



CUSTOM 3D PRINTED HYDROXYAPATITE BONE BLOCK GRAFT
FOR ALVEOLAR RIDGE RECONSTRUCTION :
A PILOT CLINICAL STUDY

BY

MISS PICHAYA MEKCHA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE PROGRAM IN DENTAL
IMPLANTOLOGY

DEPARTMENT OF IMPLANTOLOGY

FACULTY OF DENTISTRY

THAMMASAT UNIVERSITY

ACADEMIC YEAR 2021

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ENTITLED

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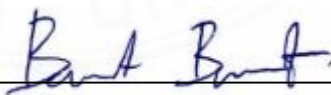
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ABSTRACT

In this clinical pilot study, custom 3D printed nanohydroxyapatite (3DHA) graft was specially fabricated to fit a patient's defects for primary bone augmentation before implant placement. This tailored design block graft was obtained from each patient's cone beam computed tomography (CBCT) which was converted into stereolithography (STL) file format. Computer-aided design (CAD) software was used to design the graft by imitating natural bone structure with cortex and trabecular patterns. Overall, 3DHA block graft was successful in 10 out of 12 patients. By incorporating concentrated growth factors (CGFs) and platelet-rich fibrin (PRF) membrane with 3DHA can reduced graft failure from early membrane exposure at the grafted site. No adjustment of the graft was needed. All 3DHA adapted and fit well in all the defect sites. Maximum mean horizontal bone gains were 3.05 ± 0.72 and 3.45 ± 0.41 mm from DICOMs and STL data set respectively. The volume gain was 223.8 ± 80.46 mm³. Low pain score after surgery was reported as 1.41 ± 0.51 , while the healing index score was increased with a maximum mean of $HI = 4.7 \pm 0.67$. Thirteen implants were placed with good primary stability ($ISQ = 65 \pm 4.08$) without additional GBR. Histomorphometric

analysis revealed new bone formation, bone tissue, residual grafts and connective tissue were 28.6 ± 1.88 , 30.48 ± 4.81 , 19.82 ± 4.07 , and $20.81 \pm 4.41\%$.

Keywords: 3D printing, Customized block graft, Nanohydroxyapatite, Concentrated growth factor



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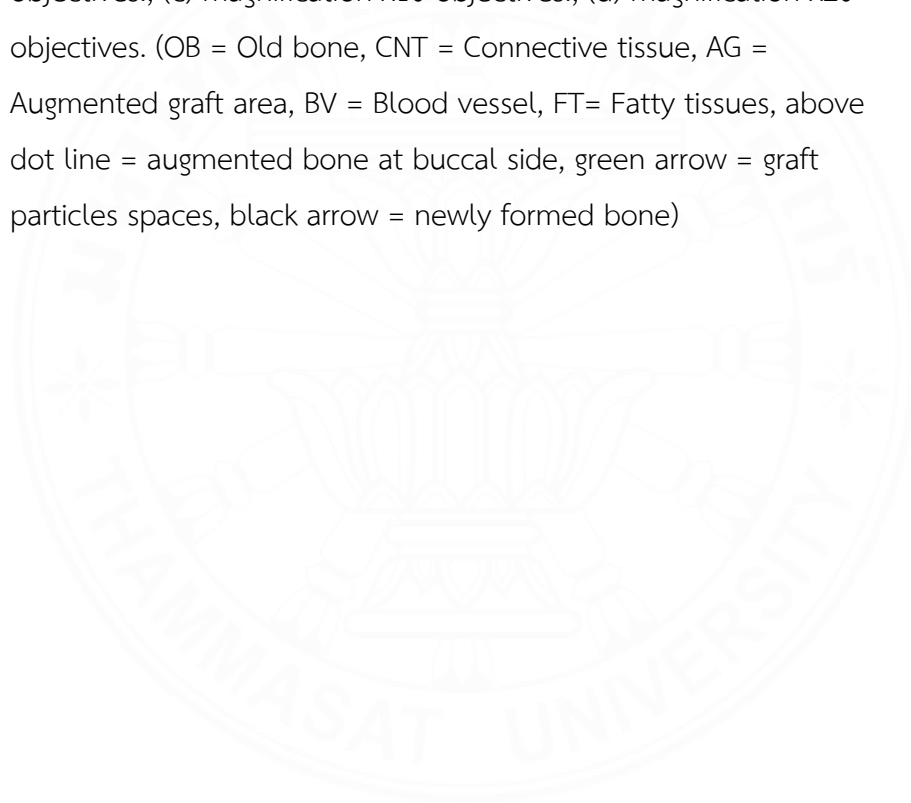
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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
α	Alpha
β	Beta
μm	Micrometer(s)/Micron(s)
%	Percentage
>	Greater-than
<	Lesser-than
$\pm$	Plus-minus
®	Registered trademark
™	Trademark
3D	Three-dimensional
3DHA	3D printed nanohydroxyapatite
3DP	Three-dimensional printing
AD	Additive manufacturing technology
BCP	Biphasic calcium phosphate
Ca	Calcium
CaP	Calcium phosphate
CAD	Computer-aided design
CAM	Computer-aided manufacturing
CBCT	Cone beam computed tomography
CGFs	Concentrated growth factors
DFDBA	Demineralized freeze-dried bone allograft
DBM	Demineralized bone matrix
d-PTFE	Dense polytetrafluoroethylene
e.g.	For example
ECM	Extracellular matrix
e-PTFE	Expanded polytetrafluoroethylene
FDBA	Freeze dried bone allograft

FDM	Fused deposition modeling
GBR	Guided bone regeneration
GTR	Guided tissue regeneration
HA	Hydroxyapatite
H ₂ O	Water
HI	Healing Index
HW	Horizontal ridge width
H&E	Hematoxylin and eosin
ISQ	Implant stability quotient
mm	Millimeter(s)
mm ²	A square millimeter
mm ³	A cubic millimeter
MTEC	National metal and materials technology center
PC	Platelet concentration
PRP	Platelet-rich plasma
L-PRP	Leukocyte platelet-rich plasma
PRF	Platelet-rich fibrin
L-PRF	Leukocyte platelet-rich fibrin
S-PRF	Standard platelet-rich fibrin
A-PRF	Advanced platelet-rich fibrin
i-PRF	Injectable platelet-rich fibrin
PO ₄	Phosphate
PDGF	Platelet-derived growth factor
IGF	Insulin-like growth factor
EGF	Epidermal growth factor
FGF	Fibroblast growth factors
VEGF	Vascular endothelial growth factor
TGF- β	Transforming growth factor beta
BMP	Bone morphogenetic protein
RP	Rapid prototyping

rpm	rounds per minute
OHIP-14	Oral Health Impact Profile-14
SO ₄	Sulphate
SFF	Solid freeform fabrication
SEM	Scanning electron microscope
STL	Stereolithography
SLS	Selective laser sintering
SD	Standard deviation
p-value	Probability value
VAS	Visual Analog Scale



CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

The dental implant is now considered to be an effective treatment options for patients who have tooth loss(1, 2). Missing teeth can be replaced by dental implant with high survival and success rate(3). Sufficient alveolar bone volume is a critical factor to facilitate subsequent dental implant placement in the most appropriated functional prosthetic position and esthetic outcomes(4).

The physiological ridge resorption is a critical consequence following tooth loss, from diseases, trauma, or tumor resection(5-7). This happens both horizontally and vertically, within the first year, there is a 25% decrease in the volume of the ridge, and the width is reduced by 40–60% in 3 years(8-11). In this situation, bone augmentation prior to or in combination with implant placement becomes a critical part of treatment(12). This has necessitated development of techniques and materials that promote predictable regenerative treatment(13).

The rationale of bone augmentation procedures is the reconstruction of bone contour and volume. The options of bone augmentation techniques or graft materials depend on several factors including the anatomical region, degree of atrophy, morphology of the osseous defect, type of prosthesis, and clinician or patient preferences. Although no studies document that one bone augmentation technique is necessarily superior, the implant surgeon should attempt to select a method that offers predictable results for the presenting clinical situation(14).

Bone substitutes are classified in four categories as autograft, allograft, xenograft and alloplast. Autograft or Autogenous bone graft is also considered as gold standard due to osteogenic properties and lack of immunological reaction(15, 16). However, limitations of autogenous bone graft in previous studies had been reported such as donor site morbidity, limited in amount of harvesting volume, or rapid resorption(17-19). For these reason, an alternative bone substitute has been widely studied in field of bone tissue engineering(20).

The developing field of tissue engineering offers a strategy to replace the traditional practice of harvesting autogenous bone from the patient. This concept involves combining the patient's own cells with growth factors and scaffold biomaterials(14). One of the most commonly used biomaterials for the treatment of bone defects is calcium phosphate (CaP). Due to its osteoconductive properties and biocompatibility, CaP can be used for various procedures such as bone augmentation, repair and regeneration(21). There are different forms of this graft materials such as hydroxyapatite(HA), beta-tricalcium phosphate (β -TCP), and biphasic calcium phosphate (BCP). The bone mineral idealized as calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a carbonate-hydroxyapatite(22). Hydroxyapatite (HA) has been used widely as bone substitute because its chemical structure and composition similar to natural alveolar bone(23). Although its limitations are low strength and resorption rate from manufacturing techniques using sintering HA(24-26). HA has a good bioaffinity and osteoconductive properties, and can create a positive environment for recruited osteoprogenitor cells to proliferate and differentiate into bone forming cells(27). Properties of HA such as pore size, configuration, and sintering temperature also affect its performance(28). HA with an optimal pore size and shape can provided bone ingrowth into three-dimensional structures and supported mechanical durability(29).

Another concern in developing efficacious bone graft substitutes or a scaffold is that significant variation in bone anatomy exists between patients. Furthermore, defect shape and size will be variable depending on the type of defect. These anatomical characteristics must be considered during graft fixation and design in order to achieve graft and host tissue integration. Thus, additive manufacturing technology(AM) including three-dimensional printing (3DP) should be considered to fabricated bone scaffold with complex shapes and structures(22). Computer-aided design(CAD) system is used to generate 3D model from patients' medical imaging techniques such as computed tomography(CT) and magnetic resonance imaging(30). The customized 3D bone printing scaffold can be created using AM with tailor design and predefined micro- as well as macrostructures(31, 32).

For these reasons, National metal and materials technology center(MTEC) develops the new material used for bone graft replacing autogenous bone. The nanohydroxyapatite bone graft materials made from MTEC by 3D printing technique with low temperature phosphorization as bone substitutes for alveolar ridge augmentation prior to implant placement. The advantage of this material is bioresorption ability, matching the required shape and size of the alveolar bone defect. To enhance the biological response, since HA only has osteoconductive properties without any osteogenic properties, incorporation of concentrated growth factors (CGFs) and platelet-rich fibrin membrane (PRF membrane) from a patient's venous blood would help tissue healing acceleration(33, 34).

The 3D-printing HA is a new customized materials to replace autogenous graft materials due to the patient morbidity from bone harvesting. This rapid prototype approach technique can create individual bone graft that fit to the defects without a gap between graft and recipient bone bed. It can also reduce the time needed to perform an operation. The reason for bone augmentation is to increase the bone volume and contour before dental implant placement that improve success of implant and prosthetic restoration(35, 36).

1.2 Research Objectives

The aims of this study are

1. To evaluate the clinical efficacy of bone reconstruction of alveolar bone defect using 3D printed nanohydroxyapatite (3DHA) block graft prior to dental implant replacement.
2. To analyze and measure bone quality and quantity of grafted areas prior to dental implant placement
3. To analyze the measure dimensional and volumetric change of bone and clinical success when dental implants placement in area of bone grafting before prosthetic reconstruction.
4. To evaluate the impact of quality of life (OHIP-14)

1.3 Research Hypothesis

H_0 : There are no significant differences in alveolar ridge dimensional change after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

H_a : There is a significant difference in alveolar ridge dimensional change after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

1.4 Statistical Hypothesis

1.4.1 Linear dimensional change

H_0 : There are no significant differences in linear horizontal bone gain after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

H_a : There is a significant difference in linear horizontal bone gain after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

1.4.2 Horizontal Ridge width

H_0 : There are no significant differences in horizontal ridge width of alveolar bone after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

H_a : There is a significant difference in horizontal ridge width of alveolar bone after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

1.4.3 Bone volumetric change

H_0 : There are no significant differences in bone volumetric change after 6 months using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

H_a : There is a significant difference in bone volumetric change after 6 months using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

1.4.4 Histomorphometric analysis

H_0 : There is no significant difference in percentage of bone tissue, new bone formation, connective tissue, and residual graft particles after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

H_a : There is a significant difference in percentage of bone tissue, new bone formation, connective tissue, and residual graft particles after using of 3D printed nanohydroxyapatite bone block graft for alveolar ridge reconstruction prior to implant placement.

1.5 Scope of the Research

This prospective clinical trial (pilot study) in 12 patients who needed alveolar ridge reconstruction with bone block graft prior to dental implant placement at the Faculty of Dentistry, Thammasat University hospital.

1.6 Expected Benefits

1. To evaluate the clinical efficacy of bone reconstruction of alveolar bone defect using 3D printed nanohydroxyapatite prior to dental implant replacement.
2. To analyze and measure bone quality and quantity of grafted areas prior to dental implant placement
3. To be the material of choice in regenerative dentistry provided for patient-tailored treatment with minimal invasive procedure and affordable in oral health care.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Dimensional changes following tooth extraction

The alveolar process is a tooth-dependent structure. The physiological ridge resorption is a critical consequence following tooth loss, from diseases, trauma, or tumor resection(6). The presence of fenestrations and bony ledging may prone to damage of bone tissue during tooth extraction procedures(7).



Figure 2.1 : An alveolar ridge resorption after 12 months of tooth extraction due to periodontal diseases. The consequential reduction of alveolar ridge in the bucco-lingual dimension.

A study conducted by Schropp et al. revealed that the resorption of the alveolar ridge occurs in both vertical and horizontal dimensions. At approximately two thirds of vertical height will be resorbed from socket wound healing process. The resorption of alveolar ridge will occur within the first 3 months after tooth extraction regardless of single or multiple tooth extractions. The resorption was pronounced on the buccal and labial than the lingual/palatal aspects of the alveolar process(4).

The dimensional changes that occur in the alveolar ridge following tooth extraction have been reported in several human studies and were determined using different methodologies, including clinical, cast model and radiographic examinations (37).

The ridge exhibits a limited vertical dimension reduction following single-tooth extraction. It is expected that the horizontal reduction will be substantial and a larger amount of alveolar bone reduction will take place in the molar regions(38).

The end of the socket-healing process is clinically observed as the closure of the socket entrance by firm epithelialized soft tissue and/or radiographic bone fill of the socket. A broad variation among individuals can be expected regarding the time needed for completion of socket healing. The socket entrance may be restored between 10 and 20 weeks and radiographic bone fill observed between 3 and 6 months post-extraction. While most of the dimensional changes that comprise socket healing occur during the first 3 months, the reorganization of the alveolar ridge may continue for up to 1 year post-extraction. The rate of socket healing is influenced by biologic differences among individuals, alveolar socket size (large vs. small sockets) and the extent of surgical trauma induced during the extraction procedure (37).

The systematic review of post extraction socket dimensional change in human showed horizontal bone loss of 29–63% and vertical bone loss of 11–22% after 6 months following tooth extraction. These studies demonstrated rapid reductions in the first 3–6 months that was followed by gradual reductions in dimensions thereafter(6).

This event may lead to an insufficient alveolar bone volume for implant placement(39). Therefore, additional bone augmentation procedures are often performed to provide the optimal width and height of the alveolar ridge prior to implant placement, especially for prosthetically driven positions.

2.2 Classification of ridge defects

The aim of ridge defect classifications related to implant placement is to help clinician to categorize the alveolar bone defects and propose the amount and clinical treatment technique for bone augmentation. Various classifications were described to identify ridge defect by many authors.

2.2.1 Seibert (1983) classified ridge defects based on the location of the deformity(40) as :

Class I – Buccolingual loss of tissue with normal apicocoronal ridge height.

Class II – Apicocoronal loss of tissue with normal buccolingual ridge width.

Class III – Combination of both buccolingual and apicocoronal loss (Both width and height).



Figure 2.2 : Siebert's classification of ridge defects

2.2.2 Allen (1985) modified Seibert's original classification by additionally describing the magnitude of the ridge defect(41).

Type A – Apicocoronal loss of ridge contour

Type B – Buccolingual loss of ridge contour

Type C – Combination of both buccolingual and apicocoronal loss

Mild < 3 mm.; Medium 3-6 mm.; Severe > 6 mm.

2.2.3 Misch and Judy (1985) presented a similar classification of ridge resorption with suggested augmentation and prosthodontic treatment modalities for each category. This classification takes into account only hard tissue defects and was stratified based on divisions that describe the natural bone resorption pattern(42).

Division A : Abundant bone

Division B : Marginally sufficient bone

Division C : Compromised bone

Division D : Deficient bone

2.2.4 Khojasteh (2013)

Characterizing the morphology of vertical alveolar defects subjected to reconstruction via different augmentation techniques eliminates the potential confounding effects of this factor on the efficacy and practicability of the examined technique(43). This classification proposes for edentulous sites in need of vertical

bone augmentation. Two major components of a vertical bony defect are considered in this classification:

The number of surrounding bony walls

Presence of bone around the teeth adjacent to the partially edentulous area should be considered while assessing the number of bony walls surrounding a defect:

- A: Two-wall defects (Figure 3a).
- B: One-wall defects (Figures 3b and 3d).
- C: A defect with no surrounding walls (Figures 3c, 3e, and 3f).

Width of the defect base

Considering the least amount of the defect base width, the bony defect can be categorized as follows (Figures 3a through 3f):

- I: A bony defect with a base width of 5 mm or more.
- II: A bony defect with a base width of 3 mm or more, but less than 5 mm.
- III: A bony defect with a base width less than 3 mm.

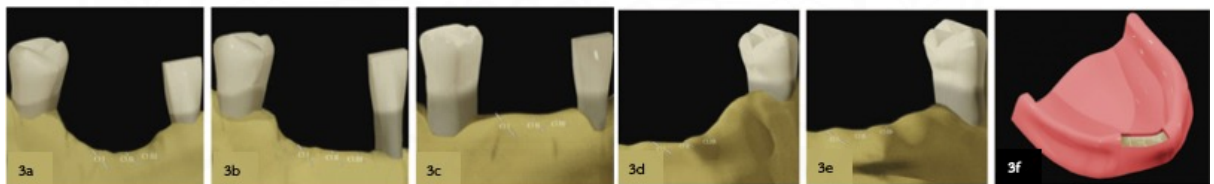


Figure 2.3 : Khojastech's classification of ridge defects

2.2.5 Benic & Hämmerle (2014)

This classification is categorized base on the optimal bone augmentation protocol and the selection of materials is primarily based on the defect morphology (Figure 4). The aim of this classification is to simplify the decision-making process regarding choice of the strategy for bone augmentation. The bone augmentations can be performed simultaneously with (combined approach) or prior to (staged approach) implant placement. The combined approach is preferred, whenever permitted by the clinical situation, as this approach results in decreased patient morbidity, treatment time and costs. The clinical and radiographic examination of the

bone at the implant site also includes assessment of the bone defect morphology, the mesio-distal size of the edentulous area and the bone level at the teeth adjacent to the gap(44, 45).

