Autologous Demineralized Tooth Matrix as Bone Grafting Material for Alveolar Ridge Preservation

Warisara Ouyyamwongs

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

Master of Science in Oral and Maxillofacial Surgery

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ชื่อเรื่อง การใช้ดีมินเนอราไลซ์ทูธเมทริกซ์ชนิดอัตพันธุ์ในการคงสภาพสันกระดูกเบ้าฟัน

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บทคัดย่อ

วัตถุประสงค์: ในช่วงระยะเวลาการหายของแผลหลังถอนฟัน จะมีการสูญเสียมิติทั้งความสูงและความกว้างของสันกระดูกตามธรรมชาติ วิธีการคงสภาพสันกระดูกภายหลังการถอนฟันจึงถูกนำมาใช้อย่างกว้างขวางเนื่องจากให้ผลที่ดีในการคงสภาพมิติของกระดูกมากกว่าการไม่ใช้วิธีดังกล่าว วัสดุทดแทนกระดูกหลายชนิดถูกนำมาใช้ในการสร้างกระดูกใหม่ผ่านวิธีการสร้างกระดูกแทนที่กระดูกปลูกที่ช่วยคงสภาพที่ว่างไม่ให้เซลล์อื่น ๆ ที่ไม่ต้องการเข้ามาบริเวณแผล แม้ว่าเกณฑ์มาตรฐานคือการใช้เนื้อเยื่อปลูกถ่ายอาตมัน แต่ในการคงสภาพกระดูกนั้นจำเป็นต้องพิจารณาถึงผลเสียเกินความจำเป็นของการผ่าตัดนำเนื้อเยื่อปลูกถ่ายอาตมันดังกล่าวมาใช้ในบริเวณแผลถอนฟันที่เล็ก จากผลการศึกษายืนยันว่าการใช้เนื้อเยื่อปลูกถ่ายเอกพันธุ์หรือเนื้อเยื่อปลูกถ่ายวิวิธพันธุ์ให้ผลที่น่าเชื่อถือและได้ผลดีในการนำมาใช้เพื่อหลีกเลี่ยงการเพิ่มแผลผ่าตัดในการใช้เนื้อเยื่อปลูกถ่ายอาตมัน เนื้อฟันเป็นส่วนที่น่าสนใจในการมาใช้เป็นวัสดุทดแทนกระดูกเนื่องจากมีส่วนประกอบของแร่ธาตุที่จำเป็นสูงมากกว่าวัสดุอื่น ๆ และยังสามารถหาได้ง่าย ทั้งยังสามารถใช้เนื้อฟันของผู้ป่วยเองมาใช้ในแผลถอนฟันของตนเองได้อีกด้วย คุณสมบัติการเหนี่ยวนำให้เกิดการสร้างกระดูกของเนื้อฟันที่ถูกดึงแร่ธาตุออกนั้นให้ผลดีต่อการหายของกระดูก ดังนั้นวัตถุประสงค์ของงานวิจัยต้องการศึกษาประสิทธิภาพของ เนื้อฟันของผู้ป่วยเองที่ถูกดึงแร่ธาตุออกต่อการคงสภาพรูปร่างของสันกระดูกภายหลังการถอนฟัน

วัสดุและวิธีการ: งานวิจัยนี้ใช้ฟันกรามน้อย 40 ซี่โดยการศึกษาแบบแบ่งส่วนช่องปาก ซึ่งจะสุ่มโดยแบ่งเป็น 2 กลุ่มคือกลุ่มที่ใช้เนื้อฟันที่ถูกดึงแร่ธาตุออกของผู้ป่วยเองปิดด้วยเพลทเลทริช เมมเบรน (กลุ่มทดลอง) และกลุ่มที่ใช้ เพลทเลทริช เมมเบรนอย่างเดียวกลุ่ม (ควบคุม) วัดขนาดการหายของปากแผล การละลายของสันกระดูก และความหนาแน่นของการหายของกระดูก จากลักษณะทางคลินิกและภาพถ่ายรังสี

ผลการศึกษาและอภิปราย: จากผลการวิจัยพบว่า การใช้เนื้อฟันที่ถูกดึงแร่ธาตุออกของผู้ป่วยเอง ไม่พบว่ามีการติดเชื้อของแผลหลังทำหัตถการและการปฏิเสธของเนื้อเยื่อเกิดขึ้น ในระยะเวลาที่ 8 สัปดาห์ค่าเฉลี่ยของความกว้างในแนวนอนของสันกระดูกที่ 3 มิลลิเมตรจากส่วนรอบปลายรากถึงบริเวณรอยต่อส่วนของซีเมนตัมกับเนื้อฟันในกลุ่มทดลอง (1.84±0.47 มม.) มากกว่ากลุ่มควบคุมอย่าง

มีนัยสำคัญทางสถิติ (2.26±0.59 มม.) ผลจากภาพถ่ายรังสีทั้งหมดของการละลายของขอบกระดูกที่ด้านใกล้กลาง ไกลกลาง และบริเวณตรงกลางของแผลถอนฟันในกลุ่มทดลอง ให้ผลไม่แตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม ในช่วง 6 สัปดาห์แรก ความหนาแน่นของการหายของกระดูกในกลุ่ม ทดลอง สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามความหนาแน่นจะคงที่และให้ผลไม่แตกต่างกันในทั้ง 2 กลุ่มที่ระยะเวลา 8 สัปดาห์

สรุป: การใช้เนื้อฟันที่ถูกดึงแร่ธาตุออกของผู้ป่วยเอง มีประโยชน์และปลอดภัยในการเป็นวัสดุทดแทนกระดูกสำหรับวิธีคงสภาพสันกระดูก แผลถอนฟันที่ใช้เนื้อฟันที่ถูกดึงแร่ธาตุออกของผู้ป่วยเอง และปิดด้วยเพลทเลทริช เมมเบรน สามารถลดการยุบของกระดูกด้านใกล้แก้ม และส่งเสริมเกิดความหนาแน่นของการหายของกระดูกดังผลที่แสดงทางคลินิกและทางภาพถ่ายรังสี

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Abstract

Background: During the healing phase following tooth extraction, dimensional loss of bone height and width is a natural occurrence. It's widely accepted that ridge preservation procedures following tooth extraction result in greater orofacial bone dimension than where no ridge preservation was performed. Numerous grafting materials have been used to facilitate the formation of new bone via osteoconduction that preserve the space and exclude unwanted cells from the wound. Although in almost the criterion standard is the autogenous bone graft, nevertheless, in socket preservation it may be considered an excessive or aggressive method to harvest autogenous bone for such small, contained defects. Studies have clearly proven the reliability and functionality of using either allografts or xenografts, which avoids the creation of an additional surgical site for bone harvesting. Dentin has been an area of interest for its potential use as a bone substitute since it has higher mineral content than any derived material. It's also a readily available graft. With the prospect of the possible use of autogenous matrix as a graft material comes the opportunity to utilize the patient's own dentin from their extracted tooth. Moreover, the osteoinductive property of demineralized dentin matrix is very valuable for bone healing defects. The aim of this study to determine the efficacy of autologous demineralized tooth matrix (auto-DTM) in the preservation of ridge shape after tooth extraction.

Material and Methods: In this study, forty symmetrical premolar extraction sockets using split-mouth design were randomly filled with auto-DTM and sealed with PRF membrane (DTM group) or PRF membrane alone (control group). The healing of socket orifices, marginal bone resorption, and bone healing density were measured clinically and radiographically.

Results: The study found that auto-DTM was well tolerated in all sites with no incidences of postoperative infection or graft rejection. At the 8th week, the mean horizontal width of the ridge at 3 mm apical to the cemento-enamel junction line in the DTM group (1.84±0.47mm) was significantly greater than that of the control group (2.26±0.59 mm). The overall radiographic resorption of marginal bone levels on the mesial side, the distal side, and at the center of the sockets in the DTM group were not significantly different from those of the control group. During the first 6 weeks, bone healing density of the DTM group was significantly higher than that of the control group. However, the density appeared more stable with no difference between the two groups at the 8th week.

Conclusion: Auto-DTM can be a useful and safe alternative graft material for alveolar ridge preservation. Grafting extraction sockets with auto-DTM covered with PRF membrane can reduce buccal bone collapse and promote bone healing density as shown clinically and radiographically.

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List of Abbreviations and Symbols

BMPs	= Bone morphogenetic proteins
PRF	= Platelet-rich fibrin
PDGF	= Platelet derived growth factor
IGF	= Insulin-like growth factor
VEGF	= Vascular endothelial growth factor
TGF-β	= Transforming growth factor beta
PDGF	= Platelet-derived growth factor
PDAF	= Platelet-derived angiogenesis factor
IGF-1	= Insulin-like growth factor -1, PF-4 - platelet factor – 4
DFDBA	= Demineralized freeze-dried bone allograft
FDBA	= Freeze-dried bone allograft
auto-DTM	= autologous-Demineralized tooth matrix
mm	= Millimeter
nm	= Nanometer
GBR	= Guided bone regeneration
PTFE	= Polytetrafluoroethylene
ePTFE	= expanded Polytetrafluoroethylene
DBBM	= Deproteinized bovine bone mineral
ICA	= Irradiated cancellous allograft
SDA	= Solvent-dehydrated allograft
FDBA	= Freeze-dried bone allograft
BPCAP	= Biphasic alloplastic graft
ABM	= Anorganic bovine bone matrix
HA	= Hydroxyapatite
TCP	= Tricalcium phosphate
OCP	= Octacalcium phosphate
ACP	= Amorphous calcium phosphate

List of Abbreviations and Symbols (Continued)

XRD	= X-ray diffraction
SEM	= Scanning Electron Microscopic
DRD	= Demineralized root dentin
DDM	= Demineralized dentin matrix
DHDM	= Demineralized human dentin matrix
HDDM	= Homogenous demineralized dentin matrix
ADDM	= Autogenous demineralized dentin matrix
rhBMP-2	= Recombinant bone morphogenetic protein-2
mg	= Milligram
ml	= Milliliter
°C	= Degree Celsius
CEJ	= Cemento-enamel junction
Href	= Horizontal reference line
Pref	= Reference point
M	= Mesial
D	= Distal

Chapter 1

Introduction

Reduction of the alveolar bone dimensions typically occurs after tooth extraction. During socket healing period, new bone grows into the extraction site while the alveolar ridge is being resorbed. Several studies demonstrated that the width and the height of the alveolar bone decreased significantly immediately after tooth extraction[1-3]. The processes mentioned in those studies led to a dimensional loss of socket bone, which obstructed dental implant placement and conventional prosthesis. Therefore, it's more common to perform ridge preservation procedures after tooth extraction, which can be performed by placing grafting materials in the extraction socket as a framework for bone deposition.

To maintain the alveolar ridge dimensions, different bone graft materials are employed for ridge preservation. Autologous graft is widely accepted as the standard in regenerative procedures because of its osteogenesis, osteoinduction and osteoconduction properties[4]. Despite these essential properties, drawbacks of the autologous bone grafting include a need for the second site surgery, donor site morbidity, and limited availability. These drawbacks have led to the challenging study for alternative biomaterial scaffolds with osteoinductive potential. Generally, xenografts or allografts are utilized with good outcomes[5, 6]. Nonetheless, the shortcomings of xenografts and allografts are that they lack osteogenic properties[7-9], tend to be expensive, and may increase the risk of disease transmission. Because of the mentioned problems, researchers have been constantly putting efforts to develop better bone substitute materials.

The patient's own tooth is a possible alternative substance for bone substitute materials. The dentin consists of inorganic and organic components. The inorganic part comprises 70% hydroxyapatite. The hydroxyapatite type is low-crystalline calcium phosphate similar to bone, which promotes bone remodeling. Unlike dentin, enamel contains a high-crystalline calcium phosphate type of hydroxyapatite[10], which is hard to be decomposed by osteoclasts, resulting in a slow resorption rate. The combination of both dentin and enamel may lead to longer retention of the socket dimension and better bone regeneration. The organic part contains various growth factors that control cellular growth, proliferation, and differentiation such as bone morphogenetic proteins (BMPs)[11-14]. Prior studies consistently concluded that dentin has osteoconductive and osteoinductive potential. Clinical trials using dentin as bone fillers or volume maintainers in sinus augmentation as well as guided bone regeneration were investigated[15-17]. Moreover, the tooth matrix material is made from the patient's own tissue through various processes and then grafted back into the same patient. As the tooth is autogenous, immunogenicity is reduced, medical waste is recycled and expense is reduced for the patient.

During ridge preservation procedures, socket sealing materials must be used to cover and hold grafting materials within the socket. In 2001, a second-generation platelet concentrate or platelet-rich fibrin (PRF) was first developed[18]. PRF has a property to promote soft tissue regeneration[19, 20]. PRF is a source and carrier of growth factors[21], and it can also act as fibrin network supporting cells and promote blood vessel proliferation[22]. Preparing PRF is affordable and it can be easily prepared as a membrane. No additional surgery is required at the donor site. Regarding to PRF characteristic, PRF membrane was selected as the sealing material in this study.

The aim of this study was to evaluate the effects of autologous demineralized tooth matrix (auto-DTM) and PRF membrane in the preservation of alveolar ridge dimension after tooth extraction.

