Healing of Buccal Dehiscence Defects at Implant Sites Using Demineralized Tooth Matrix

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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.

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ชื่อวิทยานิพนธ์ ผลการบูรณะรอยวิการชนิดดีฮิสเซนส์ด้านใกล้แก้มรอบรากฟันเทียมด้วยดีมินเนอราไลซ์ทูธเมทริกซ์
ชื่อผู้เขียน นางสาวมณทิรา แซ่เบ๊
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บทคัดย่อ

ภายหลังการถอนฟันจะเกิดการละลายของสันเหงือก ทำให้ความกว้างของสันเหงือกลดลง การฝังรากฟันเทียมในบริเวณดังกล่าวมักพบรอยวิการรอบรากฟันเทียม ซึ่งจำเป็นต้องรับการบูรณะด้วยการปลูกกระดูก กระดูกอาตมันเป็นวัสดุที่ถือเป็นมาตรฐานสูงสุดเนื่องจากมีคุณสมบัติในการสร้างกระดูก เหนี่ยวนำการสร้างกระดูก และ เป็นโครงร่างสำหรับการสร้างกระดูกใหม่ อย่างไรก็ตามที่สำคัญข้อจำกัดที่สำคัญของกระดูกอาตมันได้แก่ ความจำเป็นในการผ่าตัด 2 ตำแหน่ง ความเสี่ยงต่อการเกิดความวิการ หรือผลแทรกซ้อนในบริเวณที่ทำการผ่าตัดเพื่อเอากระดูกอาตมัน ปริมาณที่จำกัด เป็นต้น ทำให้มีความพยายามพัฒนากระดูกเทียมที่มีคุณสมบัติใกล้กระดูกอาตมันเพื่อใช้ในทางทันตกรรม สำหรับการศึกษานี้ ฟันของมนุษย์ได้ถูกเตรียมและปรับปรุงคุณสมบัติให้อยู่ในรูปแบบดีมินเนอราไลซ์ทูธเมทริกซ์ เพื่อใช้เป็นวัสดุทดแทนกระดูกชนิดอาตมันในการแก้ไขรอยวิการที่เกิดขึ้นร่วมกับการฝังรากฟันเทียม

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลการรักษาทางคลินิกและทางภาพถ่ายรังสีโคนบีมซีทีในการบูรณะรอยวิการชนิดดีฮิสเซนส์บนด้านใกล้แก้มที่เกิดขึ้นรอบรากฟันเทียมด้วยดีมินเนอราไลซ์ทูธเมทริกซ์ที่ผลิตจากฟันของผู้ป่วยเอง กับ กระดูกวิวิธพันธ์ที่ผลิตจากกระดูกวัว โดยวิธีการปลูกกระดูกร่วมกับการใช้แผ่นเยื่อกั้นกระดูก

ผู้ป่วยและวิธีการศึกษา รอยวิการชนิดดีฮิสเซนส์บนด้านใกล้แก้มรอบรากฟันเทียมที่เกิดขึ้นในขณะที่ฝังรากฟันเทียมจำนวนทั้งสิ้น 12 ตำแหน่ง ได้รับการบูรณะด้วยกระดูกวิวิธพันธ์ที่ผลิตจากกระดูกวัวจำนวน 7 ตำแหน่ง และ บูรณะด้วยด้วยดีมินเนอราไลซ์ทูธเมทริกซ์ที่ผลิตจากฟันของผู้ป่วยเอง 5 ตำแหน่ง โดยดีมินเนอราไลซ์ทูธเมทริกซ์จะถูกเตรียมก่อนเริ่มการผ่าตัดฝังรากฟันเทียมระยะที่ 1 จากฟันกรามคุดของผู้ป่วยเองด้วยกระบวนการเฉพาะที่ได้พัฒนาขึ้นโดยภาควิชาศัลยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในระหว่างการผ่าตัดฝังรากฟันเทียมระยะที่ 1 ขนาดของรอยวิการ(ความกว้าง ความสูง และพื้นที่)ที่เกิดขึ้นจะถูกบันทึกไว้ก่อนที่จะทำการปลูกกระดูกด้วยดีมินเนอราไลซ์ทูธเมทริกซ์ หรือ กระดูกวิวิธพันธ์ที่ผลิตจากกระดูกวัว ร่วมกับแผ่นเยื่อกั้นกระดูก

คอลลาเจนชนิดสลายตัวเองได้ ผู้ป่วยจะได้รับการผ่าตัดรากฟันเทียมระยะที่ 2 ภายหลังจากการผ่าตัดครั้งแรก 4 ถึง 6 เดือน ในส่วนของภาพถ่ายรังสี ผู้ป่วยจะได้รับการถ่ายภาพรังสีโคนบีมซีทีทันทีภายหลังการผ่าตัดระยะที่ 1 และที่ 3 เดือนภายหลังจากการผ่าตัด ข้อมูลที่ได้จะถูกนำไปประมวลผลด้วยโปรแกรมเฉพาะต่อไป

ตัวแปรทางคลินิกที่ทำการวัดผล ได้แก่ ลักษณะทางคลินิกของเนื้อเยื่ออ่อนที่คลุมบริเวณที่ผ่าตัด ขนาด (ความกว้าง ความสูง และพื้นที่) ของรอยวิการชนิดดีฮิสเซนส์บนด้านใกล้แก้ม และลักษณะความเข้ากันระหว่างวัสดุทดแทนกระดูกที่ใช้กับเนื้อเยื่อกระดูกโดยรอบ ตัวแปรทางภาพรังสี ได้แก่ ความหนาของกระดูกทางด้านใกล้แก้มในระดับแพลตฟอร์มของรากฟันเทียม และปริมาตรของกระดูกทางด้านใกล้แก้ม

ผลการศึกษาทางคลินิกพบว่า มีการหายของแผลเกิดขึ้นตามปกติทั้ง 2 กลุ่ม โดยลักษณะของเนื้อเยื่ออ่อนที่ปกคลุมบริเวณที่ผ่าตัดของทั้ง 2 กลุ่มไม่แตกต่างกัน ขณะที่ขนาดของรอยวิการที่เหลืออยู่ในการผ่าตัดระยะที่ 2 ลดลงอย่างมีนัยสำคัญทั้ง 2 กลุ่ม แต่ปริมาณของขนาดรอยวิการที่ลดลงนั้น ไม่แตกต่างกันระหว่างกลุ่ม และดีมินเนอราไลซ์ทูธเมทริกซ์มีความเข้ากันกับเนื้อเยื่อกระดูกโดยรอบได้ดีกว่ากระดูกวิวิธพันธ์ที่ผลิตจากกระดูกวัวอย่างมีนัยสำคัญ ผลทางภาพถ่ายรังสีโคนบีมซีทีที่ 3 เดือนหลังผ่าตัดระยะที่ 1 พบว่า ทั้งความหนาของกระดูกทางด้านใกล้แก้มในระดับแพลตฟอร์มของรากฟันเทียม และปริมาตรของกระดูกทางด้านใกล้แก้มของทั้ง 2 กลุ่มไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ

การศึกษานี้สามารถสรุปได้ว่า ดีมินเนอราไลซ์ทูธเมทริกซ์ที่เตรียมจากฟันผู้ป่วยเองสามารถนำมาใช้บูรณะรอยวิการขนาดเล็ก เช่น รอยวิการรอบรากฟันเทียมชนิดดีฮิสเซนส์ โดยให้ผลการรักษาทั้งทางคลินิกและทางภาพถ่ายรังสีที่ไม่แตกต่างกับการบูรณะด้วยกระดูกวิวิธพันธ์ที่ผลิตจากกระดูกวัว

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Abstract

Background: Following tooth extraction, the remodeling of the alveolar ridge could result in compromised dimensions for implant placement. Subsequently, the peri-implant bone defect would appear during implant placement, thus bone augmentation with bone grafting material is required to repair the defect. An autograft is considered as gold standard by its osteogenesis, osteoinductive, and osteoconductive properties. Despite the benefits associated with the use of autogenous bone, the limitations include small quantity, requiring the 2nd surgical site as well as increasing the risk of donor site morbidity, surgical costs and time. Therefore, the attempts to develop new bone graft substitutes have been conducted for several years. In this study, we develop fabricating protocols to transform the extracted human tooth to become a new bone graft material for use as an autologous graft in clinical practices, initially for small peri-implant bone defects.

Objective: The aim of this study was to compare the clinical and radiological outcomes of autologous demineralized tooth matrix(Auto-DTM) with a resorbable membrane (test group) to an anorganic bovine bone xenograft with a resorbable membrane (DBBM; control group) in the treatment of buccal dehiscence-type defect at implant site.

Materials and methods: Twelve buccal dehiscence defects during dental implant installation were repaired using DBBM (n=7) or Auto-DTM (n=5). The auto-DTM was prepared form the patient's own wisdom teeth and prepared according to PSU protocol before surgery. During implant installation at stage I surgery, the buccal dehiscence defects (width, height and area) were measured clinically and reconstructed using guided bone regeneration(GBR) technique with either DBBM or Auto-DTM and covered by a bioresorbable porcine-derived collagen membrane (Bio-Gide®). The stage II surgery was scheduled after the healing period of 4-6 months. Regarding

radiographic evaluation, cone beam computed tomography(CBCT) were performed at immediately after stage I surgery and at 3-month of follow-up. The CBCT data was collected and analyzed by mean of specific softwares including One volume viewer and ITK-SNAP.

Clinical parameters including soft tissue appearance, defect size (width, height and area) and graft integration, and radiographic parameters including midbuccal bone thickness and marginal buccal bone volume, were measured and analyzed.

Results: All patients have uneventful healings. Soft tissue color and texture were not affected by grafting materials and surgical procedures. The buccal contour improved significantly after grafting, regardless of graft materials. Significant defect reduction was observed in both groups ($P<0.05$). In addition, there was no statistically significant difference in the percentages of defect reduction between the two groups ($P>0.05$). The graft integration between the surrounding host bone and Auto-DTM particles appeared more consolidate than the DBBM significantly ($P<0.05$). Radiographically at 3-month follow-up, the marginal buccal bone thickness and buccal bone volume obtained in the Auto-DTM group were not significantly different from the DBBM group ($P>0.05$).

Conclusion: The autologous DTM can be used to repair the small-sized defects such as peri-implant dehiscence or fenestration defect reconstruction with comparable clinical and radiographic outcomes as the widely-used commercial xenograft(DBBM).

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Monthira Saebe

Content

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List of Abbreviations and Symbols

°c	=	celsius
µm	=	micrometer
A	=	autogenous bone
ABT	=	autogenous bone from bone trap
ACP	=	amorphous calcium phosphate
ADM	=	acellular dermal matrix
AP	=	autogenous particulate
Auto-DTM	=	autologous demineralize tooth matrix
BCP	=	biphasic calcium phosphate
BMPs	=	bone morphogenetic proteins
CBCT	=	cone beam computed tomography
DBBM	=	deproteinized bovine bone mineral
DDM	=	demineralized dentin matrix
DFDBA	=	demineralized freeze-dried bone allograft
DMP1	=	dentin matrix protein1
DPP	=	dentin phosphoprotein
DSP	=	dentin sialoprotein
DSPP	=	dentin sialophosphoprotein
DTM	=	demineralized tooth matrix
et al	=	and others
FDBA	=	freeze-dried bone allograft
g	=	gram
GBR	=	guided bone regeneration
HA	=	hydroxyapatite
Imm postop	=	immediate post-operation
M	=	molar
MIPs	=	mineralization inducing peptides
ml	=	millimeter

List of Abbreviations and Symbols (Continued)

mm	=	millimeter
mm2	=	square millimeter
mm3	=	cubic millimeter
NCPs	=	non-collagenous proteins
NR	=	non-resorbable membrane
NSAIDs	=	non-steroidal anti-inflammatory drugs
OCP	=	octacalcium phosphate
PSU	=	Prince of Songkla University
PTFE	=	polytetrafluoroethylene
R	=	resorbable membrane
rhBMP-2	=	recombinant human bone morphogenetic protein-2
SEM	=	scanning electron microscope
SLA	=	sandblasted, large grit, acid-etched
TCP	=	tricalcium phosphate
TGF-β	=	transforming growth factor-beta
VEGF	=	vascular endothelial growth factor

Chapter 1

Introduction

To date, dental implant restorations have become the treatment of choice for edentulous areas. Successful outcomes depend on several factors, particularly the volume and quality of bone surrounding the implant. Following tooth extraction, remodeling of the alveolar ridge could result in compromised dimensions for implant placement in the proper prosthetic-driven position. Insufficient ridge width or ridge height resulted in the presence of peri-implant bone defects. Moreover, in cases of severe atrophic ridges, implant instability or even unavailability for implant installation could be found. Thus, to provide for an appropriate ridge to form as well as to repair the defect, alveolar ridge modification or augmentation prior to or simultaneously with an implant placement is necessary.

There are several ridge modification and augmentation techniques, for instance, ridge expansion, guided bone regeneration, interpositioning grafts, and block bone grafts, etc. Technique selection depends on several factors such as the severity of the atrophic ridge, the required graft amount, and the patient and surgeon's preferences.

The materials used for bone substitutes can be autogenous, allogenous, xenogenous, or of synthetic origins. The gold standard of bone graft materials is autogenous bone due to its excellent biological properties of osteoinduction, osteoconduction, and osteogenesis cells. Despite the benefits associated with the use of autogenous bone, the limitations include donor site morbidity, surgical costs and time. Allogenous and xenogenous bone can overcome the disadvantages of autogenous bone, however, they may potentially harbor transmittable diseases, and may act to induce an immune response. Synthetic or alloplastic materials include ceramic-based bone substitutes such as calcium phosphate-based minerals, calcium sulfate, bioactive glass, titanium, polymers, and cement(1- 5) . The degradability, mechanical and osteopromotive properties of these materials depend on their fundamental elements and the fabrication process.

However, whilst synthetic materials intent to duplicate the features found in autogenous bone graft, with post-production modifications and addition of growth factors, biological materials are still considered the most effective and are the most widely-used alternative.

In addition to bone, another hard tissue is the human tooth, which also shares a similarity in inorganic composition to bone as well as contains some osteoinductive proteins, particularly bone morphogenetic proteins(BMPs)(6-14). Recently, several researches have been conducted to investigate tooth or dentin as bone substitutes with consistent results indicating their osteoconductive and osteoinductive potential regardless of different fabrication protocols(8, 11-31).

This present study was conducted to transform the human tooth, which has always been considered as medical waste, to become a new bone graft material for use as an autologous graft in clinical practices, initially for small peri-implant bone defects.

Literature Review

Alveolar ridge alteration after tooth extraction

Subsequent to tooth extraction, alveolar ridge resorption and bone remodeling usually occur and cause a dimensional change to the edentulous ridge(32). Several studies indicated that the vertical bone resorption was up to 40%, while the vertical bone resorption was as much as 63%. The alveolar ridge width reduced by approximately 50% with almost two-thirds of this reduction during the first 3 months of healing(33). The resorption of the extraction socket was found greater on the buccal wall than the lingual or palatal wall of the socket(34). The systematic review conducted by Van der Weijden et al(35) concluded that the clinical loss in alveolar ridge width was 3.87mm, whereas the loss in mid-buccal height ranged from 1.67 to 2.03mm, clinically, as well as 1.53mm, radiographically. The greater ridge resorption on the buccal aspect would result in the relocation of the ridge crest to a more palatal or lingual position and eventually lead to an incorrect three-dimensional position to install an implant without augmentation.

