horizontal ridge changes = 1.04±1.08 mm Height ridge changes = 0.46±0.46 mm	Bio-Oss = 26.34 ± 16.91	Bio-Oss = 18.46 ± 11.18
Lindhe J et al. , 2013 (39)	Bio-Oss collagen with collagen membrane	6 months	-	Mineralized bone for Bio-Oss collagen = 39.9±8.6%	Bio-Oss collagen = 19.0 ±6.5%
Jurisic M et al. , 2013 (37)	BCP(HA/β-TCP :60/40) with polylactide-co-glycolide	4 months	-	BCP+Polylactide-co-glycolide=32.2±6.8%	BCP+Polylactide-co-glycolide=31.9±8.9%

Table 2 (Continued)

Author	Material & Method	Time	Dimension change	New bone formation	Residual graft
Suttapreyasri S et al., 2013 (40)	PRF	1, 2, 4, 6, and 8 weeks	At 8 weeks, Horizontal resorption on buccal= 1.79±0.90 mm and Horizontal resorption on lingual = 0.42±0.39 mm	-	-
Toloue , S.M., et al., 2013 (33)	A: FDBA B: CS	3 months	For FDBA group , Horizontal ridge changes = 1.03±0.87 mm and Height ridge changes = 0.05±1.46 mm	FDBA = 16.7%	FDBA = 21%
Brownfield LA, Weltman RL, 2012(41)	Demineralized bone matrix with cancellous bone chips	10-12 weeks	At 3 mm level, Horizontal ridge changes = 1.6±0.8 mm and Height ridge changes = 0.8±1.2 mm	44.9% by micro-CT 37.4% by histomorphometric	2.4% by micro-CT 4.5%by histomorphometric

Introduction of bone substitutes

There is a wide range of materials that can be used as bone substitutes either natural or synthetics. In 2006 Laurencin et al(42) classified grafts and bone substitutes as follows

A. Harvested bone grafts and bone substitutes: Autogenous bone is considered as a gold-standard not only from its osteoinductive and osteoconductive properties but also its osteogenesis. However, the only disadvantage is the requirement of a donor site, with additional surgical risk and patient's discomfort(43). Human allogenic bone has been regarded as adequate bone substitutes because of their availability without additional surgery. However, the risk of transmission of infection and the immunological reaction was concerned (44, 45). Therefore, bone substitutes are considered instead of autogenous bone and allograft bone either used alone or combination to alleviate the risks of the material.

B. Growth factor-based bone graft substitutes: growth factor such as transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP) are used alone or combination with bone substitutes. This type of material stimulates new bone formation by inducing undifferentiated mesenchymal cells to migrate into the graft material and transform into living vital bone. The clinical use of growth factors is mainly limited by cost of materials(46).

C. Cell-based bone graft substitutes: cells are seeded on a scaffold or bone substitutes or used to generate new bone formation alone. Currently, the technology exists to stimulate undifferentiated stem cells in vitro to develop into bone

forming osteoblasts. These cells can then be added to ceramic scaffolds to produce bone like structures to fill bony defects (47).

D. Ceramic-based bone graft substitutes: calcium phosphate, calcium sulfate (Plaster of Paris), and bioglass are used alone or in combination. The rationale used to support the use of ceramic materials is that they mimic the inorganic component of bone and allow new bone with the connective tissue to form a bone matrix. The disadvantage of this material was brittleness, and they are not appropriate for load-bearing applications(48).

E. Polymer-based bone graft substitutes: polymers-base bone graft show some options that the other bone graft substitutes do not. Polymers-base bone grafts have different physical, mechanical, and chemical properties. The polymers can be divided into natural polymers and synthetic polymers or can be divided into degradable and non-degradable types(42).

F. Miscellaneous: such as coral(49), chitosan(50), sponge skeleton(51). The bone tissue engineering provides an alternative approach to repair bone defect or diseased that regenerate with full recovery of the site and function. However, appropriate scaffold material is required to guide tissue regeneration. On the other hand among many types of bone substitute, osteoconductive calcium phosphate ceramics have been interested in this research fields.

Bioceramics

The bioceramics have been a major advance in the development of medical materials. Biomaterials can be categorized into natural or synthetic materials introduced into living tissue as a part of the medical device. During the past 30-40 years, there has been advances in the innovation of ceramic materials for skeletal repair and reconstruction and refers to bioceramics. Biomeramics is the material that interacts with host bone. Bioceramics can be classified into two groups: bioinert or bioactive. The bioactive ceramics may be resorbable or non-resorbable. The bioceramics can be manufactured in the form of porous or dense form in bulk and granules or in the form of a coating(52).

Bioinert ceramics have almost no influence in the surrounding living tissue and absence of a toxic response. There are first ceramic to be used in clinical application aimed to replace the damaged part to restore basic function. Alumina and Zirconia are two prototypes of inert ceramics. The major application in orthopedic was a joint replacement and in the dental application as aesthetic requirements prosthesis(53).

Bioactive ceramics by contrast are capable of bonding with living osseous tissue resulting in a strong interface compared to bioinert ceramics which form a fibrous interface(52). Most bioactive ceramics are thus based on calcium phosphate materials because their compositions are close to the mineral part of the bone. According to their composition calcium phosphate can be divided into Hydroxyapatite (HA), β-Tricalciumphosphate (β-TCP) and Biphasic calcium phosphate (BCP).

Calcium Phosphates

Hydroxyapatite (HA) is one of the most widely used calcium phosphates due to the chemical similarities to the inorganic of bone. HA which a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ has Ca/P at the ratio of 2.151 and Ca/P molar ratio of 1.667(52). Synthetics HA has exhibited strong affinity to host tissue by formed a chemical bond with host tissue offers HA a greater advantage in clinical applications(54). However, in spite of chemical similarities, the disadvantage of HA are a weakness under tensile stress and very slow resorbability(55). Tricalcium phosphate (TCP) is biodegradable bioceramic. Its chemical formula is $Ca_3(PO_4)_2$ and the Ca/P molar ratio is lower than 1.50. β-tricalcium phosphate (β-TCP) and α-tricalcium phosphate (α-TCP) are the two forms of TCP that are known to exist. In articles reviewing, TCP has been generally recognized as the primary resorbable bioceramics(52). The dissolution rate of β-TCP based bioceramics is reported to be 3–12 times faster than that of stoichiometric hydroxyapatite (s-HA)(56). In addition, in vitro studies showed that α-TCP had a higher rate of dissolution than β-TCP. Therefore, the solubility of α-TCP is more than β-TCP and HA respectively (57, 58). It can be seen that TCP exhibits a higher degree of solubility. It was expected to be degraded after implantation in the host, and gradually being replaced by new bone. However, in vitro studies showed that β-TCP had excessive solubility and detrimental to cell activity(59). Although β-TCP is considered biodegradable, it has disadvantage by mechanically weak and requiring reinforcement prior to implantation. In addition, β-TCP is ineffective in inducing the formation of calcium phosphate both in vitro and in vivo, preventing it from bonding chemically to living tissues(60).

Biphasic calcium phosphate (BCP)

Biphasic calcium phosphate (BCP) was first described by Nery et al. of the bioceramic that consisted of a mixture of HA and either β-TCP or α-TCP(61). To process BCP material, a synthetic or natural of biological calcium-deficient apatite (CDA) is sintered at a temperature above 700°C according to the following reaction(8).

$$Ca_{10-x}M_y(PO_4)_{6-y}(HPO_4)_y(OH)_2 \text{ ------> } Ca_{10}(PO_4)_6(OH)_2 + Ca_3(PO_4)_3$$

CDA HA β -TCP

The concept is determined by an optimum balance of the more stable phase HA and more soluble TCP. The extent of calcium deficiency (Ca/P molar ratio < 1.67) determines the HA/β-TCP ratio in the BCP. Particle size, macroporosity and microporosity are also factors in the reactivity of BCP, sintering temperature and conditions affect these properties.

The ideal pore size for a bioceramic approximates to bone was approximately 100 μm. It had been demonstrated that microporosity (diameter < 10μm) allowed body fluid circulation whereas macroporosity (diameter > 100 μm) provided a scaffold for bone cell colonization. It was reported that BCP ceramic with an average pore size diameter of 565 μm (compared to those with average pore size diameter 300 μm) and 40% macroporosity (compared to 50% macroporosity) had greater bone ingrowth(62). It was assumed that the pore size and percent macroporosity of BCP ceramic would affect the mechanical properties(63). In vitro biodegradation was determined by suspending the material in the acidic buffer and monitoring the release of Ca^{2+} ion with the time.

Nano Biphasic calcium phosphate has been fabricated under the cooperation of between Faculty of Dentistry, Prince of Songkla University and the

National Metal and Materials Technology Center Thailand. The BCP scaffold was fabricated by using polymeric sponge method(9). Based on cell differentiation assays (alkaline phosphatase and osteocalcin synthesis assay), BCP scaffolds at the ratio of HA:β-TCP 50:50 showed the best outcomes for cell differentiation with the highest osteocalcin activity(10). Biocompatibility has been tested in vitro and showed that viable cells were attached and widely distributed within the 3D scaffolds that had presence of a large number of macroporosities, interconnected porosities and micro–nanoporosities(11). Furthermore in the rabbit model, BCP scaffolds with the high ratios of HA (90:10, 80:20) showed comparative results in bone formation and in residual graft materials. However, BCP scaffolds with the high ratios of HA presented good osteoconductive properties, biocompatibility with the living tissue and excellent space maintaining.

Selection of graft materials

Firstly, materials available for placement into extraction sites are considered either nonresorbable or resorbable. Nonresorbable materials are suggested for long-term ridge preservation because nonresorbable materials have a high modulus of elasticity and limit osteoconductive activity and the characteristics make them excellent for long-term ridge maintenance. Materials for long-term ridge preservation includes HA Porous coralline HA, Bioactive glass, Porous polymethyl methacrylate, Synthetic HA(64).

Secondly, there is another group of materials that slowly resorbed, and is considered as transitional bone grafting materials. For clinical application, these materials are beneficial for increasing bone density and bone regeneration in the medium-term ridge preservation, that patients are uncertain to receive implant treatment. Material for transitional ridge preservation such as anorganic bovine bone matrix (ABM),

resorbable calcium phosphate ceramics, and macroporous bioactive glass, deproteinized bovine bone with collagen (64).

Lastly, the third group of materials that is considered as short-term resorbable materials. The material is resorbed and gradually being replaced by new bone formation between healing periods. The objective is to maintain bone volume during the initial healing stage in 3 to 6 month period. These materials are similar to the transitional materials that they may increase bone formation, prevent early ridge resorption and facilitate implants placement. Due to the materials rapidly degrade, they should be selected for implants placement within 3 to 6 months before ridge resorption occur. Material for short-term ridge preservation are freeze-dried bone allograft (FDBA), demineralized freeze-dried bone allograft (DFDBA) or autogenous bone(64).

Plate-rich fibrin (PRF)

Platelet-rich fibrin was first developed in France by Choukroun et al. for a specific use in oral and maxillofacial surgery(14). The protocol is as follows; a blood sample is taken without anticoagulant in a 10 ml tube and is immediately centrifuged at 3000 rpm (approximately 400 g) for 10 minutes. The absence of anticoagulant implies the activation in a few minutes of most platelets of the blood sample in contact with the glass tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the high part of the tube before the circulating thrombin transforms it into fibrin. A fibrin clot is then obtained in the middle part of the tube, just between the red corpuscles at the bottom and acellular plasma at the top (Figure1,2).

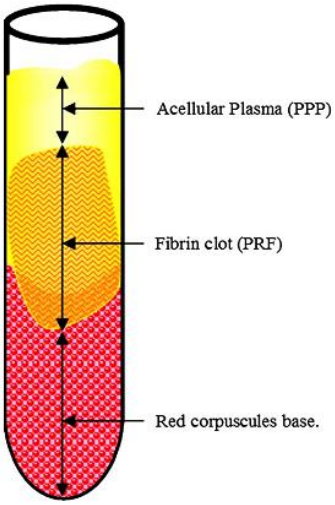

Figure 1 Blood centrifugation shows a structured fibrin clot in middle part of the tube, between the red corpuscles at the bottom and acellular plasma at the top

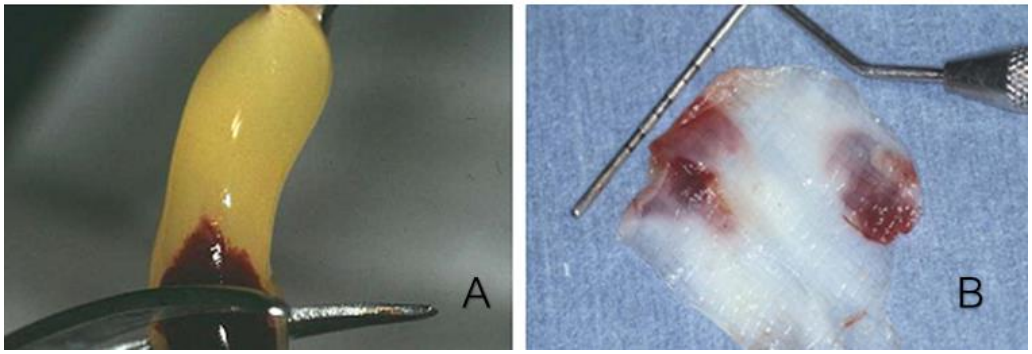

Figure 2 Picture of Platelet-rich fibrin. (A) Fibrin clot (B).PRF membranes are easily obtained by driving out the serum from the clot.