Background

i. Healing of alveolar bone after tooth extraction

Osseous deformities of the alveolar ridge, including both width and height reduction of the residual ridge, are typically caused by tooth extraction and the following healing of the socket. Bone resorption will result in a reduction of socket height in an apico-coronal and socket width in a bucco-lingual direction. During the healing phase following tooth extraction, the highly dynamic process is a natural occurrence, starting with inflammatory reactions happening immediately after tooth extraction. It occurs in five different stages[23]: Initially, a coagulum of blood cells, both red and white, casts a blood clot or coagulum from the dissected blood vessels.

This phase happens immediately after extraction. Then, on the second stage, the blood clot begins to breakdown (fibrinolysis) within a few days. The blood clot changes into granulation tissue with cords of epithelial cells associated with budding capillaries within four to five days. In the third stage, connective tissue which is rich in vessels and inflammatory cells gradually takes over the granulation tissue in 14 to 16 days. The calcification starts in the fourth stage when osteoid forms at the periphery and the base of the extraction socket. Most of the alveolus is filled with woven bone, while the soft tissue becomes keratinized. Epithelial closure of the socket is complete. This stage takes three to six weeks. In the last stage, mineral tissue inside the original socket is augmented with lamellar bone layers accumulated on the previously formed woven bone. The bone will be filled completely with little evidence of osteogenic activity by the 16th week. This last stage takes five to ten weeks.

A recently published systematic review on the changes of alveolar bone dimension of extraction sockets in humans exhibited a range of 2.6-4.6 mm in width reduction, and showed a range in height reduction between 0.4-3.9 mm[24]. The rate of alveolar ridge resorption after tooth extraction was faster in the first nine months [2, 25]. It was found that two-thirds of the resorption happened in the first three months, and half of the ridge width decreased in the first 12 months (average 6.1 mm; 2.7-12.2 mm). In a recent systematic review, Tan, Wong[26] reported a higher resorption of ridge bone horizontally (29-63%; 3.79 mm) than vertically (11-22%; 1.24 mm) at month six. Naturally, the process of alveolar ridge resorption slowly occurred throughout one’s life at the rate of 0.5- 1.0% per year

Several components may impact the changes of bone dimensions after tooth extraction, for example the tooth position in the dental arch, the number and proximity of teeth to be extracted, the condition of the socket before and after extraction, and the tissue biotype. Thin biotype with highly scalloped hard and soft tissues is more prone to display hard tissue resorption and soft tissue recession than the thick biotype. The severity of the healing pattern may establish a problem for the clinician such as an aesthetic problem in the manufacture of an implant-supported restoration, an orthodontic tooth movement into extraction site, etc. In order to eliminate or minimize extensive hard and soft tissue regenerative surgical procedures, socket preservation can be carried out at the time of tooth extraction.

Alveolar ridge preservation is a procedure at the time of tooth extraction to control bone resorption. Alveolar ridge preservation aims to preserve the bone volume and soft tissue position of the alveolar ridge, to reduce post-extraction dimensional changes and to eliminate future bone regeneration that required for ideal implant placement[27].

ii. Surgical techniques for alveolar ridge preservation

The principles behind the practice of implant site development, such as ridge preservation and guided bone regeneration, emerged from the principles of guided tissue regeneration. At the time of tooth extraction, the socket can be augmented by several techniques such a s 1) preservation of alveolar ridge using membrane or socket sealing materials, 2) preservation of alveolar ridges using growth factor, 3) preservation of alveolar ridges using bone substitution with/without membrane

Preservation of alveolar ridges using membrane or socket sealing materials

Guided bone regeneration (GBR) technique includes the use of barrier membrane to inhibit gingival cells from moving into bone defect area. Animal and clinical studies show that alveolar socket has a tendency to heal itself[28-31]. Bone formation from the bottom of tooth socket up to alveolar crest may be the result of the existence of blood coagulum that developed into granulation tissue. Then, epithelial cells will creep along granulation tissue and seal the wound of the extraction site. It's still in the discussion whether barrier membrane has any effect on the preservation of alveolar ridge or not.

There are many types of barrier membranes that were used to seal the extraction site such as expanded polytetrafluoroethylene (ePTFE)[32], collagen membrane[33], polyglycolic acid[34], and polyglactin 910[34] etc. There are two types of barrier membranes, based on resorption properties, which are resorbable type and non-resorbable type. Pros and cons of each type are as shown in Table 1.

Lekovic et al.[35] used a non-absorbable ePTFE membrane to preserve alveolar ridge after tooth extraction for six months. They found that ridge dimensional change of the group with ePTFE membrane usage to be lower than that of the control group. However, when the membrane was exposed, ridge dimension changes were the same for both groups. Further study by Pinho et al.[36] on the usage of titanium membrane, both by the membrane alone and when used in combination with autologous bone graft from maxillary tuberosity, also found no significant dimensional change between both groups. As a consequence, Pinho summarized that maintaining the space was much more important to the healing than whether bone graft was used or not. In summary, the usage of non-resorbable barrier membrane could reduce the resorption of alveolar ridge after extraction. Nevertheless, the effect disappears if the membrane is exposed.

Table 1 Pros and cons of non-resorbable and resorbable barrier membrane

Membrane	Pros	Cons	Sample
Non-resorbable	• Stable & adjustable to desired shape using titanium reinforcement • Stay in the desired shape throughout usage life • Can be used with titanium pin or resorbable tacks to fix membrane in location • Allow more bone formation, if no exposure to membrane • Induce low immunity reaction, if no exposure to membrane	• 2nd operation needed to remove the membrane • Must be removed if the membrane is exposed • Require experienced dentists with technique sensitive	• ePTFE membrane (Gore-Tex; Gore Medical,) • High-density PTFE; (Cytoplast TXT-200 Osteogenics Biomedical) • Titanium-reinforced Gore-Tex
Resorbable	• 2nd operation not required • Promote soft tissue healing • In case of membrane exposure, immunity reaction will be mild and is not likely to get infected • Do not require removal in case of membrane exposure	• Inconsistent period of its space maintenance property due to the membrane resorption • Inflammatory may affect healing process and GBR property • Require experienced dentists with technique sensitives	• Porcine collagen matrix (Bio-Gide: Geistlich AG, Wolhusen, Switzerland) • Bovine collagen (OsteoMEND: Implandent; RTM Cytoplast; Osteogenics Biomedical))

Lekovic et al.[34] studied the use of glycolic and lactide polymer membrane. The results were in agreement with that of Pinho et al.[36] where the ridge dimensional change in the group with the membrane was smaller than in the control group both vertically (0.38 mm and 1.50 mm) and horizontally (1.32 mm and 4.56 mm) Additionally, the group with the membrane was found to has more bone formation.

There are also numerous studies on the resorbable membrane. A study on collagen resorbable membrane resulted in very small resorption and the new bone was formed enough for implant placement within 3 months after tooth extraction[33]. Luczyszyn et al.[37] reported on the use of an acellular dermal matrix membrane in combination with resorbable hydroxyapatite graft. They found that both groups that use only the membrane and the group that uses both membrane and bone graft substitutes could preserve alveolar ridge dimension. But the result of the second group was significantly better. So, it could be concluded that the use of bone graft substitution in combination with a resorbable membrane can yield a better result in preserving alveolar ridge.

Although the membrane can be beneficial, there are two main drawbacks. First, using the membrane may requires at least five to six months of healing period before implant process can be performed. Second, preparation of the soft tissue for covering the membrane requires the dentist's expertise or else may lead to aesthetics problems.

Lanndberg and Bichacho[38] demonstrated the socket seal surgery technique for ridge preservation. The extraction socket was filled with bone graft substitution materials and the soft tissue graft was place atop the bone graft. The soft tissue graft allowed primary wound closure over the socket orifice which protected bone graft from contamination of bacterial in oral cavity[39, 40], and limited soft tissue shrinkage which leaded to a better esthetic of surgical site.

The survival of free gingival graft placed on the top of a graft-filled socket does not depend only on the characteristics of the graft or the graft harvesting technique, but also depend on the blood vessels that support the free gingival graft. The vascular supply the soft tissue graft develop from the surrounding gingiva and the plasma part of blood clot in the socket.

The present of the bone graft materials in the socket may interfere the revascularization of the free gingival graft[41] that might hinder the free gingival graft healing.

Preserving alveolar ridge using biological material containing growth factor

PRF is the latest development of platelet concentration developed by Choukroun[18]. Blood is collected without any coagulant and immediately centrifuged. A natural coagulation process then occurs and allows for collection of PRF clot, without the need for any biological modification of the blood are required. The PRF is composed of fibrin membrane that trapped in platelet cytokines and various growth factors. Platelets play an important role in hemostasis and are a natural source of growth factors. These growth factors are stored in granules within the platelets. Many growth factors were found in PRF including platelet derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) (Table 2)[42].

In vitro study, PRF was found to release a significant supply of growth factors and matrix glycoproteins (such as thrombospondin-1) during first week[43]. Another in vitro study showed that the growth factor content (PDGF and TGF-β) in PRP and PRF was about the same[42]. Sanchez et al.[44] used osteoblast cell cultures to examine the effect of PRP and PRF on proliferation and differentiation of osteoblasts cell. The result demonstrated that the affinity of osteoblasts to the PRF membrane appeared to be superior. For clinical study, Choukroun et al.[45] investigated the capability of PRF in combination with freeze-dried bone allograft (FDBA) in sinus floor elevation to enhance bone formation. In various attempts, Choukroun's PRF was able to provide a possible new bone formation. Mazor et al.[46] confirmed that the use of PRF alone as filling material during a sinus floor elevation can stabilize a substantial amount of regenerated bone in the sub-sinus cavity. They also concluded that PRF was an acceptable option because it was simple and inexpensive. Simonpieri et al.[47, 48] supplemented the usage of PRF membranes combination with FDBA in reconstruction protocols. They declared that PRF membranes function as a biological matrix which supports neoangiogenesis, and migration of osteoprogenitor cells. Choukroun[19] confirmed, from his clinical experience, that PRF could be considered as a healing biomaterial. This biomaterial consists of 1) fibrin matrix polymerized in tetramolecular structure, 2) combination of platelets, leukocyte, and cytokines.

Table 2 Growth factors released from platelets and their biologic actions[42]

Growth factor	Source cells	Target	Biologic action
PDGF	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, macrophages, neutrophils	Stimulates DNA and protein synthesis in osseous tissues; mitogenic effects on mesenchymal cells; angiogenic effect on endothelial cells
TGF-β	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells endothelial cells, epithelial cells, preosteoblasts	Stimulates angiogenesis; enhanced woven bone, formation; stimulate matrix synthesis in most culture systems; chemotactic effect on osteoblastic cells; stimulates endothelial chemotaxis; stimulates bone formation by inhibitory effect on osteoclasts
PDAF	Platelets, endothelial cells	Endothelial cells	Mitogenic effect on endothelial cells; increased angiogenesis and vessel permeability
IGF-1	Osteoblasts, macrophage, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondroblasts	Stimulates proliferation of osteoblasts and matrix synthesis; increases expression of bone matrix proteins, such as osteocalcin; in combination with PDGF it enhances the rate and quality of wound healing
PF-4	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts

PDGF - platelet-derived growth factor, TGF-β - transforming growth factor β, PDAF - platelet-derived angiogenesis factor, IGF-1 - insulin-like growth factor -1, PF-4 - platelet factor – 4

Nevertheless, Suttapreyasri and Leepong[20] investigated the effect of PRF on alveolar ridge dimensional change following tooth extraction. The result indicated that PRF revealed effectiveness only on soft tissue healing in the first month and demonstrated neither alveolar ridge preservation nor enhancement of bone formation during the 8-week study.

In summary, PRF can be used in conjunction with bone grafting materials for facilitating bone graft manipulation. PRF can also be used as a biological membrane for socket sealed material. These offer several advantages including promoting soft tissue wound healing, stabilizing graft, and improving the handling properties of graft materials.

Preservation of alveolar ridges using bone substitution with/without membrane

Table 3 Types and sources of grafting materials[49]

Materials	**Sources**
Autogenous graft	Bone prepared from the patient, which may come from intraoral or extraoral
Allogeneic graft	Bone prepared from others (same species), usually from donors. The bone will be prepared through various methods to reduce immunity system response. The material will also be sterilized. The allogeneic graft is usually prepared freeze dried, either mineralized or demineralized.
Xenograft	Bone prepared from difference species. Xenograft is morphologically and structurally similar to human bone. Various thermal and chemical treatment have been used to remove antigenic protein and cellular elements of xenogeneic bone. The materials are usually bovine bone, horse bone, coral, etc.
Alloplast	Synthetic material that usually do not trigger negative immunity or tissue reaction such as group of calcium phosphate transplant materials (Hydroxyapatite, Tricalcium Phosphate), polymer group transplant materials (Chitosan, Collagen, Polycaprolactone)

The bone graft materials can be classified by their original source as follows: autograft, allograft, xenograft, and alloplast or synthetic materials[49] as demonstrate in Table 3.