Dental implant placement in a post-extraction socket

Regarding implant placement, the bone volume or ridge dimensions should be suitable for placing an implant with proper buccal and lingual bone thickness or at least without any defect around the implant. The placement of a dental implant in a post-extracted socket should be considered conscientiously since resorption of the healed socket occurs continuously following extraction(32, 33). According to the 3rd ITI Consensus by Hämmerle et al(36), timing of implant placement can be classified into 4 types: type 1 (immediate placement), type 2 (early placement with soft tissue healing), type 3 (early placement with substantial bone fill), and type 4 (delayed placement with complete bone fill). Type 1 placement benefits existing bucco-lingual volume for implant placement, while type 4 displays completed remodeling of the ridge, thus reducing the ridge dimensions.

Bone defects following implant placement

To facilitate maximum bone volume, type 1 or immediate implant placement may be chosen. However, the difference between the diameter of the tooth root and implant results in three-wall gap defects between the implant surface and buccal bone plate which is known as the "jumping distance". Several animal studies demonstrated that the gap of 1 to 2.25 mm wide at the time of dental implant installation could be healed with bone despite the absence of bone grafting, nevertheless, marginal bone reduction still occurred in particular for the gap less than approximately 2 mm(37-39). Furthermore, the thickness of the buccal plate seems to affect the resorption pattern. With the buccal bone thickness at least 1.8 to 2 mm, bone loss was found to decrease significantly and some evidence of bone gain was observed(40). It has been suggested that the critical thickness of the buccal bone that reduced the marginal bone collapse was approximately 2 mm. Therefore, if this minimal requirement is not achieved, for example, the jumping distance is more than 2 mm or buccal bone thickness is less than 2 mm, then bone grafting should be performed intrasocket or extrasocket before or simultaneously with an implant placement(41). Basically, in the most clinical situations, particularly for the anterior maxilla region, the facial bone was mostly thinner than 1mm(42). Therefore, even in immediate placement, despite the size of the jumping distance, the implant-bone gap defect was suggested to be filled with bone substitutes. The outer surface of the buccal wall should be additionally grafted in case of a small jumping gap and thin buccal plate as well.

In case of socket wall fracture during extraction in sites with periapical or periodontal diseases that result in buccal bone loss or in type 3 or 4 implant placements, peri-implant defects could occur as a result of insufficient ridge width. Peri-implant defects encountered at the time of implant placement can be classified as dehiscence-type and fenestration-type defects. The dehiscence-type bone defect could be described as a buccal or lingual bone defect in the marginal area extending apically along an implant surface, whereas a fenestration-type bone defect is a buccal or lingual window defect occurring over an implant surface(43). Placing an implant in insufficient bone volume leads to an exposed implant surface and could result in mucosal irritation, decreased bone-to-implant contact, thus reduced bone support, and eventually potential implant failure(44). The presence of a dehiscence-type peri-implant defect was found to associate with a clinically decreased marginal bone level, which increased the risk of bone

overload(45). Buccal dehiscence-type defect also affects esthetic outcomes such as gingival recession subsequent to lacking buccal bone support and marginal bone loss. The residual defect height would affect the long-term stability of the implant. Gingival recession and peri-implant disease could occur if the residual defect height is over 1 mm(46).

To avoid further bone loss as well as to obtain optimal peri-implant bone support, bone grafting should be performed to fill the defect.

Correction of buccal dehiscence-type defects

The goal of treatment is to accomplish complete bone fill over the defect. Nonetheless, complete bone regeneration at dehiscence and fenestration-type defects cannot be predictably achieved, irrespective of grafting procedures. In spite of this, the use of a barrier membrane in combination with bone grafting materials seemed to increase bone fill over the defect(43, 47-54). Accordingly, guided bone regeneration(GBR) is the recommended technique used for repairing dehiscence and fenestration-type defects.

Guided bone regeneration(GBR)

Guided bone regeneration(GBR) is a technique using a barrier membrane for the regeneration of bone defects. The concepts of GBR are based on the exclusion of undesirable cells from the wound environment while enabling cells (e.g. osteoblasts, angiogenic cells) from bone tissue to proliferate into the space provided underneath the membrane(55). In other words, GBR provides an appropriate environment for bone regeneration at the site of healing. GBR can be used for reconstruction of peri-implant defects, horizontal bone defects, and vertical bone defects using either a non-resorbable or resorbable membrane in combination with or without several grafting materials(52, 53, 55-61).

The membranes for GBR can be divided into 2 major types: non-resorbable and resorbable membranes. Non-resorbable membranes, including polytetrafluoroethylene (PTFE) and titanium mesh, are commonly used in cases of non-space containing defects, such as horizontal and vertical bone defects, due to their ability in space creation and maintenance. On the other hand, bioresorbable membranes, for example, collagen membrane and acellular dermal matrices(ADM), are used in space-containing defects such as peri-implant dehiscence or fenestration-type defects,

and in combination with non-resorbable membranes or block bone grafts in horizontal and vertical defects(43, 47, 48, 50, 55-59, 61-72).

A systematic review regarding clinical outcomes of GBR procedures to correct peri-implant defects by Chiapasco and Zaniboni(53) demonstrated that grafting materials placed between the exposed implant surface and the resorbable membrane can promote new bone regeneration. However, the same review also indicated that autogenous bone has not provided better bone formation when compared to non-autogenous grafting materials(53). Deproteinized natural bovine cancellous bone mineral(DBBM) is also widely used in GBR. This material could be incorporated into the newly formed bone and also as this xenograft is slowly degraded, it would remain in the defect as a filler or space-maintainer itself(39). The systematic review by Esposito et al in 2006(73) concluded that sites treated with barrier membrane and DBBM showed a higher position of gingival margin than sites without DBBM. The use of other bone substitutes, such as freeze-dried bone allograft(FDBA), demineralized freeze-dried bone allograft(DFDBA), biphasic calcium phosphate(BCP), and bioactive agents especially rhBMP-2, have been reported for the treatment of bone defects in combination with various barrier membranes(74-76). DFDBA alone was found to not significantly enhance bone regeneration in an infrabony defect compared to using resorbable membrane alone(74). However, DFDBA mixed with FDBA in the treatment of dehiscence and fenestration defects around dental implants achieved approximately 90% complete defect coverage(76). Biphasic calcium phosphate (BCP) with adjunct recombinant human bone morphogenetic protein-2 (rhBMP-2) and collagen membrane demonstrated a greater amount of new bone formation in calvarial defects compared to the group with no treatment(75).

GBR complications during the healing phase include wound dehiscence with or without membrane exposure, infection or abscess, and loss of graft integration. Membrane exposure is the most common complication particularly in non-resorbable membrane(55, 77, 78). Premature exposure of barrier membrane resulted in a reduction of bone gain at the defect sites(79, 80). A systematic review conducted by Jensen et al in 2009 evaluated the treatment outcomes following augmentation in various types of defects using different bone substitute materials(54). Some augmentation outcomes in the treatment of dehiscence and fenestration are shown in Table 1.

Meanwhile, barrier membrane in combination with a bone graft was widely used in defect repairing and augmentation; some studies showed no significant difference when using barrier membranes(70, 81). In sites of implant placements with at least a soft tissue thickness of 2 mm, the correction of the dehiscence- type defect by overgrafting of biphasic calcium phosphate(BCP) without barrier membranes demonstrated predictable outcomes, both clinically and radiographically, using low voxel CBCT(81). Five-year follow-up of patients who received a bone substitute with or without a resorbable membrane for the treatment of peri- implantitis, showed clinical and radiographic improvements in both groups with no significance(70).

Table 1 Augmentation of dehiscence and fenestration defects(47-52, 58, 82, 83) (*Modified from Jensen et al. 2009(54))*

Study	Defect types	No.of augmentation	Bone graft materials	Membran	Healing periods(m)	Defect fill (%)	Implant survival (%)
Hammerle et al.2001 (49)	Dehiscence	10	DBBM	R	6.5	86	-
Fugazzotto. 1997(82)	Dehiscence Fenestration	172 77	DFDBA+TCP	NR	7.5	No data	99 100
Carpio et al. 2000(48)	Dehiscence and fenestration Dehiscence and fenestration	25 23	A+DBBM A+DBBM	NR R	6	54 61	-
Tawill et al. 2001(50)	Dehiscence	14	AP	R	6	87	-
Zitzmann et al. 2001(83)	Dehiscence Dehiscence	41 43	DBBM	NR R	5	78 92	- -
De boever. 2005(58)	Dehiscence	16	DBBM	NR	4	97	94
Llambés et al. 2007	Dehiscence	14	ABT	R	4.4	83	94

A=autogenous bone; ABT=autogenous bone from bone trap; AP=autogenous particulate; DBBM=deproteinized bovine bone mineral; DFDBA=demineralized freeze-dried bone allograft; TCP=tricalcium phosphate; NR=Non-resorbable membrane; R=Resorbable membrane

Dentin as a bone graft substitution

From the perspective of bone grafting, the ideal bone substitutes should have osteopromotive properties; osteoconduction, osteoinduction, and osteogenesis, as well as the overall properties comparable with the bone being replaced.

Both bone and teeth are hard tissues in the body. During the developmental period, alveolar bone, as well as dental tissues including enamel, dentin, cementum, pulp, and periodontal ligaments, are derived from neural crest cells. Dentin, a part of the tooth, is almost similar in chemical components to bone. Mature dentin components are by weight, 70% mineralized inorganic material, 20% organic material, and 10% water, whereas those of the alveolar bone, are 60%, 25%, and 15%, respectively(84).

The major component of the inorganic compartment of the tooth contains 4 types of calcium phosphate including hydroxyapatite(HA) , beta- tricalcium phosphate(β- TCP) , amorphous calcium phosphate(ACP), and octacalcium phosphate(OCP)(85). The presence of the inorganic part is responsible for the physicochemical and strength of the tissues. Previous studies show comparable physicochemical properties including compositions or phases of calcium phosphate and their crystal arrangement between bone and tooth(23, 85).

Regarding the organic component, type I collagen constitutes approximately 90% of the dentin organic matrix, while the remaining are non-collagenous proteins(NCPs). The NCPs consist of several proteins including dentin phosphoprotein(DPP) , dentin sialoprotein(DSP) , dentin matrix protein1(Dmp1), and bone morphogenetic proteins(BMPs)(86, 87).

Dentin sialophosphoprotein（DSPP) plays a primary role in the formation and growth of hydroxyapatite crystals in an extracellular matrix of hard tissue such as bone and teeth. Mineralization inducing peptides（MIPs) within DSPP have been reported to support the human bone marrow stromal cell differentiation into osteoblastic cells as well as HA nucleation activity(87, 88). DPP and DSP are the cleavage products of dentin sialophosphoprotein（DSPP). DPP is an important initiator and modulator for the formation and growth of hydroxyapatite crystals. The negatively charged regions of DPP are believed to promote mineralization by binding calcium and presenting it to collagen fibers at the mineralization front during the formation of dentin(89).

Dentin matrix acidic phosphoprotein (DMP) is an extracellular matrix protein with potentially high calcium ion-binding capacity, which is essential in mineralization of bone and dentin. DMP1 acts as a hydroxyapatite nucleator and also controls cell differentiation and maturation of odontoblasts and osteoblasts(90).

Bone morphogenetic proteins (BMPs) are multi-functional growth factors belonging to the transforming growth factor-beta (TGF-β) superfamily. Bone morphogenetic protein (BMP), extracted from bone and dentin, plays an important role in bone formation. The osteoinductive capacity of demineralized bone and the dentin matrix was attributed to the activity of BMPs(59). The BMPs with greatest osteogenic capacity are BMP-2, -4, -5, -6, -7, and -9. In bone formation, BMP-2 and BMP-7 induce the expression of the transcription factors Runx2 and Osterix in mesenchymal stem cells leading to osteoblast differentiation. They are the only signaling molecules that can induce de novo bone formation at orthotopic and heterotopic sites, and their osteoinductive potency makes them clinically valuable as alternatives to a bone graft(91).

Vascular endothelial growth factor (VEGF) was originally identified as an endothelial cell-specific growth factor stimulating angiogenesis and vascular permeability(92). VEGF plays an important role in the early phase of wound healing as well as in the bone healing process. Demineralized dentin matrices (DDM) were also observed to increase the expression of vascular endothelial growth factors (VEGF) and accelerate bone healing by stimulating bone deposition and also vessel formation(28, 93).

In vivo study of DDM as a bone graft substitution

Recently, several animal experimental studies have demonstrated the potential of dentin in different forms, for instance, non-decalcified dentin and decalcified dentin, when used as bone graft substitutes. Boiled bovine dentin stimulated the formation of new bone and was incorporated into those bones(94). Several others consistently reported a bone healing process including resorption, osteogenesis, and incorporation between newly formed bone and the dentin graft particles. Fresh perforated autogenous dentin slices showed neovascularization with osseointegration(26). In repairing of articular cartilage, it was demonstrated that the demineralized dentin matrix(DDM) acts as a scaffold for osteochondral regeneration, yielding active new bone formation early in the postoperative period(16). Liquid nitrogen–treated non-decalcified

autogenous dentin was also found to accelerate bone regeneration in bone defects in a similar way to that of autogenous bone grafts(25).

Clinical studies of DDM as a bone graft substitution

Some recent clinical trials and case reports used demineralized dentin matrix in maxillary sinus grafting, socket preservation, ridge augmentation, and guided bone regeneration with implant placements(14, 20, 21, 27, 69) . However, the study in the healing of buccal dehiscence defects when using a demineralized tooth matrix as a bone graft substitution is still limited with various implant characteristics, the presence of barrier membranes, and demineralized tooth matrix preparation.

Fabrication, physicochemical properties, and in vitro biocompatibility of a demineralized tooth matrix (DTM)

According to our preliminary study during 2013- 2014(95, 96) , the DTM fabrication protocols were developed including mechanical tooth pulverization, defatting, demineralization, lyophilization, and sterilization. The chemical compositions, regarding the amount of calcium phosphate and its characteristics or phases, found in a demineralized tooth matrix(DTM) compared to human bone and teeth are shown in Table 2. Lower calcium and phosphorous but higher crystallinity of DTM, as well as the presence of brushite and monetite in addition to hydroxyapatite(HA), indicated chemical transfiguration due to the fabrication process. These could be beneficial for using as bone substitutes since brushite and monetite were found during bone mineralization and is more soluble than HA(97) . Additionally, the surface characteristics of DTM through the scanning electron microscope represented widening of dentinal tubules with partial exposure of collagen fibers(Figure 1) , which may be assumed as a ground substance for bone mineralization. Moreover, this preliminary study indicated the stimulatory effect of DTM on cell proliferation and early differentiation of mouse pre-osteoblastic cell lines as well.

Table 2 Chemical compositions found in human bone, human teeth, and DTM(96)

Sample	Calcium (%)	Phosphorous (%)	Crystallinity (%)	Apatite Calcium Phosphate
Human bone	51.41	10.65	50.27	Hydroxyapatite(HA)
Teeth	56.719	18.651	58.51	Hydroxyapatite(HA)
DTM	37.528	14.272	58.31	Hydroxyapatite(HA) Brushite Monetite

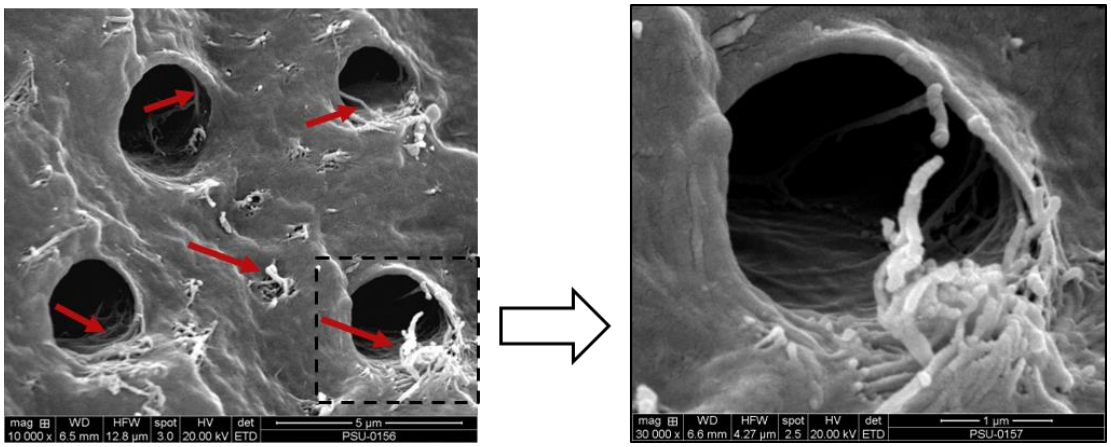

Figure 1 Scanning electron microscopic images of DTM at 10,000x and 30,000x magnification. Partial exposure of collagen fibers(red arrows) over the surface were found particularly around the dentinal tubules(96).