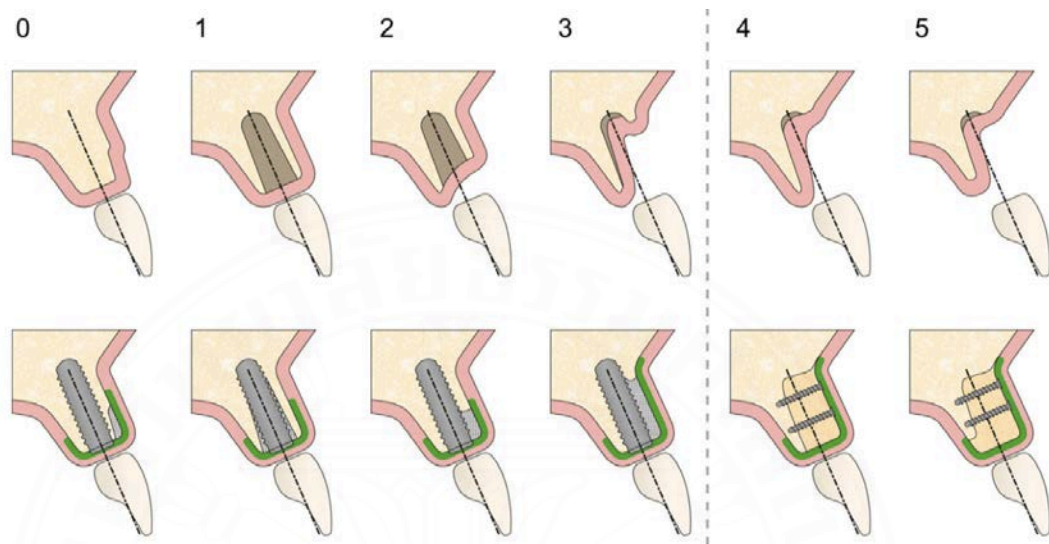
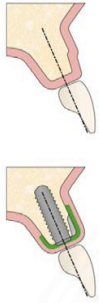
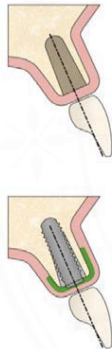
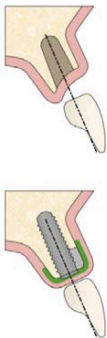
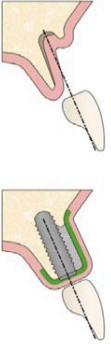
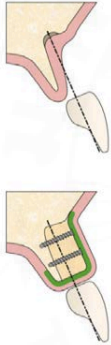
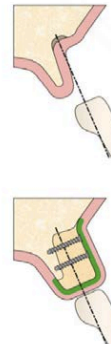


Figure 2.4 : Schema displaying bone defect Classes 0–5 and the corresponding bone augmentation procedures.

In the case of intra-alveolar defects and peri-implant dehiscences, in which the volume stability of the region to be augmented is provided by the adjacent bone walls, a bioresorbable membrane, in combination with a particulate bone substitute, represents the treatment of choice. Where the volume stability of a peri-implant dehiscence-type defect is not provided by the adjacent bone walls, an e-PTFE or d-PTFE membrane and particulate bone substitute are used. The staged approach is preferred when the implant cannot be placed in the prosthodontically correct position or result in an unfavorable appearance of the soft tissue due to the lack of hard-tissue support. In such situation, it involves first augmenting the alveolar ridge and then placing the implant in the proper position.

Table 2.1 : Description of classification of bone defects according to Benic & Hämmerle 2014.

Bone defects	Description
Class 0 	Site with a ridge contour deficit and sufficient bone volume for standard implant placement
Class 1 	Intra-alveolar defect between the implant surface and intact bone walls
Class 2 	Peri-implant dehiscence, in which the volume stability of the area to be augmented is provided by the adjacent bone walls

Bone defects	Description
Class 3 	Peri-implant dehiscence, in which the volume stability of the area to be augmented is not provided by the adjacent bone walls
Class 4 	Horizontal ridge defect requiring bone augmentation before implant placement
Class 5 	Vertical ridge defect requiring bone augmentation before implant placement

Contour deficit: Class 0

In some situation, an implant can be placed in a prosthetically correct position within the bony envelope however, a bone augmentation procedure is indicated to improve the contour of the ridge. This is often the case in esthetically sensitive sites with a healed alveolar ridge (Type 4 placement). As a result of post-extractive ridge resorption, such sites generally present a reduced dimension of the alveolar ridge. The guided bone regeneration procedure with a resorbable membrane and particulate bone substitute, described for Class 2 dehiscence-type defects, is performed in these situations.

Intra-alveolar defect: Class 1

Class 1 defects are characterized by gaps between the implant surface and the intact bone walls. Owing to the resorptive processes starting immediately following extraction of the tooth, Class 1 defects are mostly limited to situations where immediate implant placement is performed (Type 1 placement). In some situations, the bone walls of the socket may still be intact at a later time point, when implants are placed following soft-tissue healing (Type 2 placement).

After implant placement, the site is analyzed and one of the following strategies for the management of Class 1 defects is selected: (i) no guided bone regeneration; (ii) guided bone regeneration of the residual socket; or (iii) guided bone regeneration of the residual socket and over-augmentation of the buccal bone wall.

Dehiscence-type defect: Class 2

Class 2 defects are characterized by peri-implant dehiscence, in which the volume stability of the area to be augmented is provided by the adjacent bone walls. Dehiscence of the buccal bone is the most frequently encountered situation needing bone regeneration at implants. A large number of preclinical and clinical studies demonstrated that dehiscenced implant surfaces successfully osseointegrate following combined guided bone regeneration procedures

After implant placement, analysis of the dehiscence-type defect is performed and the decision regarding the need for augmentation of the ridge contour is taken.

In posterior sites, which generally do not require augmentation of the ridge contour, a bioresorbable membrane in combination with particulate bone substitute is the treatment of choice.

Subsequent to implant placement, the cortical bone around the dehiscence defect is perforated to allow earlier vascularization and thus to improve bone repair. A particulate bone substitute material is applied onto the exposed implant surface and a resorbable membrane is shaped and adapted to extend 2 mm beyond the defect margins.

Dehiscence-type defect: Class 3

Class 3 defects are characterized by peri-implant dehiscences, in which the volume stability of the area to be augmented is not provided by the adjacent bone walls. In situations requiring optimal support of the peri-implant soft tissue, the use of titanium-reinforced PTFE membranes, in combination with a particulate bone substitute, is recommended for the treatment of Class 3 defects

The clinical protocol for guided bone regeneration at Class 3 defects includes the following steps: perforation of the cortical bone around the dehiscence defect; application of particulate bone substitute; and adaptation of a titanium-reinforced PTFE membrane over the bone dehiscence without over-building the area. The use of titanium tacks is recommended to provide adequate adaptation and stabilization of the membrane. A resorbable membrane may be applied over the PTFE membrane with the aim of facilitating spontaneous wound healing in the event of soft-tissue dehiscence. Thereafter, the flap is adapted and sutured to allow submerged healing of the regeneration site.

As early exposure of e-PTFE membranes often leads to infectious complications and failure of guided bone regeneration, attention should be paid to achieve complete and tension-free soft-tissue coverage at the regenerated area. In cases where the mucosal quantity and/or quality in the defect area are deemed deficient, a soft-tissue grafting procedure may be indicated before implant placement.

Horizontal defect: Class 4

Class 4 defects are characterized by reduced ridge width precluding the primary stability of the implant in the prosthodontically correct position. In such situations, the staged approach for bone regeneration and implant placement is chosen. Autogenous bone blocks, alone, or in combination with bone substitute and/or collagen membranes, are the most reliable and successful procedures for staged augmentations of large bone defects before implant placement.

The clinical procedure for primary horizontal ridge augmentation starts with the preparation of the site to be augmented. After elevation of the mucoperiosteal flap, the cortical bone at the recipient bed is perforated in order to allow earlier vascularization and to improve integration of the bone block. Subsequently, the autogenous bone block is harvested, adapted to achieve intimate contact between the graft and the bone at the recipient site and rigidly fixed with metal screws. To reduce its resorption, the bone block is covered with particulate bone substitute and a resorbable membrane. The donor defect in the chin region is filled with bone substitute and covered with a resorbable membrane, in order to enhance bone repair. The flap is coronally advanced by periosteal release, adapted and sutured to allow a tension-free primary closure at the augmented site. A healing time of 4–6 months before the second surgical intervention for placement of the implants is commonly accepted.

Vertical defect: Class 5

Class 5 defects are characterized by reduced ridge height. Vertical ridge augmentation is indicated in situations in which the remaining amount of vertical bone is insufficient for anchorage of the standard implant length or in which an unfavorable appearance of the soft tissue is expected owing to the lack of hard-tissue support. This procedure is recommended to perform using the staged approach for bone augmentation and implant placement. Autogenous bone block, alone, or in combination with bone substitute and/or collagen membrane, is the treatment of choice for vertical ridge defects. When performing vertical ridge augmentation, the autogenous bone block is partially or completely fixed on the

coronal surface of the alveolar ridge in order to augment the bone height. The same clinical procedure is also performed as described for horizontal augmentations with autogenous bone blocks. The rate of soft-tissue complications appears to be considerably higher for vertical ridge augmentations than for horizontal augmentations. This may be because a tension-free primary wound closure is more difficult to achieve as a result of the increased volume to be covered. The clinical use of bone-substitute materials for the regeneration of vertical ridge defects is not sufficiently documented(45).

The goal of osseous replacement is to maintain the natural shape and appearance of the alveolar region, reduce postoperative infection, and improve soft tissue healing. The insufficient quantity of bone from tooth loss which results in rapid resorption of alveolar bone due to lack of intraosseous stimulation by periodontal ligament (PDL) fibers.

Bone grafting is a surgical procedure that replaces missing bone with material from patient's own body, an artificial, synthetic, or natural substitute. Bone grafting is possible because bone tissue has the ability to regenerate completely if provided the space into which it has to grow. As natural bone grows, it generally replaces the graft material completely, resulting in a fully integrated region of new bone(20). The choice of a particular augmentation technique or graft material depends on several factors including the anatomical region, degree of atrophy, morphology of the osseous defect, type of prosthesis, and clinician or patient preferences. Although no studies document that one bone augmentation technique is necessarily superior, the implant surgeon should strive to select a method that offers predictable results for the presenting clinical situation(14).

2.3 Bone augmentation procedures

Bone augmentation procedures can be performed prior to placement of implants implant (two-stage procedure), or at the same time as implant placement (one- stage procedure). This procedure can be carried out using various materials and techniques. However, it usually requires an additional surgical procedure and then the

area is left to heal for a period of time to ensure that the grafted heals properly before placing the implants (46).

Bone augmentation procedures may be performed for the applications of extraction socket defect grafting, horizontal and vertical ridge augmentation, and sinus augmentation. To maximize the results for each of these applications, a variety of different techniques is employed. They include particulate grafting, membrane use, block grafting, and distraction osteogenesis, either alone or in combination(13).

In most cases, bone augmentation is required when standard implants can't be placed in a proper prosthetically driven position due to resorbed alveolar crest. Literatures have shown that autogenous graft is the gold standard material, especially block graft in severely resorbed ridge with large defects(47).

2.4 The choice of surgical techniques for alveolar ridge augmentation

2.4.1 Guided bone regeneration (GBR)

The concept of guided bone regeneration involves the use of a barrier membrane to create a protected space for the regeneration of the bone(48). This space can be populated by osteogenic cells and blood vessels from the bone marrow cavities and the bone surface and excludes any epithelial/soft-tissue infiltration of the area. In addition, the use of a membrane is combined with autogenous particulate bone and bone substitutes. A greater degree of stability of the membrane can be achieved by fixing the membrane using titanium pins(49).

2.4.1.1 Indications of guided bone regeneration

Guided bone regeneration with a resorbable membrane is the preferred technique for the treatment of small peri-implant defects such as a dehiscence or fenestration. This technique is indicated when the defect has an irregular shape and it would be difficult to adapt an autogenous block graft. The use of a mixed graft, composed of autogenous particulate bone and a bone substitute, is usually recommended with this type of membrane(45).

GBR membranes are categorized into two types: non-resorbable and resorbable depending on their degradation characteristics. Expanded-polytetrafluoroethylene

membranes reinforced with titanium or titanium meshes have been used as non-resorbable barriers and the barrier is removed during second-stage surgery. But for its greater stiffness is associated with a higher rate of complications, such as membrane exposure and partial or total failure of the augmentation procedure and requiring secondary surgery for removal that can cause additional pain, discomfort, and infection are major disadvantages of this membrane. In the case of exposure of a non-resorbable membrane, unlike exposure of a collagen barrier, in the majority of cases the membrane must be removed.

In recent years, the use of a polytetrafluoroethylene (d-PTFE) membrane has been successfully used for the preservation and enhancement of vertical bone and socket volume. This technique has been studied in various animal and human studies(50, 51). In studies, the d-PTFE membrane was exposed to the oral cavity, which resulted in no negative effects on bone regeneration. It was also noted that such procedures increased the amount of available keratinized tissue. Exposed d-PTFE membranes have demonstrated resistance to degradation, and have a lower risk of infection(50).

In the clinical practice, a collagen membrane has been the most widely used for GBR. It has various advantages, such as low antigenicity and high biocompatibility, and excellent cell affinity, but pure collagen has the disadvantage of being difficult to handle (49).

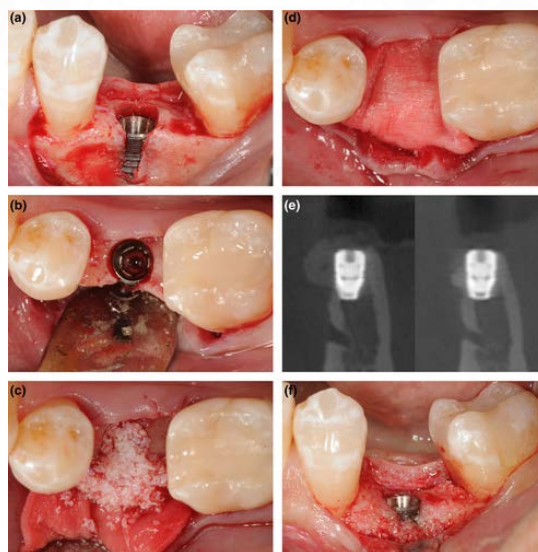


Figure 2.5 Guided bone regeneration procedure(52); (a, b) Bone dehiscence after

implant placement. Guided bone regeneration with (c) particulate deproteinized bovine-derived bone mineral and (d) collagen membrane stabilized with polylactide pins. (e) CBCT bucco-oral images (left) immediately after wound closure and (right) after 6 months. (f) Incomplete vertical defect resolution at re-entry surgery, 6 months after implant placement

Besides PTFE membranes, titanium mesh is another non-resorbable material applicable for GBR because of its high strength and rigidity, its low density and corresponding low weight, its ability to withstand high temperatures and its resistance to corrosion(53).

Titanium mesh (Ti-mesh) has excellent mechanical properties for the stabilization of bone grafts beneath the membrane. Rigidity provides extensive space maintenance and prevents contour collapse. Elasticity prevents mucosal compression. Stability prevents graft displacement. And plasticity permits bending, contouring, and adaptation to any unique bony defect(54). However, the stiffness of the Ti-mesh can cause membrane exposures, such as mechanical irritation to the mucosal flaps. In addition, the sharp edges, caused by cutting, trimming, and bending of titanium mesh, might be responsible for exposure of titanium barriers(55). Most complication of Ti-mesh are their exposure and from soft tissue ingrowth. The stiffness of Ti-mesh can maintain space better than other membrane, but may result in mucosal irritation that leads to exposure of the membrane(56).



Figure 2.6 Titanium mesh as an alternative to membrane for ridge augmentation

2.4.2 Block Graft Techniques

The bone block graft was first introduced in early 1990s to increase the horizontal width and vertical height of mandibular or maxillary edentulous ridges. In bone block augmentation technique, bone graft is usually an autologous bone block harvested from intra/extra-oral sites. The decorticated bone of recipient site is performed to increase blood clot formation and bone marrow osteoblast precursor migration into the graft. After that, bone block will be fixed with osteosynthesis screw to the recipient site. The most common donor sites for block grafts are the mandibular ramus or symphysis region (intraoral) and the iliac crest (extraoral). Several autologous bone grafting techniques have been used for the treatment of severely resorbed edentulous mandible and maxilla. In particular, the use of particulated bone graft and barrier membranes for block grafts seems to significantly improve the clinical outcome(57).

2.4.2.1 Harvested sites of autogenous bone block graft

Extraoral autogenous bone harvested from the iliac crest was used to gain ridge height with varying degrees of success, the main disadvantage being the high resorption rate before implant placement and after loading. Less common extraoral donor sites for block grafts used in ridge augmentation include the tibia and cranial vault. The disadvantages of autologous grafts are their resorption rate and high morbidity associated with the donor site(58).

Intraoral block grafts are commonly harvested from the mandibular ramus or the symphysis. However, ramus grafts are associated with significantly lower morbidity compared to symphysis grafts that may be associated with significant post-operative pain, altered sensation of mandibular anterior teeth, neurosensory disturbances in the chin region, temporary mental nerve paresthesia and mandibular fracture. Therefore, the symphysis is often reserved for cases that require thicker block grafts that cannot be obtained from other intraoral donor sites(59).

Table 2.2 Harvested sites of intraoral and extraoral autogenous graft(60).