Autogenous bone grafts can be gathered from several intraoral sites, for example, the maxillary tuberosity, edentulous ridges, post-extraction healing sites, and tori or exostoses. The origin of intraoral bone also plays a crucial role. For example, bone harvested from the area with predominantly cortex bone usually has little osteogenic potential. On the other hand, bone harvested from the area with predominantly cancellous bone has better osteogenic potential. A study that used autologous bone chips as graft materials found that it had no effect on bone formation and did not promote alveolar ridge preservation[50].

Although autogenous bone is the best candidate for repairing osseous defect, its limited volume and requisite additional surgery indicate a need for an alternative. Allografts, xenografts, and alloplasts, either in a block or particulate form, can also be used as an alternative bone graft material.

Allografts consist of tissue transferred from one individual to another within the same species. Allografts are widely used because the materials do not require a secondary surgical site and so host morbidity is decreased. The main advantage of an allograft bone graft is that it can be unlimitedly obtained. The graft materials can be classified as demineralized freeze-dried bone allograft (DFDBA) or freeze-dried bone allograft (FDBA). Urist suggested that it is possible to add an osteoinductive property to the already osteoconductive bone by demineralizing the material causing the releasing of bone morphogenic proteins (BMPs). One disadvantage of using allograft is its risk of transmitting disease, however, there have been no report of viral contamination or acquired pathology from the use of DFDBA or FDBA[51, 52]. Freezing the bone allograft can further reduce the risk of contamination to one in eight million[53].

Xenografts are tissue grafts transferred between different species. Several short-term studies indicated that the placement of xenografts in alveolar sockets could advocate bone formation and ridge preservation, but may also delay healing. A study has evaluated long-term effects on bone formation and the ridge augmentation from the usage of Bio-Oss collagen® (Geistlich Pharma North America, Inc.), a xenogeneic graft, in extraction sockets in five beagle dogs. The use of Bio-Oss collagen® showed improved preservation of the alveolar process and ridge profile when compared to the non-grafted sites[54]. Another study on human subjects comparing the use of Bio-Oss collagen® versus clot only (control group) showed that new bone formation in augmented sites (test) was merely 25% compared to 44% in the non-augmented sites (control) [55]. This result confirmed a delay in bone formation in grafted sites as mentioned in various studies.

One of the most commonly used xenografts is deproteinized bovine bone mineral (DBBM). The material was able to stay inside the extraction socket for an extended period of time. In a 9-month study, the DBBM graft material was still prevail evenly throughout the extraction socket and averaged an overall 30% residual graft at the end of the study period[56]. Another study that compared DBBM to irradiated cancellous allograft (ICA), and to solvent-dehydrated allograft (SDA) when used to preserve extraction sockets concludes DBBM as a favorable graft for the ridge. The authors also noted that DBBM grafts may be useful where new bone was desired, and a slower resorption rate of the graft was preferred[57]. These studies were only a few examples that prove xenografts as viable materials for ridge preservation.

Alloplasts are synthetic inert materials implanted into tissue. Common examples are hydroxyapatite, tricalcium phosphate, calcium sulfate and bioactive glass. These graft materials are osteoconductive, which help serve as a scaffold for new bone formation.

Hydroxyapatite is one of the synthetic graft materials. In a study on five beagle dogs by Lindhe J et al.[58], an alloplastic graft (biphasic alloplastic graft (BPCAP); α-TCP core coated with nanocrystalline biomimetic hydroxyapatite) embedded in porcine collagen was used as graft materials for the extraction socket of the premolar sites. The clinicians documented that the biphasic alloplastic graft did not undergo marked resorption, but allowed new bone formation within the post-extraction site. In another study, Shakibaie[59] compared the effectiveness of a synthetic material consisting of hydroxyapatite and silicon dioxide (NanoBone) and the Bio-Oss® (Geistlich Pharma). The result showed that the alveolar ridge was better preserved with Bio-Oss than with NanoBone or without treatment.

Several synthetic materials have also been developed and used. For example, sponge made out of collagen or polylactic/polyglycolic acid is developed as an alternative material. Serino et al.[60] performed studies with Fisiograft®, a synthetic co-polymer composed of polylactic and polyglycolic acids. The author reported that the grafted sites healed with mineralized, well-organized bone with none of graft particles left behind. In summary, many materials have been studied by clinicians in an attempt to find suitable grafting materials for ridge preservation purpose as summarized in table 4.

Table 4 Socket preservation studies

Author	Material & Method	Time (weeks)	Dimensional change (mm)		Histomorphometric analysis of residual graft
			Horizontal	Vertical	
Block MS et al .2002[61]	Human mineralized bone graft	16	-	-	
Froum et al .2002[62]	DFDBA vs .Bioactive glass	24-32	-	-	DFDBA;13.5% Bioactive glass; 5.5%
Iasella JM et al .2003[5]	DFDBA with collagen membrane	16-24	1.2±0.9	1.3±2.0	DFDBA =37%
Guarnieri et al. 2004[63]	Calcium sulfate (Surgiplaster®)	12	-	-	-
Aimetti et al .2009[64]	Calcium sulfate (Surgiplaster®)	12	2.0	0.5	-
Kesmas S et al .2010[65]	BCP (HA/β:TCP) (60/40) (BoneCeramic®) with collagen membrane	16	2.0	1.5	BCP;15.83 ±8.70%
Lindhe J et al .2013[58]	Bio-Oss collagen with collagen membrane	24			Bio-Oss collagen; 19.0 ±6.5%

Table 4 (Continued)

Author	Material & Method	Time (weeks)	Dimensional change (mm)		Histomorphometric analysis of residual graft
			Horizontal	Vertical	
Cardaropoli D et al .2012[66]	Bio-Oss collagen with collagen membrane	16	1.04±1.08	0.46±0.46	Bio-Oss; 18.46 ± 11.18%
Jurisic M et al .2013[67]	BCP(HA/βTCP:60/40) with PLA-co-PGA	16	-	-	BCP+PLA-coPGA; 31.9±8.9%
Toloue et al .2013[68]	A :FDBA B :CS	12	FDBA; 1.03±0.87	FDBA; 0.05±1.46	FDBA; 21%
Brownfield LA et al .2012[69]	Demineralized bone matrix with cancellous bone chips	12	1.6±0.8	0.8±1.2	2.4 %by micro-CT 4.5 %by histomorphometric
Suttapreyasri S et al .2013[20]	PRF	8	Buccal; 1.79±0.90 Lingual; 0.42±0.39	-	-

iii. Selection of graft materials for ridge preservation

Grafting materials for ridge preservation can be classified as for long-term, for transitional, and for short-term. Non-resorbable materials are usually discussed in the long-term preservation context because of their nonresorbable characteristic, but in fact, even the nonresorbable materials undergo some physiochemical dissolution. The nonresorable materials do not fully resorb and get replaced by a natural bone. Therefore, the nonresorbable materials are not advisable to be placed into sites with the possibility of later dental implants because the residual graft materials will prevent the integration of implant fixture to natural bone. Nevertheless, the nonresorbable characteristic makes them suitable for long-term ridge maintenance. Common materials for the purpose includes HA porous coralline HA, bioactive glass, porous polymethyl methacrylate, synthetic HA[70].

Grafting material for transitional ridge preservation is usually marketed as being resorbable but with 4-12 months period for new bone formation. These grafting materials are useful for developing bone density and are used in medium-term ridge preservation. Frequently, patients may not immediately decide to undergo implant therapy immediately after they lose their tooth but eventually desire to undergo the therapy at a later date. Grafting material for transitional ridge preservation allows patients to take some decision period before the implant. Materials in this category include anorganic bovine bone matrix (ABM), resorbable calcium phosphate ceramics, and macroporous bioactive glass, and deproteinized bovine bone with collagen[70].

Short-term resorbable materials are those that can readily be resorbed and replaced by host tissue over the typical healing period. The objective of using short-term ridge preservation is to maintain bone mass during the initial healing stage with the expectation to start implant process within 3 to 6 months. Similar to the materials for transitional ridge preservation, they increase bone density, prevent early ridge resorption, and facilitate the placement of dental implants. The material in this category includes Freeze-dried bone allograft (FDBA), Demineralized freeze-dried bone allograft(DFDBA), and autogenous bone[70].

iv. Tooth as bone graft material

The tooth has been the area of interest as a bone substitute because of its similar morphology and microstructure to bone. Tooth components have biocompatibility property and also has growth factors that encourage osteoinduction. However, a proper preparation process is required in order to preserve osteoinduction property in graft materials. Because of its many beneficial characteristics, the usage of the tooth as bone substitution has been studied in a myriad of in Vitro study, in Vivo study, and clinical applications.

Similarity between tooth and bone

Tooth, cartilages, nerves, and maxillofacial bones are all embryologically derived from the neural crest[1-4]. The compositions of these parts of the body, especially tooth (dentin) and bones, are very similar. Dentin is composed of 65% inorganic substances, 35% organic substances, and water. Cementum is also made up in a very similar ratio of 45-50% inorganic substances, 50-55% organic substances, and water. Alveolar bone is made up of an even more similar ratio of 65% inorganic and 35% organic substances.

Both bone and tooth are hard tissue with similar morphology and microstructure which can be seen in Figure 1 despite differences during the developmental period. Alveolar bone, as well as dental tissues such as enamel, dentin, cementum, pulp, and periodontal ligament, are developed from the neural crest cells. While bone is built from multiple Harversian's systems, dentin built up as a complex hydrated composite of 4 components: 1) oriented tubular 2) a high mineralized peritubular zone embedded in an intertubular matrix 3) type I collagen with embedded apatite crystals and 4) dentinal fluid.

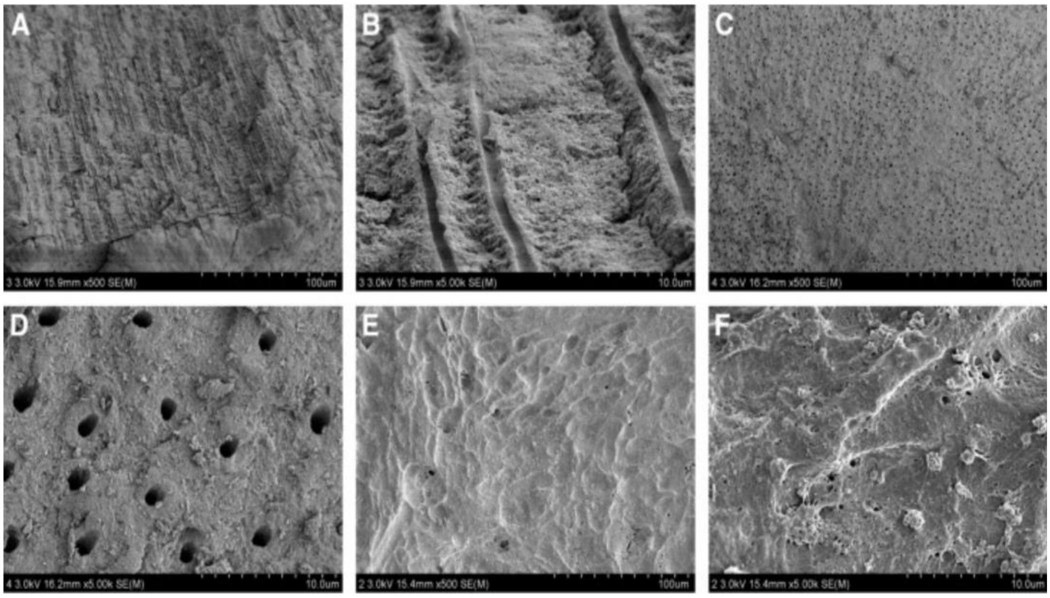

Figure 1 SEM views of the different types of graft materials. A, tooth crown (×500); B, tooth crown (×5000); C, tooth root (×500); D, tooth root (×5000); E, autogenous cortical bone (×500); F, autogenous cortical bone (×5000);[71]

Components of tooth

Inorganic component of tooth consists of 4 phases of calcium phosphate: 1) hydroxyapatite, HA; 2) tricalcium phosphate, TCP; 3) octacalcium phosphate, OCP; and 4) amorphous calcium phosphate, ACP, which interacts with each other[72]. The existence of inorganic part is responsible for the physicochemical property and strength of the tissues. The dental crown portion is made up of high-crystalline calcium phosphate minerals (mainly HA) with higher Ca/P ratio while the root section was mainly consisting of low crystalline calcium phosphates with lower Ca/P ratio compared to the crown portion.

Researchers have been studied components of tooth through various methods. The Ca/P molar ratio to determine the phase of calcium phosphate in the tooth (9). The X-ray diffraction (XRD) analysis was used to evaluate crystallinity degree. The Scanning Electron Microscopic (SEM) was used to examine the surface characteristics of the processed dentin. The similarity physicochemical properties between dentin and bone lead to the use of dentin as a scaffold material in bone substitution. The studies of physicochemical properties of dentin prepared as a bone substitute were summarized in Table 5.

Table 5 A review physicochemical studies of dentin as bone graft substitute.