Thus, we could assume that DTM itself can act as a scaffold with osteoinductive properties, as it allows more rapid bone healing and can be biocompatibly incorporate with native and newly-formed bone. In this present study, an autogenous demineralized tooth matrix (DTM) was fabricated and used for the treatment of a peri-implant dehiscence defect at the time of implant placement.

The research problem

Can a DTM be prepared in-house and be suitable as a bone graft substitution, particularly to reconstruct buccal dehiscence-type defects at implant sites?

The purpose of the study

1. To fabricate a DTM for a bone graft material
2. To investigate the clinical application of a DTM for repairing buccal dehiscence-type defects at the implant sites.

The objectives of the study

1. To compare the clinical and radiological outcomes of a DTM with resorbable membrane (test group) to an anorganic bovine bone xenograft with a resorbable membrane (control group) in the treatment of a buccal dehiscence-type defects at implant sites.
2. To compare the defect reduction, graft integration, and soft tissue outcomes at buccal peri-implant dehiscence-type defect after treated by either DTM or an anorganic bovine bone xenograft with a resorbable membrane.
3. To compare the buccal graft thickness and volume at buccal peri-implant dehiscence-type defect after treated by either DTM or an anorganic bovine bone xenograft with a resorbable membrane.

Research question

Can a DTM in association with resorbable membrane regenerate new bone at buccal dehiscence-type peri-implant defects and establish similar outcomes as anorganic deproteinized bovine bone xenografts?

Hypothesis

The use of a DTM with a resorbable membrane (test group) in the treatment of buccal dehiscence-type defects at implant sites would not be different from those using an anorganic bovine bone xenograft with a resorbable membrane (control group), either clinically or radiographically.

Chapter 2

Materials and Methods

Research design

Clinical Trial

Patient selection (Inclusion and Exclusion criteria)

The study was conducted in the Surgery clinic, Dental Hospital, Faculty of Dentistry, Prince of Songkla University.

The inclusion criteria:

- Healthy adult patients (older than 20 years)
- Required dental implant treatment with simultaneous bone augmentation with at least one tooth planned to be extracted.
- Patients who expected to have a dehiscence-type defect involving the mid-buccal aspect of the implant.

The exclusion criteria:

- Patients who can't come as scheduled for post-operative evaluation
- Smokers (patients who have smoked within 6 months of the study's onset)
- Metabolic bone disease, pregnancy, history of malignancy or radiotherapy or chemotherapy for malignancy in the past 5 years
- Autoimmune disease and long-term steroidal or antibiotic therapy
- Local or systemic infection that may compromise normal healing (eg. Extensive periapical pathology)
- Patients presenting clinical and/or radiographic signs of active periodontal disease

Sampling technique

By using PS Power and Sample Size Calculations Version 3.0 software, the sample size should be at least 5 per group (α= 0.05 and power= 80%). Eight healthy patients with a total of 12 implant sites were included and assigned into autologous demineralized tooth matrix (Auto-DTM; n=5) or deproteinized bovine bone matrix (DBBM; n=7) group based on their qualifications and requirements.

- Auto-DTM group or test group (n1=5): Peri-implant defects were augmented with autologous DTM covered with a collagen membrane(Bio-Gide®).
- DBBM group or control group (n2=7): Peri-implant defects were augmented with xenograft (Bio-Oss®) covered with a collagen membrane(Bio-Gide®).

Figure 2 Deproteinized bovine bone (Bio-Oss®)

Figure 3 Bioresorbable porcine-derived collagen bilayer membrane(Bio-Gide®)

Fabrication of the demineralized tooth matrix (DTM)

Any autologous third molar tooth or any caries-free tooth planned to be extracted for any reason, was extracted at least 7 days before the first implant surgery (stage I), and then it was processed to be a DTM ready to be used at the time of stage I implant surgery.

The DTM fabricating protocol(Figure 4) is based on our recent preliminary study(96). Following tooth extraction, the tooth was kept at -80°C. Soft tissues including the periodontal ligament and pulp were removed mechanically by rotary and hand instruments. The cleaned tooth was pulverized into small particles using a freezer mill (6770 Freezer/Mill®, SPEXSamplePrep, USA). A sieve with 500-μm apertures (Endecotts, London UK) was used to collect only the particles sized larger than 500 μm. The selected tooth particles were defatted in chloroform:methanol (1:1) for 12 hours then washed in double distilled water and left in a laminar flow cabinet for 12 hours to allow complete evaporization of the remaining chloroform. Afterward, the particles were demineralized in stirred 0.5M hydrochloric acid, where the proportion of the particles weight(mg) to acid volume(ml) was 1:20, at 4˚c for 3 hours. Then the demineralized tooth particles were washed in a large volume of distilled water before lyophilization. The freeze-dried DTM was eventually sterilized with ethylene oxide gas before being used. Figure 4 exhibits the overall process of the DTM fabrication.

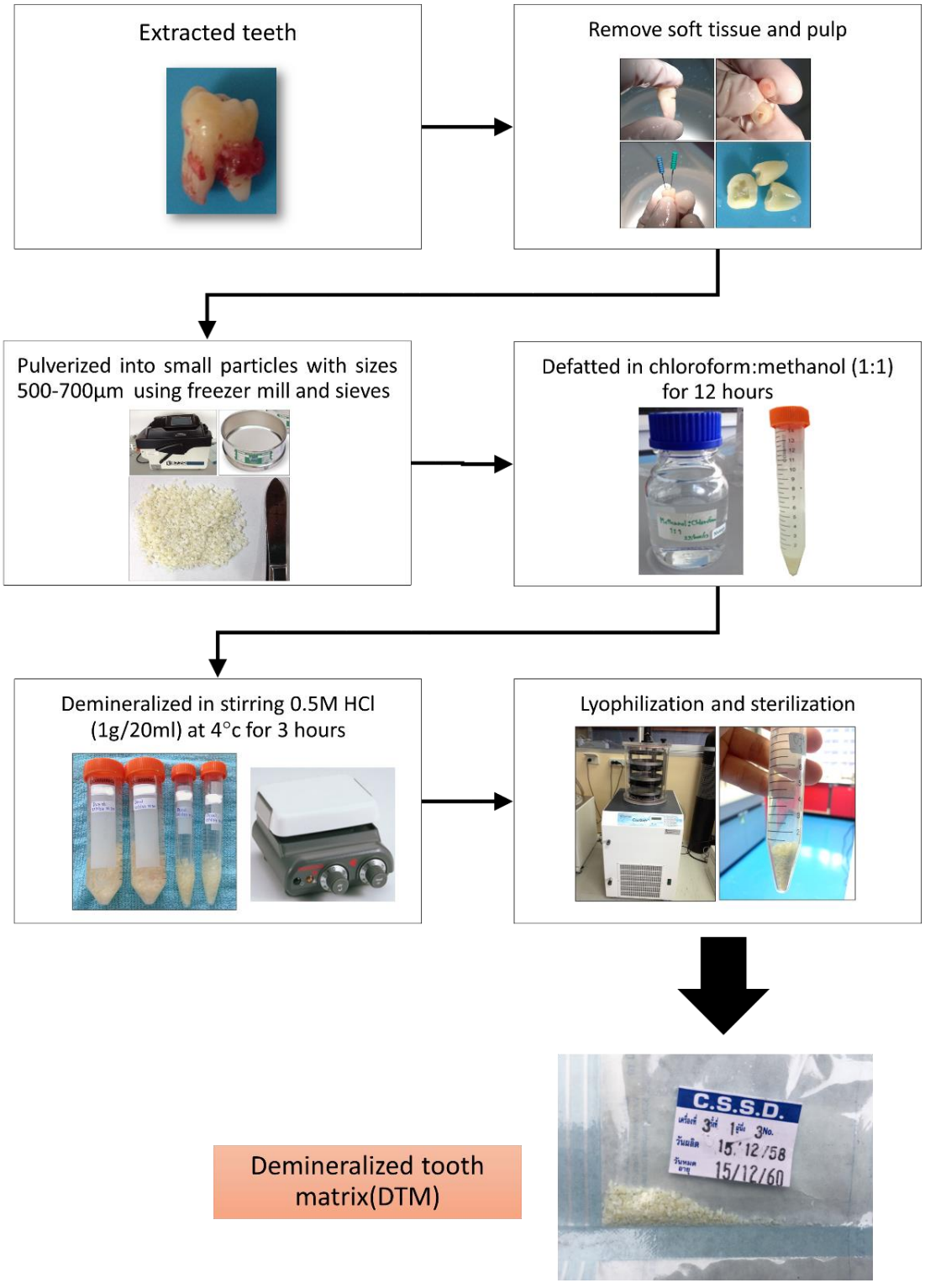

Figure 4 Fabrication of DTM.

Surgical procedures

Overall surgical procedures of both groups were illustrated in Figure 5.

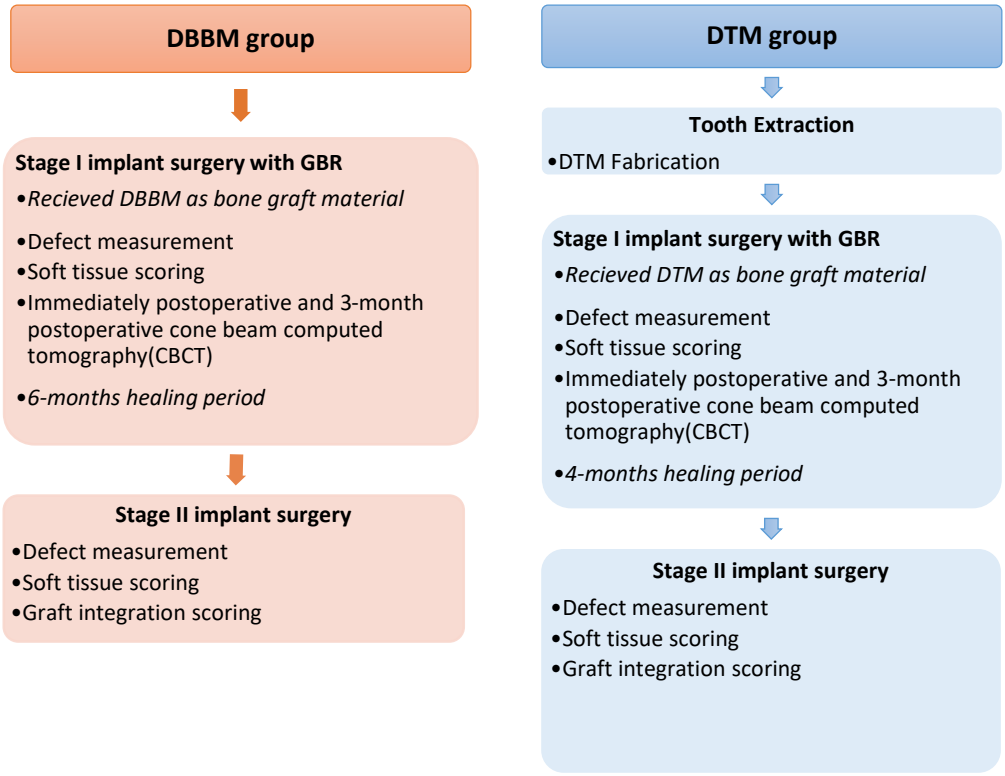

Figure 5 Overall surgical procedures in each group.

I. Dental implant placement procedures

The surgery was performed under local anesthesia. All dental implants used in this study were commercially available ITI, SLA-surfaced (ITI Dental Implant System, Institut Straumann, Waldenburg, Switzerland) (

Figure **6**). The diameter and length of implants depended on the location of the replaced tooth and mesio-distal width of the edentulous sites, but being not less than 4.1 mm in diameter and 8 mm in length. After a flap operation, the implant sites were prepared using sequence drilling as described in the manufacturer's instructions, then the implants were installed.

Figure 6 Dental implant ITI, SLA-surfaced (ITI Dental Implant System, Institut Straumann, Waldenburg, Switzerland).

II. Buccal dehiscence defect repairing using guided bone regeneration

The buccal dehiscence-type defect at the implant surface after installation was measured by a periodontal probe at each level (Figure 7). The buccal dehiscence defects were repaired with either 1) DTM granules (500-700 μm) or 2) DBBM (Bio-Oss®,Geistlich AG, Wolhusen, Switzerland) with cancellous granules 0.25-1.0 mm in size. The defects in both groups were covered by a resorbable membrane (Bio-Gide®, GeistlichPharma, Wolhusen, Switzerland) with size 25 mm x 25 mm. The membranes were fixed by suturing and/or titanium tacks to stabilize the graft. Periosteum releasing incisions were performed to allow tension-free primary closure.

Postoperative advice and home medication including non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, and 0.12% chlorhexidine mouth rinse were prescribed. Patients were followed up at 2, 6, and 12 weeks after surgery. Each time, intraoral periapical radiographic examination and photographs were taken.

III. Stage II implant surgery

Stage II implant surgery was scheduled at approximately 4-6 months after the stage I surgery; after 4 months for the Auto-DTM group and after 6 months for the DBBM group. During the operation, the defect sites were measured with the same method as for the first surgery. If the defect was filled completely with new bone, a healing abutment or provisional crown would be inserted. If the defect still remained, re-grafting with DBBM or other materials would be considered.

Clinical evaluation

I. Buccal dehiscence defect evaluation

The bone dehiscence defects observed at the first and second surgery stage were measured clinically as shown in Figure 7. The defect width (at the platform level, 2, 4, 6, and 8-mm below the platform, and at the bottom of the defect) and the defect height (distance between the lowest buccal crest at the center of the defect and implant platform) were measured using periodontal plastic probes (UNC 12 COLORVUE® PROBE TIPS, Hu- Friedy, Chicago, USA)(Figure 8). The defect area was then calculated using width and height data.

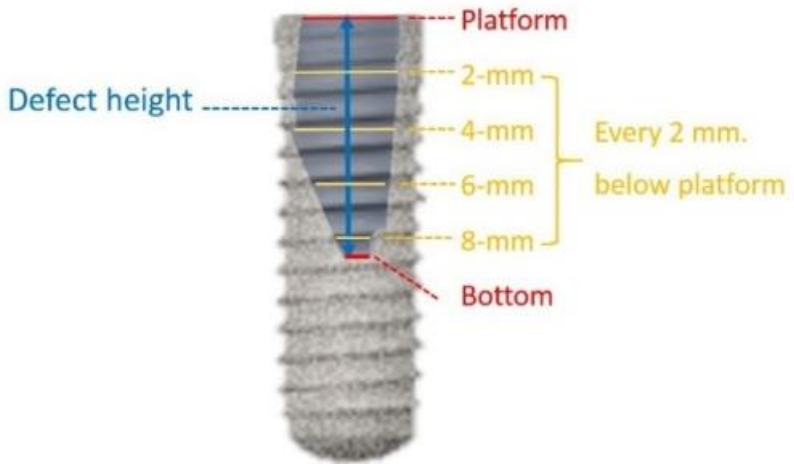

Figure 7 Defect measurement; defect width at the platform level and at every 2 mm-below the platform until reaching the bottom of the defect, and measurement of the defect's height.