The success of this technique entirely depended on the speed of blood collection and transfer to the centrifuge. The blood samples without anticoagulant started to coagulate when contact with the glass tube. And then it was taken a minimum of a few minutes of centrifugation to concentrate fibrinogen in the middle and the upper part of the tube. After that prompt usage was the key to obtain the clinical benefit of PRF fibrin. If long duration was spent in the step of blood collecting and centrifugation, the blood might not be clotted properly: The fibrin would polymerize in a diffuse way in the tube and only a small blood clot without consistency would be obtained.(14)

PRF was a rich source of autogenous cytokines and growth factors that can be considered as a biological membrane. PRF possessed the properties of angiogenesis, immunity and epithelialization (12-14). The overall architecture of the fibrin clot was examined with a scanning electron microscope and showed that RBCs were widely predominant in the red part of the PRF clot, and the leukocytes were distributed at the junction between the red and yellow parts of the clot. Only a few RBCs were identified in the rest of the clot. Platelet morphology was totally modified by aggregation and clotting processes (65). Simonpieri and coworkers(66, 67) reported and confirmed

the validate usage of PRF membranes in reconstruction protocols along with FDBA, 0.5% metronidazole solution in 20 patients and followed for 1 to 5 years, and 184 dental implants were placed and there was no implant or graft loss in during follow-up period(66). PRF membranes protected the surgical site; promoted soft tissue healing; and when its fragments mixed with graft materials, it functioned as a “biological connector” between the different elements of graft and acted as a matrix which supported neoangiogenesis, capture of stem cells, and migration of osteoprogenitor cells to the center of graft(67). The use of fibrin from PRF was simple and able to promote soft tissue healing since it has been used for socket preservation in Faculty of Dentistry, Prince of Songkla University regularly. Furthermore, Kawase and coworkers used heat-compression technique to prolong the rate of biodegradation of the PRF membrane without sacrificing its biocompatibility(15).

Choukroun and coworkers(68) confirmed that PRF can be considered as a healing biomaterial. Clinical example dealed with the filling of a tooth socket by PRF. Rapid healing of the wound was observed without pain, dryness, or purulent complication. Sinus floor augmentation as performed with freeze-dried bone allograft (FDBA) and PRF led to a reduction of healing time prior to implant placement. From a histologic point of view, this healing time could be reduced to 4 months. After complete maxillary cystic removal, a cystic cavity filled with PRF were completely healed in two and a half months, the osseous defect was replaced by a dense cortical bone. This physiologic healing phenomenon was accelerated because normal physiologic healing time of the cystic cavity lies between 6 months and 1 year.

And then, releasate or the fluid leaked from PRF could be used for mixing with grafting material to enrich growth factor and also as a fibrin to cover the graft

as a growth factor carrier(69). Thus in this study, PRF membranes were used for socket seal in socket preservation.

Growth factors in platelet-rich fibrin

Platelets are small and irregularly shape anuclear cells and 2-4 μm in diameter. They were derived from fragmentation of precursor megakaryocytes. Platelets contain angiogenic, mitogenic, and vascular growth factor in their granules. Platelets were very important in the wound healing process. They arrived quickly at the wound site and began coagulation. Activation of platelets induces α granules to release their stored coagulation factors, platelet activating factors, adhesion molecules, cell-activating molecules, cytokines, integrins, inflammatory molecules, and growth factors. They released multiple wound healing growth factors and cytokines, including platelet-derived growth factor (PDGF), transforming growth factor (TGF-β1 and TGF- β2), vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor (PDEGF), interleukin-1 (IL-1), basic fibroblast growth factor (bFGF) and platelet activating factor-4 (PAF-4). One of the highest concentrations of PDGF and TGF-β in the body is found within blood platelet(70). A previous study(71) had investigated the quantity of growth factor cytokine (PDGF-BB and TGF ß-1) and inflammatory cytokine (IL-1ß, IL-6 and TNF-α, anti-inflammatory cytokines such as IL-4) in the components of blood products such as platelet poor plasma, exudates from PRF, serum or plasma and platelet rich plasma. It was found that the exudate from PRF clot leaving in a sterile metal cup for 10 minutes contained a low level of PDGF-BB and TGF-ß1 which was significantly lower than those obtained from the several concentrated platelet-rich plasma (cPRP) protocols. On the other hand, IGF-I was found in the supernatant and the exudates in a significant higher level than in the cPRP of the various protocol(71). In 2009, Dohan presented that when the platelet-rich fibrin has been pressed into a

membrane, there was a slow release of growth factors in the exudates such as PDGF, TGF-β and VEGF for at least 7 days in vitro(72).

Hypothesis

- A nano BCP with PRF membrane can preserve socket volume both in hard and soft tissue aspect not different from Freeze-dried bone allograft with PRF membrane

General objective

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

Special objective

- To compare soft tissue maturation after socket preservation with BCP or FDBA sealed with PRF.

- To compare dimensional change after socket preservation with BCP or FDBA sealed with PRF by using cast-based measurement, and cone beam computed tomogram.

- To compare bone formation after socket preservation with BCP or FDBA sealed with PRF by using Micro-CT, Histology.

- To compare degradation rate of standard PRF membrane and warm PRF membrane.

Benefit of the study

-To use a nano biphasic calcium phosphate with PRF membrane as a bone substitute material for socket preservation.

-To apply PRF membrane for socket sealing in socket preservation.

Chapter 2

Materials and Methods

Scope of the study

The study was divided into 2 parts. Part I, the study of PRF morphology and degradation rate in vitro, PRF membrane were prepared into 2 types, Standard PRF membrane and Warm PRF membrane (warm in the water bath at 80°C for 15 seconds). The characteristic and morphology of both types were assessed by SEM observation. The degradation rate of PRF membrane was assessed by measuring weight loss of PRF membrane for 14.days. Part II, the study of the efficacy of BCP or FDBA in socket preservation, the randomized clinical controlled trial was conducted in patients whom socket preservation and later implantation were performed. The study protocol was approved by the Ethic Committee of the Faculty of Dentistry, Prince of Songkla University, Songkla, Thailand (no. EC5609-21-P-HR).

Table 3 Part I : Scope of the study

	Investigation	Method of measurement
Part I	- Characteristic and morphology - Degradation rate of PRF membrane	- SEM observation - Weight loss
Part II	- Soft tissue healing - Dimension change - Newly formed bone and Residual graft	- Reduction of socket orifice - Cast-based measurement - CBCT - Micro-CT - Histomorphometry

Part I In vitro experiment

Part 1, Morphology and Degradation rate of PRF. Six healthy volunteers, three male and three female without any systemic disease, above 20 years of age were participated in the study. Twenty milliliters of blood from each volunteer were divided into 2 tubes. Then, the blood was centrifuged at 3000 rpm for 10 minutes to prepare PRF fibrin. Sample size and investigations were summarized in Table 4

Preparation of standard PRF membranes and warm PRF membranes

Twenty milliliters of blood were used for preparing 2 sets of PRF (10 ml each), one was used as a standard PRF membrane and the other one was used as a warm PRF membrane. In brief, 10 ml of autologous whole blood were collected from the median cubital vein (forearm) by using needle gauge no. 22 connected with a 10 ml

sterile plastic 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 between the red corpuscles at the bottom and acellular plasma at the top and used as a standard one. For warm PRF membranes after centrifugation, the tube was warm in a water bath at 80°C for 15 seconds. After that, from both groups PRF was picked up with straight non-toothed forceps and the red thrombus were eliminated with scissors along the border between this fraction and the PRF. Then the PRFs were compressed by sterile spoons to get a membrane.

Morphology scanning electron microscopy (SEM) observation

The PRF membrane samples were immediately immersed in 2.5% glutaraldehyde in 0.2 N phosphate-buffered fixative for 2 hours at room temperature and post-fixed in 1% osmium tetroxide for 2 hours. Subsequently, samples were dehydrated by serial transfers in ascending concentrations of ethanol (50-100%) and infiltrated with liquid carbon dioxide before the critical drying point. Finally, the sample was made electrically conductive by mounting on aluminum slabs with a silver point, followed by sputter coating with gold/palladium using the SPI-Module Sputter Coater & Carbon-Coater(SPI Supplier, Division of Structure Probe, Inc., West Chester, PA, USA) to a thickness of approximately 250 A^{o}. The above specimens were attached to an acrylic plate with glue tape. Finally, PRF membranes were examined with a JSM-5800 scanning electron microscope (QUANTA 400, FEI, Czech Republic) at The Scientific Equipment Center, Prince of Songkla University.

Degradation rate of PRF membrane

Two types of PRF membranes (standard vs warm) were freshly prepared and were inserted in 24-well plates (Nunc™, Denmark) and incubated in a CO_2 incubator with Dulbecco's Modified Eagle's Medium(DMEM, Gibco®, USA). The PRF membranes were weighed by using digital weigh scales and examined once every 24 h for 14 days for measuring weight loss. The medium was changed every 7 days.

Table 4 Study groups of PRF membrane

Groups	SEM(n)	Degradation rate of PRF membrane(n)
Standard PRF membrane	3	3
Warm PRF membrane	3	3

Part II Clinical study of socket preservation

Study groups

Part II, the study was a randomized clinical trial. 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 (Figure 3). 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 5. 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.

Table 5 Study groups and patients used

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

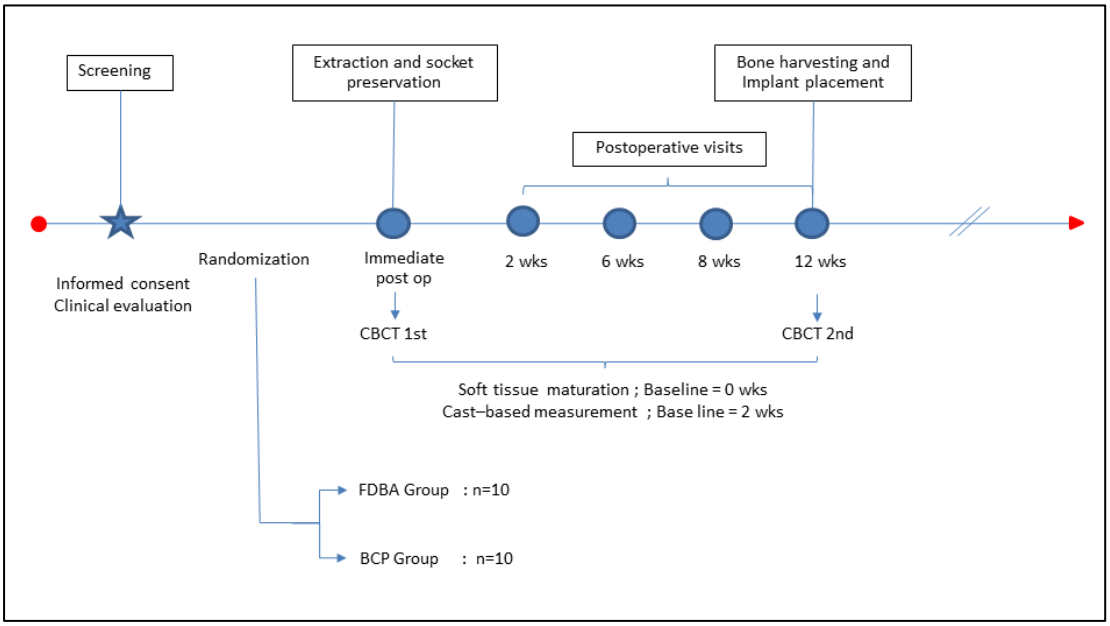

Figure 3 Timeline of the study.

Sample size

Estimate sample size for two-sample comparison of means was computed by using data from Toloue and coworker study (33) in Table 6.

Table 6 Histomorphometric analysis of Calcium sulfate and FDBA samples

	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

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

σ^2 = Pooled variance

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

Assumption :

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

β = 0.2 → $Z_{(1-\beta)} = 0.84$

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 cast 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.

Selection PRF membrane

The standard PRF membrane was selected to use in clinical study due to slow degradation than warm PRF membrane. And the upper parts of PRF membrane was selected to use for sealing socket because it lost the least weight.

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 Local anesthesia (4% articaine hydrochloride, Ubistesin 1:100,000; 3M ESPE, Platz, Seefeld, Germany) in the extraction site. 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. 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 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. Then a fibrin clot was obtained in the middle of the tube. The fibrin clot was collected with straight non-toothed forceps. Then the PRF membrane was 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 (Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand) was in the block form. The FDBA bone was crushed by using the Bone morsellizer to get particles size into 100-1000 μm. 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), BCP (HA:β-TCP = 50 : 50) was fabricated at the National Metal and Materials Technology Center of Thailand (MTEC) with gain size 200-500 um. 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 (Coamox, Community Pharmacy Public Co. Ltd, Thailand) 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 for 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 4). The measurements were carried out by 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.

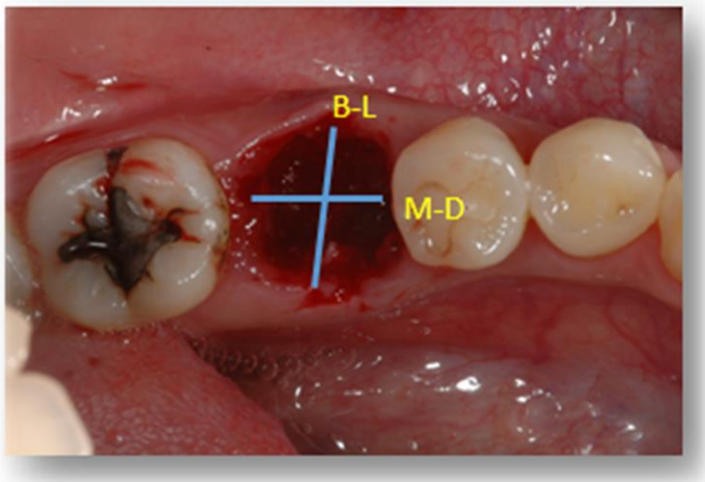

Figure 4 Measurements of socket orifice

B: Cast-based measurements for dimension change

Changes in the residual ridge dimensions were assessed on the dental casts obtained at 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 Analzer™ software (3Shape, Copenhagen, Denmark) as in Figure 5. 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 6). Ridge reduction was calculated as a mean dimensional change between baseline (T2) and each time point.