Donor Site	Type of Graft	Volume Available	Possible Complications
Ascending Ramus	Cortical	5–10 cc	- Damage to the neurovascular bundle with resulting temporary or permanent neurosensory disturbance
Symphysis	Corticocancellous	5–10 cc	- Ectropion of the lower lip and chin ptosis. - Damage to the teeth and mental nerve with neurosensory disturbance
Tuberosity	Cancellous	2 cc	- Oroantral communication
Tibia	Cancellous	20–40 cc	- Tibial plateau fracture Interference with growth plate in children
Cranium	Cortical	20–40 cc	- Damage to dura and brain
Anterior Iliac Crest	Corticocancellous	50–70 cc	- Gait disturbance - Paresthesia of the lateral thigh - Hernia - Hematoma
Posterior Iliac Crest	Corticocancellous	80–140 cc	- Gait disturbance - Paresthesia of the posterior regions - Hematoma

2.4.2.2 Indication of bone block graft

This technique is recommended with predictable outcome for treatment of class 3 advanced horizontal atrophy) and class 4 (advanced horizontal atrophy with advanced vertical atrophy)

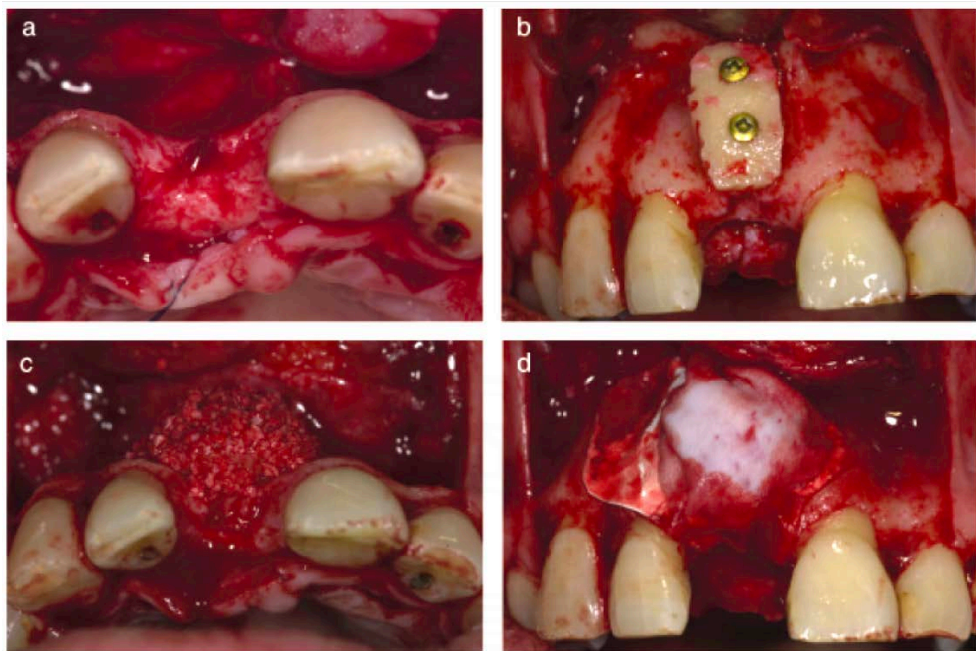


Figure 2.7 Clinical photographs of horizontal bone deficiency at right central maxillary incisor from Von Arx, T. and Buser, D. (2006) (61); (a) Marked concavity of the facial bone contour. (b) A block graft harvested in the symphysis was fixed in a vertical position for horizontal ridge augmentation and convex recontouring of the future implant site. (c) The block graft was fully covered with anorganic bovine bone mineral particles. (d) The augmented site was further-protected with a collagen membrane using the double-layer technique

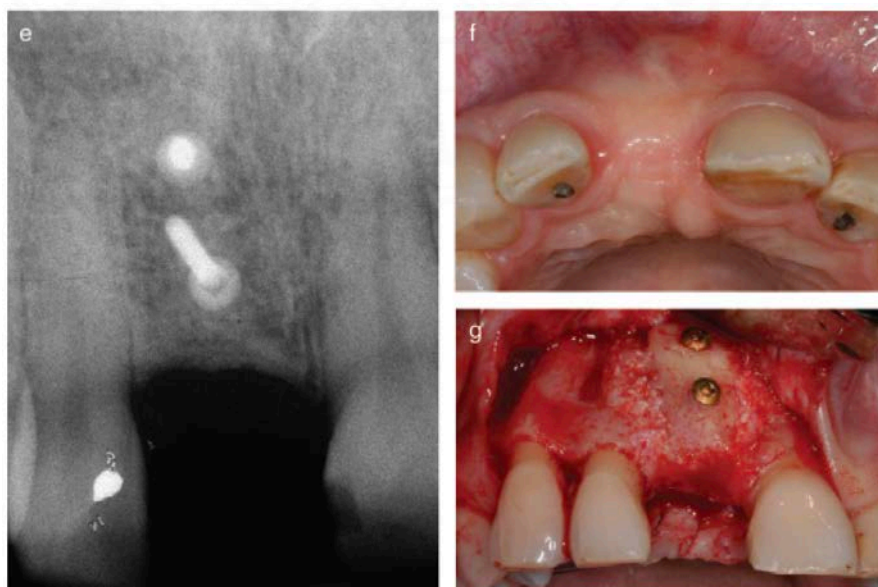


Figure 2.7 cont. (e) The post-operative radiograph shows the two fixation screws. (f) Healing was uneventful, and the new convex profile of the vestibule is visible. (g) Upon re-entry (6 months after the augmentation surgery), the block graft shows no surface resorption.

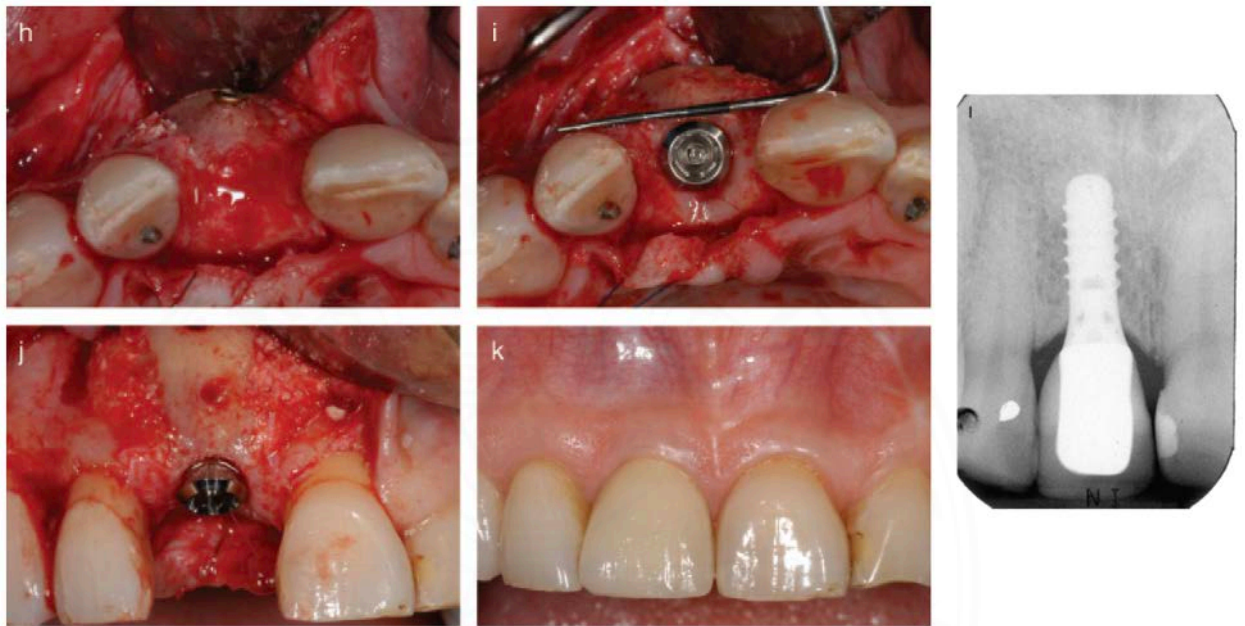


Figure 2.7 cont. (h) The occlusal view clearly demonstrates the horizontal gain of bone width. (i) A Straumann implant was placed in a correct oro-facial position. (j) The implant shoulder was aligned slightly apical to the cemento-enamel junction of the adjacent central incisor. (k) The clinical situation 17 months after implant placement. (l) The radiograph taken at the same time depicts stable peri-implant bone structures.

2.4.2.3 Incorporation of bone grafts into the recipient site

Incorporation of bone grafts within the recipient bone bed depends on several factors such as optimal revascularization, source and structure of the graft (62, 63). Integration of the graft into the recipient site is a procedure that includes the following stages: inflammation, revascularization, osteoinduction, osteoconduction and finally remodeling. The biological process leading to graft incorporation is very similar to that of fracture repair.

The viability and vascularity of the bone graft bed are the factors that are considered when it comes to the formation of a graft. The important factors for graft incorporation are vascularity and viability of the graft bed. The bone graft typically

needs to be attached to viable, bleeding bone edges. Cells from the recipient site of the graft carry out the process of neo-angiogenesis. Neovascularization supplies the avascular scaffold with oxygen and the nutrients required for cell growth and differentiation of osteoblasts and triggering the union between the graft and native bone edges (64). New blood vessels form within the granulation tissue and begin tunneling their way into the graft. Too much reaming or excessive heat generation during saw cutting can cause necrosis of the bone edges and delay union to the graft(65).

Other factors related to the graft incorporation include the graft type, porosity, and mechanism of action. Porous architecture of the graft allows easy cellular and vascular invasion. The graft trabeculae have a large surface area that is covered by osteoblasts, making it osteogenic as well as osteoconductive for three-dimensional bone growth. Additionally, due to the extensive vascular invasion, the bone matrix can readily be demineralized and its proteins exposed through the actions of osteoclasts. This leads to the release of growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming growth factor beta family (TGF- β), or insulin-like derived growth factor (IGF)(66).

The biologic behavior of transplanted autogenous bone blocks is combines osteoclastic degradation and osteoblastic substitution, which lead to healing and integration of the graft. Although complete bone remodeling of the graft may take several years, from a clinical point of view, after 4–6 months of healing, sufficient integration and revascularization of the graft has occurred and it is possible to place implants(44).

Table 2.3 Requirements for success bone graft(67)

Biologic requirements	Technical requirements	Additional considerations
- Good blood supply to bone graft site - Space maintenance for new bone formation	- Perforation of cortical bone to create bleeding points for blood supply	- Systemic conditions (e.g. diabetes) can alter wound healing and might impair regeneration

Biologic requirements	Technical requirements	Additional considerations
- Separation of soft tissues from bone - Migration and proliferation of osteoblasts and osteoprogenitors - Bone graft stabilization to prevent any micromovement - Primary wound closure and stabilization; important to achieve blood clot stabilization	- and source of osteoblasts and osteoprogenitors - Use of barrier membranes; important to establish intimate contact with bone and to stabilize with screws or tacks - Adequate blood supply maintained by good flap management - Bone graft stabilization with screw fixation (may include use of fixation plate or mesh) - Flap management designed to achieve primary closure over increased volume without tension; important to release periosteum	- Some medications (e.g. immunosuppressive drugs) can impair healing and regeneration - Habits (e.g. smoking) may impair wound healing and potential for bone regeneration - Any pressure (e.g. prosthesis) placed over the surgical site can cause wound dehiscence and impair potential for bone regeneration

2.4.2.4 Complications related to autologous bone augmentation

Complications related to bone augmentation using bone block graft mainly involve soft tissue dehiscence, infections, bone fractures, graft encapsulated by soft tissues, and nerve injury(68). The most common complication is the occurrence of soft tissue dehiscence leading to graft exposure and subsequent contamination of the bone graft and/or membrane, which usually results in impaired regenerative outcomes and frequent loss of the bone graft(69). The soft tissue dehiscence is usually encountered together with edema or ecchymosis. Wound infection/inflammation characterized by

pain, swelling, redness, fever and/or purulent discharge that required additional antibiotic treatment(57). However, complications of autogenous bone block graft are often observed at the donor site(70).

2.4.3 Distraction Osteogenesis

Distraction osteogenesis (DO) is a technique used in craniomaxillofacial surgery to achieve high bone volume gain of alveolar ridge. The principle of this technique is to achieve new bone formation created by gradually separating two bony surfaces after an osteotomy surrounding the callus. Since distraction osteogenesis is performed under general anesthesia and requiring a surgeon, this technique is hardly use in routine clinical practice due to sensitivity of the technique and strict anatomical requirements for predictable results(71).

2.4.4 Ridge splitting

This technique is an edentulous ridge expansion or split-crest technique for implant placement was originally described by Simion et al(72). The surgical technique involved splitting the alveolar ridge longitudinally in two parts, provoking a greenstick fracture. A special instrument was then used to make a fine cut and spread apart the two cortical plates. This is a technique-sensitive procedure that could be done with many different instruments, ranging from scalpel blades to spatula osteotomes, piezoelectric surgical systems, and ultra-fine fissure burs(73). Indication for this technique is at least 3 mm in width to perform this procedure to reduce risk of buccal plate fracture. Main disadvantage of this technique is that it does not resist significant variations in the position of the implant, which will follow the direction of the bilateral alveolar ridge(74).

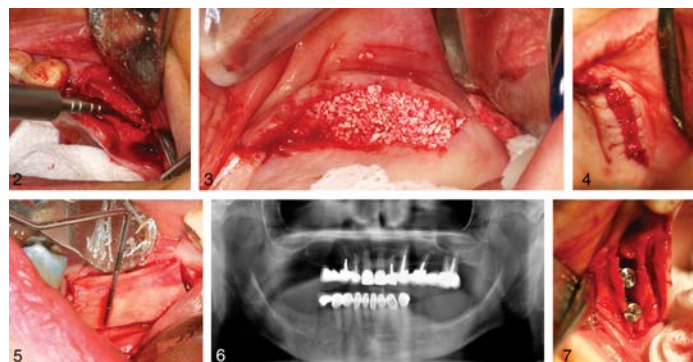


Figure 2.8 Ridge-split technique

2.5 Bone graft materials

2.5.1 The biological consideration of bone materials

1) **Osteogenesis** means the osteo-differentiation and subsequently new bone formation by donor cells derived from either the host or grafts. It occurs when vital osteoblasts originating from bone graft material contributes to the growth of new bone along with bone formation(75).

2) **Osteoinduction** means that the graft can induce the primitive, undifferentiated and pluripotent cells to develop into the bone-forming cell lineage, by which osteogenesis is induced. One proposed definition is the process by which osteogenesis is induced. Involves stimulation of osteoprogenitor cells to differentiate into osteoblasts and then begins formation of new bone. The most widely studied type of osteoinductive cell mediators are bone morphogenic proteins (BMPs). A bone graft material that is osteoconductive and osteoinductive will not only serve as a scaffold for currently existing osteoblasts but will also trigger formation of new osteoblasts, promoting faster integration of the graft(75).

3) **Osteoconduction** means the ability to support the attachment of osteoblast and osteo-progenitor cells, and allow the migration and ingrowth of these cells within the three-dimensional architecture of the graft. This term means that bone grows on a surface. An osteoconductive surface is one that permits bone growth on its surface or down into pores or channels. This occurs when bone graft material serves as a scaffold for new bone growth, which is preserved by the native bone. Osteoblasts from the margin of defect that is being grafted, utilize the bone graft material as a framework upon which to spread and generate new bone. In the very least, a bone graft material should be osteoconductive(20, 75).

Table 2.4 The Influence of bone grafts in new bone formation(76)

New Bone Formation Process	Influence of the Graft
Osteogenesis	Graft contains surviving preosteoblasts and osteoblasts that proliferate and mature into bone-producing cells

New Bone Formation Process	Influence of the Graft
Osteoinduction	Graft actively recruits pluripotent host cells that differentiate into bone-producing chondroblasts and osteoblasts
Osteoconduction	Graft serves as an inert scaffold for the ingrowth of host bone

2.5.2 Types of bone graft materials

Bone grafts and bone substitutes can be classified into four groups, according to their origins: autografts, from the same individual; allografts, from another individual within the same species; xenografts, from another species; and alloplasts, synthetically produced(57).

Table 2.4 Types of graft material, origin and examples of bone graft material

Graft material	Origin	Examples
Autograft	Patient's own tissue	Intra-orally or extra-orally harvested
Allograft	Tissue from individuals of the same species	Fresh-frozen bone, freeze-dried bone, demineralized freeze-dried bone
Xenograft	Tissue from other species	Bovine-, porcine-, equine-derived bone mineral
Alloplast	Synthetically produced material	Tricalcium phosphate, hydroxyapatite, hydroxyapatite/tricalcium phosphate composite, calcium phosphate cement, calcium sulfate, bioactive glass, polymers

In general, bone grafts are either used in block (such as from chin or ascending ramus area of lower jaw) or particulated, in order to be able to adapt it better to a defect(45).

2.5.2.1 Autograft

Autologous or autogenous bone grafting involves utilizing bone obtained from same individual receiving the graft. Autogenous bone grafts have long been used to reconstruct osseous defects, and they are still considered the gold standard of graft materials. The superior biologic properties of autogenous bone, including osteogenesis and osteoinduction, offer significant advantages over bone substitutes when reconstructing the atrophic ridge. Bone can be harvested from iliac crest, mandibular symphysis (chin area), and anterior mandibular ramus (coronoid process). For limited defects, the use of intraorally harvested cortical blocks from the chin or ramus may be considered. When a block graft will be performed, autogeneous bone is the most preferred because there is less risk of graft rejection as the graft is originated from the patient's body. Autogenous grafts offer several advantages for bone augmentation including short healing time, favorable bone quality, limited cost, and predictable results. However, the inherent disadvantages are morbidity from bone harvest, added surgical time, and a limited supply of bone. Graft resorption and donor site morbidity are clinical concerns associated with autogenous grafting procedures(20, 76).

2.5.2.2 Allografts

Allograft is derived from humans. It's taken from the bone of a deceased individual who has already donated their own bone. It is typically sourced from a bone bank.

There are three types of bone allograft available :

1. Fresh or fresh-frozen bone allograft
2. Freeze-dried Bone Allograft (FDBA)
3. Demineralized freeze-dried bone allograft (DFDBA)

The use of allografts for bone repair often requires sterilization and deactivation of proteins normally found in healthy bone. Contained in the extracellular matrix of bone tissue are combinations of bone growth factors, proteins, and other bioactive materials necessary for osteoinduction and successful bone healing; the desired factors and proteins are removed from the mineralized tissue by using a demineralizing agent such as hydrochloric acid. The mineral content of the bone is degraded, and the osteoinductive agents remain in a demineralized bone matrix (DBM) (20, 77).

Horizontal ridge augmentation using allogeneic bone block has previously been evaluated in systematic reviews and meta-analyses reporting high implant survival rates and gain in alveolar ridge width(78, 79). However, high incidence of biologic complications including soft tissue dehiscence, exposure of allogeneic bone block, partial or total loss of the block graft were reported(80).

2.5.2.3 Xenograft

Xenografts are bone grafts from a species other than human, such as bovine-, porcine-, equine-derived bone mineral. Inorganic bone of bovine origin comprises of a HA skeleton(20). Advantages of xenografts are ease of harvesting large samples of bone with the desired microstructure, which improves compatibility at the intended surgical site and having osteoconductive properties(81). Disadvantage of xenogenic origin blocks used in bone augmentation studies are very brittle and lack toughness(57).

2.5.2.4 Alloplast

The first documented use of a synthetic bone graft was reported in 1892 by Van Meekeren, who treated a large bone defect with calcium sulfate. Since then, materials classified as bioceramics have been used extensively as bone graft substitutes. The most widely used and documented bioceramics material for bone grafting in humans is hydroxyapatite (HA), which has a similar chemical composition and crystalline structure similar to human bone(21).

Alloplastic grafts can be made from hydroxyapatite, a naturally occurring mineral (main mineral component of bone), made from bioactive glass. A synthetic Hydroxyapatite is the most used now due to its osteoconduction, hardness, and acceptability by host bone. Other types of synthetic bone graft are made from calcium sulfate or calcium carbonate. They tend to decrease in usage due to their property that completely resorb in a short period of time. In addition, the composite graft of

Other synthetic bone grafts are made of calcium carbonate or calcium sulfate which start to decrease in usage because they completely resorbable in short time and makes breaking of the bone easier. Finally used is the tricalcium phosphate in combination with hydroxyapatite and thus giving effect of both, osteoconduction and resorbability(20).

Table 2.5 The Commercially available biomaterials in dentistry(82).

Biomaterial	Commercial name
Allografts	
Demineralized freeze- dried bone allograft	Distributed under various names by tissue banks
Freeze-dried bone allograft	Distributed under various names by tissue banks
Xenografts	
Anorganic bovine bone	Bio-Oss [®] , OsteoGraf [®] , Pep-Gen P-15 [®]
Alloplasts	
Tricalcium phosphate	Synthograft [®]
Hydroxyapatite	Periograft [®] , Osteogen [®] , ProOsteone [®]
Bioactive glass polymers	PerioGlas [®] , BioGran [®]
Polymers and Collagens	
Collagen	Collaplug [®] , Collacote [®] , Gelfoam [®] , Helistat [®]
Hard-tissue replacement polymer	Bioplant [®]
Coraline calcium carbonate	Biocoral [®]
Poly(lactide-co- polyglycolide)	
Methylcellulose	
Hyaluronic acid ester	HY [®]
Chitosan	

2.5.3 Ceramic-based bone graft substitutes

Majority of bone grafts available involve ceramics, either alone or in combination with another material (e.g., calcium sulfate, bioactive glass, and calcium phosphate). Calcium phosphates (CaPs) biomaterials including biphasic calcium phosphate (BCP) ceramics have proven efficacy in numerous clinical indications. Their specific physico-chemical properties (HA/TCP ratio, dual porosity and subsequent

interconnected architecture) control (regulate/condition) the progressive resorption and the bone substitution process(83). Different types of calcium phosphates are tricalcium phosphate, synthetic hydroxyapatite, and coralline hydroxyapatite; available in pastes, putties, solid matrices, and granules(20).