Figure 8 periodontal plastic probes (UNC 12 COLORVUE® PROBE TIPS, Hu-Friedy, Chicago, USA)

II. Integration of grafting materials (DTM or xenograft) and bone at defect sites

At stage II implant surgery, the clinical appearance of grafting materials at the defect area was described and recorded by a score of 0-1-2 as follows:

- Score 0: totally not integrated or easily separated from the defect site as grafted granules
- Score 1: partially integrated with some separated granule with soft consistency
- Score 2: totally integrated with bony consistency.

III. Evaluation of the soft tissue around the dental implant

Soft tissue at the implant site was evaluated preoperatively at stages I and II of the implant surgery. The soft tissue parameters consist of soft tissue colour, texture, and contour (Figure 9). Each variable was assessed with a 2-1-0 score with 2 being the best and 0 being the worst score compared to the adjacent area (Table 3).

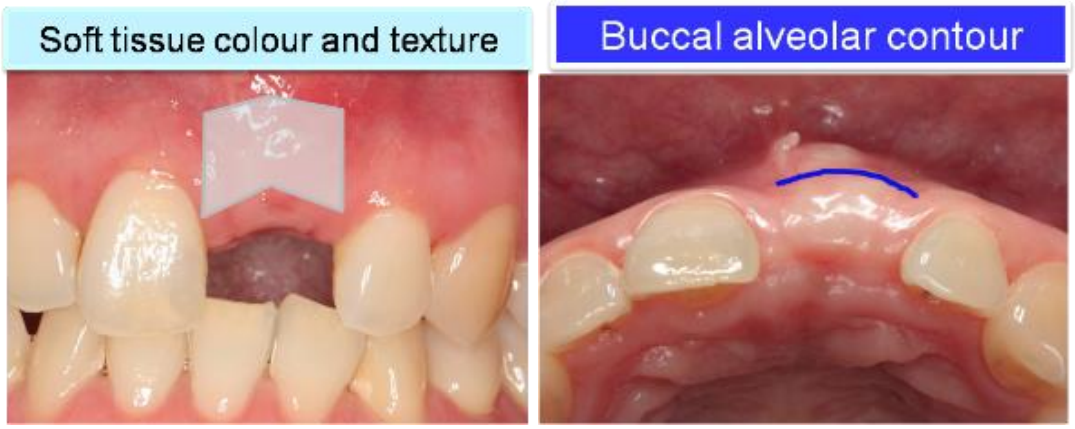

Figure 9 Soft tissue parameters

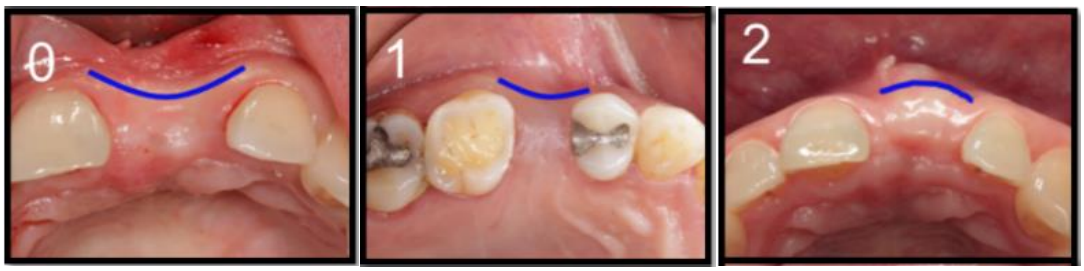

Figure 10 Buccal contour scores

Table 3 Definition of all soft tissue rating scores

	Soft tissue color	Soft tissue texture	Buccal contour
Score 0	Obvious difference with or without metal color reflection	Obvious difference	Obvious concavity
Score 1	Moderate difference	Moderate difference	Flattened or slightly having concavity
Score 2	No difference or similar to adjacent area	No difference or similar to adjacent area	Natural contour

Cone-beam computed tomography (CBCT) evaluation

All patients received CBCT (3D Accuitomo, J.Morita, Kyoto, Japan) immediately after the surgery (as baseline) and at 3-months follow-up before the stage II surgery.

IV. Mid-buccal thickness evaluation

One Volume Viewer software（J.Morita, Kyoto, Japan）was used for measuring the mid-buccal marginal bone thickness at implant sites. The mid-buccal bone thickness at the platform level of the implant was measured at baseline and at 3-months follow-up. At baseline or at immediate post-stage I surgery, the mid-buccal marginal thickness would represent the initial graft thickness, whereas at 3-months follow-up, the thickness would represent the buccal bone thickness gain. Marginal graft thickness reduction（%）at the platform level was calculated and compared between the DBBM and Auto-DTM group. Additionally, the mid-buccal bone thickness at 1-mm, 2-mm, and 3-mm apically to the implant platform were measured at 3-months follow-up to determine the buccal bone thickness gained at each level (Figure 11).

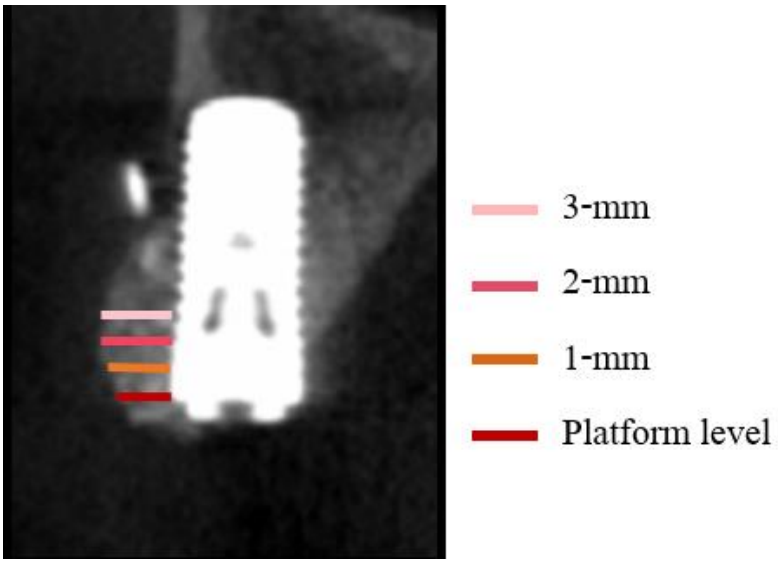

Figure 11 Buccal bone thickness measurement.

V. Buccal bone volume evaluation

The buccal bone volume including the graft material was identified and measured using CBCT data and ITK-SNAP software version 3.4.0 by the contour segmentation method(98). The CBCT data in ".dcm" extension was imported to the ITK-SNAP software. Contour segmentation of buccal bone volume was conducted at the area buccally to the implant exposed surface. The width and height of the segmentation area depended on the implant diameter and defect height (**Error! Reference source not found.**). The volume data obtained from ITK-SNAP were described in Table 4.

Table 4 Definitions of each volume parameter

Volume parameters		Definitions
Total buccal graft volume(mm^3)	At baseline	Total volume of graft material buccally to the implant surface immediately after stage I surgery
	At 3-month	Total volume of healed grafted bone buccally to the implant surface at 3-months post-stage I surgery
Total buccal volume reduction(%)		Percentages of volume reduction from immediately after stage I surgery to 3-months post-stage I surgery
Marginal buccal bone volumes (VH;mm^3)	1-mm height (VH1)	Marginal buccal bone volume at 3-months post-stage I surgery measured from the platform level to 1-mm below
	2-mm height (VH2)	Marginal buccal bone volume at 3-months post-stage I surgery measured from the platform level to 2-mm below
	3-mm height (VH3)	Marginal buccal bone volume at 3-months post-stage I surgery measured from the platform level to 3-mm below

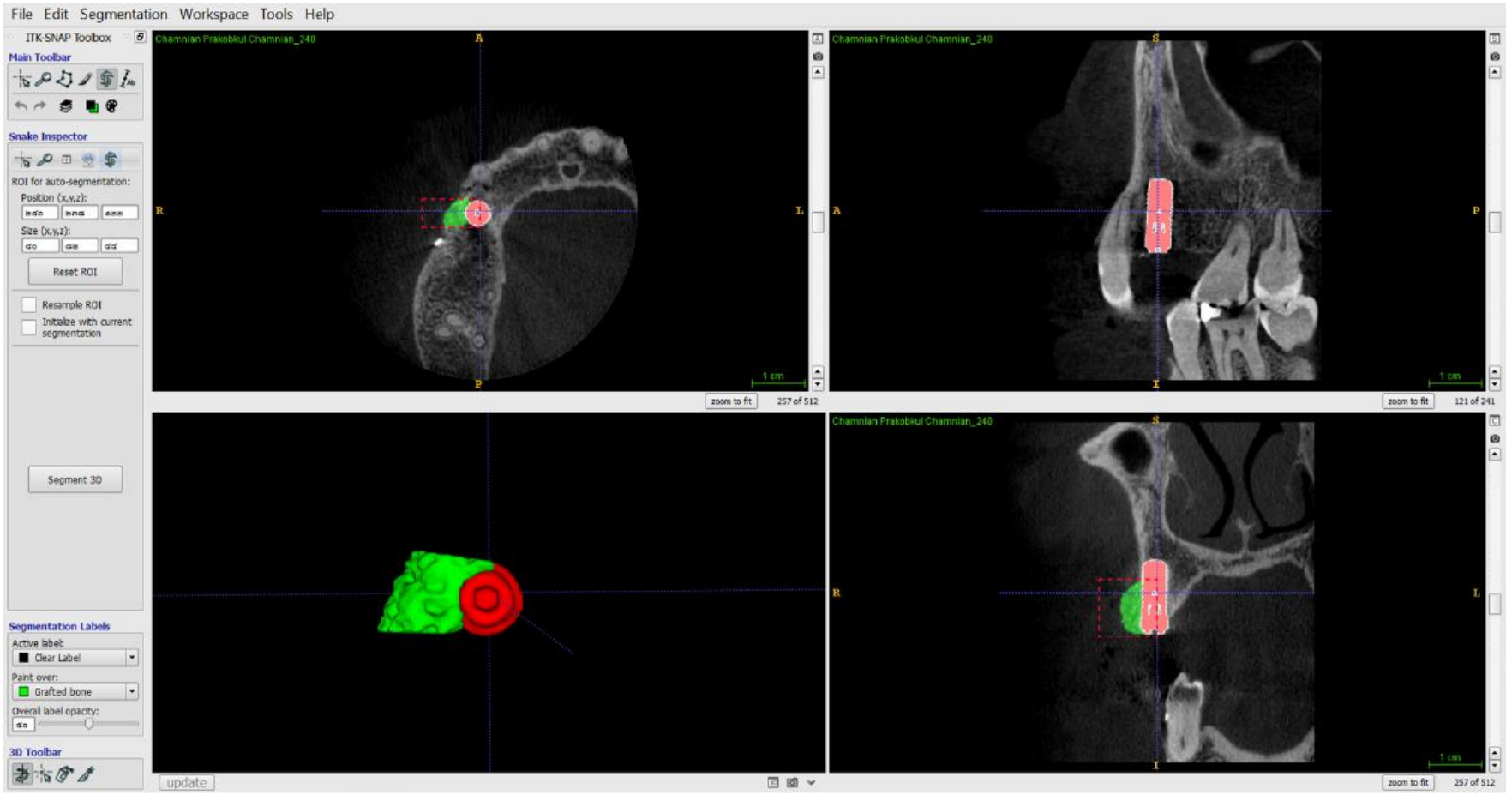

Figure 12 ITK-SNAP software interface demonstrated the area of interest (red dash box) buccally to the implant exposed surface. The width and height depended on the implant diameter and defect height, respectively. In the lower left window, a 3D rendering from the contouring segmentation of the implant and buccal graft could be illustrated.

Since the volume depends on width, height, and thickness, the marginal buccal bone volumes (VH) were measured at 1, 2, and 3 mm height with their widths equal to the implant diameters (Figure 12). Due to different implant diameters, these data were eventually standardized by comparing with the estimate of the 2-mm thickness volumes (100%): 10 mm^3 for every 1-mm height when the implant diameter is 4.1 mm, and 11 mm^3 for every 1-mm height when the implant diameter is 4.8 mm. Thus the buccal marginal volume levels were compared and analyzed in data percentages (Table 5).

Table 5 Standard buccal bone volumes with 2-mm thickness as 100% of required peri-implant buccal bone volume.

Implant Diameter / Height from implant platform	4.1mm	4.8mm
1-mm height (VH1)	$10mm^3$	$11mm^3$
2-mm height (VH2)	$20mm^3$	$22mm^3$
3-mm height (VH3)	$30mm^3$	$33mm^3$

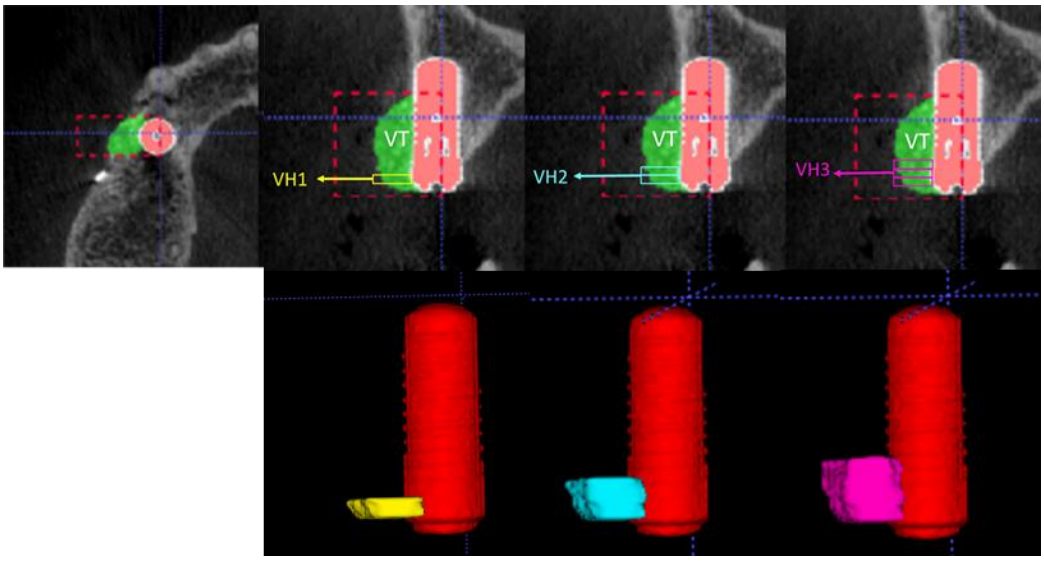

Figure 123 Volume parameters as labeled. Marginal bone volumes at 1-, 2-, and 3-mm height (VH1, VH2, VH3). The width of the measured segment was equal to the implant diameter.

Data analysis and interpretation

The statistical analysis was performed using IBM SPSS software (version 15, SPSS, Chicago, IL, USA). The $P<0.05$ was considered statistically significant.

- The Wilcoxon signed ranks test was used to determine the difference in clinical defect sizes and soft tissue parameters between the 2-time intervals for each group.
- The paired t-test was used to determine the difference in marginal buccal bone thickness and buccal bone (graft) volume between the 2-time intervals for each group.
- The Mann-Whitney U test was used to detect differences in clinical defect size reduction, soft tissue final contour, and graft integration between the two groups.
- The independent t-test was used to analyze the difference of marginal buccal bone thickness reduction (%), volume reduction (%), and marginal buccal bone volume (%) between the two groups.

Chapter 3

Results

Patients' demographic data and mean baseline defect size were summarized in Table 6. A total of 12 dental implants in both groups were installed simultaneously with guided bone regeneration (Figure and Figure). All sites healed uneventfully and were similar in both groups with neither infectious incidence nor graft rejection. Soft tissue dehiscence without exposure of collagen membrane was observed at 2-week follow-up in two patients; one in the Auto-DTM group and one in the DBBM group. At 6 weeks, wound dehiscence in the Auto-DTM group completely disappeared leaving a full soft tissue coverage, while partial exposure of the cover screw was present in the DBBM group.