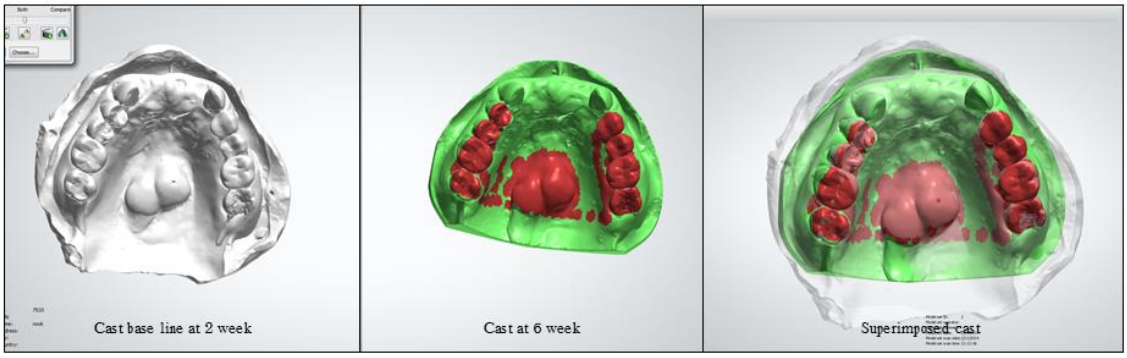

Figure 5 Superimposed cast at the baseline 2 weeks(T2) with the cast at 6

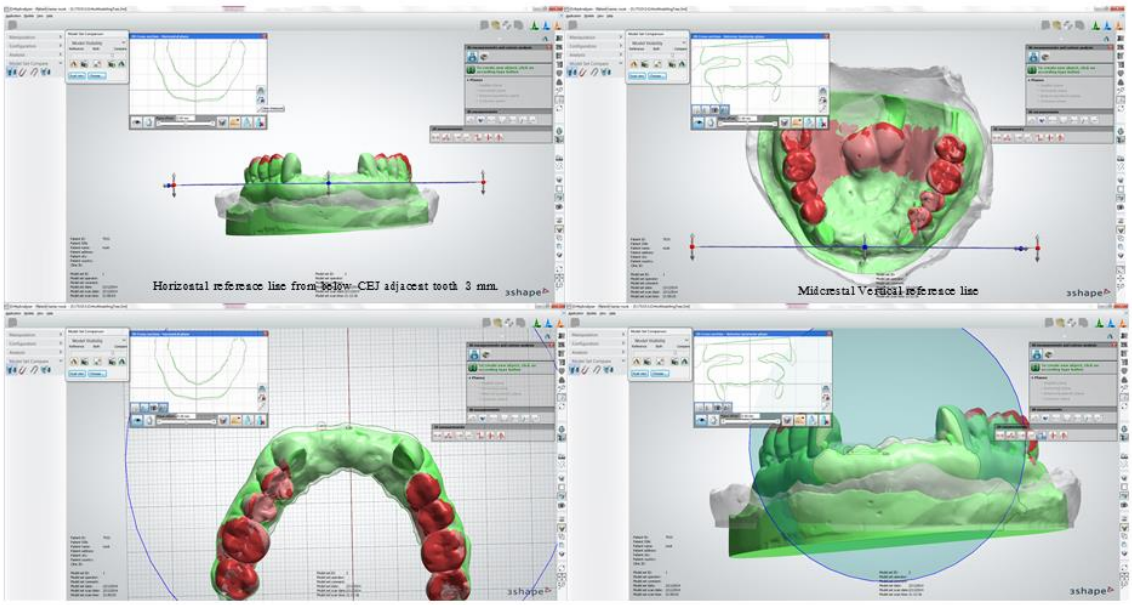

Figure 6 Measurement of the dimensional change of width and height from the reference line by using superimposed casts

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 7) 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.

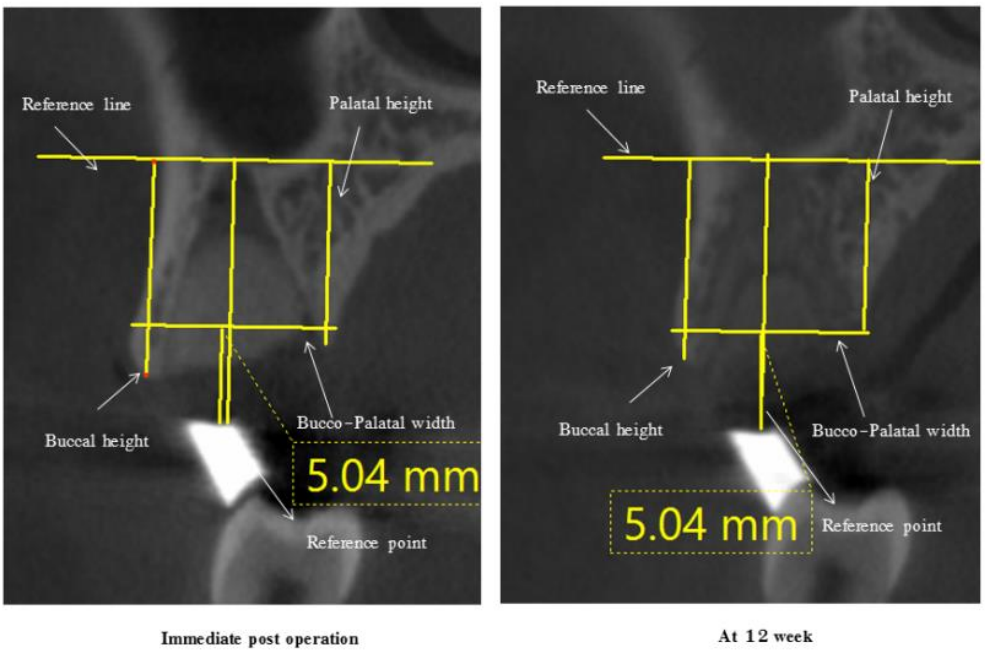

Figure 7 Dimensional measurement in CBCT

Clinical evaluation of socket healing and specimen preparation

At 12 weeks, local anesthesia was administered and full mucoperiosteal buccal and lingual flaps were reflected. The clinical evaluation of grafting material were assessed visually with grading on 3 – point scores as followed : 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 the socket (Figure 8).

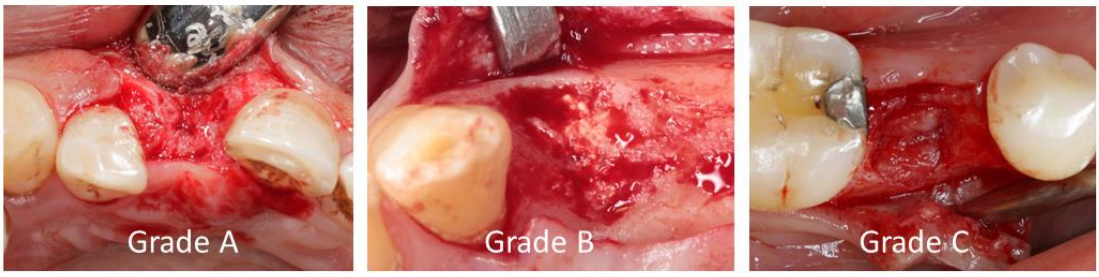

Figure 8 Clinical grading score. Grade A = graft particles blended to healing socket ; grade B = graft particles were predominate in the healing socket ; grade C = soft tissue healing filled in the healing socket.

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 (73). 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 9).

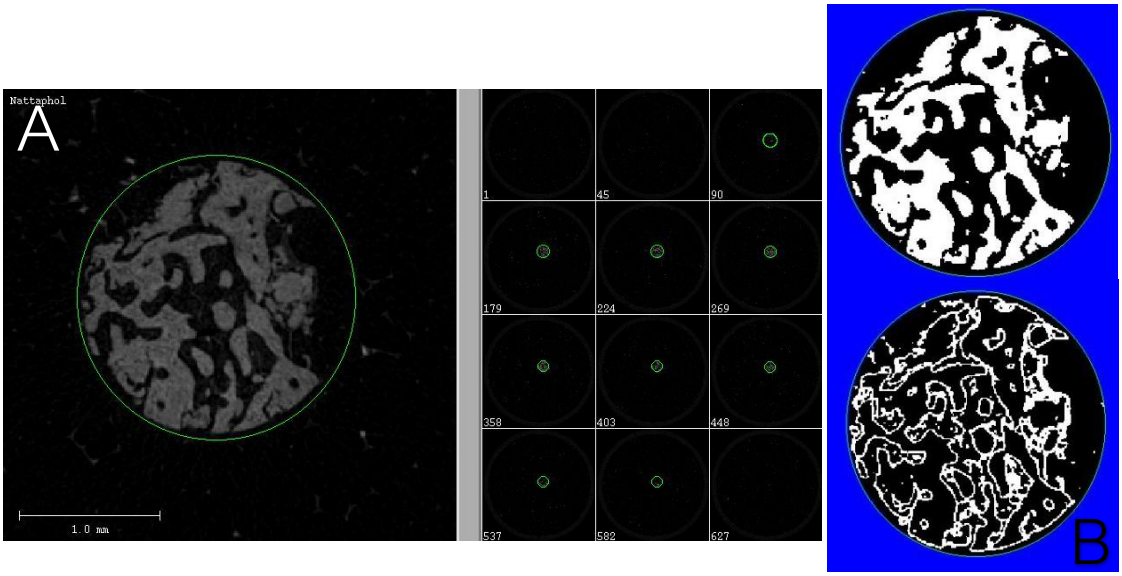

Figure 9 Micro CT analysis; (A) Margins of ROI of the defect.
(B) The high threshold and the low threshold.

E: Histological and histomorphometric analysis

After completing the image analysis, the amount of bone tissue formation was analyzed by standard histology. The specimens were processed to obtain thin ground sections, according to the technique of Donath and Breune(74). 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 150 µm and ground down to approximately 15-25 µm with a specially designed grinding machine (EXAKT® cutting and grinding system, 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 $P < 0.05$.

Chapter 3

Results

Part I Characteristic of PRF membrane and degradation rate of PRF membrane

Characteristic of the standard PRF membrane

The SEM images of standard PRF membrane were demonstrated. The PRF was divided into three parts equally in length (Figure 10A). In the lower part, activated platelets aggregated on the PRF membrane surface and some white blood cells were observed (Figure 10B). In the middle part, fibrin fibers could be observed and the number of platelets decreased from the red thrombus (Figure 10C). In the upper part, PRF membranes resemble the fibrin meshwork was clearly observed (Figure 10D).

Characteristic of the warm PRF membrane

The SEM images of warm PRF membrane and standard PRF membrane were shared similar SEM image features. The PRF was divided into three parts. In the lower part, activated platelets aggregated and some white blood cells were observed (Figure 11A). In the middle part, fibrin fibers with covered by platelet aggregates and some white blood cell (Figure 11B). In the upper part, PRF membrane presented weaving fibrin meshwork (Figure 11C).

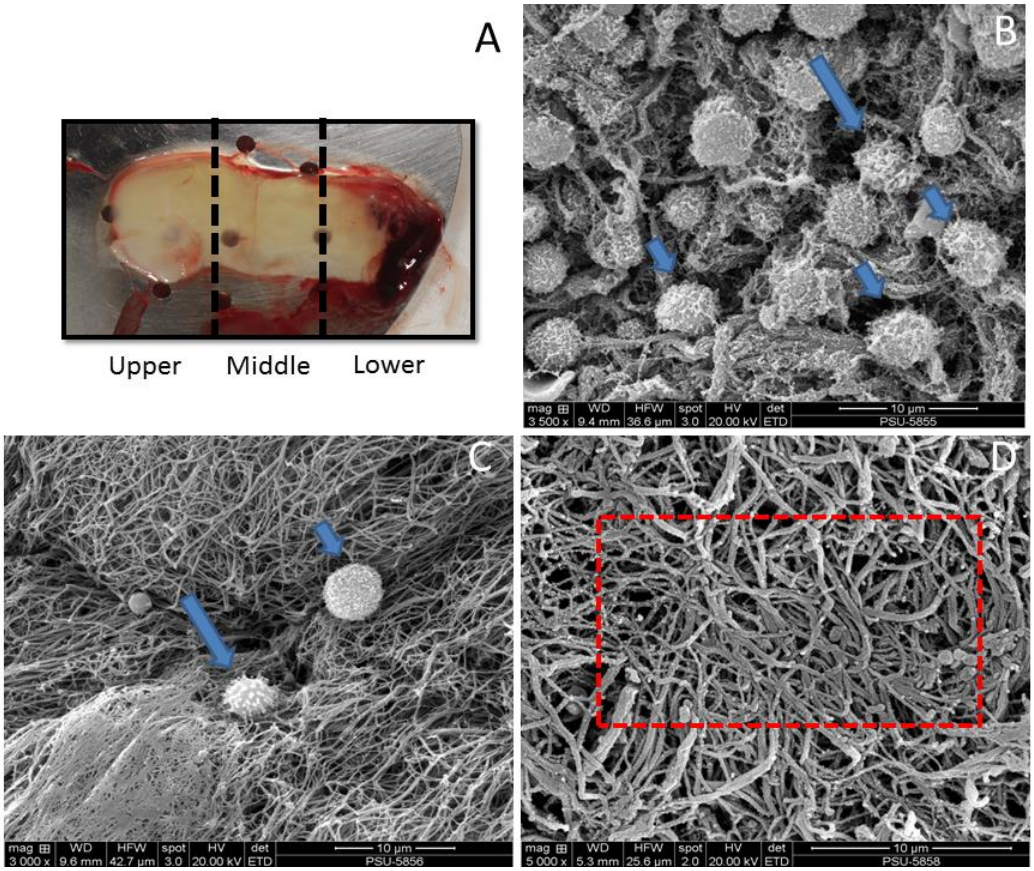

Figure 10 SEM observation of the standard PRF membrane. (A)The three parts of the standard PRF membrane. (B) The lower part were adjacent to the red thrombus and showed activated platelets(long arrow) and white blood cells(short arrow). (C) The middle part showed platelets(long arrow), lymphocytes and white blood cells(short arrow) and fibrin could be observed. (D) The upper part showed dense fibrin mesh(square mark) with a very few cells.