Techniques	Measurement and characteristics	Results
X-ray diffraction (XRD)	Analysis of inorganic components (level of crystallization)	● The level of crystallization and the amount of HA differed depending on the area of the tooth[73]. ● The crown portion was formed by high-crystalline calcium phosphate (mainly HA) while the root portion mainly contained low-crystalline calcium phosphates [73]. ● Autogenous tooth dentin, allogeneic bone, and autogenous cortical bone showed patterns that were relatively similar low crystalline HA structures [71].
Energy dispersive spectroscopy (EDS)	Surface composition (C/P ratio)	● The total tooth was in the range of 1.24-1.46 (similar to TCP and OCP values) ● A crown portion (higher Ca/P ratio) was 1.75 . (similar to HA value) ● Root portion (relatively low Ca/P ratio) was 1.32. (similar to ACP value)[10]
Scanning electron microscopy (SEM)	Surface topography (surface structure)	● The dentinal tubules were reported to be 900-2,500 nm [73] and 1000-2000 nm in diameter[71]. ● Relatively similar in density, roughness, and homogeneity of the autogenous tooth to autogenous cortical bones[71]. ● Dentinal tubules were well exposed thoroughly and loosening fiber bundles of intertubular and peritubular dentin, provided channels for releasing proteins and factors from the dentin matrix[73].

The organic component of dentin matrix consists approximately 90% of collagenous proteins; the remaining includes growth factors such as endogenous BMP, phosphoproteins, osteocalcin, proteoglycans, dentin sialophosphoprotein, etc[74]. BMPs play a key role in new bone formation. At least 20 members of the BMP family have been identified and studied. Some members of BMP family provide a promising possibility of enhancing bone regeneration such as BMP-2 and BMP-7[75]. Sialophosphoprotein is identified as having an important role in the formation and growth of hydroxyapatite (HA) crystals in an extracellular matrix of hard tissue such as bone and teeth.

Preparation by Demineralization method

Because the release of the growth factors is sometimes prevented by hydroxyapatite crystals, the demineralization process is needed to release various growth factors and proteins. The induction of heterotrophic bones was observed when DDM was used as graft materials in animal muscle study model. As such, demineralization process is believed to induce the release of beneficial growth factors which then lead to osteoinduction[76-78].

Various demineralization protocol and chemical have been utilized for preparing demineralized bone or tooth matrix. 0.6N HCl was widely used in many study for demineralizing and the result products could stimulate connective tissue cells and form ectopic bone in muscle[77, 79, 80]. In one study, dentin was treated with 2% HNO3, then rinsed in cold distilled water before lyophilized. The materials implanted in animal models demonstrated favorable results, with the observed new bone formation[81, 82].

Despite many positive results, some authors reported negative outcomes. Ike and Urist[83] found that when human partially demineralized dentin granules were used as graft material in the intramuscular pockets, no osteoinduction was observed, but cellular adhesion and proliferation of the MG-63 cell were found. With different results from the use of graft materials prepared from different demineralization methods, it is possible to conclude that graft properties, especially osteoinductive properties, may partly depend on different demineralization methods.

Biocompatibility and Osteoinduction property of tooth

Dentin has been proposed as bioinert bone substitutes providing osteoconductive scaffolding similar to those of autogenous bone. Another advantage of dentin over hydroxyapatite is that it contains organic matrix which induce bone formation.

Moharamzadeh et al.[84] revealed that in vivo implantation of prepared dentin into rat femurs exhibited biocompatibility without fibrous connective tissue layer and inflammatory reaction. New bone was formed between the implant and surrounding bone.

Both homogenous demineralized dentin matrix (HDDM) and autogenous demineralized dentin matrix (ADDM) are biocompatible and are able to promote osteoinduction since both materials can induce ectopic bone formation without fibrous encapsulation and host immune rejection[76]. During the preparation of HDDM, the demineralization process does not denature its osteopromotive ability. So, HDDM stays as a reservoir of biochemical factors that induce cell differentiation, cellular proliferation, and chemotaxis[85]. Gomes et al.[86] studied the bone reconstruction process after the implantation of HDDM slices in surgical defects in rabbit parietal bones. The author reported that HDDM was biocompatible and can stimulate bone tissue formation. The result showed that HDDM was well accepted by the rabbits and is completely fused into the newly formed bone tissue.

Osteoinductive cannot be exerted only by BMPs alone without carriers. Scaffolds, which functions as a carrier, are used to contain BMPs at the graft sites[87]. An optimal carrier should be able to control release growth factors as well as prevent degradation and inactivation[88]. Clinicians use different carrier materials for different purposes. The most widely used materials are Collagen and TCP. For the specific purpose of delivering BMPs and growth factors, collagen, calcium phosphates, and polyesters such as polycaprolactone have been used[89, 90].

DDM is another scaffold material for the releasing of BMPs62-64. Ike and Urist[83] recycled extracted teeth by using root portion of the tooth as a carrier for recombinant bone morphogenetic protein-2 (rhBMP-2). New bone formation was observed when DDM was used as carrier although the quantity of BMP in teeth is very limited[91]. Through many studies on the biochemical and histomorphometric properties of bone and cartilage induced by human DDM and BMP-2, researchers found that human DDM could induce bone formation, and BMP-2 can significantly accelerate bone formation in the DDM carrier system[76, 92].

In Vitro study, in Vivo study and clinical application

Several studies demonstrated the potential of dentin in different preparation forms as bone grafts substitutes (Table 6-7). The results were consistent in yielding or promoting new bone formation. Several clinical studies indicate that dentin has the potential to be used as a bone substitute in bone regeneration regardless of the differences in preparation form or processes.

Table 6 The In vitro and in vivo studies demonstrated the potential of dentin in different preparation forms used as bone grafts substitutes

Authors/Year	Dentin preparation forms	Results
Gomez et al. 2002[93]	Demineralized dentin matrix (ADDM)	• ADDM slices showed osteoconductive properties. • Resorbed during the bone remodeling process. • Accelerated bone repair process
Moharamzadeh et al. 2008[84]	Non-demineralized dentin (Processed boiled dentin)	• Excellent biocompatibility in Vitro • Stimulated formation of new bone completely incorporated into the new bone in vivo
Yagihashi et al. 2009[94]	Demineralized dentin matrix (DDM)	• DDM acts as a scaffold for osteochondral regeneration • Yielding active new bone formation early in the postoperative period.
Murata et al. 2010[95]	• Human demineralized dentin matrix (DDM) • Human demineralized root dentin (DRD)	• Both Humans recycled DDM and DRD might be effective materials as osteoinductive collagenous carriers of BMP-2 for bone engineering
Murata et al. 2012[74]	Human demineralized dentin matrix (DDM)	• Human DDM should be an effective carrier for delivering BMP-2 and superior scaffold for bone-forming cells.
Bormann et al. 2012[96]	Fresh perforated autogenous dentin slices	• Neovascularization response • Osteointegration with new bone

Table 6 (Continued)

Authors/Year	Dentin preparation forms	Results
Reis-Filho et al 2012[97]	Demineralized human dentin matrix (DHDM)	• Accelerates the bone healing, by stimulating bone deposition and neovascularization
de Oliveira et al 2013[75]	Demineralized human dentine matrix (DHDM)	• DHDM acted as a scaffold for osteoblast differentiation • Actively yielding new bone formation
Atiya et al. 2014[98]	Liquid nitrogen- treated calcified autogenous dentin	• Accelerating bone regeneration in bone defects in a manner similar to that of autogenous bone grafts

Table 7 The clinical studies demonstrated the potential of dentin in different preparation forms used as bone grafts substitutes

Authors/Year	Clinical uses	Results
Mônica M et al. 2006[99]	Socket preservation with PTFE membrane	• ADDM with membrane in 90th-day socket had Radiographic bone density similar to normal surrounding bone
Jeong et al. 2011[15]	Maxillary sinus augmentation	• Gradual resorption • New bone formation through osteoconduction and osteoinduction.
Kim et al. 2011[100]	Auto-tooth transplantation with autogenous tooth as graft material used between the root and the alveolar socket	• Reattachment completed after 10 months • Autogenous tooth-bone graft material induces bone formation in autotransplantation.

Table 7 (Continued)

Authors/Year	Clinical uses	Results
Park et al. 2012[101]	• Implant placement with GBR • Maxillary sinus graft • Socket preservation • Ridge augmentation	• No genetic and infectious risks • As strong as other graft materials • Providing good bone generation through osteoinduction and osteoconduction • Excellent initial bone remodeling capacity
Lee et al. 2013[102]	Implant placement with simultaneous GBR (with or without membrane)	• Significant bone gain in vertical bone defect sites regardless in use of membranes
Kim 2015[103]	Reconstruct defects at the osteotomy site simultaneously with or before implant placement.	• Favorable wound healing • No implant was lost after 12 months of prosthesis loading • New bone formation induced by the graft material.

The research problem

Can the demineralized tooth matrix be prepared in house and be used as bone graft substitution especially for ridge preservation?

The purposes of the study

1. To fabricate the demineralized tooth matrix used as bone graft material.
2. To investigate the clinical application of the use of demineralized tooth matrix for ridge preservation

The objective of the study

Primary objective

To evaluate the effects of autologous demineralized tooth matrix (auto-DTM) and platelet-rich fibrin (PRF) membrane in the preservation of alveolar ridge dimension after tooth extraction.

Secondary objectives

- To evaluate tissue response to the use of auto-DTM and PRF membrane for alveolar ridge preservation.
- To compare socket orifice closure between the auto-DTM and PRF membrane group (test group) to the PRF membrane only group (control group) in alveolar ridge preservation
- To alveolar ridge dimension changes between the auto-DTM and PRF membrane group (test group) to the PRF membrane only group (control group) in alveolar ridge preservation
- To radiographically compare marginal bone resorption and bone density between the auto-DTM and PRF membrane group (test group) to the PRF membrane only group (control group) in alveolar ridge preservation

Benefit of the study

The alveolar ridge preservation protocol using autologous DTM and PRF membrane could be utilized in Surgery Clinic, Dental hospital, Faculty of Dentistry, Prince of Songkla University

Chapter 2

Materials and Methods

Research design

The study was the split-mouth randomized clinical trial. The patients were selected according to criteria, then their wisdom tooth was extracted for demineralizing tooth matrix (DTM) preparation. After their premolar teeth were extracted, several measurements were performed for evaluations. The measurements continued for the next 8 weeks as scheduled. The framework is shown in Figure 2.

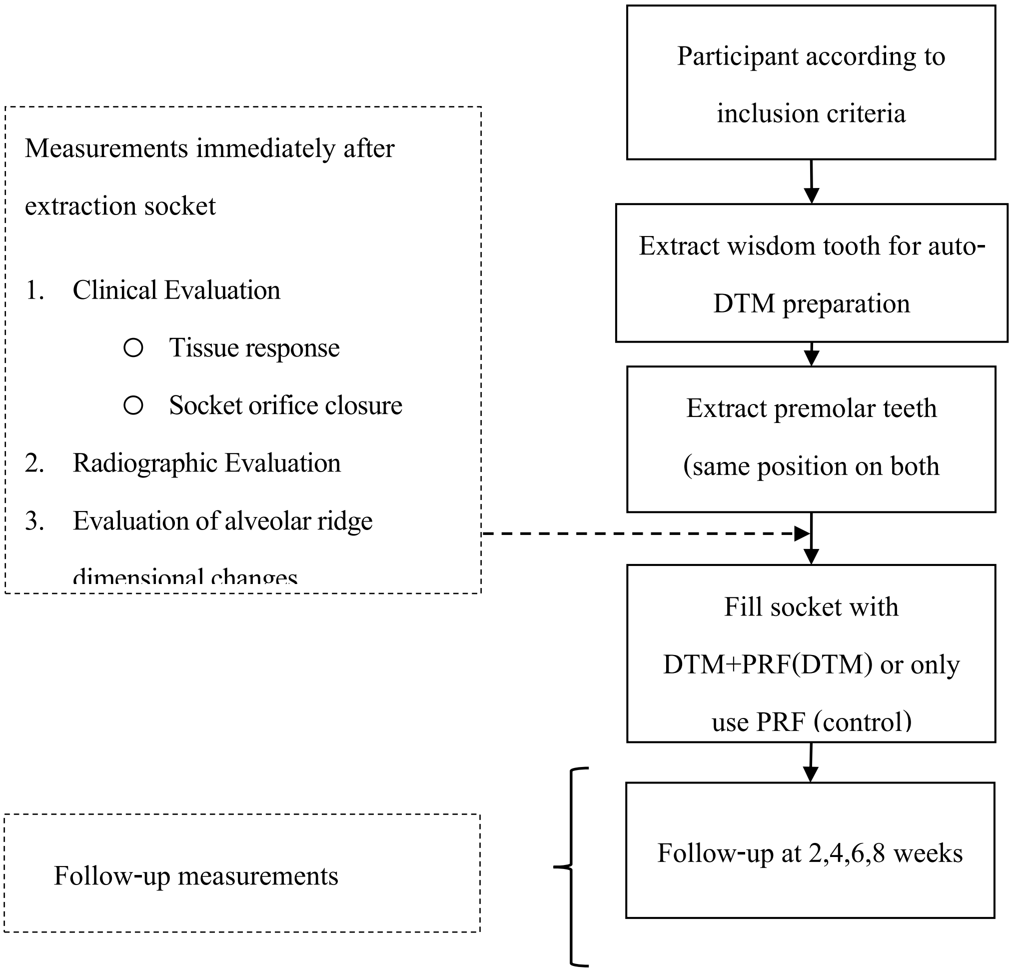

Figure 2 Framework of the study

Patients

Patients were randomly selected from the Surgery clinic, Dental Hospital, Faculty of Dentistry, Prince of Songkla University. The recruited patients were healthy adults (>20 years old) in need of symmetrical teeth extraction (1^{st} or 2^{nd} premolar) for orthodontic treatment and present 3^{rd} molar impaction. The patients' gingiva must be of thin gingival biotype. Characteristics of thin gingival biotype (104) are described as the followings: narrow zone of keratinized tissue, less than 1.5mm gingival thickness, 3.5-5mm gingival width, pronounced scalloped soft tissue and bony architecture, slight gingival recession, and thin marginal bone. Exclusion criteria were as follow: 1) patient who cannot come as scheduled post-operative evaluation, 2) smokers (patients who have smoked within 6 month), 3) patients with uncontrolled osteoporosis or other bone diseases, 4) patients with autoimmune disease, 5) patients under long-term steroidal or antibiotic therapy, 6) patients with local or systemic infection that may compromise normal healing (eg. extensive periapical pathology), 7) patients who present clinical and/or radiographic signs of active periodontal disease, 8) patients with pregnancy, 9) patients with history of malignancy.