Table 6 Patients' demographic data and mean baseline defect sizes.(Mean±SD)

Group	N	Area	Age (years)	Baseline defect width(mm)	Baseline defect height(mm)	Baseline defect area(mm^2)
DBBM	7	Premolar 4 Molar 3	58.29±5.9	3.29±0.70	3.29±2.21	8.21±7.20
Auto-DTM	5	Incisor 1 Premolar 2 Molar 2	46.80±17.38	3.06±1.06	4.60±3.29	10.34±8.04
*P-Value				>0.05		

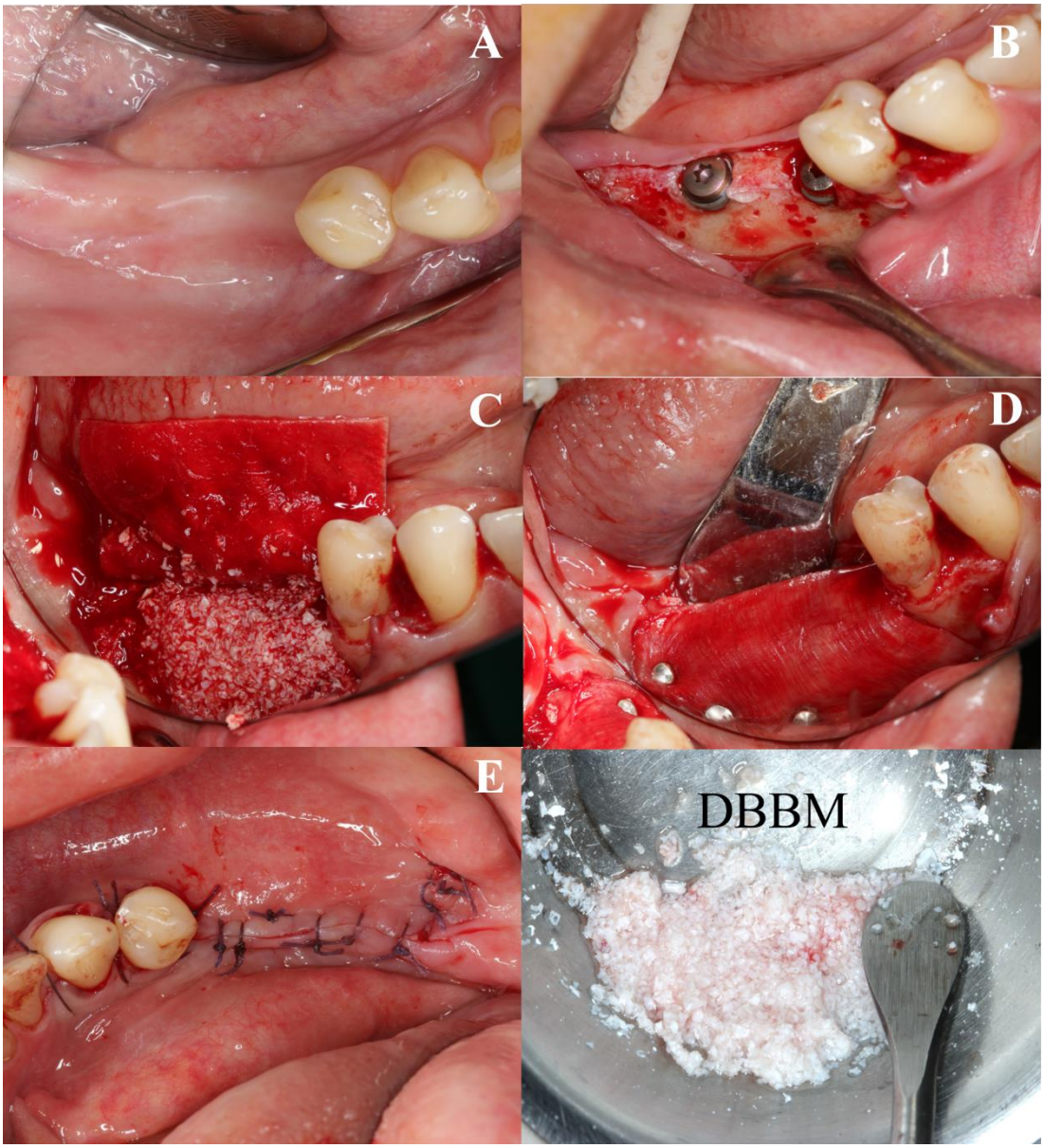

Figure 14 DBBM group: GBR was performed simultaneously with the implant placement.

(A) Pre-operative images showed buccal concavity at the sites.

(B) Immediately after implant installation, buccal dehiscence- type defects were observed and measured.

(C) DBBM was filled over the defects.

(D) Resorbable collagen membranes were applied and stabilized over the grafted areas.

(E) Tension-free primary closures were achieved.

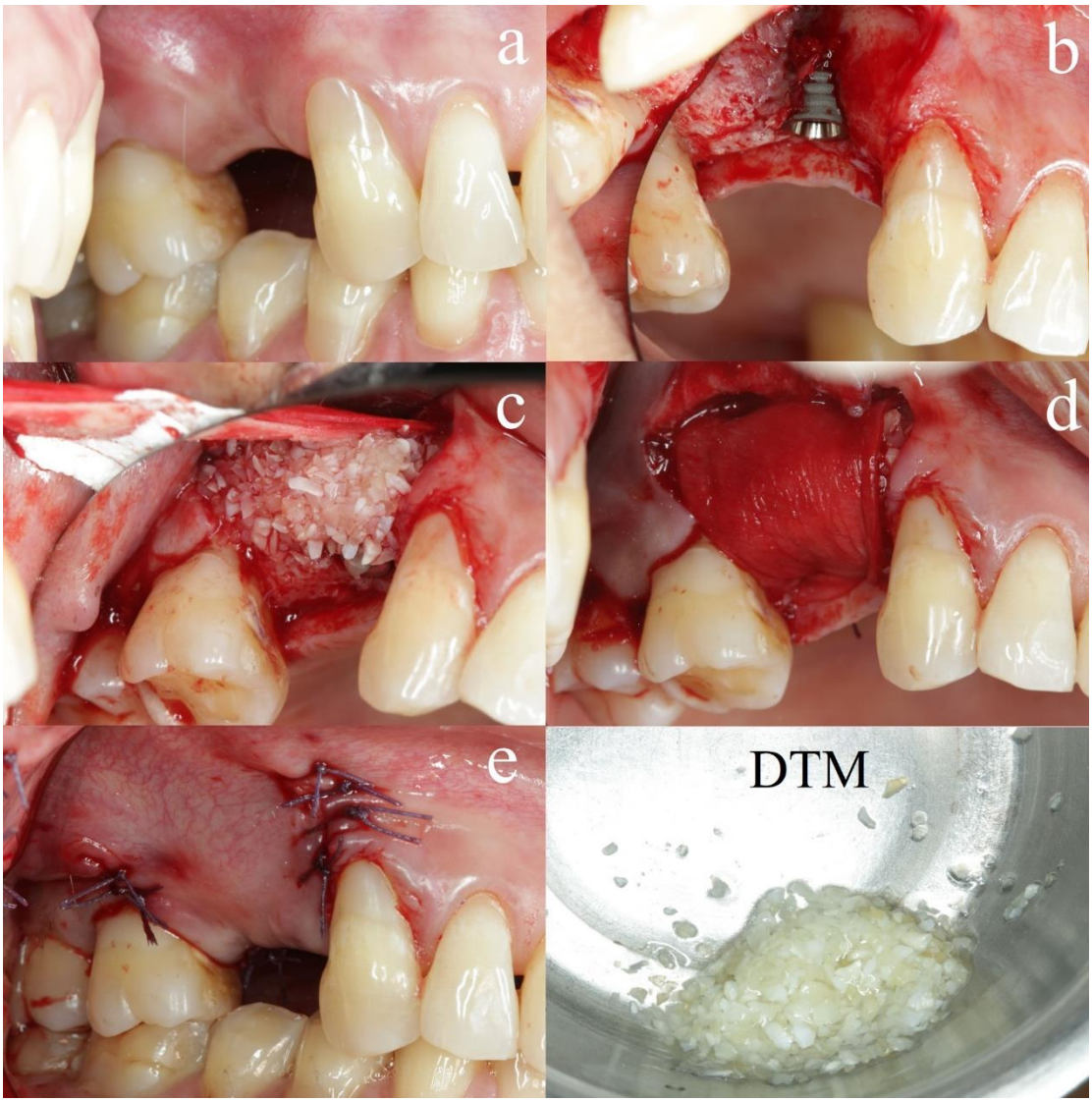

Figure 15 Auto-DTM group: GBR was performed simultaneously with the implant placement.

(a) Pre-operative images showed buccal concavity at the sites.

(b) Immediately after implant installation, buccal dehiscence- type defects were observed and measured.

(c) Auto-DTM was filled over the defects.

(d) Resorbable collagen membranes were applied and stabilized over the grafted areas.

(e) Tension-free primary closures were achieved.

Clinical evaluation

VI. Buccal dehiscence defect evaluation

At baseline, no statistically significant differences between DTM and DBBM were found for any of the parameters assessed (P>0.05). All defects were significantly lessened in height, width, and area compared to those at baseline (Table 7, Figure and Figure). The percentages of defect width, height, and area reduction were shown in Figure . No statistical significant differences was found between the two groups (P>0.05).

One defect in the Auto-DTM group showed a remaining defect width of 3.5 mm and height of 2 mm resulting in a defect area of approximately 5 mm^2. In this case, re-grafting was performed using DBBM and resorbable membrane as the GBR technique. The healing abutment connection was postponed.

Table 7 Clinical parameters comparing between two groups.

Clinical parameters		DBBM	DTM	#P-value
Marginal defect width (mm)	Baseline	3.00; 1.50	3.00; 1.65	0.639
	2nd surgery	0; 2.50	0; 2.75	1.000
	†P-value	0.018	0.043	
	Reduction (%)	100; 62.50	100; 69.80	1.000
Defect height (mm)	Baseline	2.00; 2.00	4.00; 6.50	0.639
	2nd surgery	0; 1.50	0; 1.50	1.000
	†P-value	0.016	0.042	
	Reduction (%)	100; 50	100; 31.25	0.876
Defect area(mm^2)	Baseline	5.50; 7.00	11.68; 15.50	0.876
	2nd surgery	0; 1.13	0; 3.00	1.000
	†P-value	0.018	0.018	
	Reduction (%)	100; 10.27	100; 24.74	1.000
Graft integration(score)		1; 1	2; 0	0.048
Soft tissue color(score)	Baseline	2; 0	2; 0	1.000
	2nd surgery	2; 0	2; 0	1.000
Soft tissue texture(score)	Baseline	2; 0	2; 0	1.000
	2nd surgery	2; 0	2; 0	1.000
†P-value		1.000	1.000	
Soft tissue contour(score)	Baseline	1; 0	1; 1	0.530
	2nd surgery	2; 0	2; 1	0.876
†P-value		0.008	0.034	

All Values are displayed as the median; interquartile range (IQR)

#Mann-Whitney U test, †Wilcoxon Signed Rank test, *Significant level < 0.05

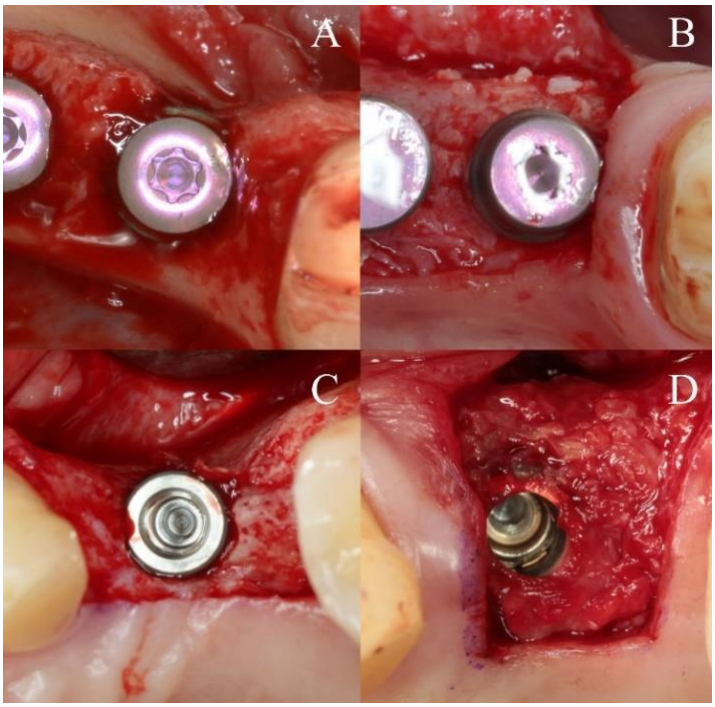

Figure 16 Defect reduction.

(A,B) DBBM group: A = Baseline defect; B = Re-entry at 6 months.

(C,D) Auto-DTM group: C = Baseline defect; D = Re-entry at 4 months.

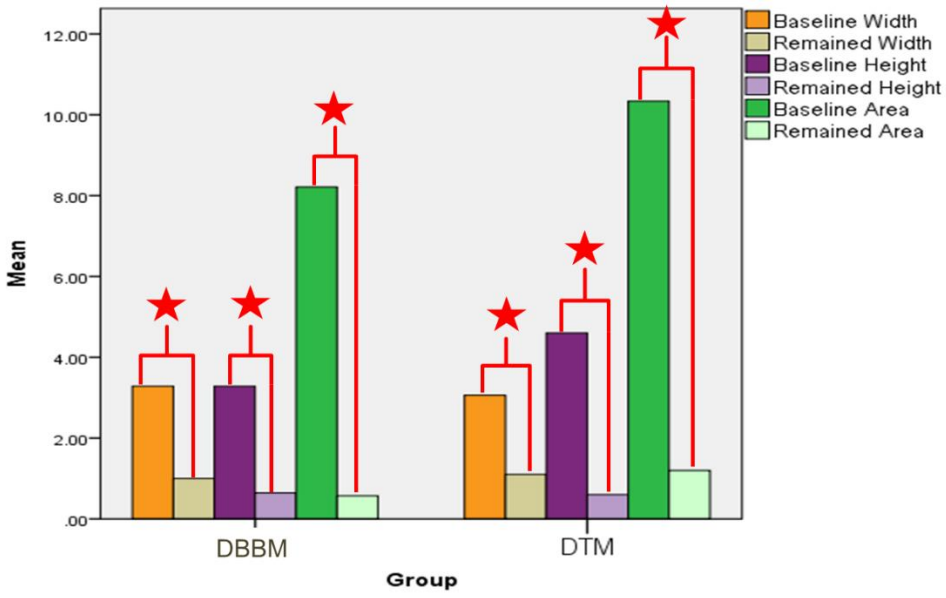

Figure 17 Illustration of defect sizes in width, height, and area comparing between at baseline and at the time of re-entry. ★ Significant reductions were detected for all parameters in both groups.