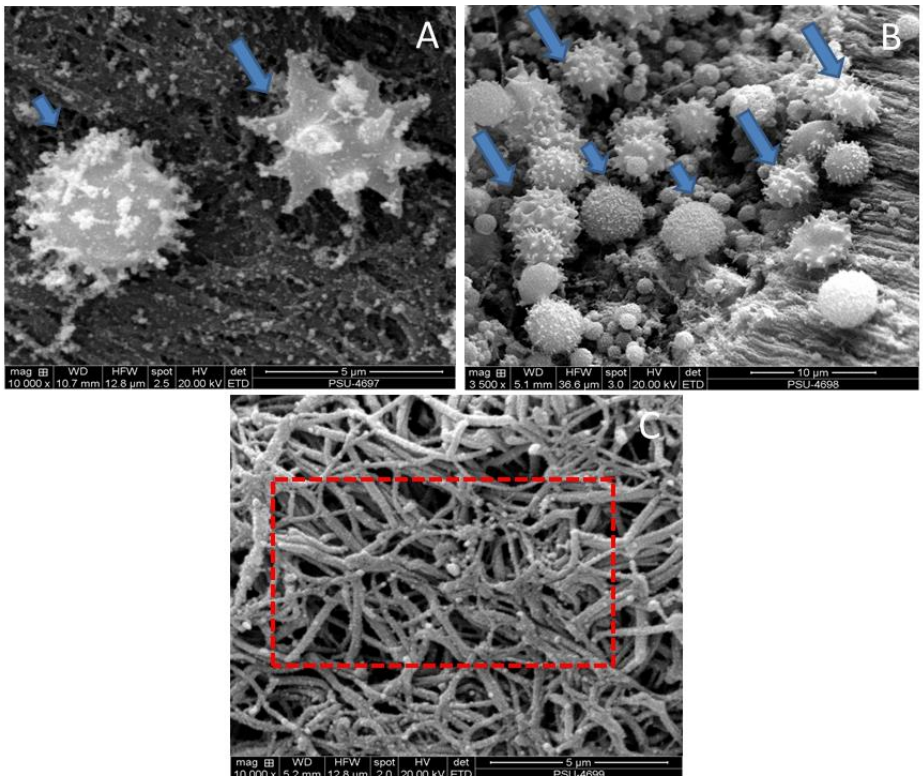

Figure 11 SEM observation of the three parts of the warm PRF membrane. (A) The lower part showed activated platelets (long arrow) and white blood cells (short arrow). (B)The middle part showed fibrin fibers with covered by platelet aggregates (long arrow) and some white blood cell (short arrow). (C)Upper part show dense fibrin meshwork (square mark).

Degradation rate of PRF membrane

The degradation rate of the standard and the warm PRF membrane were evaluated by measuring weight loss and were demonstrated in Figure 12. In the standard PRF membrane, the weight reduction of the upper part was significantly less than the lower parts from day 10 to day 14. The upper part showed the least weight loss, followed by the middle and the lower part respectively. The upper part showed weight loss less than 50% by weight, the middle part showed weight loss more than 60% and the lower part showed weight loss more than 80% by weight in 14 days. In the warm PRF membrane lose weight loss more than the standard PRF membrane in all three parts and lose 60% to 80% in the second weeks. The weight reduction of warm PRF membrane was significant different detected within the group from day 1 and day 2.

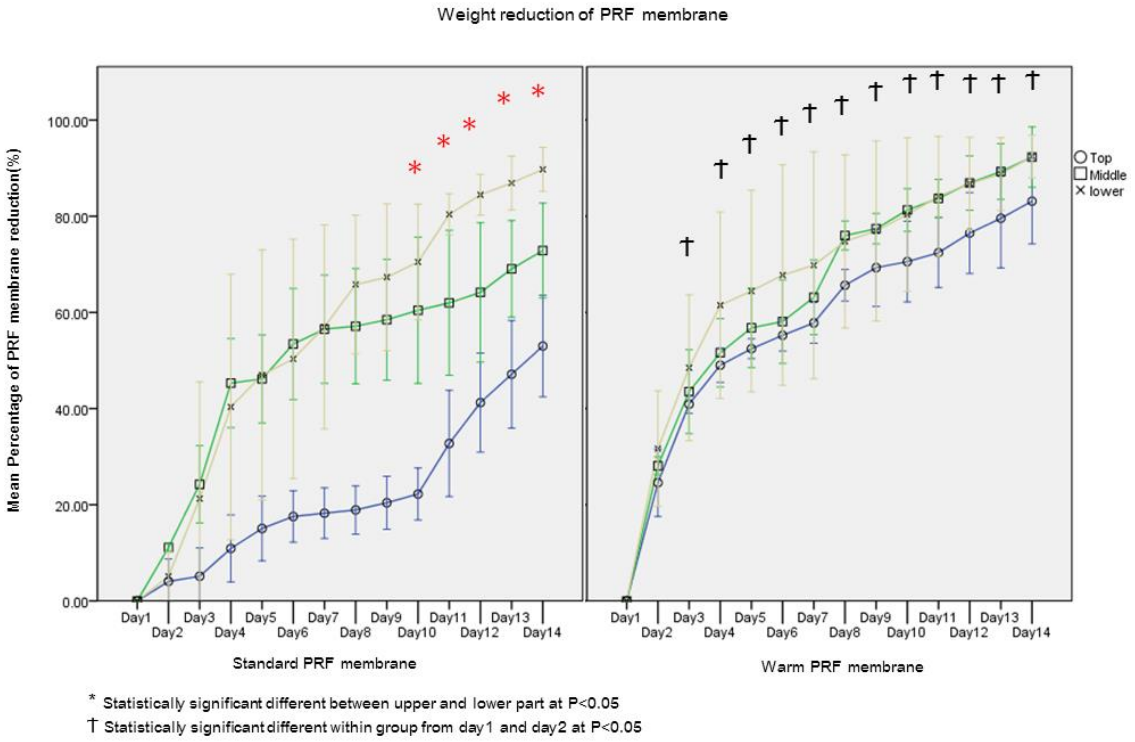

Figure 12 The mean percentage of PRF membrane weight reduction by measuring weight loss. The standard PRF membrane (left) and the warm PRF (right).

Part II Clinical study of socket preservation

Sixteen patients aged 52.87±15.61 years with 20 socket preservation participated in the study. 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 month after socket preservation. At stage I implant surgery, the grafting sites were reentry, the FDBA group showed blended grafted material to the surrounding bone whereas the BCP particles were clearly seen in all cases of the BCP group. Demographic data were presented in Table 7.

Table 7 Demographic data

Case	Groups	Age	Gender		Position	Bone assessment	Additional graft	Area		Buccal plate(mm)	Implant system	Implant size(mm)
			M	F				Anterior	Posterior			
1	FDBA	55.67	/		22	A		/		0.76	Biohorizon®	3.0x12
2	FDBA	54.83		/	35	A	/		/	0.93	Straumann®	4.1x12
3	BCP	31.33		/	25	B			/	1.83	Biohorizon®	3.8x12
4	BCP	53.67	/		15	A			/	1.30	Zimmer®	4.5x11.5
5	FDBA	65.67		/	12	C	/	/		0.80	Neoss®	3.5x13
6	BCP	65.67		/	22	B	/	/		0.75	Neoss®	3.5x13
7	BCP	63.33		/	11	B	/	/		0.66	Straumann®	4.1x12
8	BCP	51.83	/		14	A			/	0.67	Straumann®	4.1x12
9	FDBA	26.16	/		11	A	/	/		0.00	Intralock®	4.0x13
10	BCP	26.16	/		21	B	/	/		0.00	Intralock®	4.0x13
11	BCP	40.50	/		14	B	/		/	0.67	Osstem®	4.5x11.5
12	FDBA	40.50	/		15	C	/		/	0.69	Osstem®	4.5x11.5
13	FDBA	45.50		/	35	C			/	0.73	Pw plus®	4.2x12
14	BCP	63.41		/	14	B	/		/	0.53	Ankylos®	4.5x11
15	BCP	62.33	/		34	B			/	0.49	Osstem®	4.5x11.5
16	FDBA	59.16		/	44	A			/	0.51	Osstem®	4.0x13
17	FDBA	78.83	/		21	A	/	/		0.56	Osstem®	3.5x13
18	BCP	78.83	/		22	B	/	/		0.52	Osstem®	3.5x13
19	FDBA	58.67	/		44	-			/	0.79	-	-
20	FDBA	35.35	/		11	C		/		0.60	Osstem®	4.0x13
		52.87±15.61	9	7			11	9	11			

Biohorizon®(BioHorizons IPH Inc, Birmingham, AL, USA), Straumann®(Institut Straumann AG, Basel, Switzerland), Zimmer®(Zimmer Inc, CA, USA), Neoss®(Neoss Ltd., Harrogate, North Yorkshire England), Intralock®(Intra-Lock® International Inc, Boca Raton, Florida, USA), Pw plus®(PW PLUS CO.,LTD., Nakhon Pathom, Thailand), Osstem®(Osstem Co., Ltd., South Korea)

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

Platelet-rich fibrin membrane could not 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 13). The mean different percentage of socket orifice reduction between baseline (TO) and the follow-up time in mesial-distal and buccal-lingual directions were presented in Figure 14 and Table 8. 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 ($P < 0.05$).

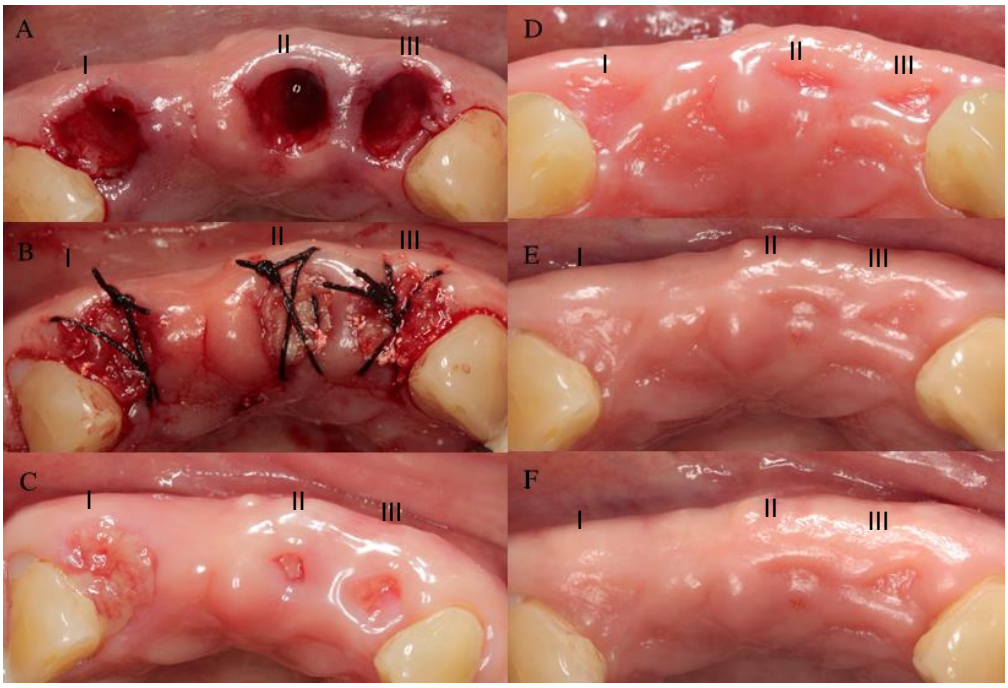

Figure 13 A comparative of socket healing in a case of socket preservation. Tooth 12 was grafted with FDBA(I) and sealed with PRF membrane, tooth 11 was filled with PRF gel(II) and tooth 22 was grafted with BCP(III) and sealed with PRF membrane A) Immediate extraction B) Socket preservation C) Follow-up 2 weeks D) Follow-up 6 weeks E) Follow-up 8 weeks F) Follow-up 12 weeks

Table 8 The mean Percentage of socket orifice reduction(%)