The common use of ceramic-based bone graft substitutes is calcium hydroxyapatite which is osteoconductive and osteointegrative; and in some cases, osteoinductive. The fabrication technique by sintering require high temperatures for scaffold formation and have brittle properties(84).

Table 2.6 Calcium phosphate ceramics bone graft in different ratio of Ca/P (85)

Name	Abbreviation	Formula	Ca/P ratio
Monocalcium phosphate monohydrate	MCPM	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	0.5
Dicalcium phosphate anhydrate (monetite)	DCPA	CaHPO_4	1.0
Dicalcium phosphate dihydrate (brushite)	DCPD	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	1.0
Octacalcium phosphate	OCP	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	1.33
β -Tricalcium phosphate	β -TCP	$\text{Ca}_3(\text{PO}_4)_2$	1.5
Amorphous calcium phosphate	ACP	$\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$	1.5
α -Tricalcium phosphate	α -TCP	$\alpha\text{-Ca}_3(\text{PO}_4)_2$	1.5
Hydroxyapatite	HA	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	1.67
Tetracalcium phosphate	TetCP	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	2.0

2.5.3.1 Hydroxyapatite bone graft

The bone mineral idealized as calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a carbonate-hydroxyapatite(22), approximated by the formula:

$(\text{Ca},\text{X})_{10}(\text{PO}_4,\text{HPO}_4\text{CO}_3)_6(\text{OH},\text{Y})_2$ where X are cations (magnesium, sodium, strontium

ions) that can substitute for the calcium ions, and Y are anions (chloride or fluoride ions) that can substitute for the hydroxyl group(21).

Hydroxyapatite (HA) has been used widely as bone substitute, although its low strength and resorption rate limits its clinical indications. HA has a good bioaffinity and osteoconductive properties, and can create a positive environment for recruited osteoprogenitor cells to proliferate and differentiate into bone forming cells. Properties of HA such as pore size, configuration, and sintering temperature also affect its performance. HA with an optimal pore size of 100–400 μm favors bone ingrowth into three-dimensional structures(29).

2.6 Bone tissue engineering

The developing field of tissue engineering offers a strategy to replace the need for harvesting autogenous bone from the patient. Tissue engineering may be used to regenerate bone by combining cells from the body with growth factors and scaffold biomaterials(14). Bone tissue engineering is focused on methods to synthesize and/or regenerate bone, maintain or improve its functions. Successful application of bone tissue engineering can avoid challenges related to other treatment options involving different materials such as autografts or allografts. Apart from material issues, a clear understanding of biology involving cells, extracellular matrix (ECM) and growth factors are important in bone tissue engineering(86).

Tissue engineering components consisted of three general components (Figure 2.9) : 1) A scaffold is a 3D structure that is manufactured from biodegradable biomaterials and in which cells are seeded; 2) cells are the basic constituent of a tissue by synthesizing the matrix of the tissue; and 3) growth factors are polypeptides that regulate the activities of cells.

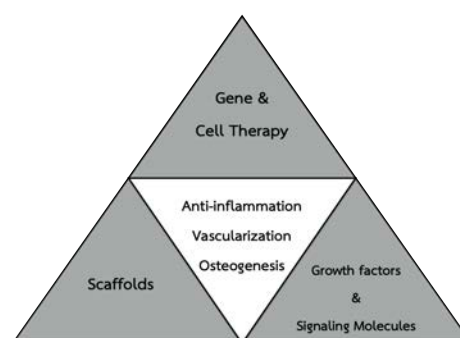


Figure 2.9 Tissue Engineering components

2.6.1 Scaffold

Scaffold or supporting matrices of bone or soft tissue engineering acts like a framework to provide space for cell or vascular ingrowth. The major roles for the supporting matrices are to provide space and having proper mechanical strength to endure collapse of the surrounding tissue into the grafted area, to serve as a scaffold for cellular migration and proliferation, and serve as a time-release mechanism for signaling molecules(82).

2.6.1.1 Scaffold features for bone regeneration

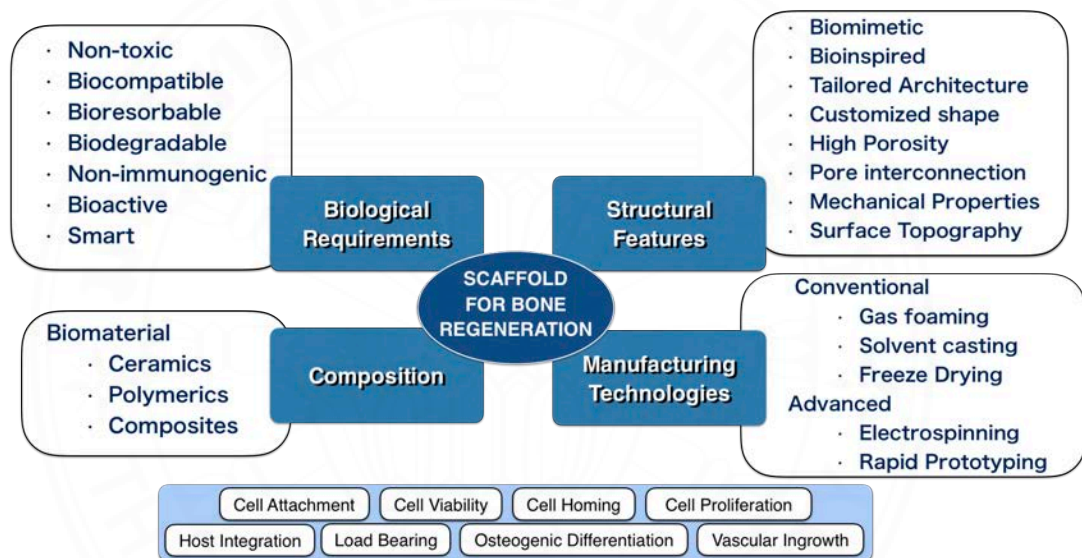


Figure 2.10 An ideal scaffold properties for Bone Tissue Engineering applications

The required characteristics of scaffold features for bone reconstruction consisted of biological requirements, structural features, manufacturing technologies and biomaterial composition.

1) Biological requirements : An ideal scaffold should be biocompatible, non-toxic, biodegradable and able to integrate and interact with the surrounding tissue, and having minimal risk of any adverse reaction.

2) Structural features : Scaffolds structure should be imitate the anatomical and physiological hierarchical 3D network structure of native bone extracellular matrix.

3) Manufacturing technologies: Nowadays, there are various fabrication technologies for reconstruction 3D scaffolds by conventional or advanced techniques.

4) Composition: the majority of scaffolds currently in use are naturally derived or synthetic polymers or a combination of them (composite materials).

The main purposes of bone regeneration applications by means of proper scaffolds are indicated: to allow cell engraftment and restore physiological structure and functions(87). The ideal 3D printed composite bone tissue engineering scaffold should have regular pores, mechanical properties similar to bones, good biocompatibility, and osteoconductive/osteogenic capacity.

Scaffolds are an integral part of bone tissue engineering. Scaffolds are three dimensional (3D) biocompatible structures which can mimic the ECM properties (such as mechanical support, cellular activity and protein production through biochemical and mechanical interactions), and provide a template for cell attachment and stimulate bone tissue formation. In addition to utilizing a scaffold that more closely replicates natural bone, it is also important to create a cell environment that more closely replicates the cellular processes that occurs during the natural bone healing, where multiple cells types migrate into the grafted area.

2.6.1.2 Porosity of scaffold

Pore size, pore volume and mechanical strength are critical parameters which define a scaffold's performance(86). At an early stage, bone ingrowth happens at the periphery of scaffolds with a negative gradient in mineralization toward the inner parts(88). For continuous ingrowth of bone tissue, interconnected porosity is important. Open and interconnected pores allow nutrients and molecules to transport to inner parts of a scaffold to facilitate cell ingrowth, vascularization, as well as waste material removal(89). Because higher porosity increases surface area per unit volume, the biodegradation kinetics of scaffolds can be influenced by varying pore parameters. Biodegradation through a cell-mediated process or chemical dissolution are both important to ascertain stabilized repair and scaffold replacement with new bone without any remnant. A minimum pore size between 100 and 150 μm is needed for bone formation; however, enhanced bone formation and vascularization are reported for scaffolds with pore size larger than 300 μm . Pore size also plays an important role in ECM production and organization(90).

Macroporosity (pore size $>80\text{--}100\text{ }\mu\text{m}$) is defined by its capacity to be colonized by cells. It can be induced in the material by the addition of organic substances that are sublimated or calcinated before sintering at higher temperatures. Both macroporosity (pore size $>80\text{--}100\text{ }\mu\text{m}$) and interconnectivity support the cellular invasion on ceramics. Interconnection size of $15\text{ }\mu\text{m}$ appeared to be effective to promote cellular colonization without bringing down mechanical strength(91). It appeared from histological observations that osteoblasts were able to cross interconnections measuring less than $5\text{ }\mu\text{m}$. By consequent, larger connections would optimize cellular penetration. Increasing the size of macroporosity would reduce the number of interconnections to cross and therefore would accelerate the cellular colonization. However, that would extremely compromise the mechanical resistance of ceramics(83).

The pore size of calcium phosphate scaffolds is an important factor for vascularization and osseointegration. Although smaller pore sizes have more surface area, pore sizes greater than $210\text{ }\mu\text{m}$ have been found to have increased amounts of bone and vessel formation than scaffolds with smaller pores(92).

Porous bone scaffolds can be made by a variety of methods(93). Chemical/gas foaming, solvent casting, particle/salt leaching, freeze drying, thermally induced phase separation, and foam-gel are some of those that have been used extensively(94). However, pore size, shape, and its interconnectivity cannot be fully controlled in these approaches. Moreover scaffolds with tailored porosity for specific defects are difficult to manufacture with most of these approaches.

2.6.1.3 Fabrication techniques of tissue engineering scaffold

Techniques for tissue engineering scaffold in the reconstruction of bone defects have been widely utilized to construct three-dimensional (3D) porous scaffolds(95, 96). The most frequently used technique to fabrication porous scaffold for bone regeneration is additive manufacturing (AM). The advantage of this technique was to construct complex structures. The structural, mechanical, biodegradation, biocompatibility, osteogenic and angiogenic features have been lean on the properties of biomaterial and techniques(22). Different AM approaches, for example, 3D printing (3DP), solid freeform fabrication (SFF), rapid prototyping (88), are approaches that allow

complex shapes for scaffolds' fabrication directly from a computer aided design (CAD) file. The concept of AM was first introduced by Chuck Hull in 1986 via a process known as 'stereolithography (SLA)'(97).

Some of the commercially available AM techniques are 3DP, fused deposition modeling (FDM), selective laser sintering (SLS), stereolithography, as well as various forms of direct writing. In all these AM approaches, 3D scaffolds are created layer-by-layer without any part specific tooling or dies. These AM techniques can be classified as (a) extrusion (deformation + solidification), (b) polymerization, (c) laser-assisted sintering, and (d) direct writing-based processes. Some of the AM techniques toward bone tissue engineering applications including their advantages and disadvantages(86).

2.6.1.3. Additive Manufacturing (22)

The different techniques of additive manufacturing(AM) approaches know as 3D printing (3DP), solid freeform fabrication (SFF), rapid prototyping (88) are allow to fabricate complicated shape of scaffold directly from computer design(22, 86).

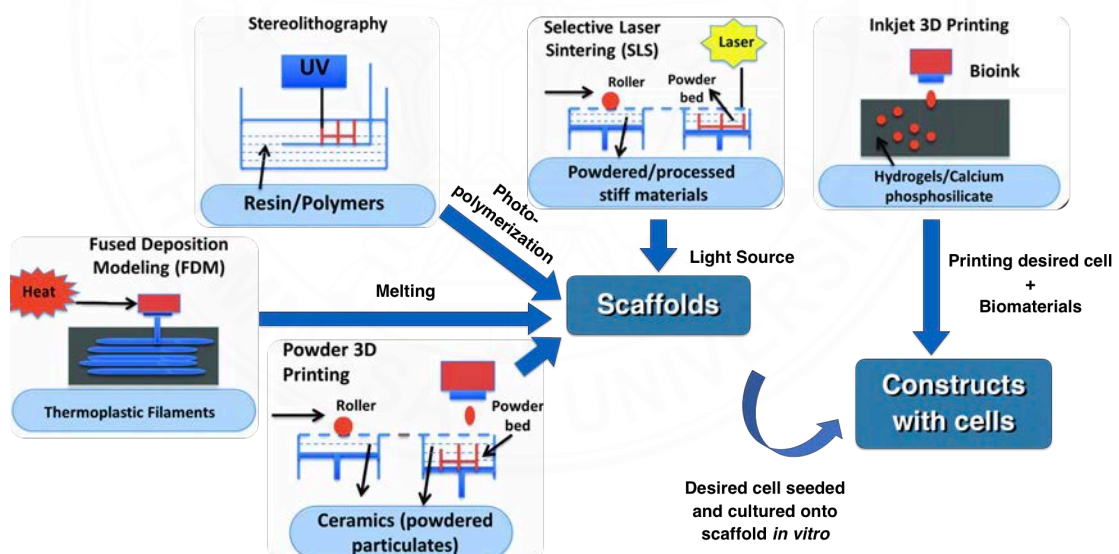


Figure 2.11 Various additive manufacturing approaches(22)

Stereolithography uses light (UV) to cross-link photopolymer liquids that polymerizes layer-by-layer by immersion of a platform downward into the liquid. Fused deposition modeling (FDM), on the other hand, involves deposition of a melted thermoplastic (filaments) by extrusion through a nozzle. Selective laser sintering modality uses long-wavelength lasers (or a high-energy light source) to fuse beads of

material one layer at a time, whereas powder 3DP does the same using a binder solution that locally hardens the powder bed where the binder is deposited. Ink-jet 3DP involves direct printing of liquid (polymer, cells, and biomolecules) using a nozzle and a print head in order to fabricate tissue engineering constructs. 3DP, three-dimensional printing(31).

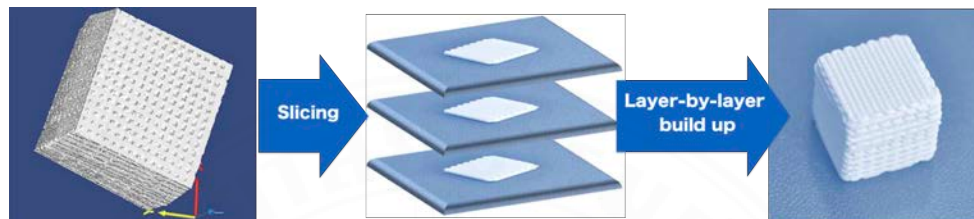


Figure 2.12 A flow chart illustrating the Rapid Prototyping process steps.

A computer-based 3D model is generated using imaging scan data. The model is then imported into a manufacturing system software to be sliced into thin horizontal layers finally, the 3D scaffold is built layer by layer based on the shape of the computer model for the manufacturing of tailored 3D structures(87).

2.6.1.4 Steps in the powder-based 3DP principle(98)

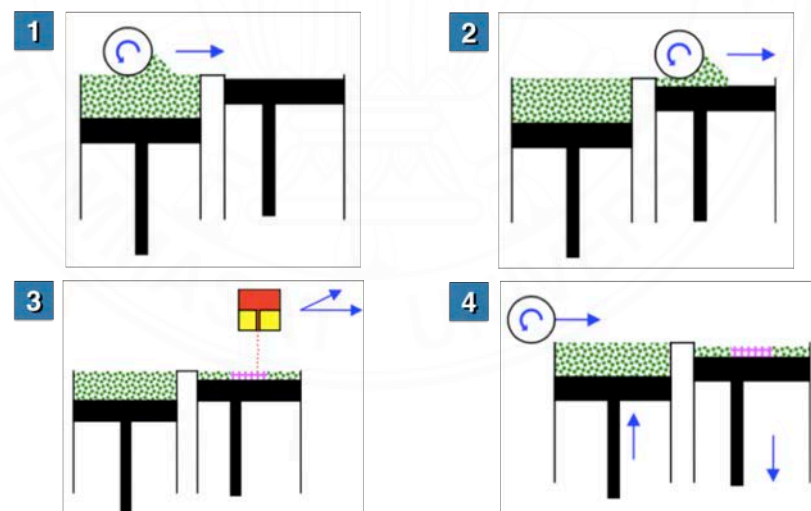


Figure 2.13 Schematic illustration in steps of the powder-based 3DP technique

Steps 1 and 2 : The roller places a thin layer of powder in the build area.

Step 3 : The inkjet head prints droplets of a binder fluid on the powder bed and thus locally solidifies part of the solid cross-section.

Step 4 : This process is repeated for every layer until the 3D structure is printed and the remaining powder is removed.

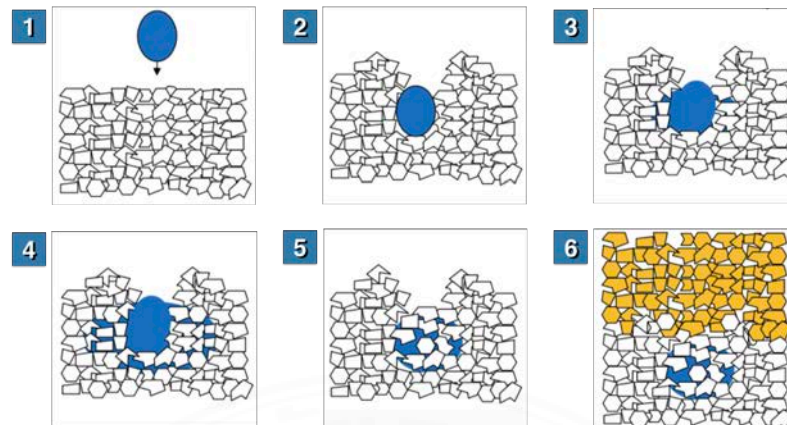


Figure 2.14 Schematic illustration on the main stages of single binder drop powder interaction during 3DP

- 1) build-up a homogeneous thin powder layer
- 2) binder droplet delivery on the powder bed while maintaining its integrity
- 3) wetting of the powder by the binder
- 4) spreading of the drop within the powder
- 5) binder/ powder reaction and hardening
- 6) recoating with a new powder layer
- 7) extraction of the green specimen from the powder bed
- 8) removal of loose powder within the green specimen(98)

2.6.2 Concentrated growth factors(CGFs) and Platelet-rich fibrin (PRF) membrane

Platelets have been introduced as biological substitutes in tissue engineering derived from a blood sample of patient to accelerate soft tissue healing in addition to bone healing(99). Several techniques for platelet concentrations have been suggested for a long time(100).

The platelet concentrations can be classified into 4 types as pure platelet-rich plasma (P-PRP), leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF) and leucocyte- and platelet-rich fibrin (L-PRF) depending on their content of leukocyte and fibrin (101).