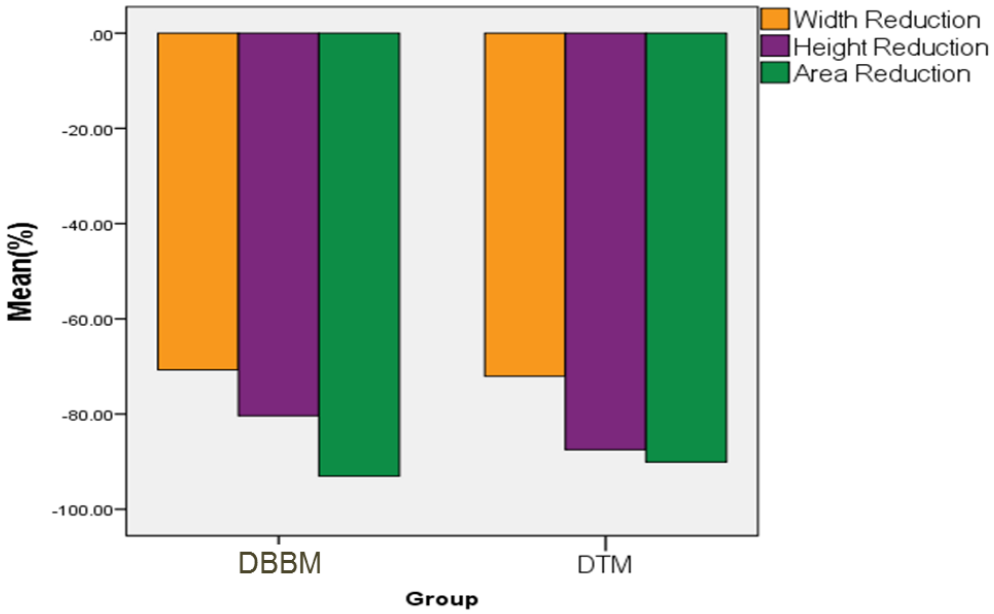

Figure 18 The percentages of defect reduction in width, height, and area compared between two groups. No significant difference was found in all three parameters.

VII. Integration of grafting materials (DBBM or DTM) at bone defect sites

No graft rejection or total disintegration was observed in either group. The integration scores obtained in the DBBM group were either score 1 or score 2 (Figure), whereas, only score 2 was observed in the Auto-DTM group (Figure). The graft integration between the surrounding host bone and DTM appeared significantly more consolidated than with DBBM($P<0.05$) as shown in Figure .

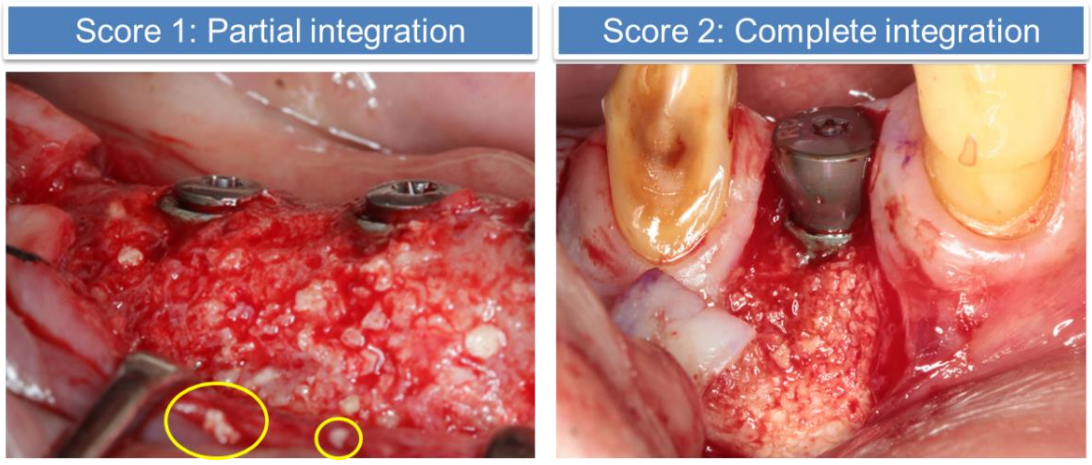

Figure 19 Graft integration in DBBM group. Yellow circles indicated some DBBM granules detached with the flap at the grafted site with an integration score of 1.

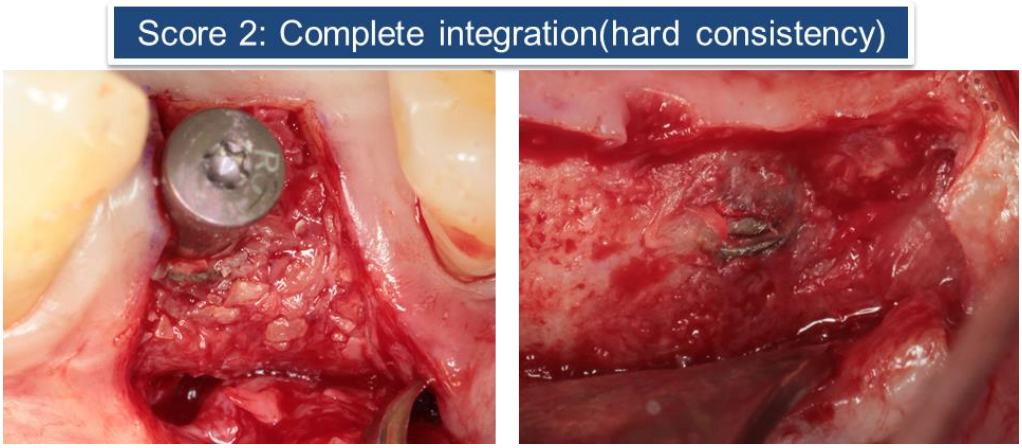

Figure 20 Graft integration in Auto-DTM group. Complete graft integration with score of 2 was observed in all sites.

VIII. Evaluation of the soft tissue around the dental implants

In both groups, soft tissue color and texture were not affected by grafting materials and surgical procedures (Figure 13 and Figure 14) . However, buccal contours improved significantly after grafting, regardless of the graft materials($P<0.05$). The final buccal contour was not different in either group($P>0.05$).

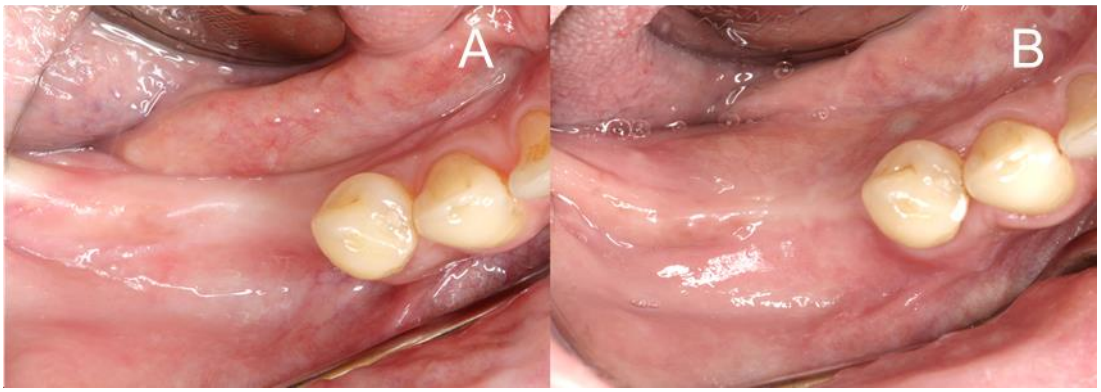

Figure 13 Soft tissue color, texture, and contour in the DBBM group. (A) Pre-operation; (B) At the time of re-entry(6 months of healing).

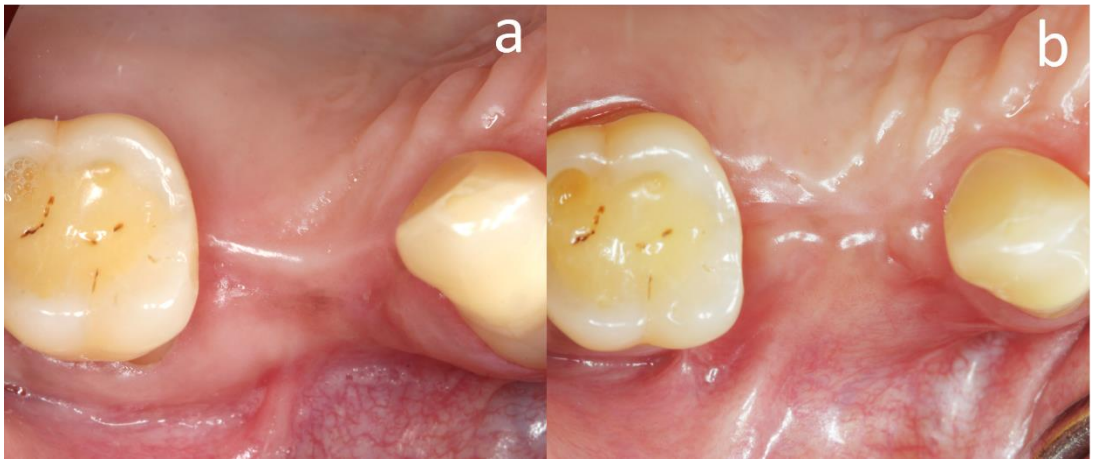

Figure 14 Soft tissue color, texture, and contour in the Auto-DTM group. (a) Pre-operation; (b) At the time of re-entry (4 months of healing).

Cone-beam computed tomography(CBCT) evaluation

IX. Mid-buccal thickness evaluation

The marginal mid buccal thickness at each level in both groups were illustrated in Figure 15 and Figure 16. After 3-months of healing, a graft collapse particularly at the implant platform was observed in both groups(Figure 17, Figure 19, and Figure 20) but it was significantly obvious in the DBBM group($P<0.05$). Irrespective of the initial graft amount, the marginal buccal bone thickness obtained in the Auto-DTM group was not significantly different from that of the DBBM group ($P>0.05$).

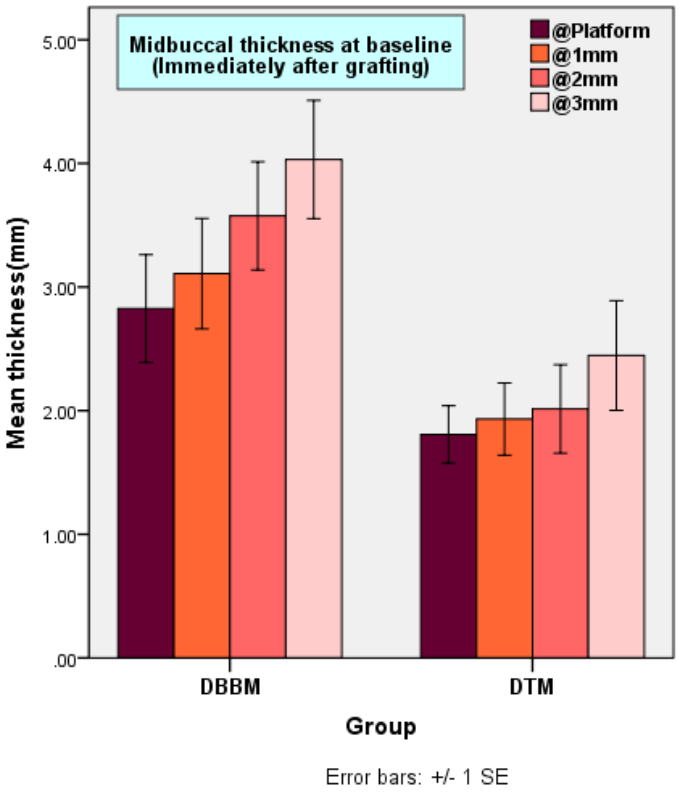

Figure 15 The mean of the mid-buccal bone thickness at each level from the implant platform measured immediately after grafting(P>0.05).

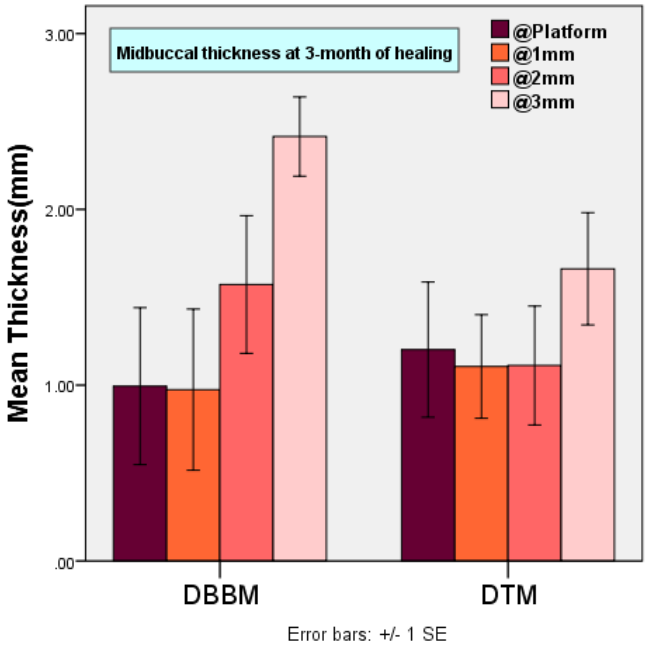

Figure 16 The mean of the mid-buccal bone thickness at each level from the implant platform measured at 3-month of healing(P>0.05).

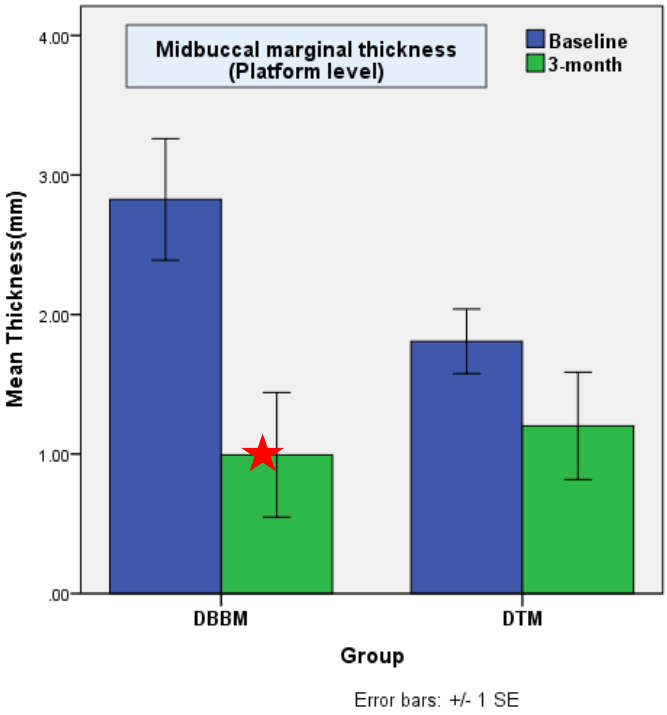

Figure 17 The mean of the mid-buccal bone thickness at the implant platform compared between baseline and 3-months of healing. ★ Significant reduction was observed only for the DBBM group.

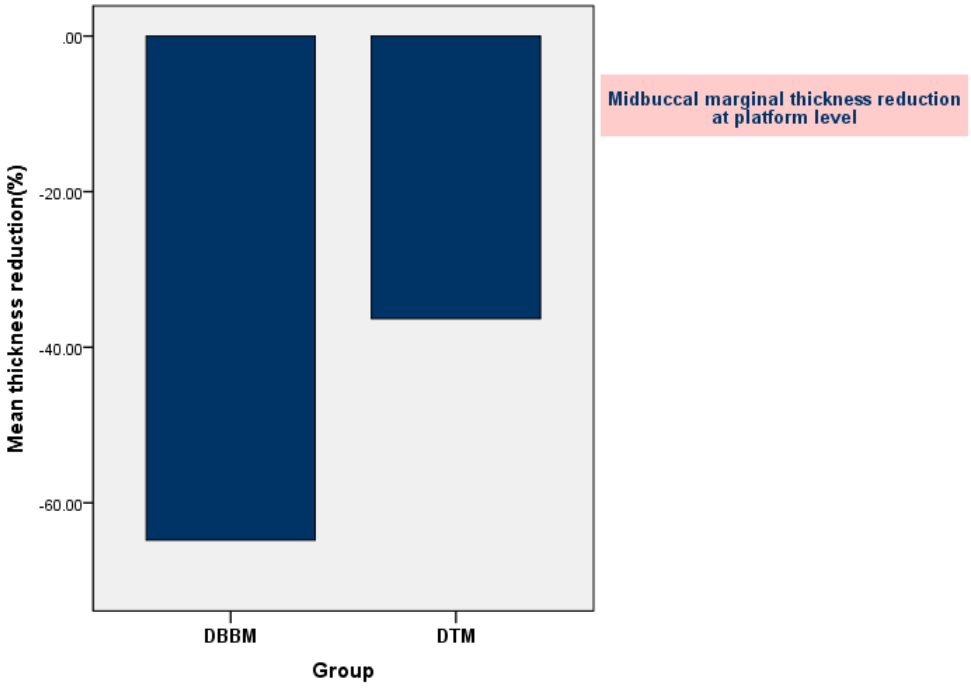

Figure 18 The percentages of mid buccal marginal thickness reduction in both groups.