	Bucco-lingual		Mesio-Distal	
	FDBA	**BCP**	**FDBA**	**BCP**
T0 vs T2	52.76±23.31	50.33±17.12	36.63±20.25	45.49±13.16
T0 vs T6	93.41±17.12*	95.97±6.62*	84.58±23.24*	90.95±16.10*
T0 vs T8	98.00±6.32*	100.00±0.00*	100.00±0.00*	100.00±0.00*
T0 vs T12	100.00±0.00*	100.00±0.00*	100.00±0.00*	100.00±0.00*
Values are presented as mean±SD. ***Significant different from ΔT2 in each group**				

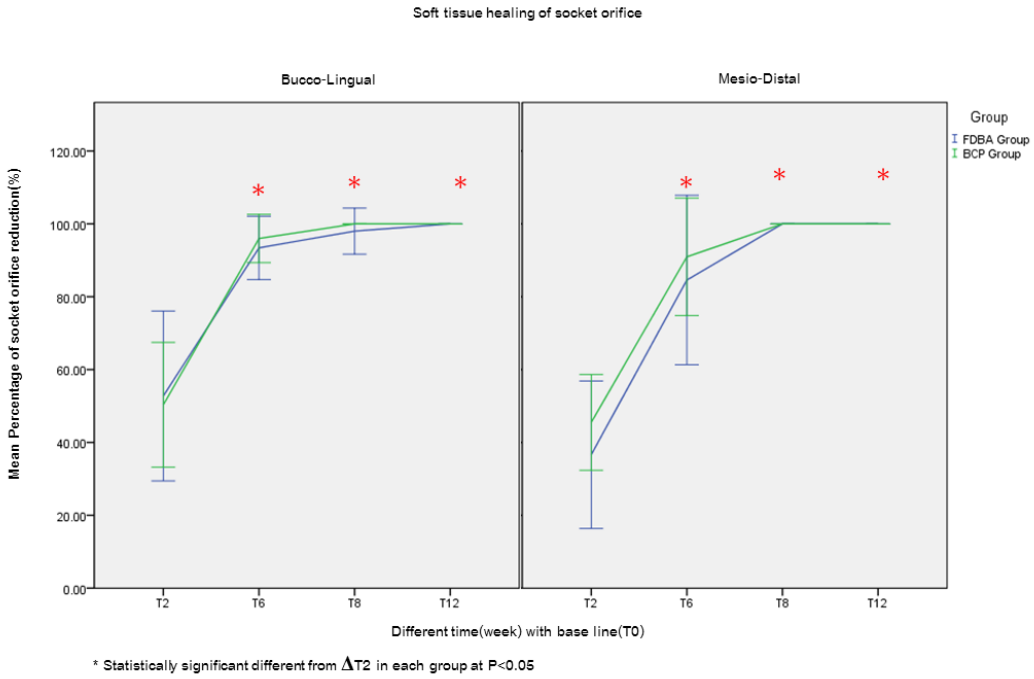

Figure 14 The mean different percentage of socket orifice reduction from immediate post extraction (T0) to each time point (T2, T6, T8, T12) in the bucco-lingual direction (left) and the mesio-distal direction (right)

B: Cast-based measurements

The cast-based measurements of the dimensional change at the buccal side, lingual/palatal side and height were shown in Figure 15 and Table 9. 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 all sides of both groups. Ridge width reduction at the buccal side of FDBA group was most pronounced at 12 weeks postoperatively (1.26±0.48 mm) and more than the BCP group (1.02±0.68 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 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 very similar and limited and less than 1 mm at 12 weeks.

Table 9 The cast-based measurements of the dimensional change

Cast-based measurements of the dimensional change from 2 weeks(baseline) to 12 weeks (mm.)						
	Buccal		Lingual/palatal		Height	
Time	FDBA	BCP	FDBA	BCP	FDBA	BCP
T2-T6	0.76±0.38	0.65±0.64	0.37±0.20	0.34±0.15	0.40±0.21	0.43±0.19
T2-T8	1.00±0.34[a]	0.81±0.71[a]	0.47±0.21[a]	0.45±0.19[a]	0.56±0.20[a]	0.57±0.19[a]
T2-T12	1.26±0.48[ab]	1.02±0.68[ab]	0.71±0.38[ab]	0.68±0.44[ab]	0.66±0.21[ab]	0.72±0.21[ab]
Values are presented as mean±SD. **[a]Significant different from ΔT6 in each group** **[b]Significant different from ΔT8 in each group**						

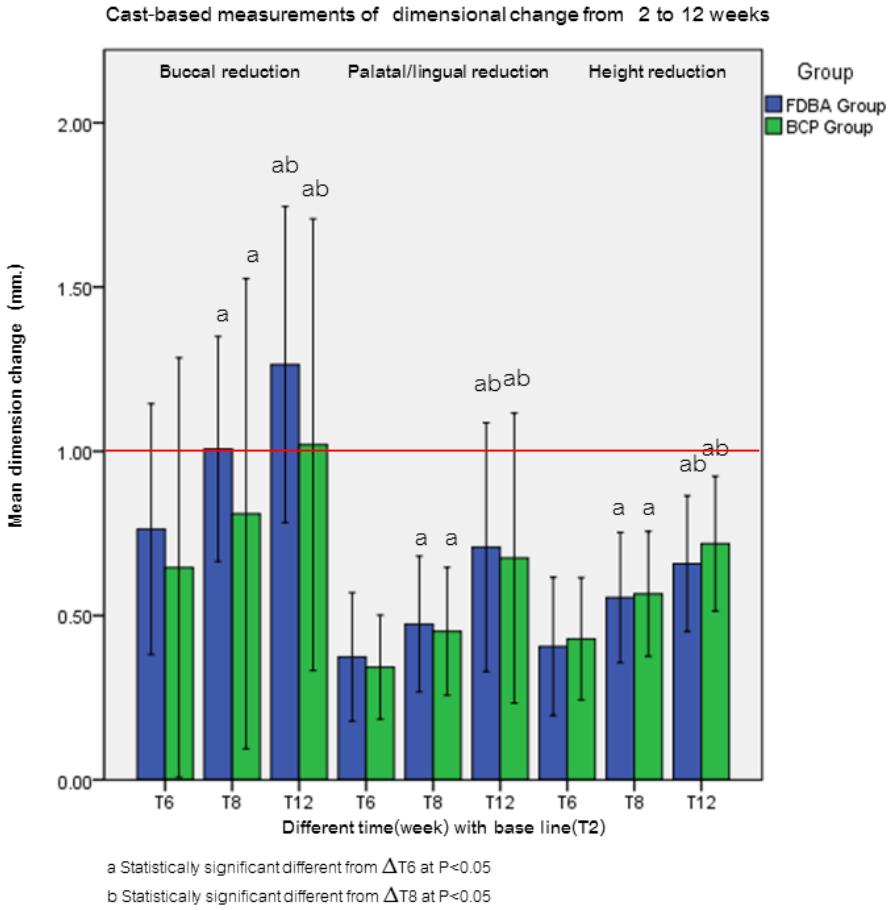

Figure 15 The dimensional change in the reduction buccal, lingual sides and the height of the extraction site from cast-based measurements.

C: Cone beam CT measurements

The morphological ridge width and height from the cone beam CT were presented in Figure 16 and Table 10. At the level of 5 mm from the stent, the FDBA group showed 0.98±0.15 mm ridge width reduction, 0.34±0.25 mm height reduction at buccal side, and 0.35±0.12 mm height reduction at palatal/lingual side while the BCP group showed 0.92±0.34 mm ridge width reduction, 0.49±0.25 mm height reduction at buccal side, 0.42±0.13 mm height reduction at palatal/lingual side respectively. No statistically significant differences were detected between the groups in width reduction and also no statistically significant differences were detected between the groups in buccal ridge height and palatal/lingual ridge height reduction.

Table 10 The CBCT measurements of dimensional change

Dimensional change measured in CBCT from immediate post-op to 12 weeks follow-up period						
	Ridge width		Buccal ridge height		Palatal or lingual ridge height	
Time	FDBA	BCP	FDBA	BCP	FDBA	BCP
T0-T12	0.98±0.15	0.92±0.34	0.34±0.25	0.49±0.25	0.35±0.12	0.42±0.13
Values are presented as mean±SD.						

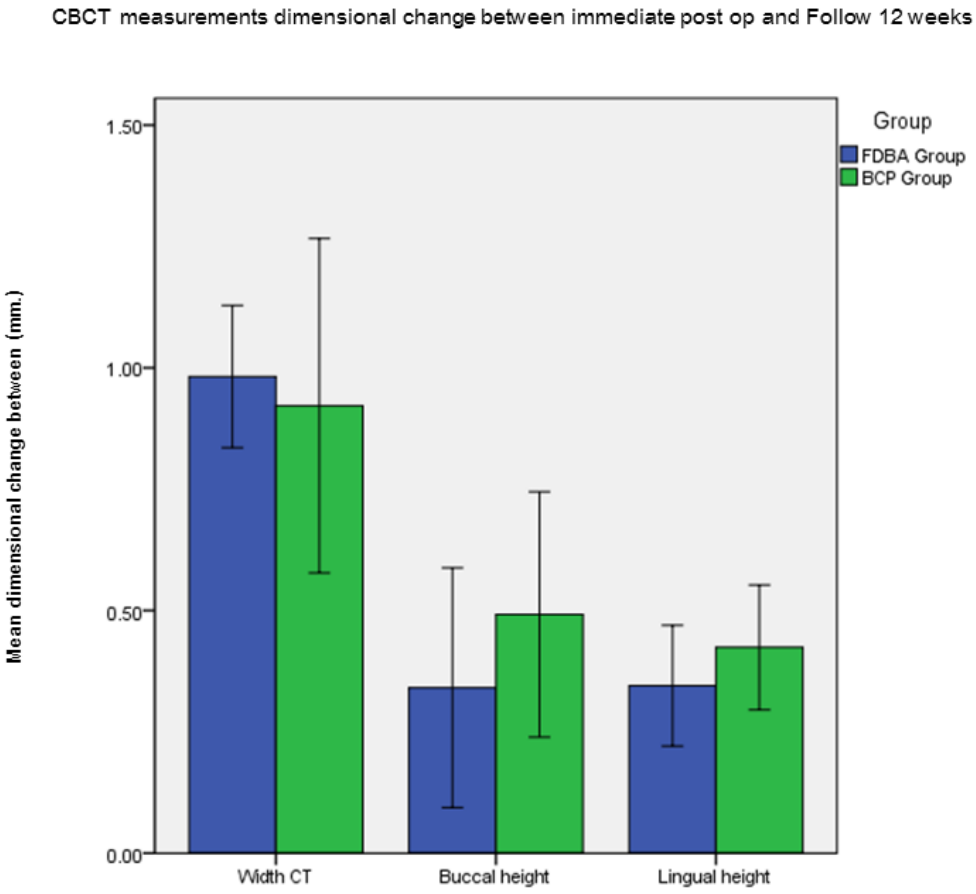

Figure 16 The dimensional change measurement in the CBCT view of FDBA and BCP from immediate post-op to follow-up period of 12 weeks.

D: Micro-CT Analysis

The total volumes of newly formed bone as well as residual grafting materials were summarized in Figure 17 and Table 11. At 3 months, the percentage of new bone volume fraction for FDBA and BCP were 22.37±9.61 and 16.89±7.46 respectively. Percentage of residual graft volume fraction for FDBA and BCP were 17.31±14.53 and 15.94±7.39 respectively. Nevertheless, no significant different were detected between both groups. And 3D-reconstruction of new bone volume fraction and residual graft volume fraction of each group at 3 months were shown in Figure 18 and Figure 19

Table 11 Micro-CT analysis residual graft and new bone volume fraction

Study group	Micro-CT analysis mean percentage of residual graft area and new bone volume(%)	
	Residual graft	New bone
FDBA	17.31±14.53	22.37±9.61
BCP	15.94±7.39	16.89±7.46
Values are presented as mean±SD.		

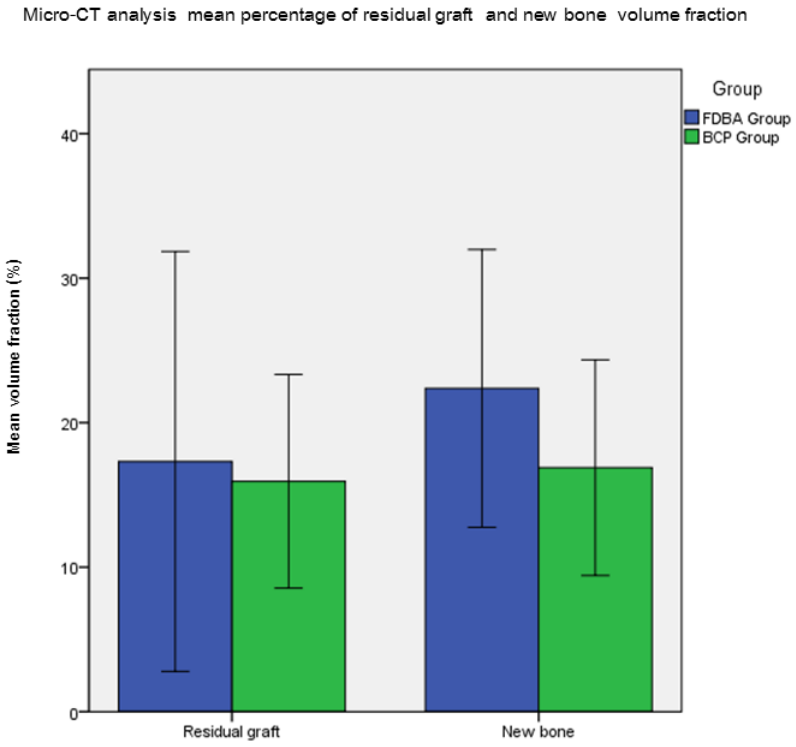

Figure 17 Micro-CT analysis; Residual graft and new bone volume fraction at 12-week postoperative period.