Sample size calculation and sampling techniques

Estimated sample size for two-sample comparison of means was calculated using data from Suttapreyasri and coworker's study[20]. The output of the sample size calculation for testing two dependent means (two-tailed test) are calculated from the formula:

$$n = \frac{(z_{1-\frac{\alpha}{2}} - z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

SD.(σ) = 0.26, Delta (Δ) = 0.19

Alpha (α) = 0.05, Z(0.975) = 1.959964

Beta (β) = 0.20, Z(0.800) = 0.841621

Calculated sample size (n) = 15, however, the study enrolled more subjects to account for potential dropouts, therefore, actual sample size for each group was 20.

The extraction sites were randomly assigned into two groups as shown in Table 8.

Table 8 Study group categorization and sample size

Study Group	Details	Number of socket (n)
Group 1 DTM	Socket grafted with DTM and sealed with PRF membrane	20
Group 2 Control	Socket sealed with PRF membrane	20

Fabrication of auto-DTM

The autogenous caries-free wisdom tooth was extracted prior to the alveolar ridge preservation at least one week. Soft tissues including the periodontal ligament and pulp tissue were removed mechanically by hands and rotary instruments. The tooth was stored in liquid nitrogen at -196 °C before use, then the tooth was pulverized into small particles by a freezer mill (6770 Freezer/Mill®, SPEX SamplePrep, USA). Sieves with 500 µm and 700 µm aperture (Endecotts, London UK) were used to select desired particle size (Figure 3). The particle was defatted in chloroform:methanol (1:1) solution for 12 hours followed by washing in double-distilled water. Then, the particle was demineralized in stirring 0.5M hydrochloric acid at 1:20 weight(mg) to volume(ml) ratio, at 4°C for 3 hours. The auto-DTM particle was washed in a large volume of distilled water and lyophilized. The freeze-dried auto-DTM particle was sterilized by ethylene oxide gas. Before application, auto-DTM was mixed with a saline solution. The particle characteristics were shown in Figure 4.

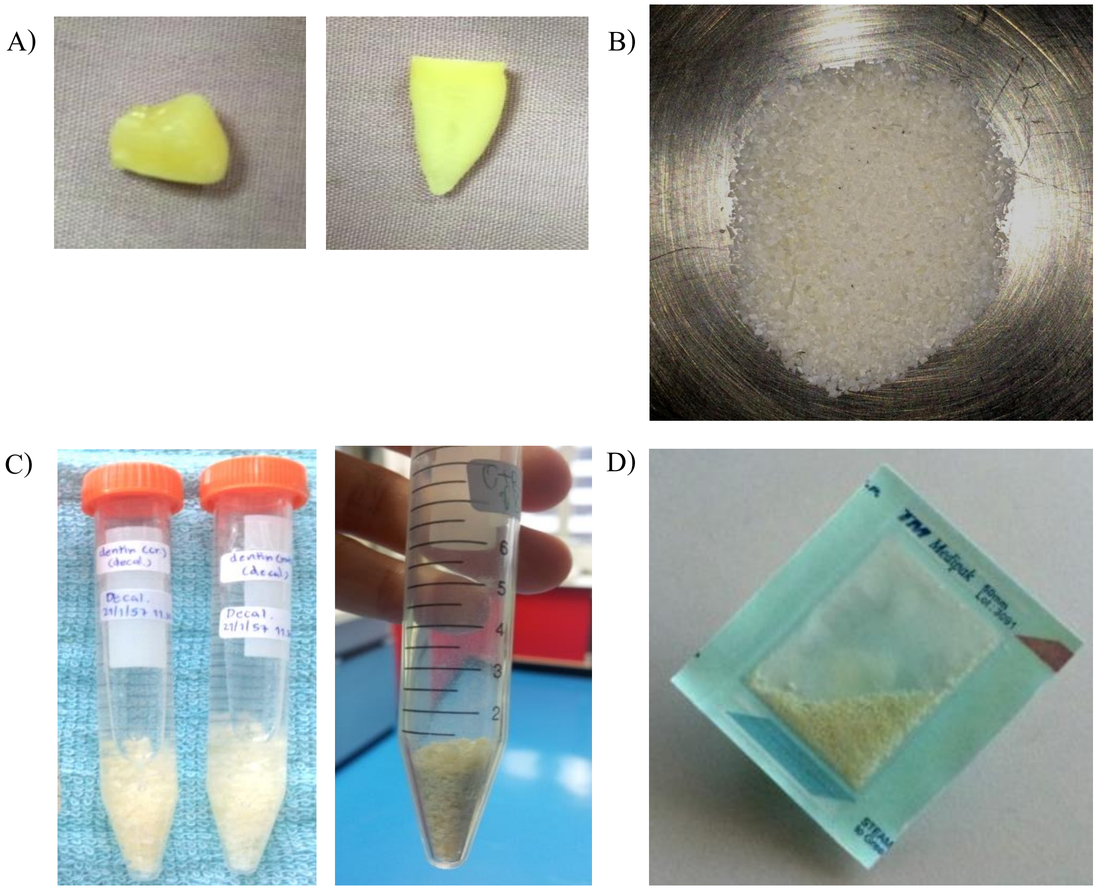

Figure 3 Demineralize tooth matrix fabrication procedure. A) The tooth was section and the soft tissue including dental pulp and periodontal ligament was removed. B) The tooth was pulverized into small particle sized 500-700 µm using freezer mill and sieves. C) The tooth particles were defatted (left), demineralized (right) and freeze-dried. D) The demineralized tooth matrix was sterilized using ethylene oxide gas before use.

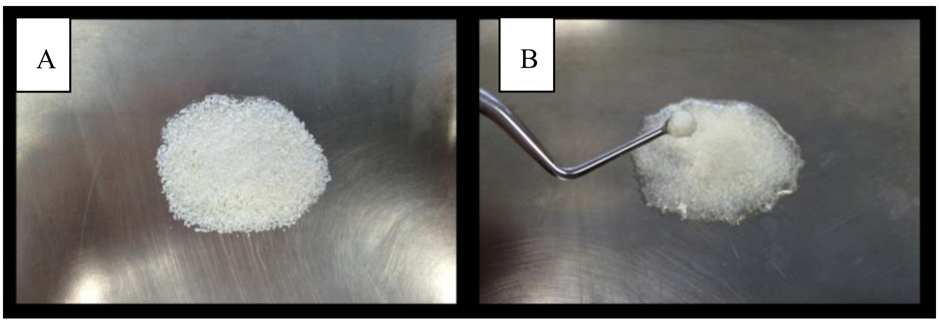

Figure 4 Auto-DTM characteristics A) Auto-DTM particles; Dry condition, B) Auto-DTM; Soaked with normal saline solution

PRF membrane preparation

Ten milliliters of autologous whole blood were obtained using needle gauge no.20 and a 10-ml syringe without anticoagulant from a median cubital vein (forearm). The collected blood was transferred into a 10-ml glass tube. Then, it was immediately processed with a centrifuge (Hettich Zentrifugen centrifuge EBA 20, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 3,000 revolutions/min for 10 minutes. In the middle of the 10-ml glass tube between the acellular plasma at the top and the red corpuscles at the bottom, the fibrin clot was then obtained. The fibrin clot collected was compressed by sterile spoons to create PRF membrane, which was sectioned longitudinally for further usage (Figure 5).

Extraction and post-extraction Protocol

A local anesthesia (4% articaine hydrochloride, Ubistesin 1:200,000; 3M ESPE, Platz, Seefeld, Germany) was applied at the extraction sites. The tooth then was gently luxated with an elevator and carefully extracted with extraction forceps with an intention to lessen the trauma occurred to the surrounding bone as much as possible. The two alveoli of each jaw were randomly filled with either auto-DTM particle and sealed with PRF (DTM Group) or only sealed with PRF (control group). The randomization was done using sealed envelopes prepared by an independent party

In the DTM group, the extraction sockets were packed with auto-DTM in layers until the extraction sockets were filled up to one mm below the marginal bone and then covered with PRF membrane. On the other hand, the control site the sockets were only sealed with PRF membrane. Figure-of-eight suture with resorbable suture material (Vicryl 4-0; Ethicon, Norderstedt, Germany), were used to secure the graft material in the socket during the early healing period (Figure 6). Postoperatively, patients were prescribed antibiotics and anti-inflammatory medicines. Sutures were removed two weeks after the operation. Clinical and radiographic evaluation of the extraction sites were performed at baseline (T0, immediately after tooth extraction), 2 (T2), 4 (T4), 6 (T6), and 8 (T8) weeks following tooth extraction.

A)

B)

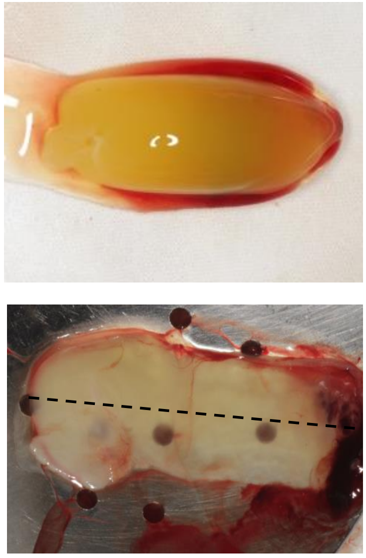

Figure 5 PRF membrane preparation A) 10-ml blood was drawn and centrifuge for 10 minutes at 3,000 rev/min. B) PRF membrane was prepared by compressing with sterile spoons and then sectioning longitudinally

A)

B)

C)

Figure 6 Extracted socket A) immediate after extraction. B) ridge preservation using PRF membrane (control group) C) alveolar ridge preservation using auto-DTM and PRF membrane

Clinical evaluation

Socket characteristic after tooth extraction

After the tooth was removed, the buccal and lingual bone wall was carefully investigated for bone wall fracture, bone dehiscence, and bone plate loss.

Tissue response evaluation

Several signs of tissue response were evaluated including, the texture and color of the soft tissue covering the extraction site, the appearance of the gingival tissues at the buccal and lingual aspects, and the existence of graft particle migration outside of the socket.

Socket orifice closure evaluation

The measurement of the dimensions of the socket orifice (mesial-distal [M-D] and buccal-lingual [B-L]) width were done directly from the midpoint of the inner socket orifice of the extraction site (Figure 7). The measurements were performed by one investigator using a UNC-15 periodontal probe (Hu-Friedy, Hu-Friedy Mfg. Co., Chicago, IL, US). Data were collected at immediate post operation (T0), follow-up time of 2 weeks (T2), 4 weeks (T4), 6 weeks (T6), and 8 weeks (T8). The socket orifice closure (%) was calculated from orifice dimension reduction from the baseline (T0) at each time point.

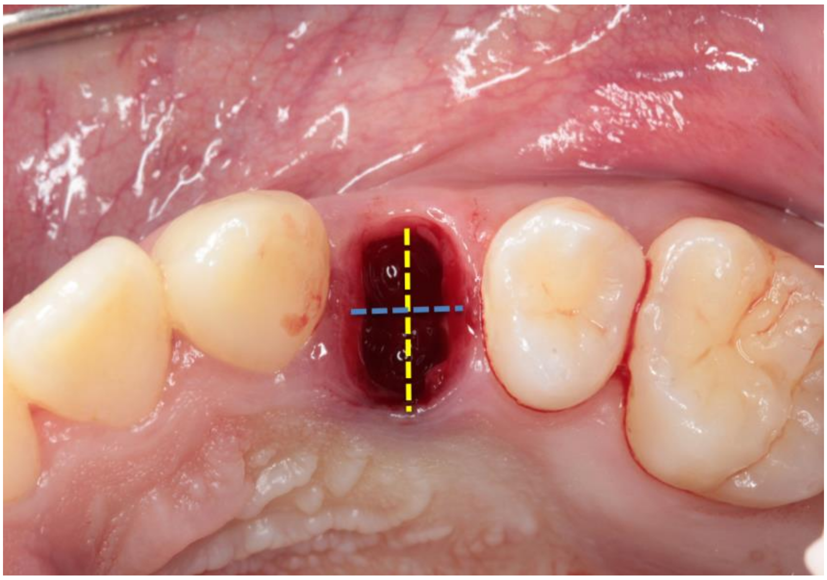

Figure 7 The dimensions of the socket orifice (mesial-distal [M-D] and buccal-lingual [B-L]) width

Cast-based evaluation

The alveolar ridge dimensional change was assessed on a 2D digital model. Each cast was made with dental stone (GC Fujirock type 4; GC Corp, Tokyo, Japan) immediately after tooth extraction (T0) and at each of the follow-up times (T2, T4, T6, and T8 at 2, 4, 6, and 8 weeks after the extraction respectively).