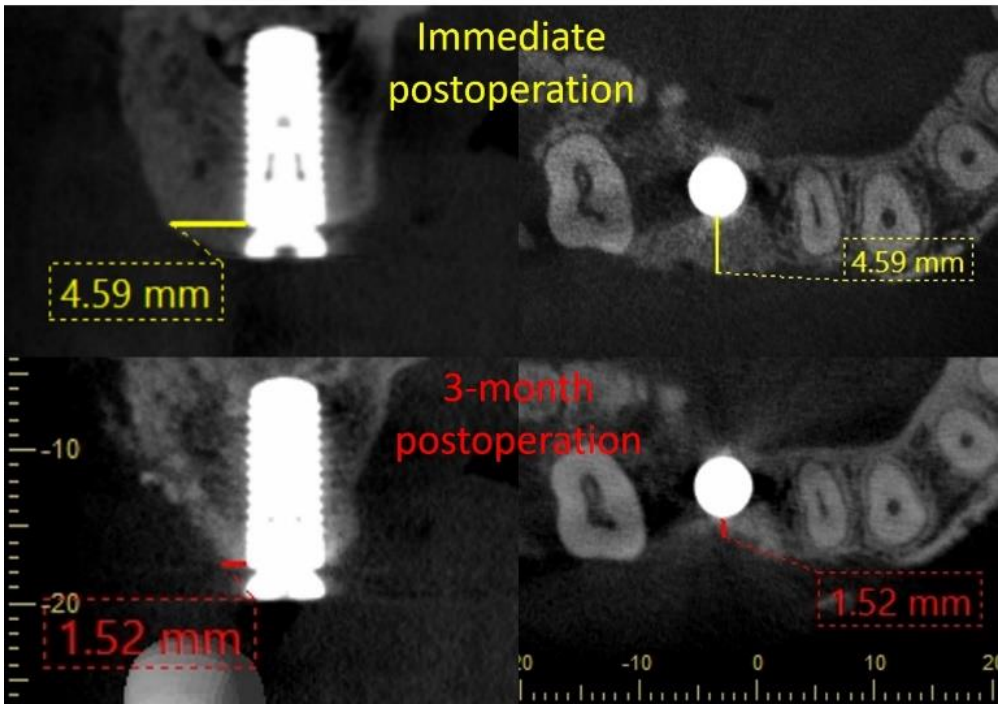

Figure 19 CBCT images represented buccal graft and bone thickness immediately postoperation and at 3-months postoperation in the DBBM group.

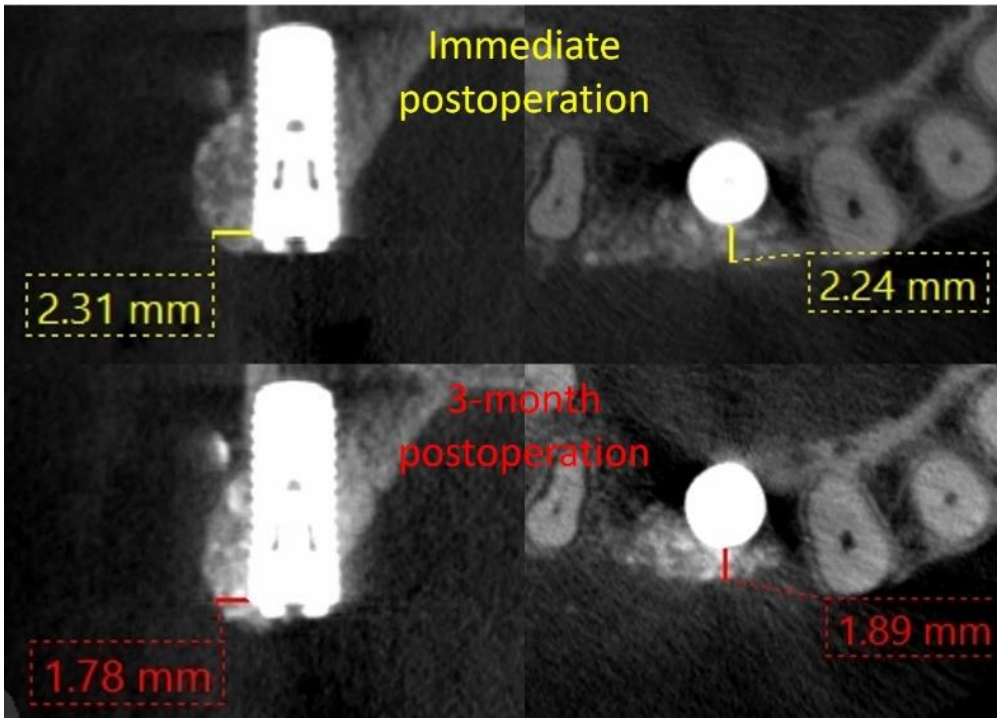

Figure 20 CBCT images represented the buccal graft and bone thickness immediately postoperation and at 3-months postoperation in the Auto-DTM group.

X. Buccal bone (graft) volume evaluation

Figure 21 and Figure illustrated the use of ITK-SNAP software for volumetric analysis of buccal bone(graft) at the exposed implant surface. The three-dimensional images rendered from ITK-SNAP representing total buccal graft (bone) volume were shown in Figure 22 and Figure 23.

Figure 21 Volumetric assessment of buccal bone using ITK-SNAP. Green represents the total volume of bone(graft) buccally to the implant exposed defect.

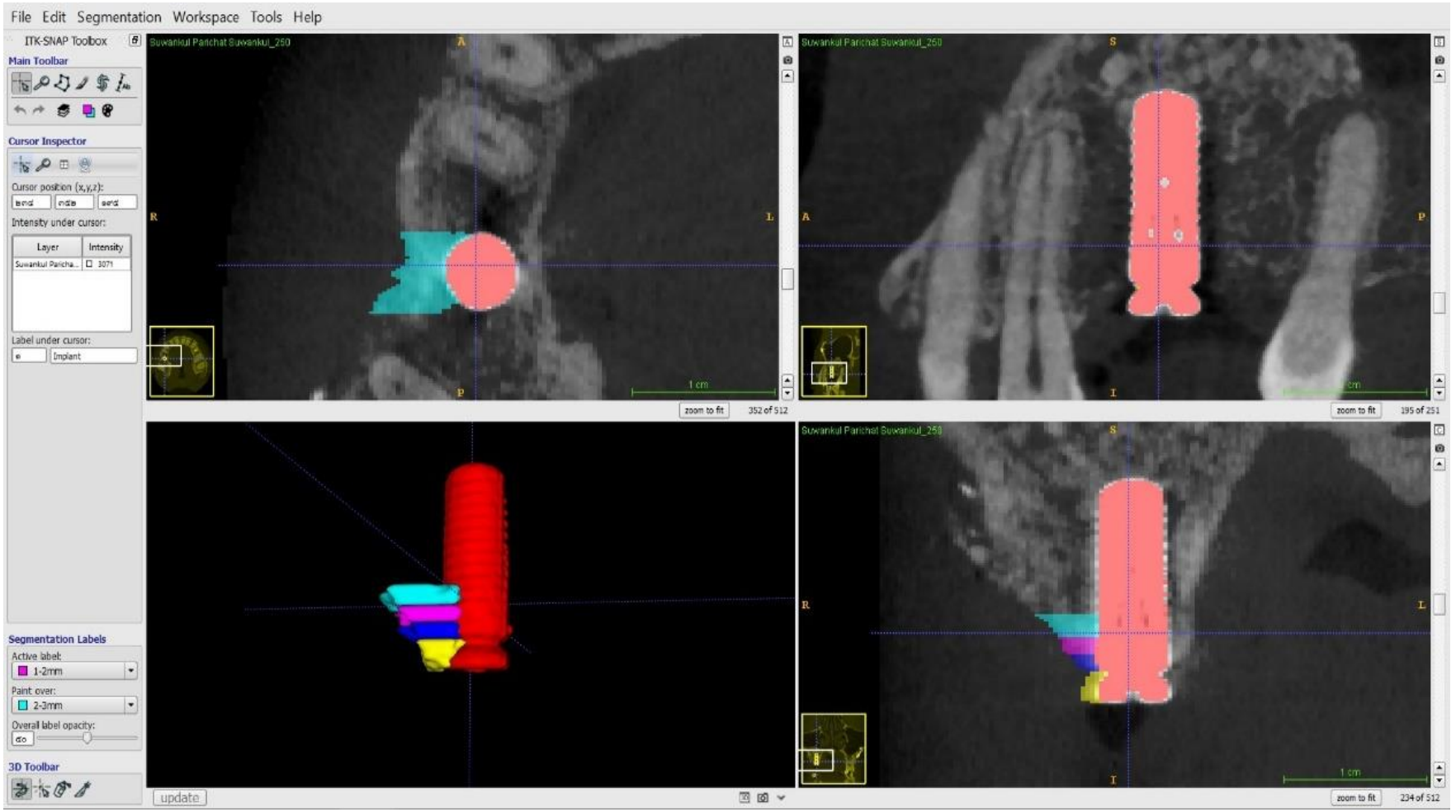

Figure 30 Volumetric assessment of buccal bone using ITK-SNAP. Yellow represents volume beyond the implant platform level; Navy, pink, and blue represent volumes at each 1-mm below extending from the implant platform.

The total volume of buccal bone was significantly lower than initial total graft volume in both groups($P<0.05$). The percentages of volume reduction and marginal buccal bone volume at each level were not significantly different between two groups($P>0.05$) as illustrated in Figure 24 and Figure 25.

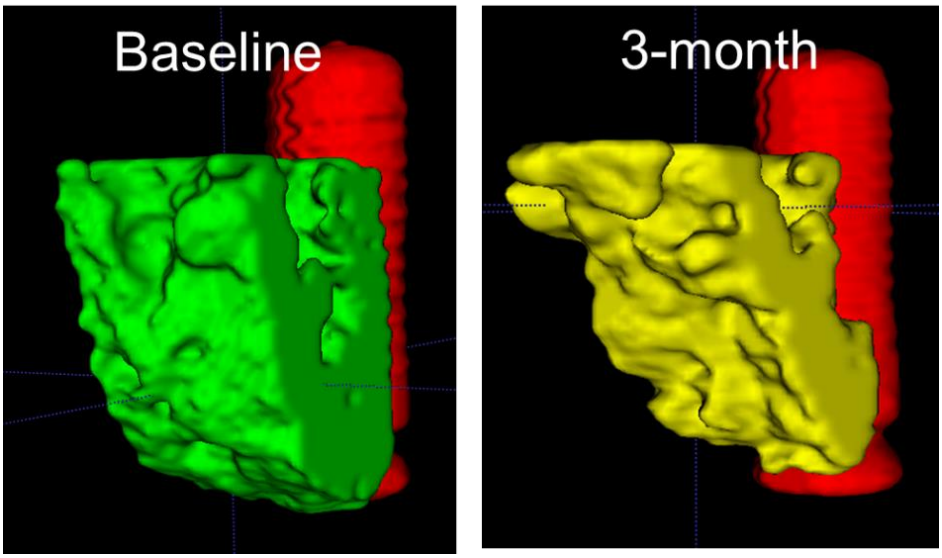

Figure 22 Three-dimensional images of one grafted site in the DBBM group obtained from ITK-SNAP.

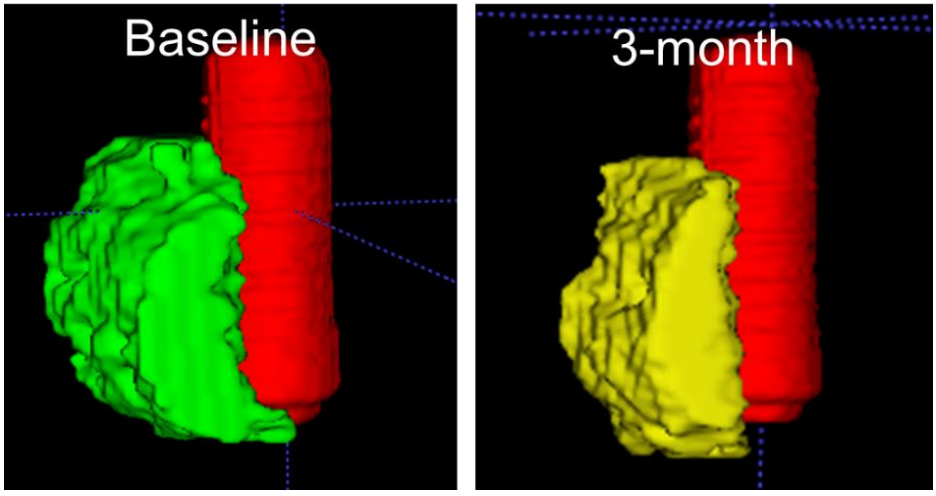

Figure 23 Three-dimensional image of one grafted site in the Auto-DTM group obtained from ITK-SNAP.

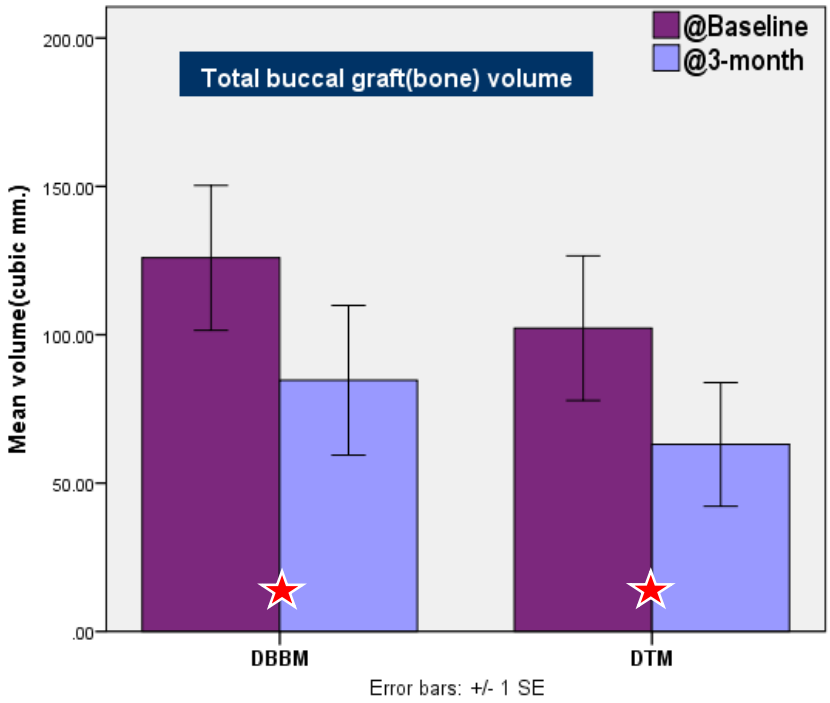

Figure 33 Mean total buccal volume at baseline and at 3-months follow-up. Significant volume reduction presented in both groups(P<0.05).