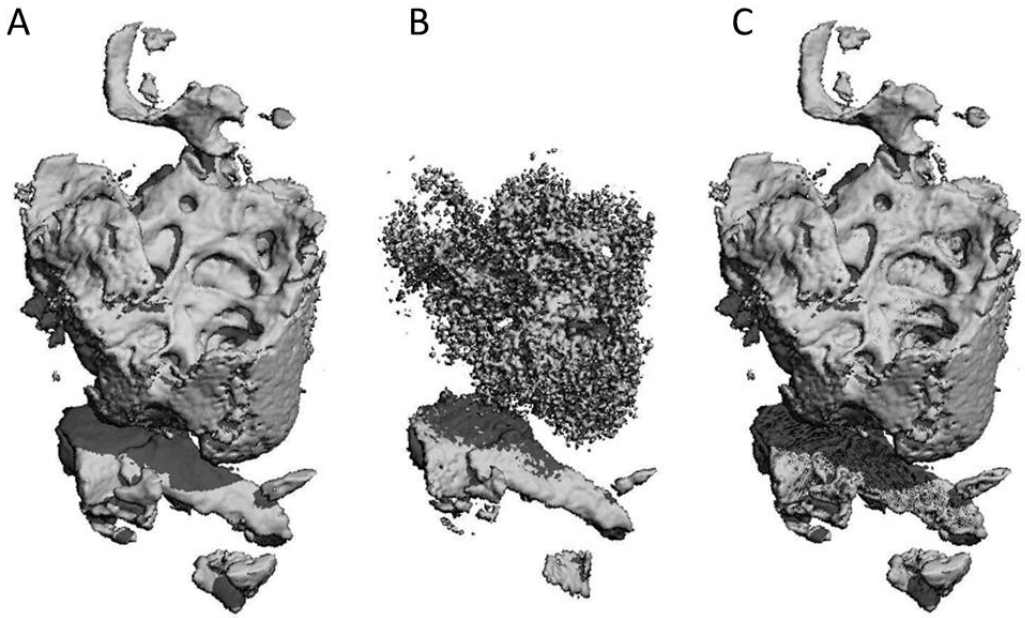

Figure 18 3D-reconstruction of FDBA at 3 months (A,B,C) ; (A)Total volume of bone core, (B) Residual graft volume fraction, (C) New bone volume fraction

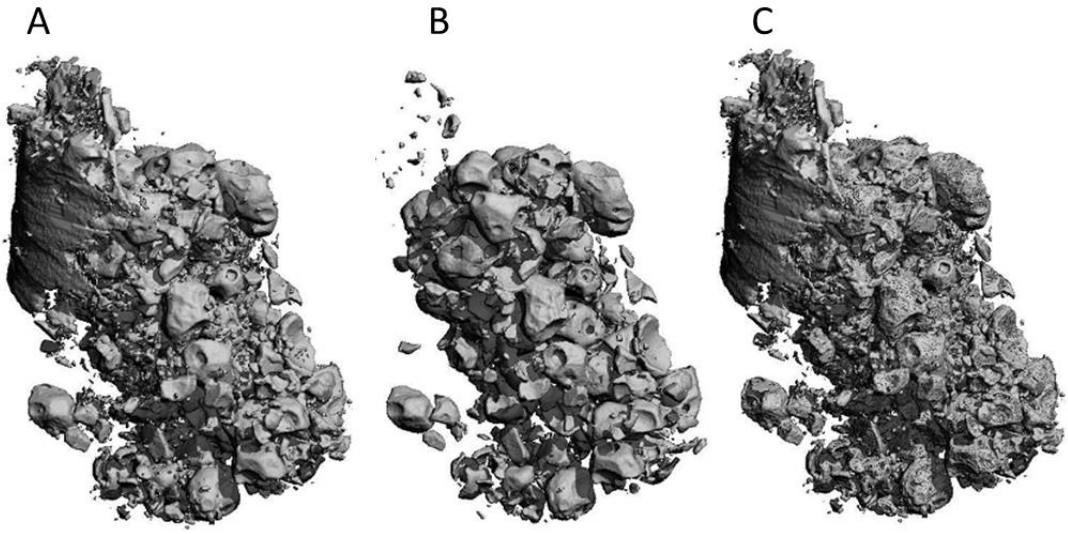

Figure 19 3D-reconstruction of BCP at 3 months (A,B,C) ; (A)Total volume of bone core, (B) Residual graft volume fraction, (C) New bone volume fraction

E: Histology

Photomicrograph of undecalcified specimens were shown in Figure 20-24. In the FDBA group, the characteristic of newly formed bone showed good continuity, and irregular trabecular bone was well incorporated with FDBA particles (Figure 23). In addition, bone matrix, osteoid and the active osteoblast-like cell could be seen on FDBA with surrounding newly formed bone (Figure 20-21) and showed bone formation aligned with osteoblast at the rim of woven bone(Figure 20B). While in the BCP group, BCP particles were visual. Newly formed bone could be seen by fusing with BCP particles(Figure 22). However, surface resorption of BCP particles with active osteoclasts and bone formation was also evident with the appearance of osteoid in Figure 24B.

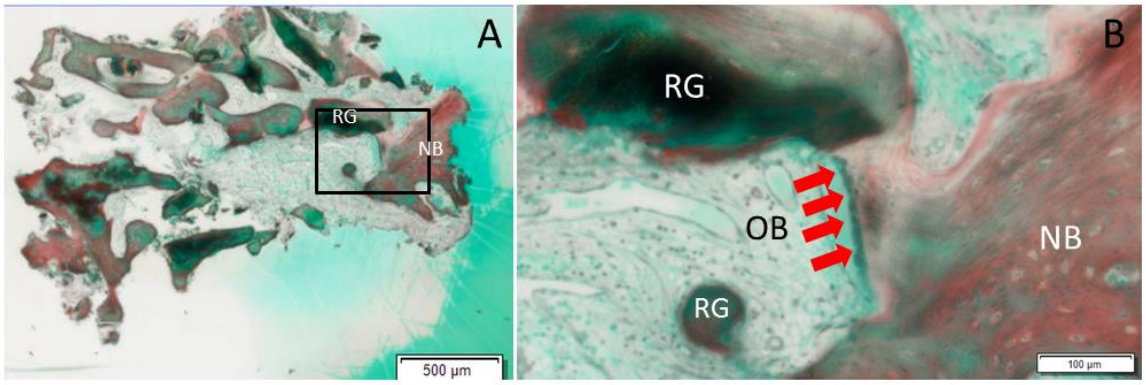

Figure 20 FDBA specimens at 3 months; (A) FDBA, original magnification (B) FDBA shows that bone formation is aligned with osteoblast ; OB = osteoblast, RG = residual graft, NB = newly formed bone. Goldner's Masson trichrome original x 10

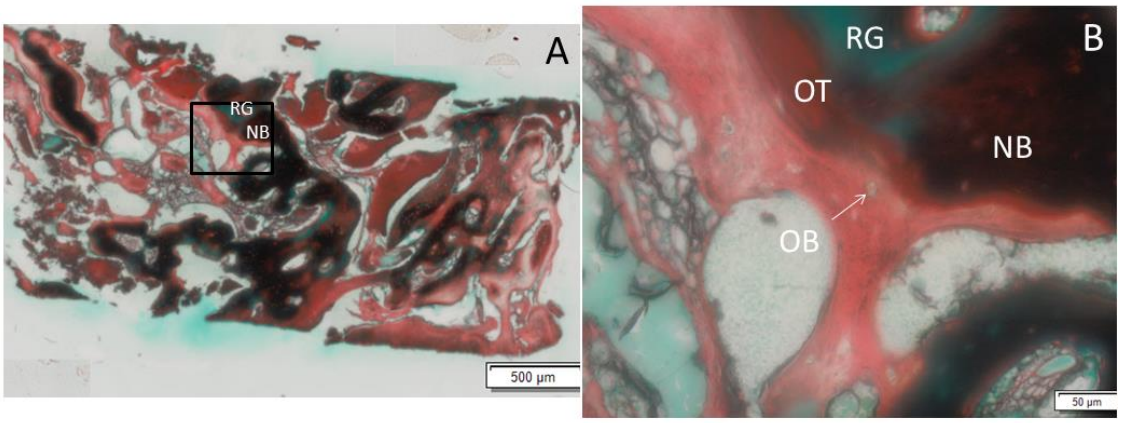

Figure 21 FDBA specimens at 3 months; (A) FDBA, original magnification (B) FDBA shows that bone formation is also evident with the appearance of osteoid; OB = osteoblast, RG = residual graft, NB = newly formed bone. Goldner's Masson trichrome original x 10

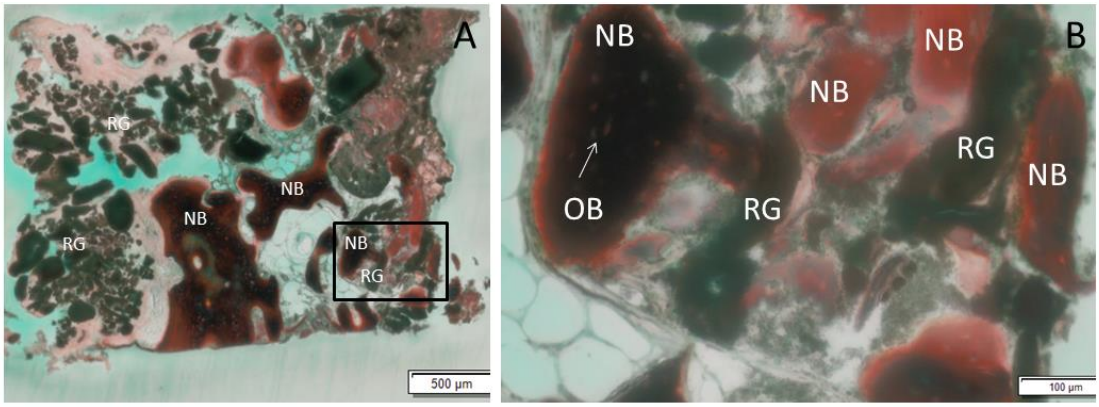

Figure 22 BCP specimens at 3 months; (A) BCP,original magnification (B) BCP shows that bone formation is fused with the BCP particles; OB = osteoblast, RG = residual graft, NB = newly formed bone; Goldner's Masson trichrome original x 10

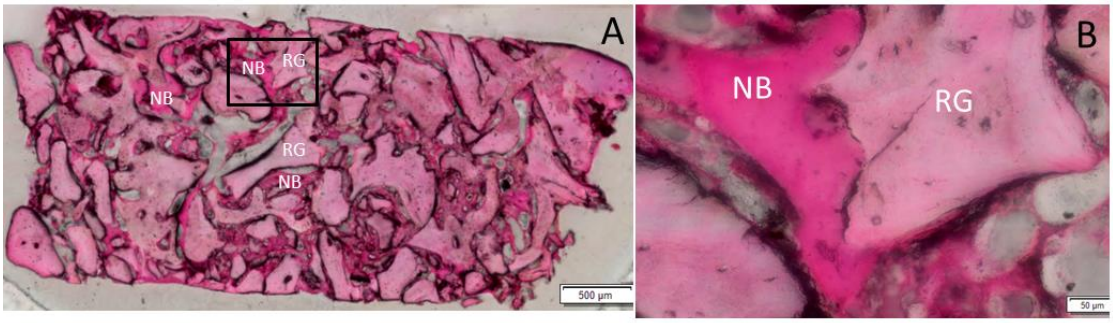

Figure 23 FDBA specimens at 3 months; (A) FDBA, original magnification. (B) FDBA shows that newly formed bone with FDBA particles on the bone core; RG = residual graft, NB = newly formed bone; Hematoxylin and eosin, original magnification x 10

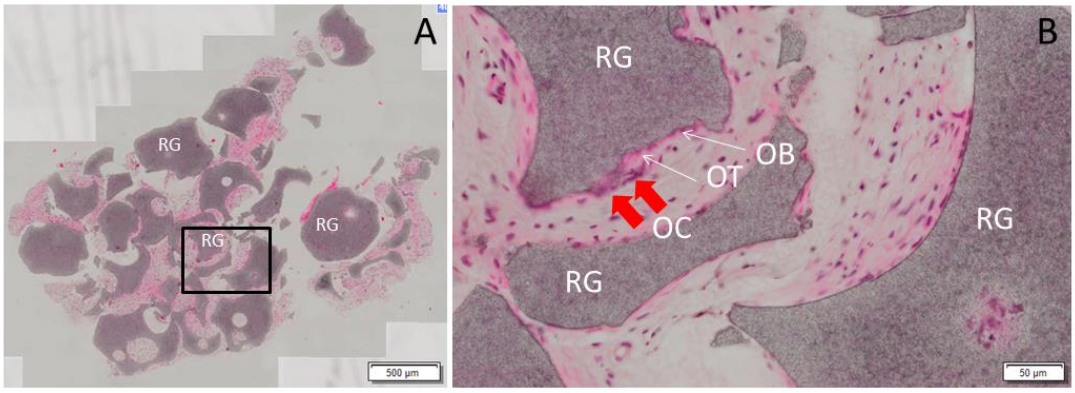

Figure 24 BCP specimens at 3 months; (A) BCP,original magnification. (B)BCP shows surface resorption of BCP particles with active osteoclast and bone formation is also evident with the appearance of osteoid; RG = residual graft, OC = osteoclast, OT = osteoid, OB = osteoblast; Hematoxylin and eosin, original magnification x 10

F: Histomorphometric Analysis

The percentage of the new bone area and percentage of residual graft particle area were presented in Figure 25 and Table 12. At 3 months, the percentage of new bone area for FDBA and BCP groups were 20.17±4.59 and 18.40±7.20 respectively. Percentage of residual graft area for FDBA and BCP groups were 22.50±2.64 and 29.38±7.96 respectively. Statistic significant different were detected between the group for residual graft showed more BCP particles than FDBA particles.