The cast at each time point was scanned with model scanners (3Shape D700, Copenhagen, Denmark). To determine the alveolar ridge dimensional change, the 2D model at T2, T4, T6 and T8 were superimposed with that of T0 using Ortho AnalzerTM software (3Shape, Copenhagen, Denmark).

A test for model measurement error was performed by measuring and compare the dimension of a tooth adjacent to the socket location. The dimension of the tooth measured at each period during the study were compared and were found to be consistent which lead to the conclusion that measurement was accurate.

Then each edentulous site with the superimposed cast was measured for the dimension change of ridge width. The buccal and lingual tissue contours were measured using Ortho AnalyzerTM software in the following way (Figure 8-10): a horizontal reference line (Href) was drawn to connect the cemento-enamel junction (CEJ) of the adjacent teeth, a vertical line perpendicular to Href line was drawn 3mm apically down from the middle of the socket. The end of the line was used as a reference point (Pref) to measure both buccal and lingual ridge resorption.

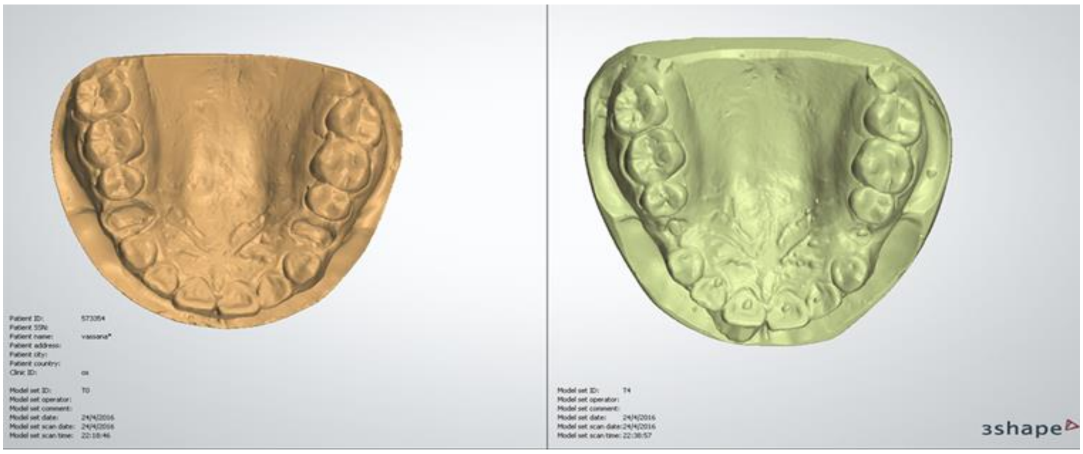

Figure 8 Cast at T0 (yellow) and T4 (green) captured by Ortho Analyzer™ software

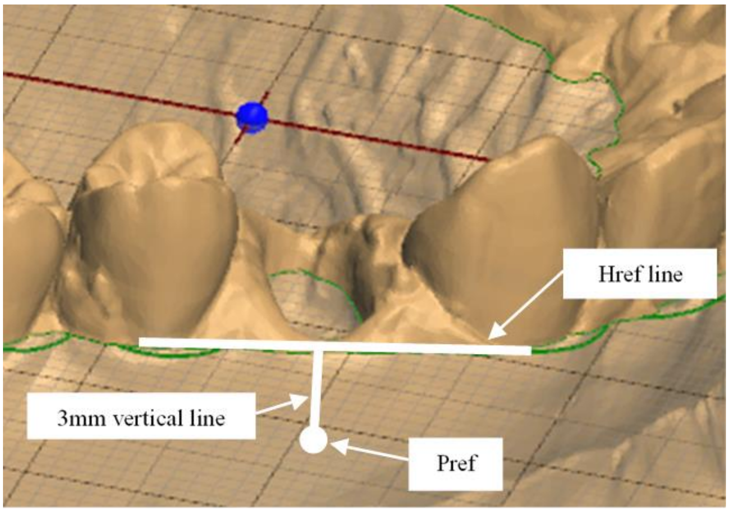

Figure 9 Horizontal reference line (Href) and Pref point

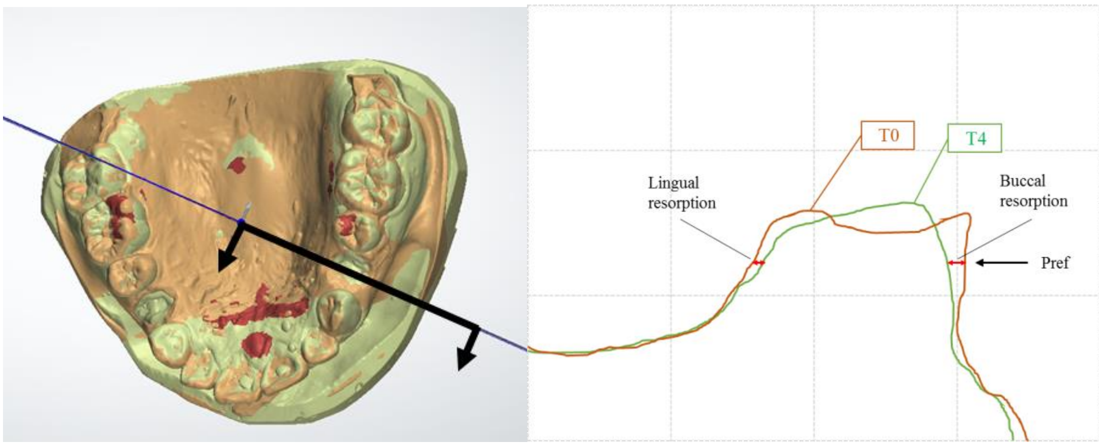

Figure 10 Measurement of alveolar ridge dimensional change from superimposed cast, ridge resorption values were then measured at the level of Pref on both buccal and lingual side.

Radiographic evaluation

The standardized periapical radiograph was taken digitally using a wireless portable dental x-ray system with a digital sensor size 2 (BPD-I, BEMEMS Co. Ltd., South Korea) with a standardized custom lead step wedge attached to the sensor holder (XCP-DS®, Rinn, Dentsply, IL, USA).

A custom lead step wedge preparation was arranged by using a lead strip from film No.2. The first stripe was cut 5 mm in width and 30 mm in length. The remaining 5 strips must be cut progressively 5 mm shorter in length but with the same width. The stripes were placed one on top of the other, starting with the longest and getting progressively smaller until a series of even steps were built up, then the strips were glued together and the films were sealed.

To achieve reproducible periapical images, the paralleling technique was used with an occlusal bite index prepare from pattern resin (DuraLay, Reliance, Dental Mfg Co, IL, USA) fixed to a trollbiten film holder. The bite index was save for use at all visits. The exposure time was set to be 0.35 seconds for every participant.

The resorption of marginal bones at the extraction site was determined using image analysis software (Apexia Digital Imaging Software 3.0, Masterlink, LLC., Glendale, California) in the following way (Figure 11A). A radiographic cemento-enamel junction (xCEJ) line was drawn to connect the CEJ of the adjacent teeth. Then, the vertical lines perpendicular to the reference line were drawn and measured from the most coronal prominent point mesially (M), distally (D), and from the center of the sockets (C).

The density of the socket preservation site was measured from a 2x2 mm area located at the end of the 5-mm line drawn perpendicularly from the reference line to the middle of the socket (Figure 10B).

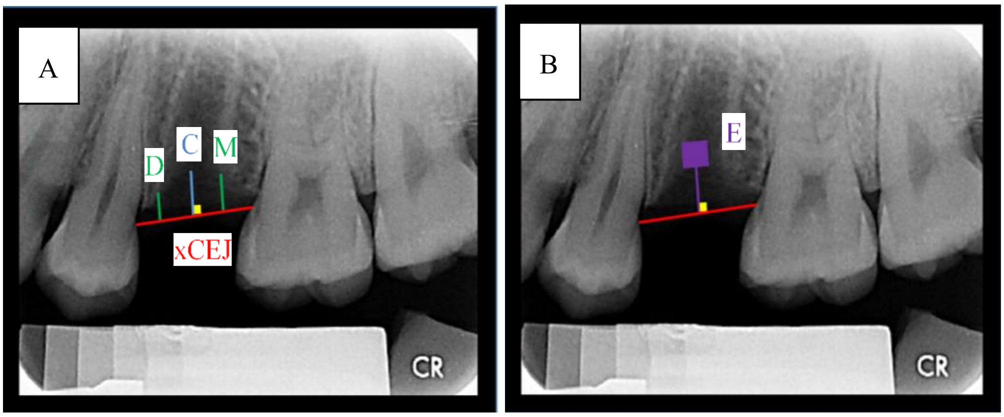

Figure 11 The resorption of marginal bones and the width of the socket orifice at the extraction site. xCEJ-line; radiographic cemento-enamel junction line, C; bone resorption distance at the center of the socket orifice, D; bone resorption distance at the distal side, M; bone resorption distance at the mesial side, E; density measurement area

Data analysis and interpretation

All data were present in means and standard deviations (SD). One-way analysis of variance and a Post hoc test with the Scheffé test was applied to detect differences among groups, when appropriate. The paired T-Test was used to analyze the difference between the 2 groups (control/DTM). The statistical analysis was performed using SPSS (version 13, SPSS, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Ethical considerations

The study protocol was approved by the Ethic Committee of the Faculty of Dentistry, Prince of Songkla University, Songkla, Thailand (MOE 0521.1.03/709). All included subjects were informed consent before participation. and required to read, understand, and sign the consent form, which included a thorough explanation of expected benefits and possible risks

Chapter 3

Result

Demographic data

A total of 40 extraction sites (24 maxillary premolars, 16 mandibular premolars) from 12 subjects (10 women, 2 men), aged 20.0 to 22.0 (20.5 ±0.80 years), were included in the study. Every patient attended the study regularly.

Clinical Evaluation

Socket characteristic after tooth extraction

All extraction sites healed uneventfully. After tooth removal, the socket was carefully investigated. Three incomplete buccal plate fractures were found (2 sites in the DTM group and one site in the control group). However, neither bone dehiscence nor buccal bone plate loss was observed in either groups. No infection or complication of any kind was found after the operation.

Tissue response to auto-DTM

Soft tissue that covered the extraction site was normal in terms of texture and color, and the gingival tissues around the extraction site and at adjacent teeth appeared to be clinically healthy. There was no graft particle migration outside of the socket.

Socket orifice closure evaluation

Soft tissue healing at the socket orifice was completed by 6 weeks after the extraction in both groups. The socket orifice closure (in percentage, where 100% represents a complete closure of the orifice) between baseline (T0) and the follow-up time in the M-D and B-L directions were presented in Figure 12, 13 and Table 9. At 2 and 4 weeks after tooth extraction, the B-L width and the M-D width of socket orifice closure in the control were significantly better than in the DTM.

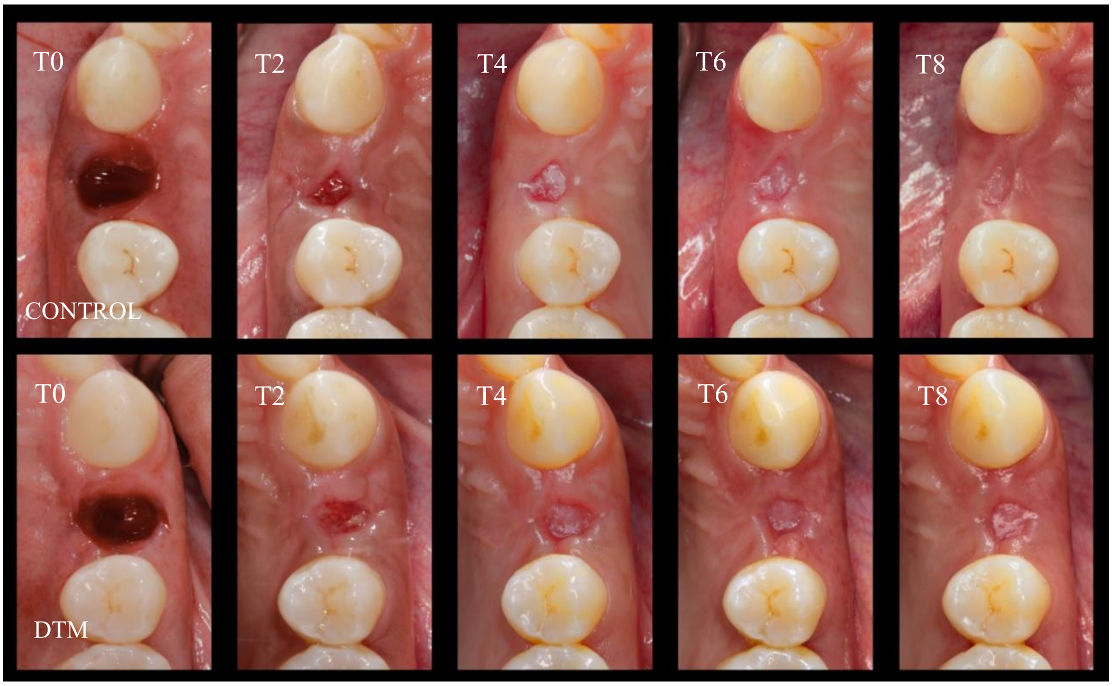

Figure 12 The clinical appearance of the healing socket from immediately after extraction (T0) and each follow-up period (T2, T4, T6, and T8: 2, 4, 6, and 8 weeks after the extraction, respectively).