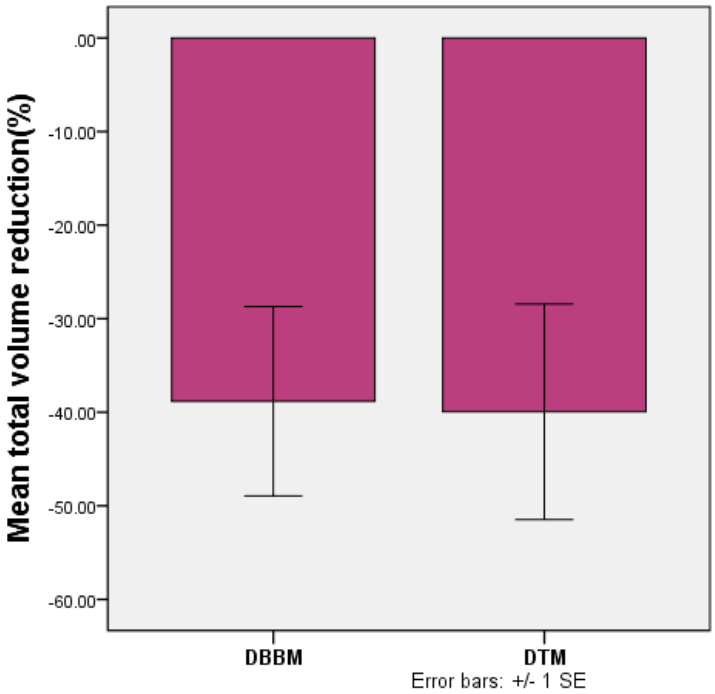

Figure 24 Mean total buccal volume reduction(%) were not significant different between the two groups(P>0.05).

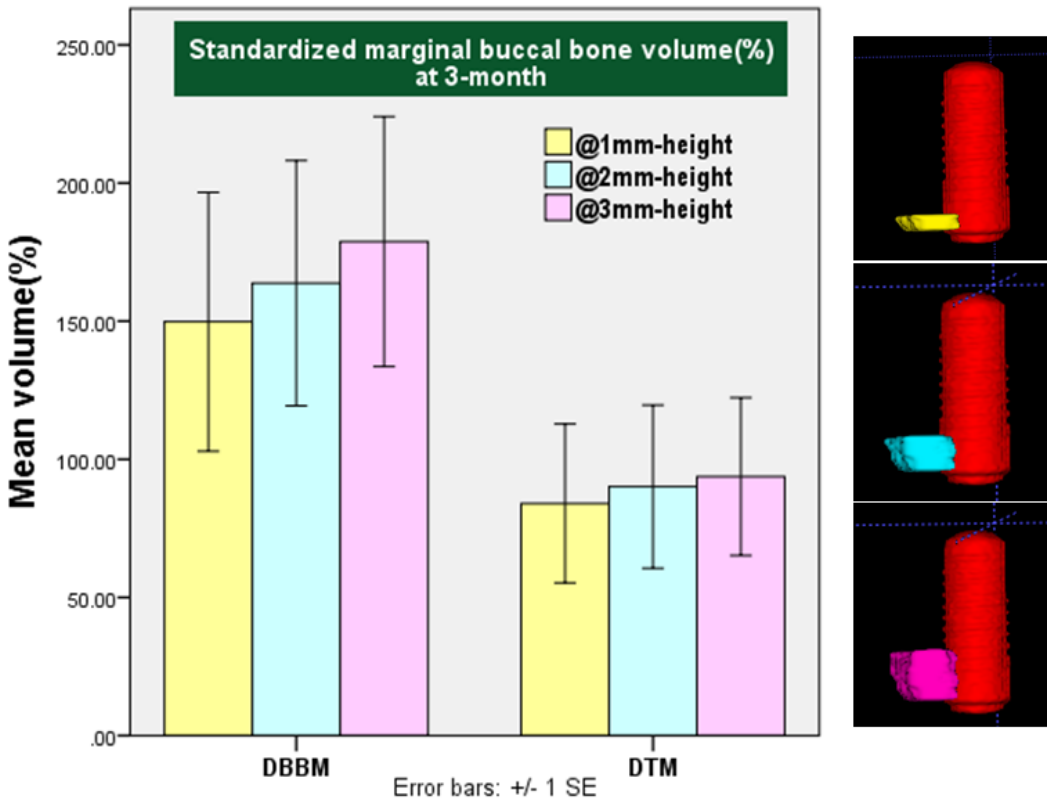

Figure 25 Marginal buccal bone volume(%) gained after 3 months of healing.

The buccal thickness and volume statistical data are summarized in Table 8.

Table 8 Buccal bone thickness and volume comparing between the two groups.

CBCT parameters		DBBM	DTM	†P-value
Marginal graft thickness (mm)	[a]Imm. postop	2.83 ± 1.15	1.81 ± 0.52	0.097
	[b]3-month postop	0.99 ± 1.18	1.20 ± 0.86	0.746
‡P-value		0.005	0.084	
Total buccal graft volume(mm^3)	[a]Imm. postop (VT0)	125.93 ± 64.60	102.20 ± 54.55	0.520
	[b]3-month postop (VT1)	84.69 ± 66.74	63.05 ± 46.57	0.548
‡P-value		0.002	0.016	
[c]Buccal thickness reduction(%)		64.80 ± 37.91	36.33 ± 36.90	0.224
[c]Total buccal volume reduction(%)		38.83 ± 26.77	39.95 ± 25.75	0.944
Marginal buccal bone volume(%)	1-mm height(%VH1)	149.78 ± 123.96	84.02 ± 64.30	0.307
	2-mm height(%VH2)	163.78 ± 117.47	90.10 ± 66.02	0.237
	3-mm height(%VH3)	178.81 ± 119.63	93.76 ± 63.86	0.181

All values are displayed as Mean ± SD

c = graft thickness/volume reduction calculated from $\left(\frac{\square-\square}{\square}\right)$ □□□ %

†Independent t-test; ‡Paired t-test , *Significant level < 0.05

Chapter 4

Discussion

From the present study, we clinically and radiologically evaluated the bone formation over the implant surface as well as surrounding soft tissue appearance following GBR procedures using DBBM and DTM for repairing buccal dehiscence defects around a dental implant.

For second surgery to expose the implant and deliver the abutment, the soft tissue and bone healing are required. Various factors affect the healing of bone around dental implant. The re-entry time depended on the degradation rate of grafting materials and membranes. In addition, the selection of grafting materials and membranes also depends on the size of the defect. Since, the growth rate of woven bone hovers near 60μm a day, the augmentation of <5mm defect would take 4-6 months of healing. A bigger defect requires more time for bone regeneration than a smaller defect(55). According to the present study, DTM required a shorter time for integration and regeneration of bone tissue. As DBBM is a pure anorganic bovine bone composed of natural hydroxyapatite, its degradation is limited. From other literature, the re-entry time was suggested to be approximately at least 6-7 months(49, 60, 72, 99). One study compared the effect of DBBM (Bio-Oss®) and biphasic calcium phosphate (Straumann BoneCeramic®), which is composed of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (β-TCP), in treatment of a bony dehiscence defect around an implant. It was also allowed 6.5 months of healing time before re-entry for abutment connection(100). In contrast to DBBM, DTM could be defined as a composite matrix containing inorganic calcium phosphate crystals and as a collagen fiber network acting as a ground substance, and as organic osteogenic growth factors which could probably indicate both osteoconductive and osteoinductive potency. Several experimental studies previously demonstrated that a demineralized tooth matrix can accelerate bone formation during the bone healing process(16, 26, 28, 29, 95, 101). Accordingly, in this present study, the re-entry surgery was conducted at 4 months of healing for the Auto-DTM group and at 6 months for the DBBM group.

The consensus statements obtained from the 4th ITI Consensus Conference by Stephen et al(43) also recommended the uses of particulate autogenous, allogenous, or xenogenous bone in combination with a membrane to treat dehiscence and fenestration defects around the implant. With regard to a systematic review conducted by Jensen et al(54), the best-documented grafting protocols for the treatment of dehiscence-type bone defects are by using DBBM covered with a membrane, particulate autogenous bone with or without a resorbable membrane, and a resorbable membrane alone. In this review, the mean defect fill was 81.7% with a complete defect fill of 68.5%, regardless of grafting materials, whereas, in this present study, both DBBM and Auto-DTM can reduce defect sizes in all dimensions with 93.05% and 90.11% of the mean defect area filled, respectively, without compromising soft tissue appearance. However, at stage II surgery, one defect on the lower posterior area in the Auto-DTM group, required re-grafting. This could be due to the limited amount of Auto-DTM, which was not enough to cover the defect in the case of a lower posterior free-end position.

Cone beam computed tomography(CBCT) is typically used for treatment planning, and follow-up in implant dentistry for evaluating the buccal plate thickness at the implant site(102, 103). To conduct volumetric analysis, the data obtained in the dicom series can be analyzed by using several softwares, for example, Ez3D2009, Simplant, InVivoDental5.0, Accurex, or Dolphin, for evaluation of intraoral bone graft volume in cleft sites and pre-implant sites (104-106). However, due to limitations in the license requirements of those mentioned softwares, this study used ITK-SNAP software to measure volumetric parameters instead. ITK-SNAP is a free software application used to segment structures in 3D medical images and was used in biomedical science to analyze anatomical structures from medical CT, CBCT and MRI data(98, 107).

Regarding the buccal bone thickness around an implant, the thickness should be at least 1 mm in posterior region and 2 mm in anterior region to ensure stability of the buccal plate(108). In this study, after 3-months healing, the mid-buccal bone thickness in the DBBM group presented greater reduction than the Auto-DTM group, however, the minimum 1mm of the buccal bone thickness remained in the two groups. Additionally, the 3-dimensional buccal bone volumes demonstrated over 100% in the DBBM group and approximately 90% in the Auto-DTM group which could be referred that the buccal thickness adjacent to mid buccal area could exceed

2mm in the DBBM group, and 1.5mm in the Auto-DTM group. Graft collapse, particularly at the first 2-mm apical to the implant platform, was more evident in the DBBM group (Figure 19, Figure 22) indicating the migration of the graft particles during the healing period. Graft stability of the Auto-DTM was more noticeable despite its limited quantity (Figure 20, Figure 23), and was consistently with the clinical appearance of the Auto-DTM grafted site at the time of re-entry. Therefore, it could be assumed that graft migration, in the DBBM group, and small graft quantity with graft resorption, in the Auto-DTM group, were the two major factors leading to the deficit of buccal thickness during the bone healing process.

The limitation of the autologous DTM is the limited quantity of graft is available for harvest. Nevertheless, the cost for the DTM fabrication is lower than the price of a commercial xenograft with the same amount. Hence, Auto-DTM should be used to repair only at a small defect where its amount could be covered.

Although the results showed that 90% of defect area fill occurred in both groups with significant defect reduction from baseline, long-term follow-up should be considered to investigate further buccal bone resorption.

The limitation of this present study is the small sample size with various diameters of implants. Additionally, the volume evaluation using contour segmentation from CBCT data is not as precise as medical CT, as well as the negative effects of beam-hardening from the bone-implant interface. However, this study used only one operator for all measurements to reduce technical error as much as possible.

Chapter 5

Conclusion

From this study, we can conclude that autologous DTM can be used to repair small-sized defects such as peri-implant dehiscence or fenestration defect reconstruction with comparable clinical and radiological outcomes, similar to widely-used commercial xenograft (DBBM). However, due to limited quantity of graft, autologous DTM may not be adequate for large defects such as horizontal or vertical defects unless multiple teeth could be used to achieve the required graft amount. Further research in DTM for use as allogenous materials should be performed to overcome the limitations.

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Appendix

Appendix I : Funding and Ethical approval

Funding: This study was supported by Graduate School Research Support Funding. Some clinical expenses were waived by the Dental Hospital, Faculty of Dentistry, Prince of Songkla University. All implants in the test group were supported by Straumann Thailand, DKSH.

Ethical approval: The experimental protocol was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Prince of Songkla University（MOE 0521.1.03/750）. Thailand. All included subjects gave informed consent before participation.

Appendix II

1. Implant and membrane
    A. Bioresorbable porcine-derived collagen bilayer membrane (Bio-Gide®, GeistlichPharma, Wolhusen, Switzerland).
    B. Deproteinized bovine bone mineral (DBBM; Bio-Oss®,Geistlich AG, Wolhusen, Switzerland).
    C. Demineralized tooth matrix (DTM, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Prince of Songkla University, Thailand).
    D. Straumann® Bone Level, Titanium, SLA-surfaced fixtures (ITI Dental Implant System, Institut Straumann, Waldenburg, Switzerland).
2. Medications
    A. Amoxicillin 500 mg (Coamox, Community Pharmacy Public Co. Ltd, Thailand)
    B. Chlorhexidine gluconate mouthwash 0.12%, Faculty of Dentistry, Prince of Songkla University, Thailand
    C. Ibuprofen 400 mg (Cefen, Central Poly Trading Co., Ltd., Nonthaburi, Thailand).
    D. Paracetamol 500 mg (Cemol, Central Poly Trading Co., Ltd., Nonthaburi, Thailand).
3. Softwares
    A. EndNote X5 for Windows, Thomson Reuters, Philadelphia, USA
    B. i-Dixel and One Volume Viewer, J.Morita MFG.Corp., Kyoto, Japan
    C. ITK-SNAP Version 3.4.0, the U.S. National Institute of Biomedical Imaging and BioEngineering
    D. SPSS version 15, SPSS Inc., Chicago, USA

ที่ ศธ 0521.1.03/ 750

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

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง "ผลการบูรณะรอยวิการชนิดดีฮิสเซนส์ด้านใกล้แก้มรอบรากฟันเทียมด้วยดีมินเนอราไลซ์ทูธเมทริกซ์"

รหัสโครงการ EC5804-16-P-**HR**

หัวหน้าโครงการ ทันตแพทย์หญิงมณฑิรา แซ่เบ๊

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 5/2558 **เมื่อวันที่** 4 มิถุนายน 2558

ให้ไว้ ณ วันที่ 2 5 มิ.ย. 2558

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Documentary Proof of Ethical Clearance

Research Ethics Committee (REC)

Faculty of Dentistry, Prince of Songkla University

The Project Entitled Healing of Buccal Dehiscence Defect at Implant Site Using Demineralized Tooth Matrix

REC Project No. : EC5804-16-P-**HR**

Principal Investigator : Miss Monthira Saebe

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 : 25 JUNE 2015 **No. of Approval** : MOE 0521.1.03/ 750

(Asst. Prof.Surapong Vongvatchranon)
Acting on behalf of Chairman of Research Ethics Committee

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(Mr. Boonsit Buaban)

ราชวิทยาลัยทันตแพทย์แห่งประเทศไทย

ร่วมกับ

สมาคมศัลยศาสตร์ช่องปากและแม็กซิลโลเฟเชียลแห่งประเทศไทยในพระบรมราชูปถัมภ์

ชมรมเอ็นโดดอนติกส์แห่งประเทศไทย

ขอมอบเกียรติบัตรนี้แด่

ทันตแพทย์หญิงมณทิรา แซ่โป๊

เพื่อประกาศเกียรติคุณ ที่ได้ทำคุณประโยชน์ในการนำเสนอผลงานวิชาการ

ในการประชุมวิชาการ ประจำปี 2559

มอบไว้ ณ วันพฤหัสบดีที่ 15 เดือนกันยายน พุทธศักราช 2559

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List of Publication and Proceeding

Publication:

Saebe M, Suttapreyasri S. Mini- review: Dentin as bone graft substitution. Songklanakarin Dental Journal. 2014;2(1):40-7

Proceeding (Oral presentation):

Saebe M, Leepong N, Suttapreyasri S. "Treatment of Buccal Dehiscence Defects around Dental Implant using Autologous Demineralized Tooth Matrix". 2016 Annual academic conference "Expanding Knowledge for Better Dental Practices" by The Royal College of Dental Surgeons of Thailand during 14th - 16th September 2016 at Centara Grand at Central World, Bangkok, Thailand.