Table 12 Histomorphometric analysis of the mean percentage of residual graft area and new bone area

Study group	Histomorphometric analysis mean percentage of residual graft area and new bone area(%)	
	Residual graft	New bone
FDBA	22.50±2.64*	20.17±4.59
BCP	29.38±7.96*	18.40±7.20
Values are presented as mean±SD.		
***Significant different between group at P<0.05**		

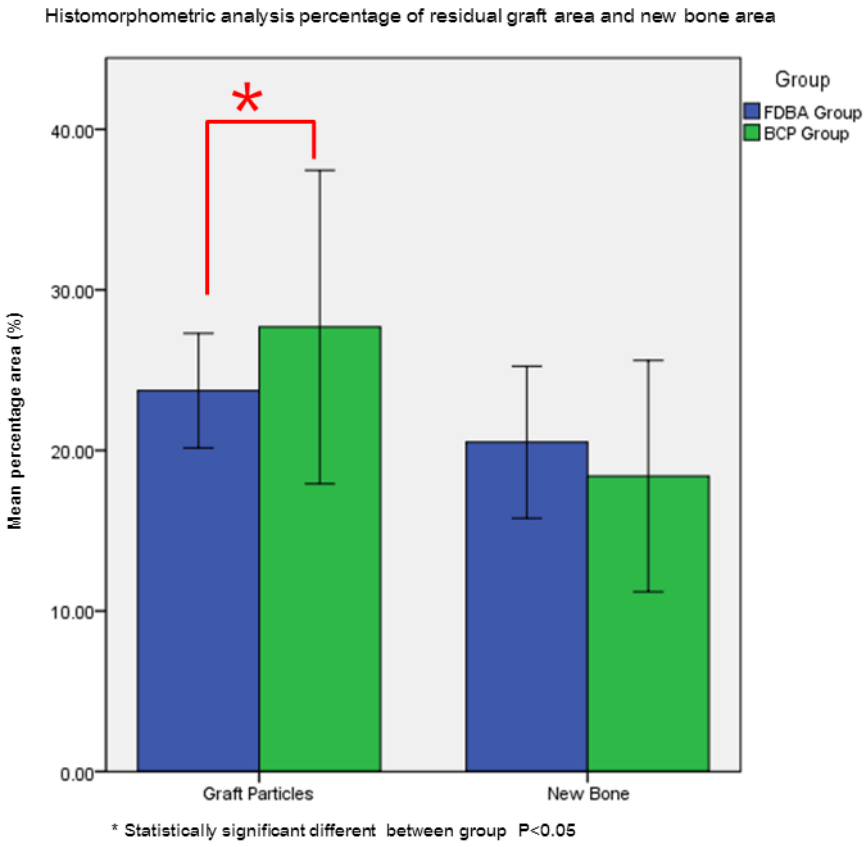

Figure 25 Histomorphometric analysis; the percentage of residual graft area and the percentage of new bone area at 12-week postoperative period.

Chapter 4

Discussion

This study investigated the characteristic and morphology of PRF membrane by SEM observations and the degradation rate of PRF membrane by weight loss. In SME observations, the standard PRF membrane and the warm PRF membrane are shared similar SEM images features. The findings confirmed that platelets were not equally distributed along the fibrin length both inside and on the surface of the membranes. Therefore, in a clinical situation, when growth factors provided by platelets are expected and desired, the platelet-rich region adjacent to the red thrombus should be used. In addition, the degradation rate of standard PRF membrane showed result the upper part lose the least weight in 14 days and the middle and the lower part showed more weight loss than 60% and 80% by weight. It is presumable that the faster degradation might due to the concentrated platelets, leukocytes and proteolytic enzymes. And also the higher levels of growth factors released by activated platelets(75). It is important for clinical use that the upper parts resorbed slowly with high fibrin mesh feature but less quantity of growth factors while the lower part resorbed fastly but might contain more quantity of growth factors. Therefore, the results of the study support that the upper part of PRF membrane which prosesses high fibrin mesh and slow resorption might be used in a clinical situation that working time needs more than 2 weeks such as using for socket sealing. Also, the lower part which contains more growth factors is suitable for mixing with grafting material and use at implant sites as a source of growth factors for enhancing tissue regeneration.

The warm PRF membrane showed more weight loss than the standard PRF membrane in the early period. Kawase and coworkers suggested that heat-compression technique prolonged the rate of biodegradation of a PRF membrane. They hypothesized that heat increased crosslinking density among individual fibrin fiber within a PRF and prolong the preservation of the PRF membrane (15). This study made warm PRF membrane by using water bath at 80°C for 15 seconds but found opposite reports. It might be that the heat denature fibrin fiber, therefore warm PRF membrane had fast degradation. So that, in this study, it's noted that warm PRF membrane did not have a positive effect on the microstructure of the PRF surface but affected on faster degradation.

From soft tissue healing, it is evidence that PRF membrane used as a socket seal was appropriate and reasonable. From this study, PRF membrane showed that the socket healed within 6 weeks and was clearly shown the complete maturation of mucosa covering the socket orifice within 8 weeks. Suttapreyasri et al (40) used PRF alone for socket preservation and reported that it accelerated soft-tissue healing on the first 4 weeks. In this study, the use of PRF membrane for socket seal obtained better result for complete soft tissue healing as the surgical wound was closed by within 6 weeks.

Regarding clinical study, 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 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 width at

the buccal and the lingual side and the ridge height which showed no statistically significant differences between the groups. In each group, there was significant loss of ridge at all sides especially in the ridge width at the buccal side that resorbed more than 1.00 mm in both groups. Regarding the cast-based measurement, the results indicated that the BCP performed as well as FDBA in preserving ridge height and width and keeping the ridge contour after 3 months. Although the 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 (1, 2). Iasella et al (22) 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 was 1.3 mm in ridge height and 1.17 mm in ridge width during 4-6 months. Furthermore, Eskow and Mealey(76) 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 which might due to shorter time frame, and more reduction still progress afterward especially after 3 to 6 months.

Regarding non-grafting site from the previous study, Cardaropoli and coworkers (38), 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, Wolhusen, Switzerland). Also from the study by Lekovic et al (1) who compared the non-grafting site to the

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 (77). 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 1.00 mm in both groups and the FDBA group (0.98±0.15) showed more reduction than the BCP group (0.92±0.34). 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.

In our 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. However, BCP group showed different patterns in the group. It was found that the granule of BCP was mainly composed of loosely arranged connective tissue with some mineralized bone. Because the core bone biopsy was only 2 mm in diameter and located at the center of the socket, therefore it represented the least healing part of the socket. It can be summarized that the socket with grafting material healed incompletely during 3 month time frame.

For the evaluation of new bone formation and residual graft material, most studies have been performed using histomorphometric analysis, which is limited to 2-dimension information from a representative slide. Histomorphometric was reliable and widely used in the assessment of bone tissue regeneration or healing(78). The micro-CT techniques offer some advantages, such as represent 3-D features, fast calculation and multiple analysis opportunities. Therefore, recent reports have also performed micro-CT, observing newly formed bone and residual graft material of the bone core using 3-dimensionally reconstructed images. Results from Micro-CT revealed that newly form bone of the FDBA group(22.37±9.61) is more than the BCP group(16.89±7.46). However, residual graft particle of the FDBA group(17.31±14.53) is more than the BCP group (16.89±7.46). Due to, micro-CT imaging data has limitation in discrimination of FDBA materials that show similar density of the bone but the bone volume in the BCP groups represented actual new bone formed voxels that could be discriminated from BCP particles.

The histomorphometry result supported the micro-CT analysis that the FDBA group had higher newly bone formed than the BCP group. In contrast, histomorphometry result for residual graft is different from the micro-CT analysis that possibly due to micro-CT measured the volume of bone in 3-dimension while histomorphometry represented only a section of 15-25μm in 2-dimension.Therefore, the micro-CT and histomorphometry of the FDBA and the BCP results showed that both had effective space maintaining in socket preservation. 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% (33).

Regarding the 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. But the BCP particles showed lower residual graft than FDBA group (15.94±7.39 vs 17.31±14.53) in micro-CT analysis. It is noted that the BCP particles may be fastly resorbed and then was replaced with connective tissue instead of bone. Therefore the BCP particles at the ratio 1:1 of HA to TCP might be quickly resorbed in the early stage and the sockets were infiltrated with soft tissue in the area of socket orifice and blended with the BCP particles. Theoretically, the TCP part was dissolved into Ca^{2+} and PO_4^{3-} ions, whereas HA retained its form and structure and was not resorbed (79). This BCP were fabricated at HA:β-TCP = 50:50. The ratio of HA: β-TCP showed that β-TCP in this study is too high. It is suggested that higher HA than 50% and β-TCP less than 50% might be appropriate for bone regeneration in 3 month period.

The same specimen in this study were investigated both histomorphometric analysis and micro-CT analysis. The volume fraction measured by the micro-CT is the most accurate value for implying bone formation in 3-D. The area fraction measured by the histomorphometric analysis is less accurate for evaluating the total bone formation within the defects because the specimen represents only the center of the socket. However, the histomorphometric data is still the best method for cellular determination and maturity of the new bone. They showed correlation between the micro-CT and histomorphometric analyzes as shown in Table 13. The correlation between micro-CT and histomorphometric analyzes had been proven by Park et al. and Yeom et al. (80, 81). They showed that micro-CT is a very convenient tool for evaluating the bone micro-architectures in intramembranous bone regeneration. However, the correlation between micro-CT measurement and histomorphometry were varied according to the features measured(81).

Table 13 Micro-CT analysis and Histomorphometric analysis

Study group	Micro-CT analysis		Histomorphometric analysis	
	Residual graft	New bone	Residual graft	New bone
FDBA	17.31±14.53	22.37±9.61	22.50±2.64	20.17±4.59
BCP	15.94±7.39	16.89±7.46	29.38±7.96	18.40±7.20
Values are presented as mean±SD.				

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.

Chapter 5

Conclusion

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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Appendix

1. Material
    1.1 Biphasic calcium phosphate (BCP), HA:β-TCP=50:50 from Metal and material Technology Center,Thailand
    1.2 Freeze Dried Bone Allograft (FDBA) from Siriraj hostipal
2. Equipments and instruments
    2.1 Bone morselizer, Bone crusher titanium stainless , Pacific Dental @ imaging Ltd, Australia
    2.2 Centrifuge EBA 20, Hettich Zentrifugen, Andreas Hettich GmbH & Co, KG, Tuttlingen, Germany
    2.3 CO2 incubator, Thermo Forma series II, USA
    2.4 Cone beam CT, 3D Accuitomo 170, J Morita, Kyoto, Japan
    2.5 Digital Water Bath, Whip Mix, Whip Mix Corp, Louisville, KY
    2.6 Dry bath incubator, EL-02-220, MS major science, Taiwan
    2.7 Grinding machine, cutting and grinding system, EXAKT® Apparatebau, Norderstedt, Hamburg, Germany
    2.8 Micro-CT, Micro-CT35, SCANCO Medical AG, Brüttisellen, *Switzerland*
    2.9 Microscope VS120 Virtual Slide, Olympus, Tokyo, Japan
    2.10 Periodontal probe, Hu-Friedy, *Hu-Friedy* Mfg. Co., Chicago, IL, USA
    2.11 Scanners (3Shape D700, Copenhagen, Denmark
    2.12 Scanning electron microscope, Model JSM-5800LV, JEOL LTD, Japan
    2.13 Sputter Coater, SPI-Module TM, Model 11425, SPI supplies, USA
    2.14 Trephine bur, 3i Implant Innovations Inc., USA
    2.15 Test tube 16 mm x 125 mm, Pyrex®, VISTA™, Sigma-Aldrich Ltd, Singapore
    2.16 Weight meter, Adventurer™, Electric balance Ohaus, Model AR2140, Ohaus Corp., USA