Table 9 The mean Percentage of socket orifice reduction (%)

Time	**Bucco-lingual**			**Mesio-distal**		
	Control	**DTM**	**p-value**	**Control**	**DTM**	**p-value**
T2	50.4±16.3	45.0±15.7	0.031*	48.2±13.1	41.9±12.3	0.102*
T4	63.6±15.0	58.7±17.8	0.042*	59.9±14.3	51.8±17.1	0.018*
T6	100.0±0	100.0±0		100.0±0	1.000±0	
T8	100.0±0	100.0±0		100.0±0	1.000±0	

Values are presented as mean ± SD.

*Significant different between group (control/DTM)

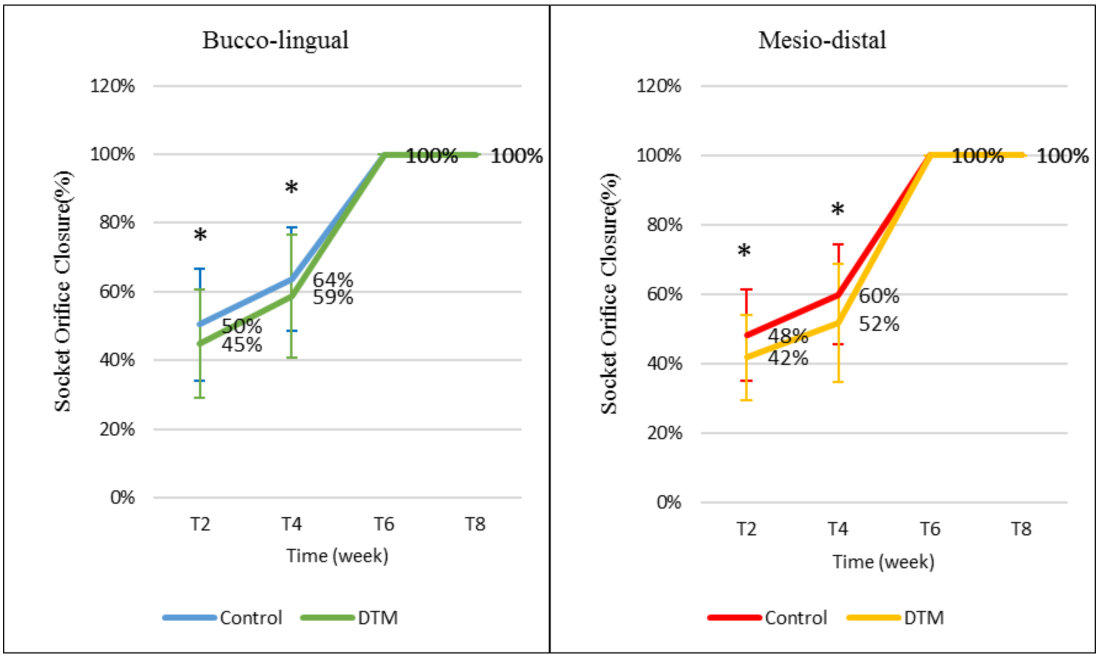

Figure 13 The socket orifice closure in percentage in the bucco-lingual direction (left) and the mesio-distal direction (right) *Statistically significant different at P<0.0

Cast-based evaluation

The dimensional changes at the buccal side and lingual/palatal side were shown in Figure 14 and Table 10. At baseline, no statistically significant difference between the control and the DTM group was found in any parameters assessed (p>0.05). In both groups, the buccal contours reduction was more pronounced than the lingual side. The buccal contraction in the DTM group was significantly less than the control group at every time point. The lingual resorption in auto-DTM was also less than the control, however, no statistically significant differences were found among groups for all time frames. All statistical tests were done at P<0.05.

Table 10 The dimensional change in the reduction buccal and lingual/palatal sides of the extraction site from cast-based measurements.

Time	Lingual-Resorption			Buccal-Resorption		
	Control	DTM	p-value	Control	DTM	p-value
T2-T0	0.43±0.18	0.40±0.14	0.441	0.53±0.17	0.39±0.11	0.004*
T4-T0	0.62±0.23	0.55±0.13	0.154	0.858±0.29[a]	0.66±0.24	0.004*
T6-T0	0.79±0.25[a]	0.67±0.16[a]	0.050	1.14±0.28[a]	0.89±0.34[a]	0.002*
T8-T0	0.92±0.29[ab]	0.80±0.14[ab]	0.072	1.34±0.37[ab]	1.04±0.40[ab]	0.001*

Values are presented as mean ± SD.

*Significant different between group (Control-DTM)

[a]Significant different from ΔT2 in each group

[b]Significant different from ΔT4 in each group

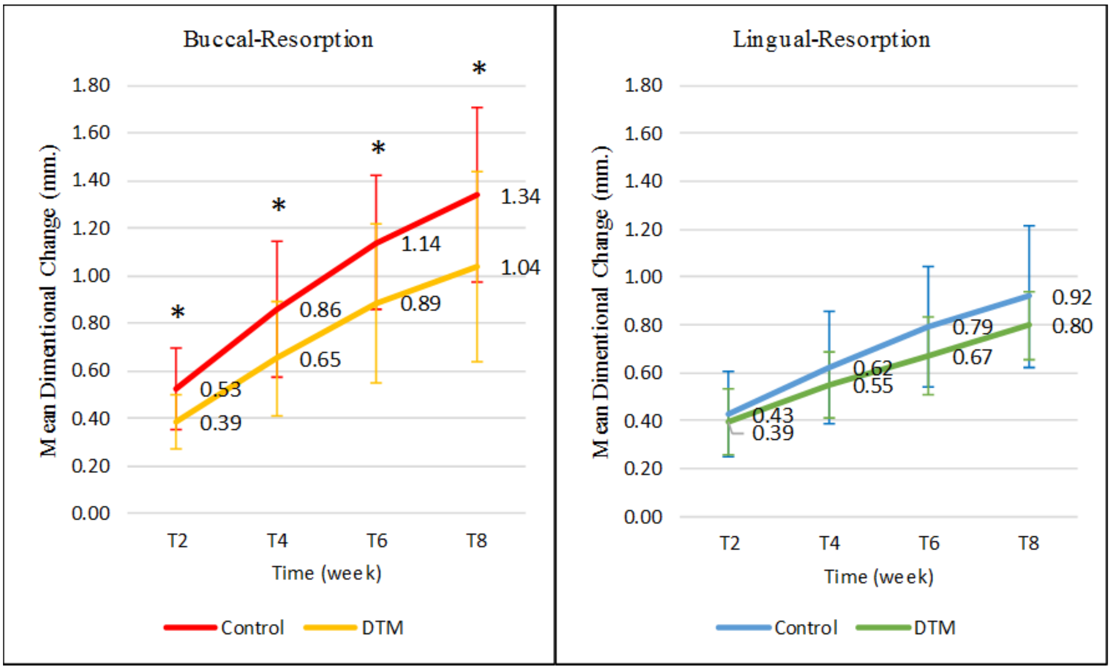

Figure 14 The buccal and lingual contour reduction of the extraction sites.

*Statistically significant different at $P<0.05$

Radiographic evaluation

The accumulated radiographic resorption distances of marginal bone on the mesial side, distal side, as well as at the center of the sockets in the DTM group were not significantly different from those of the control group. No statistically significant differences were detected between the groups throughout all time frames at 2, 4, 6, and 8 weeks after extraction ($P > 0.05$) (Figure 16, Table 11-13).

Throughout the 8-weeks period, bone healing density in the DTM group was higher than in the control group. However, the result was only statistically significant during the first 6 weeks ($P < 0.05$) (Figure 15,17, Table 14).

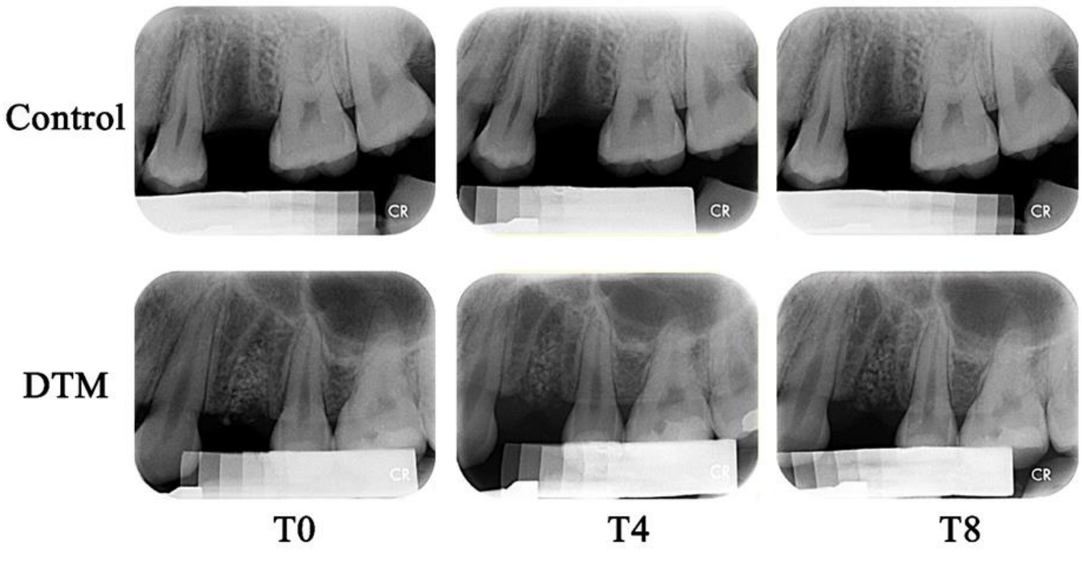

Figure 15 The radiographs of the socket sites after extraction (T0), at 4th week (T4), and at 8th week (T8)

Table 11 Radiographic resorption of marginal bone levels at mesial to the extraction site

Time	Mesial marginal bone		
	Control	**DTM**	**p-value**
T2-T0	-0.27±0.23	-0.20±0.20	0.330
T4-T0	-0.48±0.25	-0.45±0.26	0.707
T6-T0	-0.71±0.28	-0.57±0.28	0.168
T8-T0	-0.86±0.31	-0.67±0.47	0.202
Values are presented as mean ± SD .			

Table 12 Radiographic resorption of marginal bone levels at distal to the extraction site

Time	Distal marginal bone		
	Control	**DTM**	**p-value**
T2-T0	-0.23±0.22	-0.22±0.17	0.967
T4-T0	-0.48±0.34	-0.52±0.30	0.682
T6-T0	-0.70±0.36	-0.77±0.35	0.474
T8-T0	-0.81±0.42	-0.93±0.39	0.378
Values are presented as mean ± SD .			

Table 13 Radiographic resorption of marginal bone levels at center to the extraction site

Time	Height of the socket		
	Control	**DTM**	**p-value**
T2-T0	-0.20±0.17	-0.26±0.24	0.409
T4-T0	-0.35±0.21	-0.50±0.37	0.143
T6-T0	-0.47±0.23	-0.64±0.39	0.120
T8-T0	-0.70±0.28	-0.79±0.47	0.451
Values are presented as mean ± SD .			