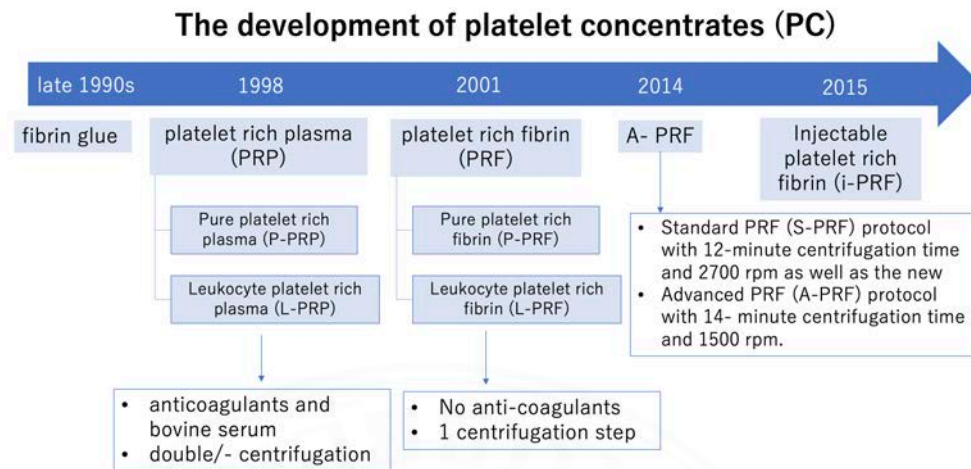


Figure 2.15 Development of platelet concentrations

In 1997, the first generation of PRP was used in oral and maxillofacial surgery. For the preparation, anticoagulated whole blood is centrifuged to separate red blood cells from platelets by mixing collected blood with thrombin and excess calcium, resulting in activated platelets trapped within a fibrin network(101). PRP require chemical additives such as anticoagulants and thrombin or calcium chloride to induce fibrin polymerization before applying to the surgical site and a double centrifugation technique for preparation(102).

In 2006, Choukroun et al. described the use of another platelet preparation obtained in a single centrifugation step and called it platelet-rich fibrin (PRF) which consists of releasing various growth factors (33). (103) PRF and concentrated growth factors (CGF) are second generation of platelet concentrate used patient's venous blood alone to activate platelet function and fibrin polymerization. Venous blood is drawn from patient's vein and collected in silica-coated vacutainers without anticoagulants. The Vacutainers are immediately centrifuged at 2,700 rpm for 12 minutes. A natural coagulation of fibrin layer is obtained with separation of the red blood cell layer, platelet-rich fibrin can be easily collected from the tube. Sacco's concentrated growth factors (CGF)(104) utilizes altered centrifugation from 2,400 – 2,700 rpm for 12 minutes to obtain much larger, denser and richer growth factors fibrin matrix. PRF or CGF layers contain concentrated autologous growth factors, but it has limited utilization for ridge augmentation as PRF/CGF layer cannot stabilize particulate

or powder bone(105). Unlike PRF using constant centrifugation speed, CGF utilizes altered centrifugation speed to produce much larger, denser and richer fibrin matrix containing growth factors. The growth factors enriched bone graft matrix or sticky bone using autologous fibrin glue has been demonstrated since 2010(106). Sticky bone provides stabilization of bone graft in the defect, and therefore, accelerates tissue healing and minimizes bone loss during healing period. CGF releases TGF- β , Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Bone Morphogenetic Protein 2 (BMP2) and Insulin-like Growth Factor (IGF-1) that increase proliferation and extracellular matrix mineralization via the BMP2/SMAD5/RUNX2 signaling pathway.

Table 2.7 Activities of growth factors

Growth factors	Fibroblast proliferation	Pre-osteoblast proliferation	Extracellular matrix synthesis	Mesenchymal cell differentiation	Vascularization
PDGF	++	++	-	-	+a
IGF	+	++	++	-	-
EGF	+	++	++	-	-
FGF	++	++	-	-	++
VEGF	$\pm$	-	-	-	++
TGF-B	+	+	++	-	+a
BMP	-	+	$\pm$	++	++a

++=greatly increased, +=increased, - =no effect or negative effect. a=indirect effect

In 2014, Choukroun introduced an advanced PRF called A-PRF with the low-speed centrifugation of the autologous blood at 1,300 rpm for 14 minutes (107). A-PRF shows results of earlier soft tissue growth, more release of BMPs, greater and faster vascularization and more cytokine release than conventional PRF.

In 2015 Mourão et al (108) described preparation of i-PRF where injectable form of platelet concentrate is required. Blood collected in 9 mL tube without any additive, centrifuged at 3300 rpm for 2 minutes, the resultant orange color fluid in the tube is the i-PRF.

PRF has shown enhancing effects on stem cell proliferation, differentiation, migration, and mineralization during bone formation; however, PRF alone may have an unstable effect on osteogenesis in vivo(109). PRF and CGF can be used as alternative to traditional barrier membrane over bone graft, therefore acceleration of tissue regeneration is acquired(110, 111).

2.7 Novel hydroxyapatite bone graft materials

The concern in developing efficacious bone graft substitutes or a scaffold is that significant variation in bone anatomy exists between patients. Furthermore, defect shape and size will be variable depending on the type of defect. These anatomical characteristics must be considered during graft fixation and design in order to achieve graft and host tissue integration. This suggests the need for using additive manufacturing (22) technology along with computer-aided design (CAD) so that bone grafts or scaffolds can be fabricated with complex shapes. Identified in patients by medical imaging techniques such as computed tomography (CT) and magnetic resonance imaging. This custom to defect graft can be easily created. These grafts or scaffolds could then be used in biomedical applications ranging from customized medical implant design to tissue engineering. AM also has the distinct advantage of enabling scaffolds or artificial constructs to be built with predefined micro- as well as macrostructures(31).

To overcome the drawbacks of routinely employed grafting materials, bone graft substitutes such as 3D scaffolds have been recently investigated in the dental field. Different biomaterials suitable for 3D scaffold fabrication, with a focus on “3D-

printed” ones as bone graft substitutes that might be convenient for various applications related to implant therapy(32).

2.8 3D printed nanohydroxyapatite (3DHA) by National Metal and Materials Technology Center (MTEC)

National Metal and Materials Technology Center (MTEC) has developed a 3D Printed Microporous Nanohydroxyapatite as bone substitute materials for orthopedic and dentistry applications for the last decade. The process of the synthetic hydroxyapatite using additive manufacturing (22) also known as 3D printing technique. It is a computerized fabrication technology that additively builds three dimensional physical objects layer by layer using digital data generated by computer. MTEC 3D printed microporous hydroxyapatite is now being used for reconstruction of bone defect in various clinical applications including(35, 36) :

- Orthopaedic surgery e.g. osteomyelitis treatment using antibiotic loaded hydroxyapatite in spine and long bone of upper and lower extremities.
- Scaffold and bone replacement material of skull
- Dental surgery e.g. augmentation/ reconstruction of alveolar ridge, filling of extraction sockets, filling of bone defect

2.8.1 The manufacturing of 3D printed nanohydroxyapatite

3D Printed nanohydroxyapatite layer thickness of 0.10 mm and saturation ratio of 1 was found to yield the highest transformation rate, density and greatest flexural modulus and strength. This was related to the sufficiently low-density printed structure suitable for the low-temperature phosphorization process. The balance between density and mechanical properties of as-fabricated 3DP samples is the crucial factor for efficient phosphorization and high-flexural properties after conversion(35).

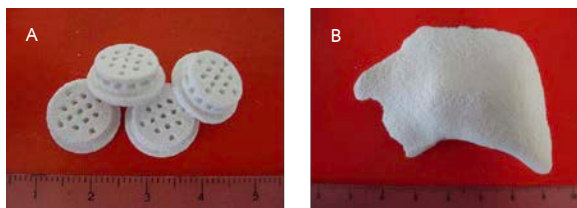


Figure 2.16 (A) Images showing bone tissue engineered scaffold which were fabricated

by the developed process using the layer thickness of 0.10 mm and saturation ratio of 1 (B) Custom or tailored cranial implant in various shapes to match the patient's defects.

The compositions of hydroxyapatite fabricated by phosphorization of three dimensionally printed calcium sulfate hemihydrate based materials at low temperature give the fastest transformation rate and greatest mechanical properties comprised 85% $\text{CaSO}_4 \cdot 0.5 \text{H}_2\text{O}$ and 15% pregelatinized starch(35).

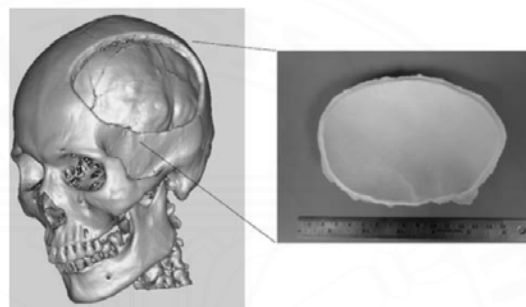


Figure 2.17 Hydroxyapatite skull implant made of transformed 3DP85 sample

The advantage of this fabrication technique is that the structure can be tailor made to fit the required shape and size with virtually no limitation.

2.8.2 Clinical study

From previous clinical study results using 3D printed Hydroxyapatite granules in alveolar ridge preservation concluded that 3D printed hydroxyapatite could potentially be used as a suitable bone grafting material for alveolar ridge preservation(112). However, further study in larger number of patients is still needed to ensure its performance. Other bone grafting procedures guided bone regeneration (GBR) in different types of defects should also be investigated.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

A Prospective clinical trial (Pilot study) to evaluate a clinical efficacy of 3D printed HA block graft for alveolar ridge reconstruction prior to dental implant placement.

3.2 Study Population

Twelve patients (113) who required dental implant therapy with horizontal bone defect during June 2019 to Dec 2020 at Faculty of Dentistry, Thammasat University hospital were participated in this study. The clinical protocol was registered in the Thai Clinical Trials Registry (Study ID: TCTR20190704004) and approved by Thammasat University Research Ethical Committee (Approval No. 039/2562) (Appendix A). Written informed consent about the details and purpose of the study were obtained in all patients before they signed informed consent (Appendix B).

3.2.1 Inclusion criteria

1. Adult patients (Age 20-65 years) (114)
2. Good general health (Physical status classification 1,2 according to the American Society of Anesthesiologists (ASA1,2) and not contraindication for minor surgery
3. The diagnosis and indications for bone block graft are including :
 - a. Horizontal ridge deficiency classification 4 (Benic & Hämmerle 2014).
 - b. The edentulous area allow placement of at least one standard implant and not exceeded a two-tooth span.
4. Patients who required primary bone augmentation prior to implant placement.
5. Controlled periodontitis.

6. Smoking history <10 cigarettes per day. For subject who smokes but less than 10 cigarettes per day is requested to stop smoking two weeks before and after bone block graft surgery and implant placement.
7. No history of allergy or hypersensitivity to any of the materials to be used in the study.
8. Signed informed consent.

3.2.2 Exclusion Criteria:

1. Age <20 or > 65 years
2. Presence of relevant medical conditions: Patients with bone disease, osteoporosis, or uncontrolled diabetes mellitus. Patients with medication of drugs influencing the bone metabolism or use of bisphosphonates.
3. Smoking status of more than 10 cigarettes/day.
4. Pregnancy or lactation.
5. Patients who have poor oral hygiene.
6. Patients with active infection or pathologic lesions prior to operation
7. Patients who cannot take impression (both conventional and digital) and CBCT
8. History of malignancy, radiotherapy, or chemotherapy for malignancy in the past 5 years.
9. History of autoimmune disease or long-term prescribed of nonsteroidal anti-inflammatory drugs
10. Unwillingness to return for the follow-up examination.

3.3 Materials

3.3.1 Data collections

Clinical record form of the Implantology clinic, Faculty of Dentistry, Thammasat University will be employed, including:

1. General information: patient's demographic information (e.g., name, age, gender, race, occupation, address, telephone number).

2. Medical record: past and present medical history and treatment, medication.
3. Dental record: chief complaint, dental history, clinical and radiographic examinations, procedures and outcomes.
4. Clinical examinations including:
 - Baseline clinical records: plaque index, bleeding on probing, hard and soft tissues dimensions, width of attached gingiva and keratinized tissue, etc.
 - Peri-operative clinical measurements: hard and soft tissue dimensions
 - Postoperative clinical measurements: postoperative hard and soft tissue dimensions, wound healing (Healing index; HI according to Landry, Turnbull and Howley; Appendix D) etc.
 - Follow up clinical record: postoperative wound healing, pain visual analog scale (115), complications, etc.
 - i. Healing index (HI) classified into 5 levels:
 1. 1-very bad
 2. 2-bad
 3. 3-good
 4. 4-very good
 5. 5-excellent

At follow-up visits of 2 weeks, 1 month, 3 and 6 months.

- ii. 10-unit VAS score ranged from 0 (no pain) to 10 (worst pain) at time of 3DHA block graft and follow-up visits of 2 weeks, 1 month, 3 and 6 months.
- Oral Health Impact Profile (OHIP-14): All patients attended and replied to the Oral Health Impact Profile (OHIP-14)-questionnaire before and after treatment. The seven dimensions are functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap. The five categories of response for each item are never (= 0), hardly ever (= 1), occasionally (= 2), fairly often (= 3) and very often (= 4).

5. Radiological examination record, including:
 - Pre-operative and immediately postoperative periapical film record
 - Pre-operative CBCT record: measurement of bone dimension
 - Final CBCT record after 6 months follow up: measurement of bone dimension
6. Conventional and digital impression using Cerec 3 (Dentsply Sirona, Germany) intraoral scanner to evaluate dimensional change of soft tissue before and after bone alveolar ridge augmentation.
7. Evaluate quality and quantity of bone at implant site : at the time of implant placement
 - a. Quality of bone : Insertion torque measurement to evaluate implant stability and Implant Stability Quotient (ISQ) was measured using Osstell® system, (Osstell AB Stampgatan, Gotemborg, Sweden)
 - b. Quantity of bone : evaluate further bone augmentation needed.
8. Histology and Histomorphometric analysis : at the time of implant placement to evaluate percentage of bone tissue, new bone formation, residual graft particles and connective tissue.

3.3.2 Control and testing Materials:

1. 3D printed bone block nanohydroxyapatite (MTEC, Thailand)
2. Collagen membrane (Cytoplast® RTM, Sybron Implant Solutions, Orange, CA, USA)
3. Bovine bone substitutes (Cerabone®, Botiss Dental GmbH, Germany)
4. Titanium pins (Master-Pin-Control Bone System, Meisinger, Germany)
5. Suturing material (VICRYL® 4-0 & Nylon ETHILON® 5-0, Ethicon Sutures, Belgium)
6. Implant fixtures (IS-III, Neobiotech, Korea) (BLT implant, Straumann, Switzerland) (NR line, Dentium, Korea)

3.3.3 Instrumentations and software :

1. Surgical instrument used for bone block graft surgery and implant placement
2. Centrifuge (Duo Quattro centrifuge, Nice, France), vacuum silica-coated tubes

(A-PRF™ tube), non-silica-coated tubes (Plastic S-PRF™ Tubes for Sticky Bone), butterfly needle (gauge 21, 7.5 inch in tube length), and tourniquet.

3. CBCT x-ray and imaging system (NSTDA, Thailand)
4. Periapical x-ray (SEARCHER 70 DX-073, Belmont, USA) and plate scanner imaging system (Apixia digital imaging, USA)
5. Cerec 3 imaging system (Dentsply Sirona, Germany)
6. Imaging analysis software of CBCT (DentiPLAN, NSTDA, Thailand), Periapical file (Apixia digital imaging, USA) and STL file (Geomagic, 3D Systems, USA)
7. Devices used for reconstruction of defect (The Touch™ Haptic Device, Geomagic freeform, 3D Systems, USA)
8. 3D printer for printing HA block graft (Projet160, 3D Systems, USA)
9. Volumetric analysis software (Geomagic ControlX, 3D Systems, USA)
10. Dimensional change analysis (Geomagic ControlX, 3D Systems, USA)
11. Implant stability (Osstell system, Osstell AB, Gothenburg, Sweden)
12. Chemical and materials for histology and histomorphology in laboratory (10% formalin, hematoxylin and eosin (H&E))
13. Light microscope equipped with camera (Nikon DS-U3, USA)
14. Histomorphometric analysis software (ImageJ software, NIH, Bethesda, MD, USA)
15. Statistical analysis software (GraphPad Prism 8.0.0, GraphPad, California, USA)

3.4 Conceptual Framework

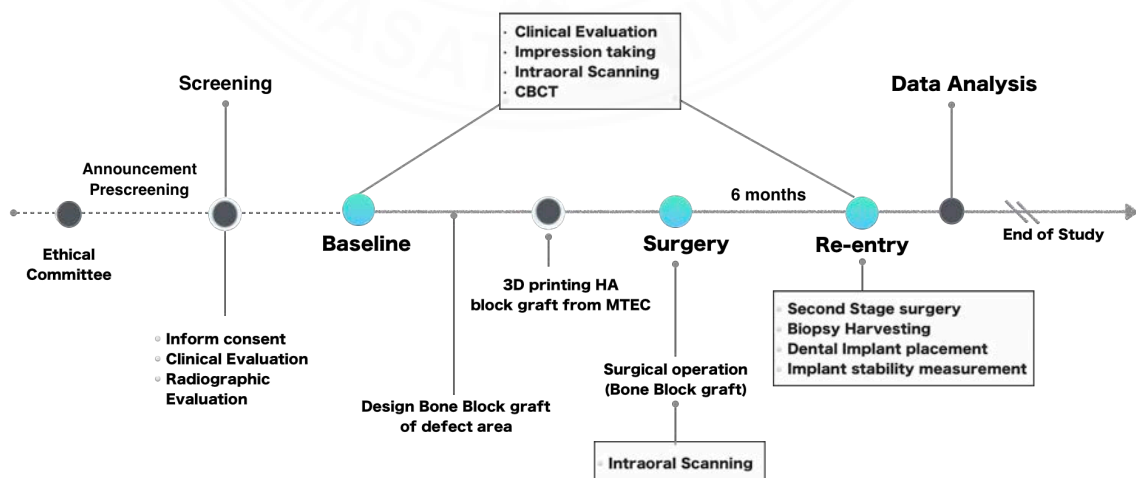


Figure 3.1 Conceptual framework

3.5 Variables and Measurements

3.5.1 Independent Variable : 3D printed nanohydroxyapatite bone block graft

3.5.2 Dependent Variables :

1. Clinical variable: hard and soft tissue dimensions, wound healing, complication.
2. Radiographic variable for bone tissue: Horizontal bone width (HW) of the alveolar ridge from CBCT scan at baseline and 6 months were measured perpendicular to a vertical line representing the planned implants or central axis at levels of 0-9 mm (HW0-9) apically 1 mm to the bone crest
3. Bone dimensional change: Horizontal bone gain from DICOMs and STL data set
4. Implant stability: Insertion torque of implant placement, Implant Stability Quotient (ISQ)
5. Histomorphometry: - Percentage of bone tissue, new bone formation, residual graft particles, and connective tissue.

3.6 Methods

3.6.1 Ethical Approval

- A clinical pilot study was conducted following the ethical principles founded in the Declaration of Helsinki and the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP).
- The clinical protocol was registered in the Thai Clinical Trials Registry (Study ID: TCTR20190704004) and approved by Ethical Review Sub-Committee Board for Human Research Involving Sciences, Thammasat University, No. 3 of Thammasat University, (Approval No. 039/2562).