3. Disposable materials

3.1 Alginate Jeltrate®, Dentsply, Toronto, Canada

3.2 Articaine hydrochloride 4%, Ubistesin 1:100,000; 3M ESPE, Platz, Seefeld, Germany

3.3 Dental stone , GC Fujirock type 4; GC Corp, Tokyo, Japan

3.4 Disposable 24 Well plate, Costar, Corning Incorporation, USA

3.5 Disposable hypodermic needle, 21G X 1", Nipro Medical Corporation, Japan

3.6 Disposable syringe 10 ml, Terumo® Syringe, Laguna, Philippines

3.7 Disposable syringe 20 ml, Terumo® Syringe, Laguna, Philippines

3.8 Vicryl 4-0, ETHICON, Johnson & Johnson Medical Limited, Livingston, Scotland

4. Chemical and reagents

4.1 Absolute ethanol, Cat. No. 39420483, Merk, Germany

4.2 Amoxicillin 500 mg, Coamox, Community Pharmacy Public Co. Ltd, Thailand

4.3 Chlorhexidine gluconate mouthwash 0.12%, Faculty of Dentistry, Prince of songkla university

4.4 Dulbecco's Modified Eagle's Medium,Gibco, Invitrigen Corporation, USA

4.5 Formalin solution, neutral buffered 10% , Sigma-Aldrich™, USA

4.6 Glutaraldehyde, Cat No. 111380, Sigma-Aldrich™, USA

4.7 Glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany

4.8 Goldner's Masson trichrome , Bio-optica, Milano, Italy

4.9 Hematoxylin stains, H&E staining, Sigma-Aldrich™, USA

4.10 Osmium tetroxide, Cat on 20816-12-0 , Sigma-Aldrich™, USA

4.11 Paracetamol 500 mg (Cemol, Central Poly Trading Co., Ltd., Nonthaburi, Thailand

4.12 Sterile water 100 ml, General Hospital Products Public Co.,Ltd, Thailand

5. Software

5.1 EndNote X5 for Windows, Thomson Reuters, Philadelphia, USA

5.2 Image Pro Plus 7.0, Media Cybernetics, MD, USA

5.3 OlyVIA 2.8, Olympus software, Tokyo, Japan

5.4 One Volume Viewer, J.Morita MFG.Corp., Kyoto, Japan

5.5 Ortho Analzer™ software, 3Shape, Copenhagen, Denmark

5.6 SPSS version 15, SPSS Inc., Chicago, USA

RESEARCH ETHICS COMMITTEE (REC)
BUILDING 1 5TH FLOOR ROOM 504
TEL. 66-74-287533, 66-74-287504
FAX. 66-74-287533

FACULTY OF DENTISTRY
PRINCE OF SONGKLA UNIVERSITY
HADYAI, SONGKHLA 90112, THAILAND
TEL. 66-74-212914, 66-74-429871, 66-74-287500
FAX. 66-74-429871, 66-74-212922

Documentary Proof of Ethical Clearance

Research Ethics Committee (REC)

Faculty of Dentistry, Prince of Songkla University

The Project Entitled Efficacy of Nano Biphasic Calcium Phosphate Compared with Freeze Dried Bone Allograft in Socket Preservation

REC Project No. : EC5609-21-P-HR

Principal Investigator : Mr. Nattaphol Boonsri

Approved by Research Ethics Committee (REC), Faculty of Dentistry, Prince of Songkla University.

This is to certify that REC is in full Compliance with International Guidelines for Human Research Protection such as the Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and The International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Date of Approval : 19 DECEMBER 2013 **No. of Approval** : MOE 0521.1.03/1428

(Asst. Prof. Dr. Srisurang Suttapreyasri)
Chairman of Research Ethics Committee

(Asst. Prof.Surapong Vongvatchranon)

(Dr. Supatcharin Piwat)

(Assoc. Prof. Pornchai Sathirapanya)

(Mr. Kamolphan Nuangsri)

(Asst. Prof. Dr. Angkana Thearmontree)

(Mr. Wasin Suwannarat)

(Asst. Prof. Dr. Suwanna Jitpakdeebodintra)

ที่ ศธ 0521.1.03/ **1428**

คณะทันตแพทยศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ไปรษณีย์เลขที่ 17
ที่ทำการไปรษณีย์โทรเลขคอหงส์
อ.หาดใหญ่ จ.สงขลา 90112

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง "ประสิทธิผลของวัสดุไบเฟสิกแคลเซียมฟอสเฟสเปรียบเทียบกับกระดูกเอกพันธ์แห้งแบบระเหิดในการคงสภาพเบ้าฟัน"

รหัสโครงการ EC5609-21-P-HR

หัวหน้าโครงการ ทันตแพทย์ณัฐพล บุญศรี

สังกัดหน่วยงาน นักศึกษาหลังปริญญา ภาควิชาศัลยศาสตร์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ได้ผ่านการพิจารณาและได้รับความเห็นชอบจากคณะกรรมการจริยธรรมในการวิจัย (Research Ethics Committee) ซึ่งเป็นคณะกรรมการพิจารณาศึกษาการวิจัยในคนของคณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ดำเนินการให้การรับรองโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นสากล ได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guidelines และ The International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

ในคราวประชุมครั้งที่ 9/2556 **เมื่อวันที่** 24 ตุลาคม 2556

ให้ไว้ ณ วันที่ 19 S.A. 2556

(ผู้ช่วยศาสตราจารย์ ดร.ทพญ.ศรีสุรางค์ สุทธปรียาศรี)
ประธานคณะกรรมการจริยธรรมในการวิจัย

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(รองศาสตราจารย์ นพ.พรชัย สถิรปัญญา)	(อาจารย์ ทพ.กมลพันธ์ เนื่องศรี)
.....กรรมการ	.....กรรมการ
(ผู้ช่วยศาสตราจารย์ ดร.ทพญ.อังคณา เธียรมนตรี)	(อาจารย์วศิน สุวรรณรัตน์)

.....กรรมการ
(ผู้ช่วยศาสตราจารย์ ดร.ทพญ.สุวรรณา จิตภักดีบดินทร์)

HSO-211

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, & Prasertthem, 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).

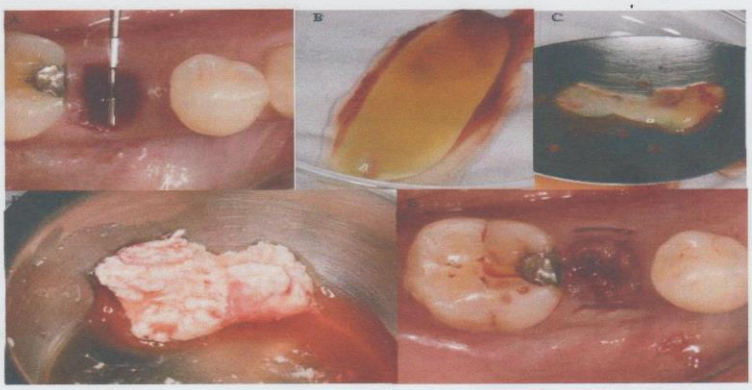

Figure 1 – Operation procedure A) Atraumatic extraction and de-epithelized socket orifice B) PRF C) PRF membrane D) Grafting material mixed with fluid leaked from PRF E) Placement of graft material and sealed with PRF membrane and sutured with criss-cross suture technique

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.

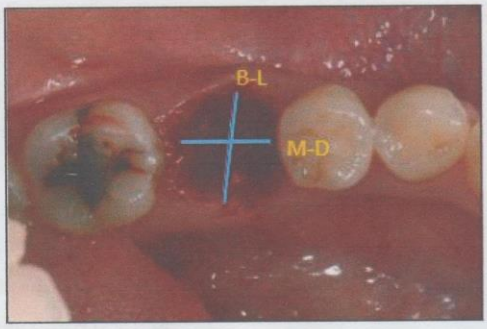

Figure 2 Measurements of socket orifice

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.

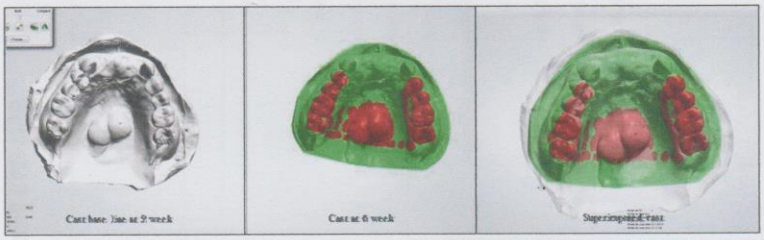

Figure 3 Superimposed cast at the baseline 2 week(T2) with the cast at 6 weeks(T6)

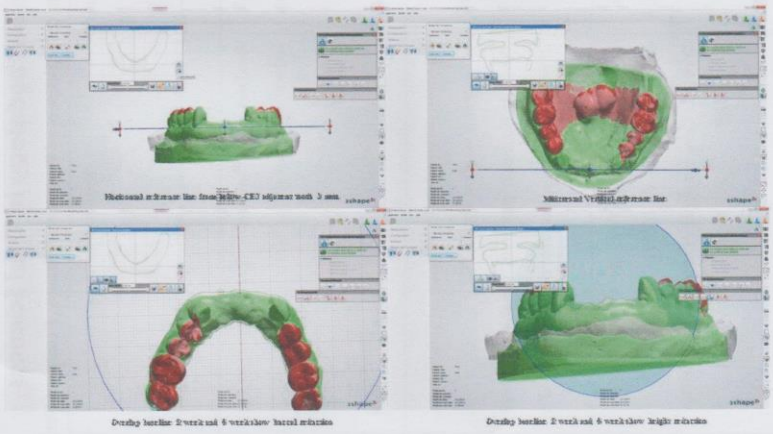

Figure 4 Measurement of the dimensional change of width and height from the reference line by using superimposed casts

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

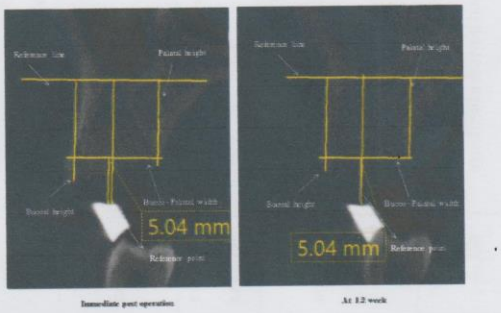

Figure 5 Dimensional measurement in CBCT

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 ($P < 0.05$).

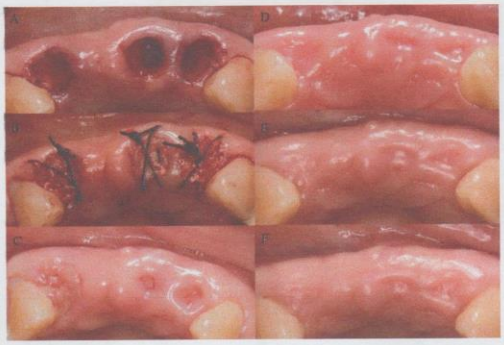

Figure 6 A case of socket preservation on the area 12 with FDBA seal with PRF membrane, the area 11 with PRF gel and the 22 with BCP seal with PRF membrane A) Immediate extraction B) Socket preservation C) Follow-up 2 weeks D) Follow-up 6 weeks E) Follow-up 8 weeks F) Follow-up 12 weeks

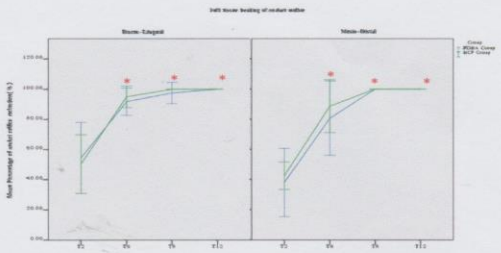

Figure 7 Mean different percentage of socket orifice reduction from immediate post extraction (T0) to each time point (T2, T6, T8, T12) in the bucco-lingual direction (left) and in the mesio-distal direction
(right)

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.

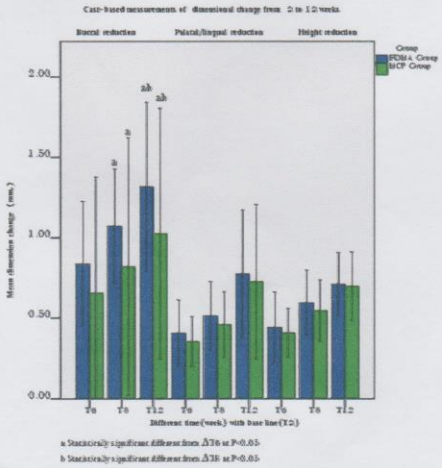

Figure 8 The buccal, lingual side and the height of the contour changes of the extraction site from cast-based measurements

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.

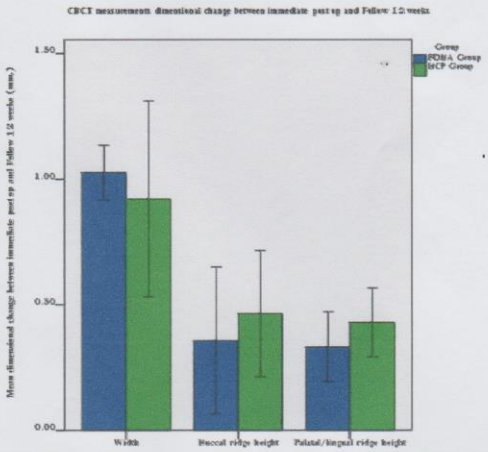

Figure 9 The dimensional change measured by CBCT of both groups. There was no statistical difference between groups

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, Wolhusen, 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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การประชุมวิชาการเสนอผลงานวิจัยระดับบัณฑิตศึกษาแห่งชาติ ครั้งที่ 38

"บัณฑิตศึกษากับการพัฒนาสู่โลกาภิวัตน์"

ขอมอบเกียรติบัตรนี้เพื่อแสดงว่า

ทันตแพทย์ณัฐพล บุญศรี

ได้เข้าร่วมนำเสนอผลงานประเภท Oral presentation

ให้ไว้ ณ วันที่ 19 กุมภาพันธ์ 2559

(รองศาสตราจารย์ ดร.เอกชัย แสงอินทร์)
คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่
ประธานที่ประชุม ทคบร.

(ศาสตราจารย์ ดร.รัตนะ บัวสนธ์)
คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยนเรศวร

Vitae

Name Nattaphol Boonsri

Student ID 5710820006

Education Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Chiangmai University	2011
Higher Graduate Diploma in Clinical Science (Oral and Maxillofacial Surgery)	Prince of Songkla University	2014

Work-Position and address

The Dental public health, Lamphun provincial Health office, Meung, Lamphun, Thailand, 51000

Proceedings (Oral presentation)

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