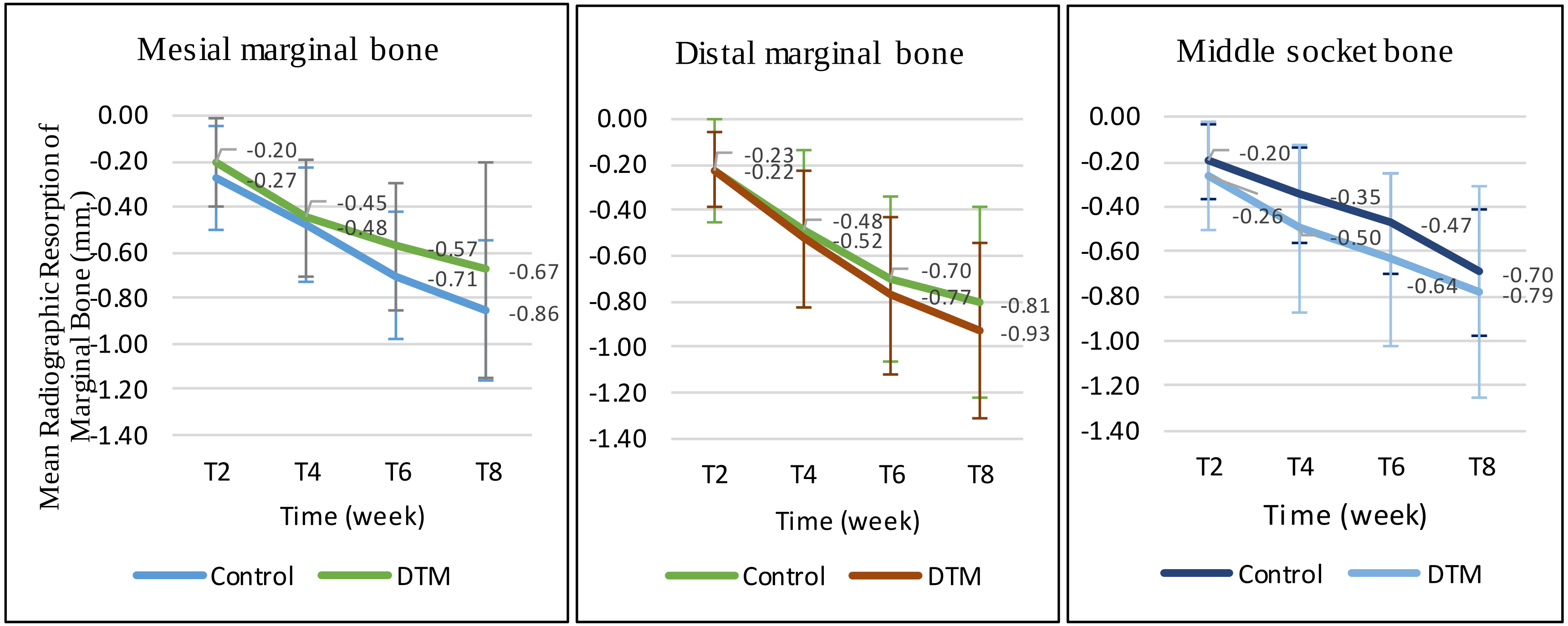

Figure 16 The marginal bone resorption at the extraction sites measured from radiographs. *Statistically significant different at P<0.05

Table 14 The radiographic of density to the extraction site

Time	Density		
	Control	**DTM**	**p-value**
T0	23.39±7.62	37.17±7.21	0.000*
T2	23.02±6.74	38.91±6.00	0.003*
T4	31.32±14.99	45.22±13.27	0.023*
T6	31.28±13.42	48.03±8.95	0.019*
T8	35.85±15.15	44.84±9.12	0.253

Values are presented as mean ± SD .

*Significant different between group (Control-DTM)

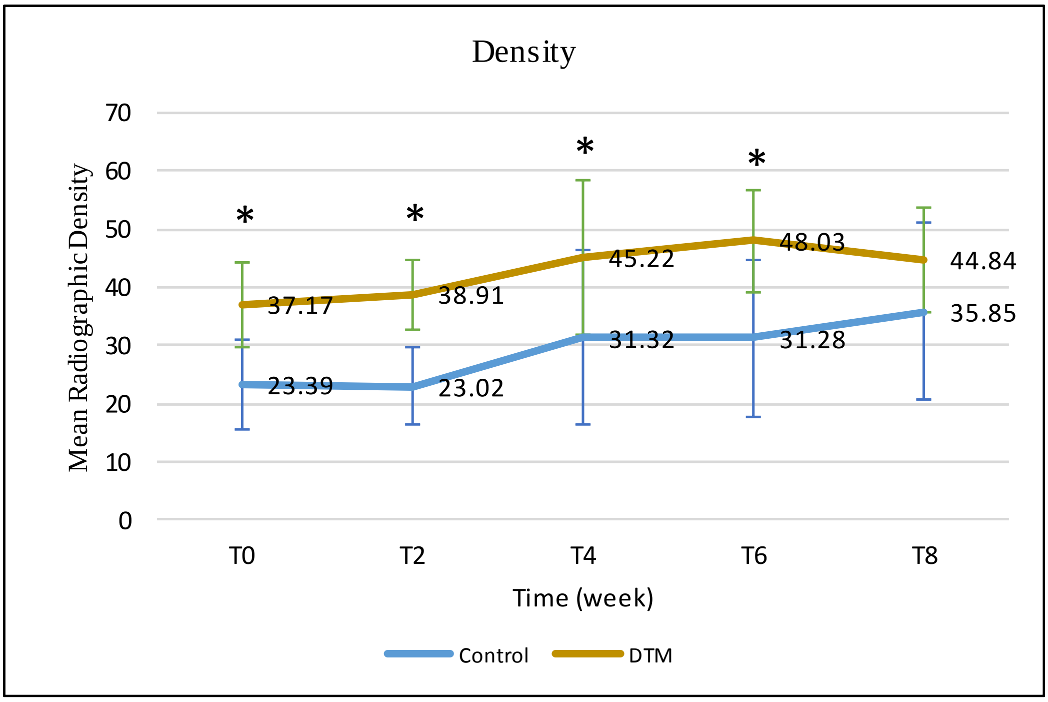

Figure 17 The radiographic density at extraction sites. *Statistically significant different at P<0.05

Chapter 4

Discussion

As there are various materials for alveolar ridge preservation, this present study investigated the use of autologous DTM in combination with PRF membrane in freshly extracted sockets. The advantages of autologous DTM over xenogeneic or alloplastic bone graft substitution are 1) the tooth matrix is autologous; thus, the possibility of graft rejection is low, 2) the graft is osteoinductive since it contains various growth factors including Bone morphogenetic proteins (BMPs), Transforming growth factor-beta (TGF-β), Insulin-like growth factor -1 and -2 (IGF-1 and-2)[105] and 3) the cost of graft is cheaper than commercially xenograft or alloplastic bone graft substitutions.

The use of autologous DTM in combination with PRF membrane showed good biocompatibility property. Starting from the 6th week of the study, socket orifice closure of the DTM group was not significantly different from the control group. There is no abnormality of the soft tissue that covered the extraction site, the gingival tissue around extraction site or the adjacent teeth. No inflammations, graft particle migrations, or any complications were found after the ridge preservation. This result demonstrates the biocompatibility property of the auto-DTM which is comparable to the use of teeth as graft material as mentioned by the previous study[106].

The study also demonstrated high osteoconductivity property of auto-DTM which helps maintain ridge dimension. The total horizontal ridge change at 8-week post extraction in the DTM group was 1.84±0.475 mm, which is comparable with the result from previous studies using various graft materials. Aimtti M et al[64] reported 2 mm of the horizontal bone resorption after 3 weeks of preserving the ridge with calcium sulfate. Kesmas et al.[65] used biphasic calcium phosphate (HA/β-TCP: 60/40) in combination with collagen membrane and demonstrated 2 mm of horizontal ridge changes at 16 weeks postoperative. In addition, Brownfield and Weltman[69] utilized the demineralized bone matrix with cancellous bone chips as graft material and demonstrated 1.6±0.8 mm of the total horizontal ridge changes at 10-12 weeks postoperative. The greater horizontal ridge contraction in this present study was possibly resulted from two reasons; 1) auto-DTM has biodegradation property leads to the reduction in ridge dimension, and 2) the inclusion of subjects with thin gingival biotype or thin buccal plate which make them more prone to buccal plate resorption.

The inclusion of subjects with thin gingival biotype or thin buccal plate may also contribute to the greater horizontal ridge contraction in this study. From a study by Kao and Pasquinelli in 2002[107] found that patients with thick bone plate usually had thick gingiva biotypes while patients with thin bone plate usually showed thin gingiva biotypes. Both biotypes respond differently to tooth extraction. Patients with thick biotypes are found to has little buccal plate resorption when compared to those with thin biotypes. Additionally, trauma during tooth extraction in patients with thin biotypes might destroy labial plate which also contribute to the resorption.

Biodegradation characteristic of the graft material is one of the important factors which contribute to the alveolar ridge resorption which is undesirable, but at the same time it provides space for new bone formation. The appropriate material should be able to prevent the resorption until the desired time to perform implant placement. Thus, the clinician should select graft materials according to the timing requirement. The present study found that the density of radiographs from DTM group was not significantly different from those of the control group at 8 weeks postoperative. These results indicated the bioresorbable property which cohere to other relevant case report[108] where the used auto-DTM was prepared from the same protocol. In the case report by Ouyyamwongs et al.[106], at the time of implant placement a trephine biopsy was harvested. Histologic analyses of the trephine biopsy showed osteoblastic rimming, graft resorption and new active bone formation.

A greater amount of resorption on buccal side was documented when comparing to that on the lingual side, which was consistent with prior studies[3, 109, 110]. The buccal bone comprises mainly of bundle bone, whereas the lingual bone, although also comprises of bundle bone, has much less in percentage. Consequently, buccal bone resorption level is higher since it contains a higher proportion of resorption-prone bundle bone. The previous study showed that even with various ridge preservation techniques, there will eventually be some loss of the bone[24].

Regarding the results of our study, the use of auto-DTM could, to some extent, maintain ridge dimension after extraction but will eventually subject to some loss as mentioned in the prior studies.

In the present study, the reduction of mesial and distal bone resorption, as well as, level of the bone in the center of socket were examined using periapical radiograph, However, there was no significant difference between two groups. Because of the examined ridge was situated between adjacent teeth, the Shapey's fiber of the adjacent teeth could anchor the periosteum and maintain the mesial and distal marginal bone level. Regarding the limitation in the plain radiographs which revealed only 2-dimension of the true 3-dimension anatomy, the level of bone height which was the average of buccal and lingual bone wall was difficult to identify. Cone-beam CT, if available, is recommended for the measurement of the delicate images.

Usage of the resorbable membrane, as well as other socket covering materials, have been actively investigated, but no consensus has been reached. The shortcoming of using a resorbable membrane in socket preservation was reported[111, 112] such as a tendency to uncover, to stimulate the inflammatory cell response, and to require longer surgical time and more treatment charge. To avoid the complications, the present study did not use occlusive membranes and a PRF membrane closure of the socket was applied instead.

Regarding PRF membrane influence, a study by Suttapreyasri and Leepong[20] found that the PRF revealed no advantage in maintaining alveolar ridge dimension but it hastened soft-tissue healing. Therefore, in this study, the PRF was utilized as the sealing material in both DTM and control group only to retain auto-DTM particles within the sockets and helps hasten the orifices closure. As PRF had no effect on ridge preservation, the conservation property found in the study was assumed to be from the auto-DTM alone. In this study, during the first four weeks the sockets of the control group close faster than the sockets of the group with the combination of auto-DTM and PRF. This may be because DTM particle hindered the healing process. However, eventually sockets from both groups are completely healed at 6th week.

This study demonstrated that PRF membrane can be used as extracting socket cover for socket preservation. The membrane can effectively hold graft material within the socket without any graft migration during the 8-week study period. Infection was not found, probably because the PRF capability to reduce a chance of postoperative infections.

With support from various studies on autogenous tooth as bone graft material, the results confirmed that autogenous tooth was a safe and effective graft material. If we can develop the preparation processes to the point that the graft materials can be used in other patients without the risk of immune rejection or disease transmission, it would allow clinicians to access an almost unlimited amount of materials. The DTM is further suggested to have become more widely used and to be developed into allogenic tooth bone graft materials, an ideal scaffold for bone tissue engineering in the future. It is possible to develop a tooth bank where extracted tooth, instead of becoming merely a medical waste, can be kept so that it can be used as bone graft for the patient or even other patients in the future. In order to make the tooth bank concept feasible, further study should be conducted in many areas, such as the appropriate conditions to keep extracted tooth in usable condition, the cost-efficient way to run the tooth bank, or the technique to adjust growth factor level in the material.

The trend of development might progress toward the path that allows alloplast, xenograft, and allograft to achieve the same level, or even, better properties as autologous graft materials. However, one aspect that will maintain the use of autologous materials as the first priority for many patients is the fact that the materials come from the patient's own body, thus giving no negative emotional feelings toward using the materials. In practice, many factors such as cultures or religious beliefs lead patients to deter from accepting the use of biomaterials from other people or some specific species of animals.

Chapter 5

Conclusion

This randomized clinical trial study demonstrated that the auto-DTM was a tissue-compatible material and could be recognized as bone graft substitution. Alveolar ridge preservation using auto-DTM and PRF membrane are a reliable method for preserving alveolar dimensions. The graft material reduced buccal collapse and accelerated the speed of bone density increment when compared to those of the control group throughout the study period.

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Appendix

ที่ ศธ 0521.1.03/ 709

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

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง "การใช้ดีมินเนอราไลซ์ทูธเมทริกซ์ชนิดอัตพันธุ์ในการคงสภาพสันกระดูกเบ้าฟัน"

รหัสโครงการ EC5804-15-P- HR

หัวหน้าโครงการ ทันตแพทย์หญิงวริศรา อุยยามวงศ์

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 1 9 มิ.ย. 2558

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Oral Presentation

Ouyyamwongs W, Suttapreyasri S, Samruajbenjakun B. Leepong N "Autologous demineralized tooth matrix as bone grafting material for alveolar ridge preservation" in The Expanding Knowledge for Better Dental Practices, The Royal College Of Dental Surgeons Of Thailand, 14-15 September 2016, Centara Grand at Central World, Bangkok, Thailand.