3.6.2 Sample Size Calculation

- Subjects were voluntarily selected from adult healthy patients with horizontal alveolar defects that should be augmented with bone block graft prior to implant placement.

- The ages range in 20-65 years old was selected (114).
- Sample size was calculated from this equation considering from 20% drop out rate.

$$n = n_0 / (1-R)$$

$$n = 10 / 1 - 0.2$$

$$n = 12.5$$

which R = 20% drop out rate

3.6.3 Preparation of Subjects

(1) Preoperative Intervention

Twelve patients were enrolled in this research from inclusion and exclusion criteria. Informed of methods, benefit and risk of procedure and voluntary consent before starting protocol of research. Written informed consent was obtained. Medical and dental history were recorded.

The radiographic examination (CBCT) was performed in DICOMs files and exported into STL files to design patient's individual 3D printed nanohydroxyapatite block graft using computer aided design (CAD) software.

Extraoral, intraoral examination and the mouth preparation were obtained prior to the time of bone graft procedure to provide a more favorable oral environment to wound healing (e.g.; extraction of hopeless teeth, filling, scaling and polishing with oral hygiene instructions until full-mouth plaque score and full-mouth bleeding score <20%).

(2) Intervention

Subjects were performed bone block graft surgery with 3D printed nanohydroxyapatite bone block (3DHA) by one surgeon (PM).

3.6.4 Design workflow

For design workflow of this study, using 6 processes to create custom 3D printed hydroxyapatite bone block graft of individual patient's defect:

1. Patient's defect: CBCT scan (DentiiScan 1.1, NSTDA, Thailand)

2. Reconstruction of 3D model: using dental implant planning software (DentiPlan version 3.5, NECTEC, Thailand)
3. Create custom 3D bone block graft: 3D design software and professional haptic device (The Touch™ Haptic Device, Geomagic Freeform®, 3D Systems, USA)
4. Custom 3D HA block graft printing process: 3D printer (Projet160, 3D Systems, USA)
5. Scanning electron microscope (SEM, HITACHI S-3400N, Japan)
6. Clinical application

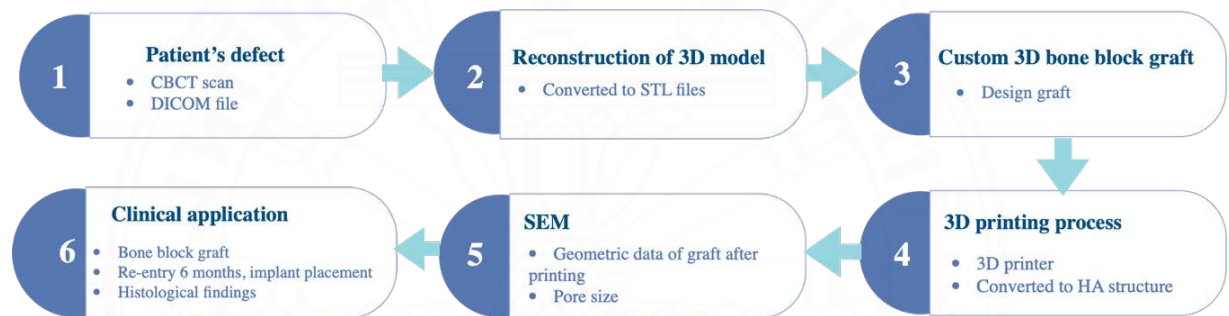


Figure 3.2 Workflow process of this study

3.6.5 Experimental Procedures

(1) Before Surgical Procedure

- a. Extraoral and intraoral examination.
- b. Intraoral photographs to being an initial data.
- c. Periodontal examination, including the assessment of plaque, probing depth (PD), bleeding on probing (BOP) at six sites (mid-buccal, mesio-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) adjacent to the block graft area at the baseline data by same calibrated examiner.
- d. Taking two impressions (upper and lower arch) with alginate for the treatment planning, diagnostic wax-up, fabrication of surgical stent
- e. Radiographic examination with intra-oral x-ray film (Periapical imaging

system) with parallel technique.

- f. Diagnostic wax up and treatment planning.
 - i. Waxed up the future restored tooth with implant on a stone cast to determine the location of the final prosthesis.
 - ii. The duplicated cast with waxed up was achieved to fabricate a surgical stent by means of the vacuum which will be applied for implant placement in correct position.
- g. All subjects underwent a baseline cone-beam computed tomography (CBCT) scan and Periapical imaging system.
- h. Analyzing all the collected data.

(2) Design and fabrication of customized 3D printed nanohydroxyapatite bone block graft

DICOM files from CBCT scans the dataset of an individual patient's defect (116) was imported to dental implant planning software (DentiPlan, NECTEC, Thailand). The DICOM file was converted into STL files, then exported to 3D design software (Geomagic Freeform®, 3D Systems, USA) and professional haptic device (The Touch™ Haptic Device, 3D Systems, USA) to create a custom 3D bone block graft. The template model of bone block graft was created by an ideal contour on the surface of the patient bone relevant to the ideal final dental implant position, then forming it into a 3D model.



Figure 3.3 DICOM files from CBCT scans were imported to dental implant

planning software (DentiPlan, NECTEC, Thailand). The DICOM file was converted into STL files

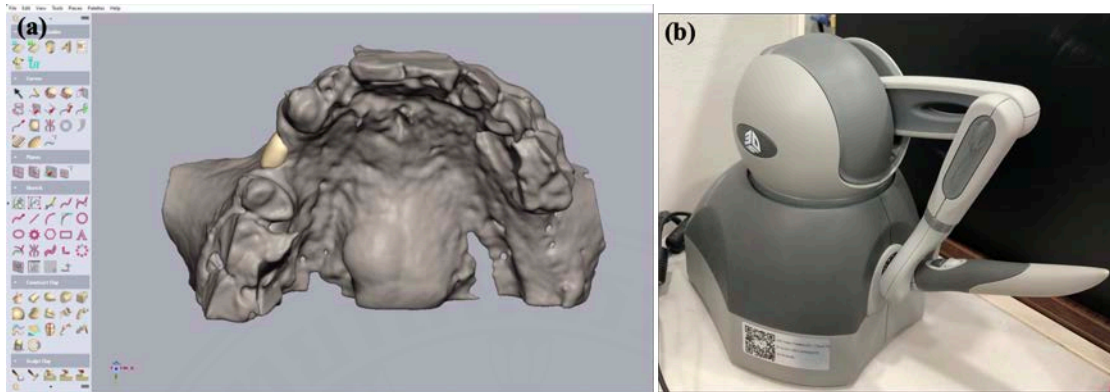


Figure 3.4 (a) Screen shot of 3D design software (Geomagic Freeform®, 3D Systems, USA).; (b) The haptic device (The Touch™ Haptic Device, 3D Systems, USA).

The 3DHA structure was designed to imitate the alveolar bone structure, which contained an internal bone trabeculae and the outer surface of the cortex bone. The pore structure configuration was created using Geomagic freeform software to create an optimal internal structure within the 3D printing pore size limit of less than 1 mm. Cell construction was completed with a spherical shape designed with 1.75 mm in diameter, 1.5 mm of the interconnected strut, and 2 mm in thickness of the cortex (117).

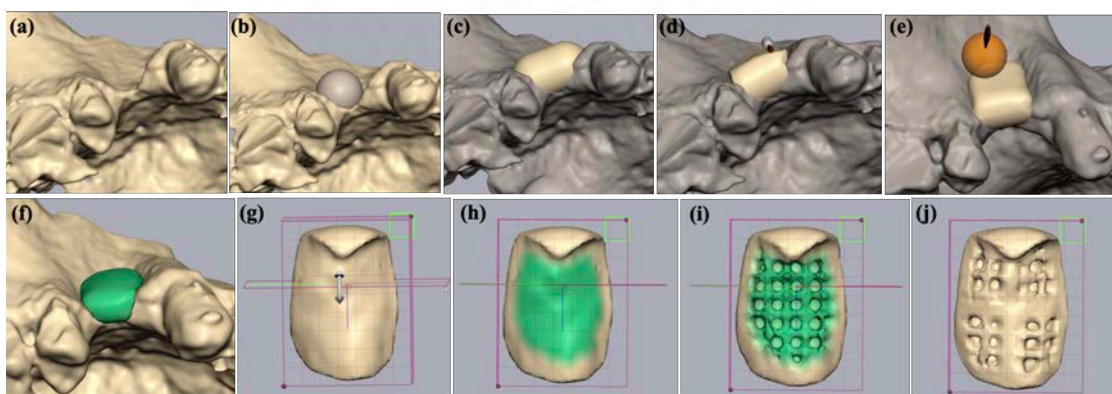


Figure 3.5 The design of block graft : (a) Defect area ; (b,c) Graft was created by adding clay using clay construction tool; (d) Clay was shaped and recontoured by carve tool; (e) Smoothed the clay; (f) Final structure of graft; (g) Reposition of clay

paralleled to the axis of pore creation; (h) Selected area of porous structure in green(active) and deselected area of cortex; (i) Porous structure was created using structural Fx tool; (j) Clay was designed with XT strut for strengthening graft after printing.

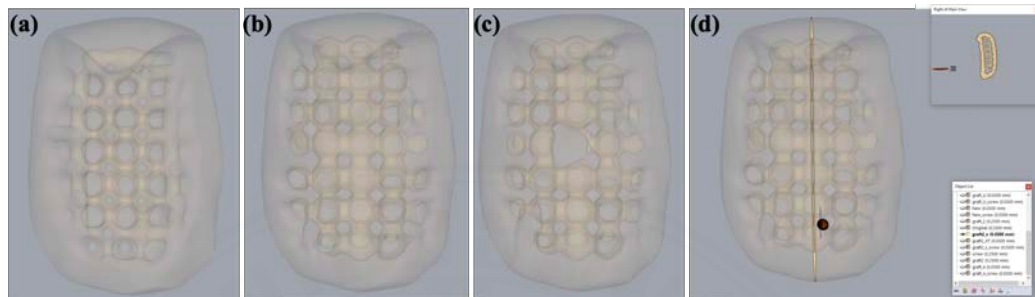


Figure 3.6 The strut of design was categorized into 3 patterns: (a) no strut; (b) cross; (c) cross combined T-shaped (xt) pattern; (d) cross-sectional view

The morphology of 3D bone block grafts was designed in cross-combined T-shaped (XT) patterns using Projet160 (3D systems, USA) with 300x450 dpi in resolution and 0.1 mm in layer thickness. The customized block graft was built layer-by-layer using calcium sulfate hemihydrate in combination with a water-base binder. After building, the printed grafts were soaking in a disodium hydrogen phosphate solution for 24 hours in order to convert into hydroxyapatite structure. All 3DHA grafts were dried and sterilized by ethylene oxide gas.

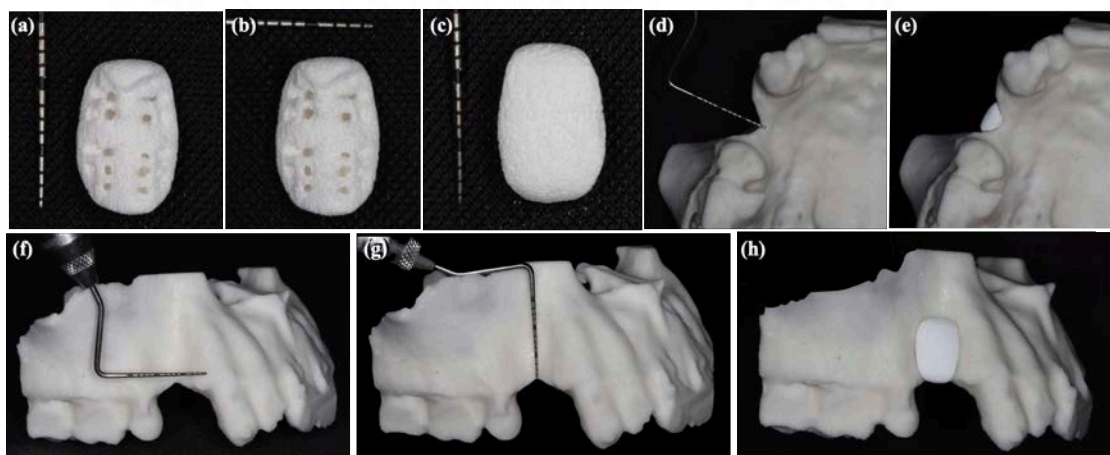


Figure 3.7 Custom 3D HA block graft after printing (cross combined T shaped pattern) : (a,b) Inner surface of block graft showing porous structure; (c) Outer surface mimicked cortex bone; (d) Occlusal view of defect model; (e) Occlusal view of graft; (f, g) Lateral view of defect model; (h) Lateral view of graft

(3) Scanning electron microscope (SEM)

The scanning electron microscope (SEM, HITACHI S-3400N, Japan) was used to examine the appropriate structural construct of the final HA bone block graft. The structure of the block graft would consist of the outer cortex and inner porous structures. The pore size of the graft ranged from 1.47-2.25 mm depending on the cutting plane of the graft.

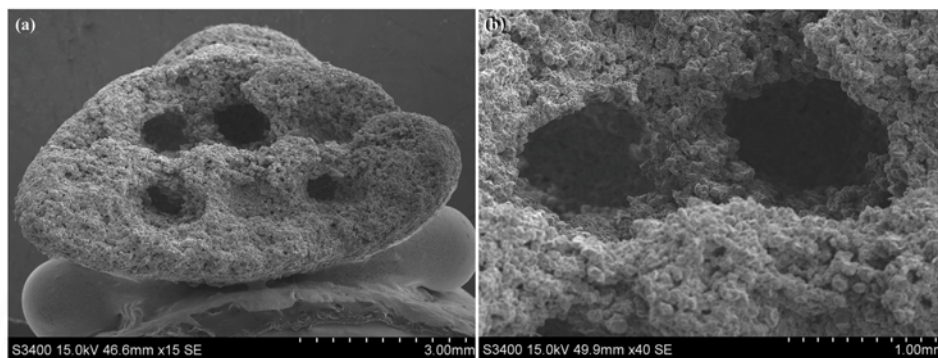


Figure 3.8 SEM micrographs of microstructure of custom 3D printed HA block graft : (a) Cross-sectional view of graft with cortex and porous structure ; (b) SEM in higher magnification showing pore in inner structure of the graft.

(4) Surgical Procedure

All the surgical procedure was performed by the one surgeon under local anesthesia.

a. Preoperative procedure :

- 1) Preoperative intraoral scanning with Cerec 3 imaging system (Sirona, Germany)
- 2) Vital signs measurement (Blood pressure, pulse rate)
- 3) Premedication with 2 g amoxicillin and 400 mg ibuprofen orally before surgery
- 4) Mouth rinsing with chlorhexidine 0.12% for 1 min.

b. CGFs and PRF preparations :

- 1) The CGFs and PRF membrane were prepared during the surgical procedure.
- 2) The patient's venous blood was drawn via a butterfly needle and collected in and collected in four vacuum tubes (40 ml).
- 3) The silica-coated tube was used for PRF membrane and non-silica-coated tubes

for CGFs.

- 4) Both tubes were placed in the centrifuge (Duo Quattro centrifuge, Nice, France) at the speed of 1,300 rpm for 14 minutes

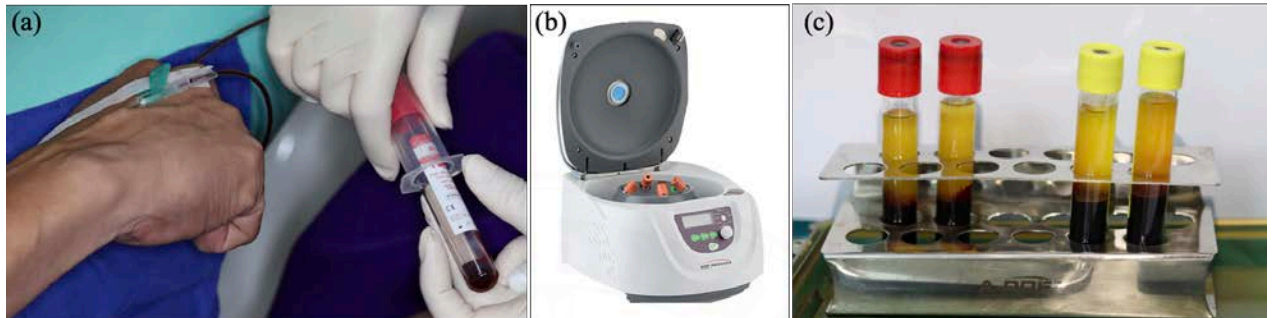


Figure 3.9 (a) patient's venous blood was drawn via a butterfly needle ; (b) Centrifuge (Duo Quattro centrifuge, Nice, France); (c) 3) The silica-coated tube (red caps) was used for PRF membrane and non-silica-coated tubes (yellow caps) for CGFs

- 5) In non-silica coated tubes, the yellow fluid as CGFs in the buffy coat layer was aspirated with a sterile syringe without the red part. The CGFs applied directly on the 3DHA graft until completely soaked.



Figure 3.10 (a) For CGF, inserted the needle near the buffy coat and used syringe to drain from the tubes to the bone graft materials; (b) 3DHA block graft was immersed in CGFs.; (d) Particulated xenograft was mixed to CGFs for sticky bone.

- 6) The silica-coated tubes were placed in the tube holder for approximately 15 mins to allow fibrin clots to form and consequently compressed into PRF

membranes according to the manufacturer's protocol

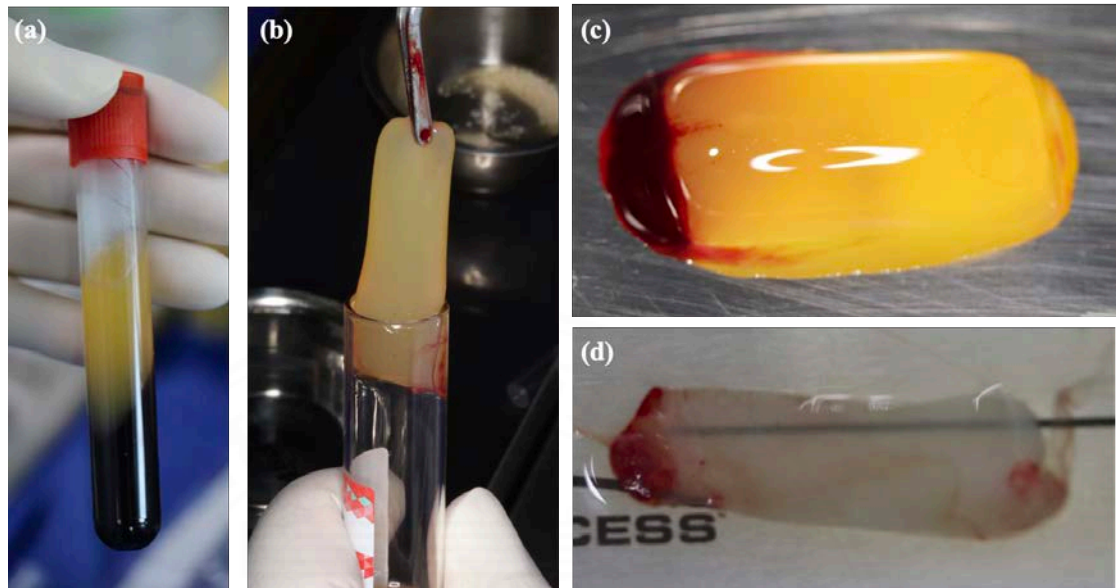


Figure 3.11 (a) Silica-coated tube without anticoagulant; (b) Remove upper layer from the tube.; (c) PRF membrane.; (d) PRF membrane after compressing with metal cover

c. 3DHA bone block graft surgery :

- 1) Administration of the local anesthesia with 4% articaine with epinephrine 1:100,000.
- 2) The muco-periosteal flap was prepared by a crestal incision together with a vertical releasing incision and gently elevated from the native bone tissue to allow complete visualization of the defect and surrounding bone.
- 3) Any granulation tissue remnants were removed from the bone surface and then native bone was decorticated with drills during irrigation with saline to ensure vascularization of the block graft and the recipient site.
- 4) The 3D printed bone block nanohydroxyapatite (MTEC, Thailand) was adapted to the defect.
- 5) For coverage of the augmentation sites, resorbable membrane (81) will be used.
- 6) Suturing with VICRYL® 4-0 & Nylon ETHILON® 5-0.
- 7) Administration of amoxicillin (500 mg ,administered orally , 1 capsule/3 times/day for 5 days), ibuprofen (400 mg., administered orally, 1 tablet/3

times/day as needed) , paracetamol (500 mg, administered orally, 1 tab every 6 hr as needed) and 0.12% chlorhexidine mouthwash (2 times/day).

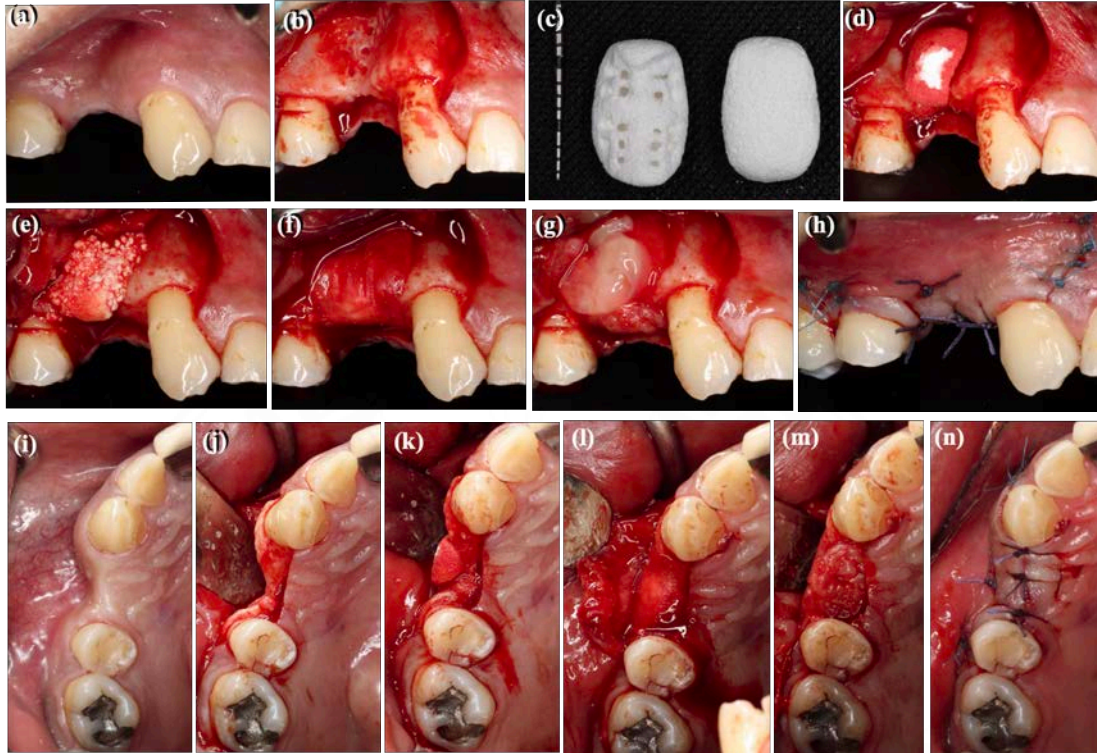


Figure 3.12 Clinical procedures of primary augmentation in (a-h) lateral and (i-n) occlusal views. Clinical image at (a,i) baseline. (b,j) Full thickness flap was reflected and (b) buccal bone was decorticated. Then (c) 3DHA block graft was placed combined with particulated bovine bone graft (d,k). Covered with collagen membrane (f,l) and PRF membrane(g,m) was placed on top. (h,n) Suturing.

d. Follow-up visits :

- 1) Subjects were followed up at day 14, 30, 90, 120 for intraoral scanning with Cerec3 imaging system (Sirona, Germany), at 2 weeks the sutures were removed. At 6 months of re-entry, titanium screws were removed and implant was placed at augmentation site. All subjects were recalled at 1,3,6 months postoperative.
- 2) After the integration period of 6 months, before the re-entry, two impressions, Periapical imaging system and CBCT scan were performed and then recorded

the aforementioned measurements scores of plaques index, bleeding index, gingival recession, and pocket depth.

e. Re-entry

After 6 months, surgical site was reopened. A biopsy was taken in progress of implant bed preparation at implant sites by replacing the first implant bur with a trephine (outer diameter: 3 mm, inner diameter: 2 mm, Meisenger, Germany). The bone cylinder will be placed in a fixative (10% formalin). Drilling at implant site preparation follow by surgical protocol. Implant was placed in an optimal position. Measurement of insertion torque to evaluate implant stability and ISQ evaluation. Administration of amoxycillin (500 mg. ,administered orally , 1 capsule/3 times/day for 5 days), ibuprofen (400 mg. , administered orally, 1 tablet/3 times/day as needed) , paracetamol (500 mg, administered orally, 1 tab every 6 hr as needed) and 0.12% chlorhexidine mouthwash (2 times/day). All patients were evaluated at 2 weeks for removing the sutures.

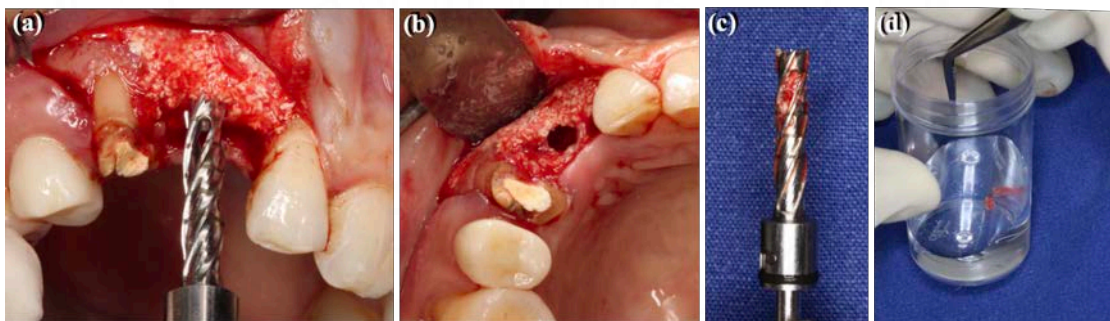


Figure 3.13 (a) Bone core biopsy collection procedure using trephine at implant site ; (b) core biopsy area for implant placement; (c) bone core sample inside trephine bur; (d) sample was fixed in 10% formalin solution.

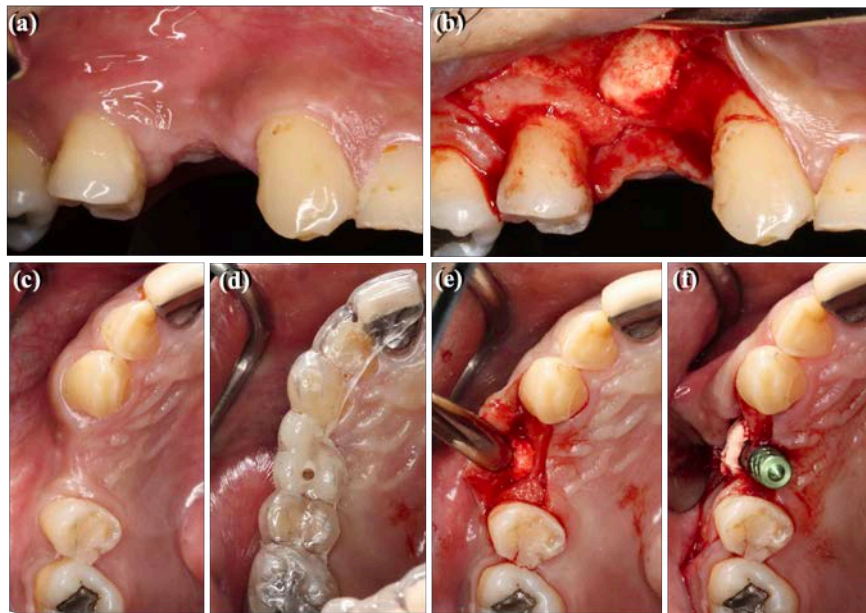


Figure 3.14 Re-entry of block graft area : Clinical situation after 6 months of block graft surgery in (a,b) lateral view; (c) occlusal view; (d) Marking implant position from surgical stent.; (b,e) HA block graft was integrated to native bone; (f) Implant placement.

(5) Histological Processing and Histomorphometry

The bone biopsy was placed in 10% buffered formalin solution for further histologic processing. The specimens were decalcified by immersing in 10% formic acid for 48 h, dehydrated in serial ethanol and embedded in paraffin blocks. After tissue processing, the histological sections of the bone biopsy were prepared parallel to the long axis of the cylindrical bone core with 3-5 μm in thickness by a microtome (Leica RM2235, Leica Biosystems Nussloch GmbH., Germany). The samples were stained with hematoxylin and eosin (H&E) and examined with a light microscope equipped with a camera (Nikon DS-U3, USA). Digital images were processed and measured in the area of bone tissues using computer software (ImageJ, NIH, Bethesda, MD, USA). The microscopic rectangular region of interests (ROI) of 3x4 mm was selected at the central part of the sections.

(6) Prostheses

The prosthesis was placed after 3 months of implant placement.

3.7 Data Collection

All measurements were performed by one examiner.

1. Characteristic of subject's demography and selected implants treatment.
2. Clinical characteristics of 3D printed nanohydroxyapatite bone block graft; peri-operative, postoperative and complications during healing process.
3. The baseline of clinical and radiographic data.
4. The final outcome after treatment with 3D printed nanohydroxyapatite bone block graft materials in clinical, radiographic and histological data.

3.8 Outcome variables

The primary outcomes of this study were horizontal bone gains assessed from DICOMs and STL data set. Secondary outcomes were pain VAS, healing index, OHIP-14, horizontal bone widths, volume and dimensions of graft, and histomorphometry.

3.8.1 Clinical evaluation

(1) Clinical features of soft tissue healing after bone block graft (Healing index) : Healing of augmented site was monitored and recorded according to Healing Index by Landry, Turnbull and Howley(16). The healing index classified into 5 levels: 1-very bad, 2-bad, 3-good, 4-very good, and 5-excellent at follow-up visits of 2 weeks, 1 month, 2, 3, and 6 months.

(2) Postoperative pain (VAS score) : recorded and evaluated using a 10-unit VAS score ranged from 0 (no pain) to 10 (worst pain imaginable) at time of HA block graft, follow-up visits of 2 weeks, 1 month, 2, 3, and 6 months.

(3) Complications were monitored including signs and symptoms of pain, swelling, ecchymosis and nerve injury. In addition, complications associated with bone block graft surgery such as incision line opening, membrane exposure, perforation of mucosa over the graft, infection, and partial and total graft failure were also monitored (17).

(4) Implant stability assessment : At 6 months, was assessed by Implant

stability quotient (ISQ) measurement.

(5) Dimension of alveolar ridge : Clinical ridge width (bucco-lingual direction), height (apico-coronal direction), and length (mesio-distal direction) in the buccal area of the blocks and measurements were obtained with a periodontal probe from the landmark from surgical stent at baseline. At follow-up visits of 2 weeks, 1, 2, 3, and 6 months after surgery, clinical examination and x-rays were assessed.

(6) Oral Health Impact Profile (OHIP-14) : To evaluate the oral health-related quality of life of patients treated with 3DHA block graft and implant supported prosthesis, all patients attended and replied to the Oral Health Impact Profile (OHIP-14) questionnaire before and after treatment. The seven dimensions are functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap. The five categories of response for each item are never (= 0), hardly ever (= 1), occasionally (= 2), fairly often (= 3) and very often (= 4). Higher OHIP scores indicate worse, and lower OHIP scores indicate better oral health-related quality of life.

3.8.2 Horizontal bone gain

The horizontal bone gains assessed from DICOMs and STL data set.

(1) CBCT analysis (DICOMs data set)

Data from CBCT scan of patients at baseline and 6 months after HA block graft were imported into dental implant planning software (DentiPlan, NECTEC, Thailand). The horizontal widths (118) of the alveolar ridge from CBCT scan at baseline and 6 months were measured perpendicular to a vertical line representing the planned implants or central axis at levels of 0-9 mm apically 1 mm to the bone crest (Figure 3.15). Horizontal bone gain was calculated by subtraction of baseline and at 6 months.

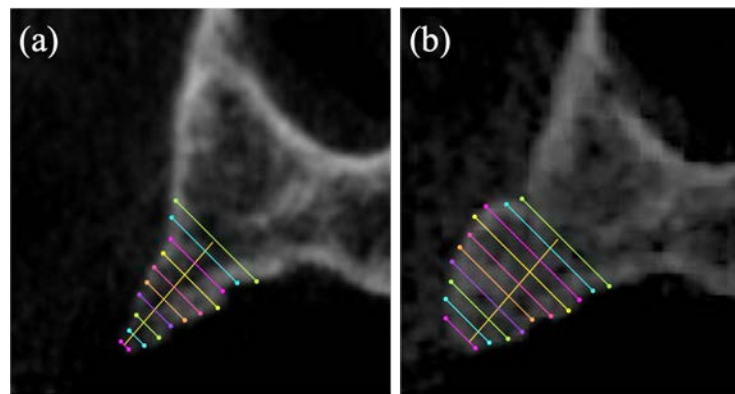


Figure 3.15 Horizontal ridge width (HW, mm) from CBCT at levels from alveolar crest (HW0-9); (a) CBCT at baseline, (b) CBCT at 6 months

(2) Linear analysis (STL data set)

The obtained DICOMs files at baseline and 6 months were converted into STL files. The two digital models of each patient were superimposed accepting values of average convergence distance of < 0.1 mm with best-fit alignment command by a software program. The 2D linear measurement from STL data set was performed by the calculated horizontal bone gain from baseline to 6 months along the cross-sectional plane at the central part of graft at each level of 0-9 mm from bone crest (Figure 3.16).

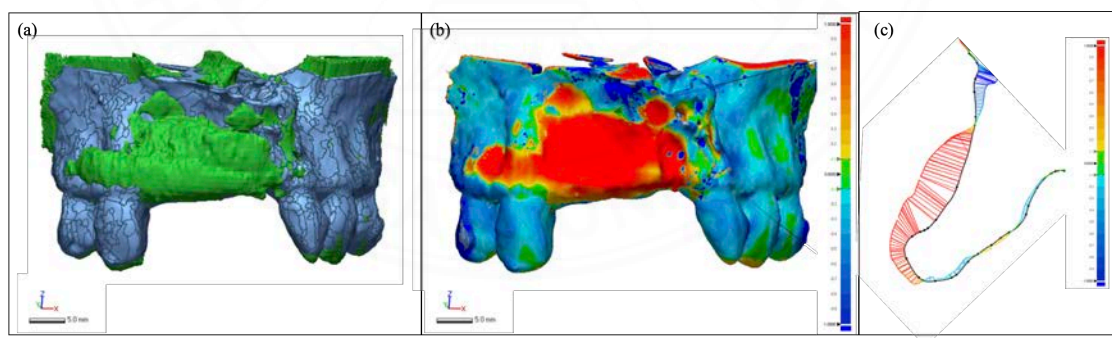


Figure 3.16 STL data set : (a) Superimposition of the STL files obtained at baseline and at 6 months. (b) 3D color map of the volume gain (red color). (c) In cross-sectional view show linear horizontal bone gain between baseline and 6 months.

(3) Volumetric changes of the augmented graft

The region of interest was selected according to the size of the grafted area.

Then two superimposed models were subtracted and calculated in mm^3 (Figure 3.17). The volume gain (mm^3) after 6 months was compared to the volume of 3D printed graft design by computer. The dimensions (width, length and height; mm) were also compared.

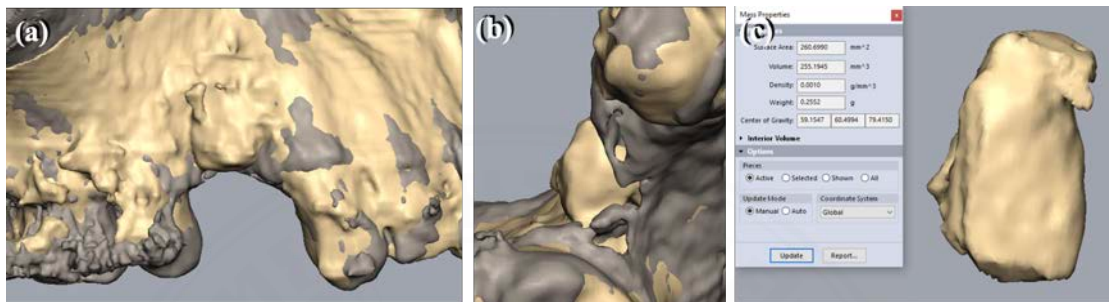


Figure 3.17 Volumetric analysis from CBCT scan by subtraction of STL files of preoperative and postoperative surgery in 6 months: (a,b) Superimposition of CBCT at baseline and 6 months; (c) Volume of bone gain after subtraction

(4) Histological Findings and Histomorphometric Analysis

The histological parameters were calculated and presented as percentage of total bone area, new bone formation, residual bone graft remaining, and connective tissue.

3.9 Statistical Analysis

All data were managed and calculated by GraphPad Prism 8.0.0 (GraphPad, California, USA). The descriptive statistics were presented as the mean and standard deviation (SD) of the parameters. The normality of the samples was tested using the Shapiro-Wilk test. Paired t-test was used to compare horizontal bone gain between DICOMs and STL data set, volume gain and dimensions of bone after 6 months and 3D printed graft. One-way analysis of variance (ANOVA) was used to compare horizontal bone gain at level of 0-9 mm and histomorphometric analysis. A statistically significant difference was considered when the p-value < 0.05. The other parameters of VAS, HI, ISQ were presented as descriptive statistics.

3.10 Budget

1. 3D printed nanohydroxyapatite bone block graft	Provided by MTEC, Thailand	
2. Collagen membrane (Cytoplast® RTM, Sybron Implant Solutions, Orange, CA, USA)		79,200
3. Bovine bone substitutes (Cerabone®, Botiss Dental GmbH, Germany)		42,000
4. Titanium pins (Meisenger, Germany)		16,560
5. Blood Centrifuge (Duo Quattro centrifuge, Nice, France)	Provided by Faculty	
6. Vacuum tubes		12,000
7. Butterfly needles		4,200
8. Osstell® system, (Osstell AB Stampgatan, Gotemborg, Sweden)	Provided by Faculty	
9. CEREC Omnicam intraoral scan imaging system (Sirona, Germany)	Provided by Faculty	
10. Geomagic software (Geomagic ControlX, Geomagic freeform, 3D Systems, USA)	Provided by MTEC, Thailand	
11. 3D printer for printing HA block graft (Projet160, 3D Systems, USA)	Provided by MTEC, Thailand	
12. CBCT imaging (NSTDA, Thailand)	Provided by Faculty	
13. Periapical films (Apixia digital imaging, USA)	Pay by Patients	
14. Alginate impression material	Provided by Faculty	
15. Prosthetic lab fees (surgical stent, crown)	Pay by Patients	
16. Implant fixtures, abutment and accessories	Pay by Patients	
17. Medication after surgery	Pay by Patients	
Total		153,960

3.11 Study Schedule

	Apr 2019	May 2019	Jun 2019	Jul 2019	Aug 2019	-----	Mar 2020	Dec 2020	Jan 2021	-----	Aug 2021	Sep 2021	Oct 2021	Nov 2021	Dec 2021	Jan 2022	Feb 2022	Apr 2022	Mar 2022
Research proposal	→																		
Ethical Approval	→																		
Study preparation		→																	
Surgical Treatment				→							→								
Data collection & Analysis									→										
Manuscript writing & Publication											→								→
Thesis Defense																	→		→

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS

4.1 Patient characteristics

Twelve patients were enrolled in this study (6 males, 6 females, with a mean of 53.5 ± 9.26 years). The demographic data of patients and implant characteristics were shown in Table 4.1 and flow diagram of included patients was shown in Figure 4.1. The sites of the patient's defect were presented in 6 anterior regions, 4 premolar regions, and 2 molar regions. Two out of twelve patients were excluded from this study due to block graft failure. Total 13 implants were placed with good primary stability (mean ISQ = 65 ± 4.08). The diameter and length of implants ranged from 3.2-4.5 and 8-10 mm respectively. All implants were restored after 3 months with provisional and screw-retained zirconia prostheses.

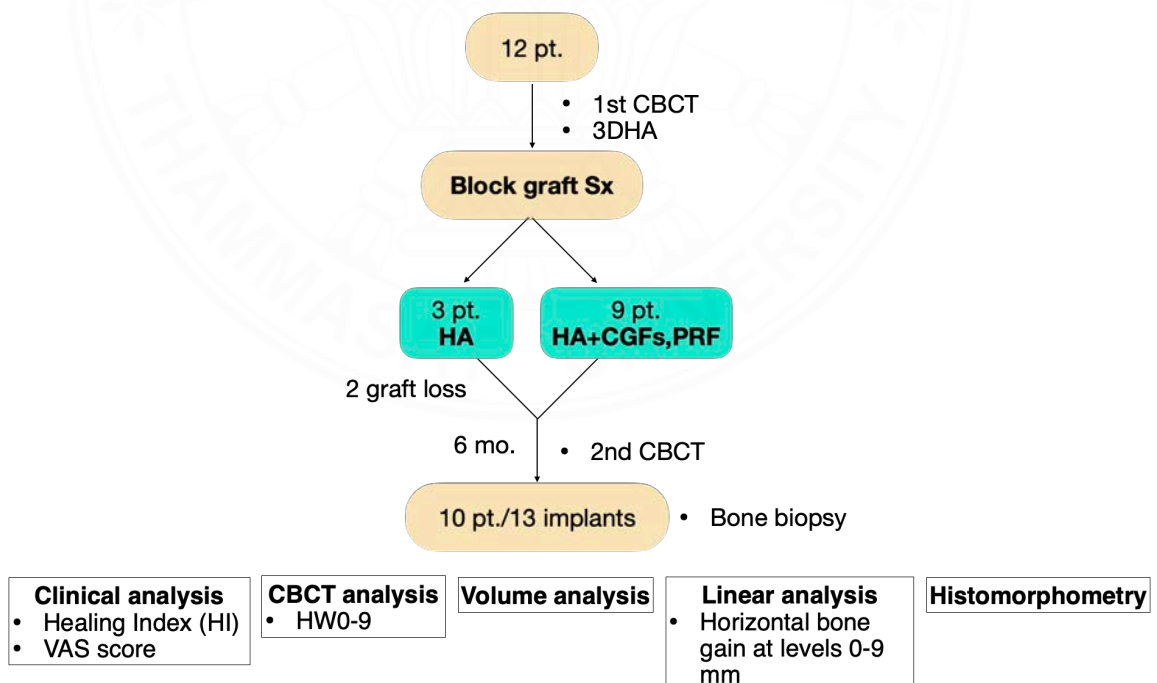


Figure 4.1 Flow diagram of included patients

Table 4.1 : Demographic data of patients, graft sites, materials, complications, implant characteristics and prosthesis type.

Patient	Gender	Age	Grafting site	Bone augmentation technique	Complications	Complication (months)	Management	Re-entry (months)	Implant type	ISQ	Prosthesis type
1	M	46	14	3DHA	Total bone block graft failure	2	Ramus block graft	-	-	-	-
2	M	53	22	3DHA	Partial bone block graft failure	2	GBR	-	-	-	-
3	M	57	13	3DHA	-	-	-	6	Neobiotech IS-III Ø 4.0*8.5 mm	65	-
4	M	53	14	3DHA + PRF	-	-	-	6	Neobiotech IS-III Ø 4.0*8.5 mm	65	Zr
5	F	65	34	3DHA + PRF	-	-	-	6	Neobiotech IS-III Ø 4.0*8.5 mm	70	Zr
6	M	65	11,21	3DHA + PRF	Partial bone block graft failure	2	-	6	Straumann BLT, Ø 3.3*8 mm	55	PR

Patient	Gender	Age	Grafting site	Bone augmentation technique	Complications	Complication (months)	Management	Re-entry (months)	Implant type	ISQ	Prosthesis type
7	M	56	14	3DHA + PRF	-	-	-	6	OsseoSpeed EV implant Ø 4.25 * 9 mm	70	-
8	F	38	12,21	3DHA + PRF	-	-	-	6	Neobiotech IS-III Ø 3.5*10 mm, 8.5 mm	65	PR
9	F	50	13	3DHA + PRF	-	-	-	6	Dentium NR line Ø 3.2*9 mm	65	PR
10	F	38	12,13	3DHA + PRF	-	-	-	6	Dentium NR line Ø 3.2*9 mm	65	PR
11	F	63	46	3DHA + PRF	-	-	-	6	Neobiotech IS-III Ø 4.5*10 mm	65	Zr
12	F	58	36	3DHA + PRF	-	-	-	6	Neobiotech IS-III Ø 4.5*10 mm	65	Zr

Note : M-Male; F-Female; GBR-Guided bone regeneration; ZR- screw-retained zirconia crown; PR- Provisional prosthesis

4.2 Clinical evaluations

4.2.1 Clinical outcomes and complications

Two patients who received only HA block grafts found early perforation of mucosa over the graft without signs of infection but resulting in partial and total bone block graft loss during first 2 months (Figure 4.3). HA block graft combined with CGFs and PRF membrane was used in nine patients resulting in enhancing of soft tissue healing and has fewer complications of HA block graft (one case of partial bone block graft failure) during the entire postoperative follow-up period and at 6 months after surgery exhibited good incorporation into the recipient sites and standard implants can be placed (Figure 4.2).

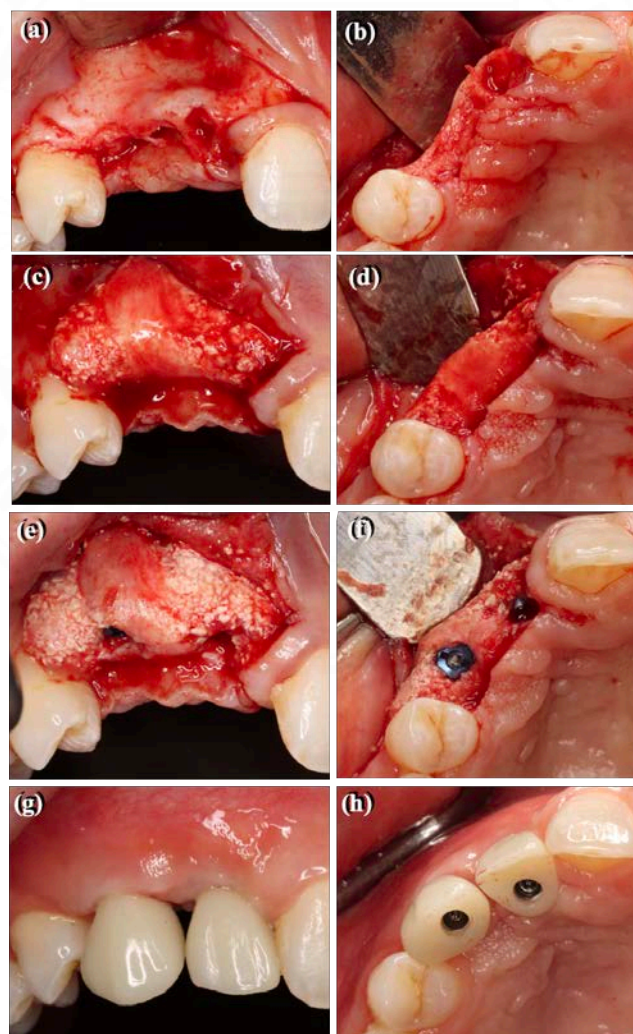


Figure 4.2 Clinical images at (a,b) baseline; (c,d) re-entry at 6 months.; (e,f) Implant placement; (g,h) provisional prosthesis.



Figure 4.3 Clinical images of grafted complications: (a) partial 3DHA graft exposed at 22 area; (b) loosening of graft at 14 area.

4.2.2 Pain VAS score and Healing index (HI)

The mean pain VAS scores were generally very low at the time of surgery (1.41 ± 0.51) and over the follow-up periods at 2 weeks (0.92 ± 0.51), 1 month (0.33 ± 0.49), 2,3,6 months (0). There were no significant differences in perception of pain at baseline of surgery and 2 weeks postoperatively (Figure 4.4 a). For tissue healing, the healing index increased over time corresponded with the means improved soft tissue healing and presented a significantly higher score in 2 months (3.75 ± 1.22) and highest score in 6 months (4.7 ± 0.67) (Figure 4.4 b).

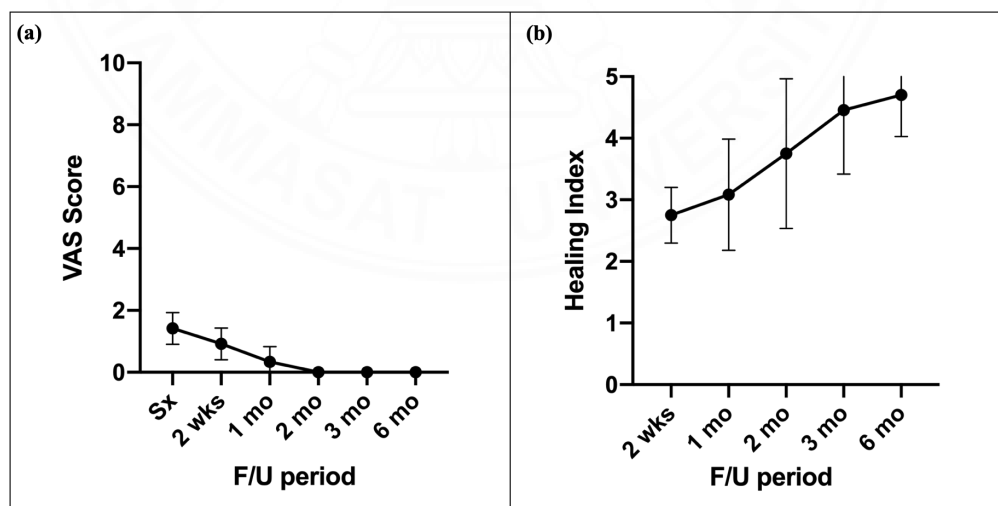


Figure 4.4 (a) Pain VAS score; and (b) Healing Index of Landry, Turnbull and Howley at follow visits.

4.2.3 Oral Health Impact Profile (OHIP-14)

10 out of 12 patients replied to the Oral Health Impact Profile (OHIP-14) questionnaire before and after treatment. The mean scores of the OHIP-14 prior to and after treatment were 2.41 and 2.00 respectively. After treatment, the mean score was significantly lower. Mean score of each question from Q1-Q14 was shown in figure 4.5.

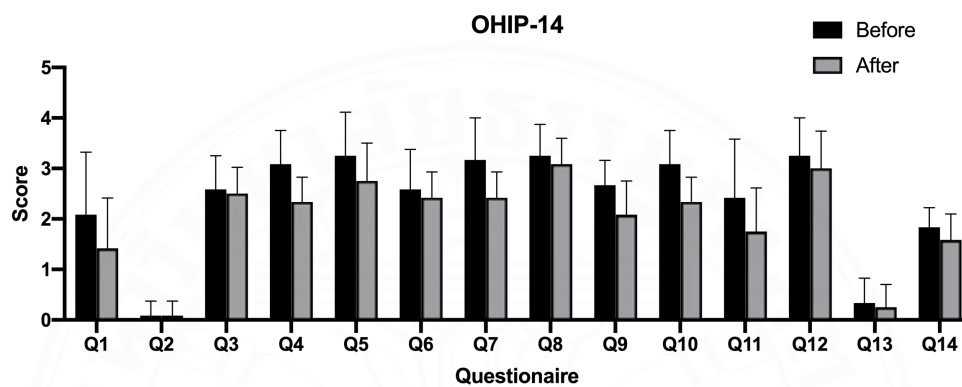


Figure 4.5 Score of OHIP-14 in each question.

4.3 CBCT analysis (DICOMs data set)

The mean horizontal width (HW0-9) of the alveolar ridge at baseline, 6 months, and bone gain was shown in Table 4.2. When comparing HW between baseline and 6 months, there was a significant difference at the level of HW0-6 but not a significant difference at HW7-9. The mean of HW at baseline and 6 months was 4.53 ± 1.71 and 6.87 ± 1.24 mm respectively. The mean of bone gain was 2.34 ± 0.52 mm (maximum and minimum bone gain of 3.05 ± 0.72 and 1.55 ± 1.15 mm respectively.)

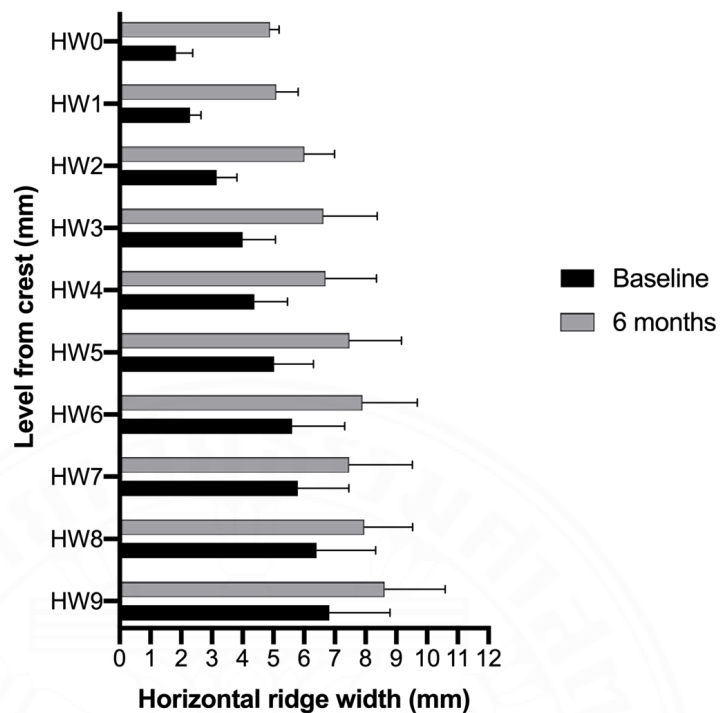


Figure 4.6 Horizontal ridge width (HW, mm) from DICOMs data set at each level from alveolar crest (HW0-9)

4.4 Linear analysis (STL data set)

The mean horizontal bone gain from baseline to 6 months were presented in Table 4.2. The mean maximum bone gain was 3.45 ± 0.41 mm at level 0 mm. and minimum bone gain was 1.61 ± 0.87 mm at level 9 mm. from alveolar crest. The letters (a, b, c) indicate the statistically different of bone gain compare to each level (Figure 4.7).

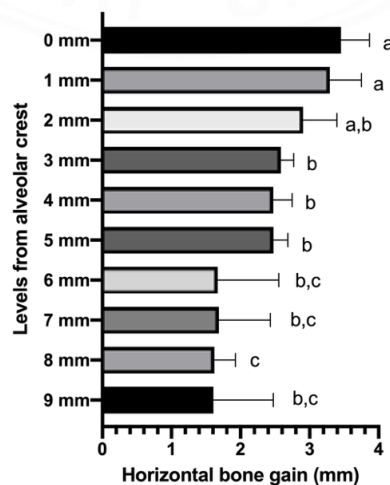


Figure 4.7 Horizontal bone gain (mm) from linear measurement using STL data at

levels of 0-9 mm from alveolar crest. ANOVA followed by Tukey's test. The different letters above the bars indicate statistically different levels (significance level at P-value < 0.05).

Table 4.2 Horizontal ridge width at baseline and 6 months. Horizontal bone gain (mm) from DICOMs and STL data set.

Levels from alveolar crest	Horizontal ridge width (mm)			Horizontal bone gain (mm)		
	Baseline	6 months	P-value	DICOMs data set	STL data set	P-value
0 mm	1.84 ± 0.54	4.89 ± 0.30	<0.0001	3.05 ± 0.72*	3.45 ± 0.41 ^a	0.1456
1 mm	2.29 ± 0.36	5.10 ± 0.71	0.0003	2.81 ± 0.85	3.29 ± 0.46 ^a	0.1306
2 mm	3.16 ± 0.66	6.01 ± 0.98	0.0002	2.85 ± 1.16	2.91 ± 0.49 ^{a,b}	0.8915
3 mm	4.00 ± 1.06	6.63 ± 1.75	0.0008	2.62 ± 1.40	2.58 ± 0.19 ^b	0.9299
4 mm	4.38 ± 1.08	6.69 ± 1.66	0.0047	2.31 ± 1.20	2.47 ± 0.27 ^b	0.6816
5 mm	5.03 ± 1.27	7.48 ± 1.69	0.0022	2.45 ± 1.15	2.48 ± 0.21 ^b	0.9366
6 mm	5.61 ± 1.71	7.89 ± 1.79	0.0055	2.28 ± 1.33	1.67 ± 0.89 ^{b,c}	0.2450
7 mm	5.80 ± 1.66	7.47 ± 2.05	0.1048	1.67 ± 1.31	1.69 ± 0.75 ^{b,c}	0.9710
8 mm	6.40 ± 1.92	7.96 ± 1.57	0.1656	1.55 ± 1.15*	1.62 ± 0.31 ^c	0.8450
9 mm	6.82 ± 1.97	8.61 ± 1.98	0.0628	1.79 ± 1.62	1.61 ± 0.87 ^{b,c}	0.7820
Mean ± SD	4.53 ± 1.71	6.87 ± 1.24	<0.0001	2.34±0.52	2.38 ± 0.85	0.6975

Note : Data represent (mm; Means ± SD). Different letters (a,b,c) and (*) indicate significant differences between levels for each variable analyzed (p < .05) within data set group.

4.5 Volumetric changes of the augmented graft

The 3-dimensional analysis and volume of the graft at the original design and 6 months after augmented was shown in table 4.3. The mean volumetric of 3DHA from computer design software was ($202.8 \pm 65.88 \text{ mm}^3$) and ($223.8 \pm 80.46 \text{ mm}^3$) at 6 months after grafted. No statistically different was found when compared in width, length, height and volume (Table 4.3).

Table 4.3 The volumetric and dimensional changes of the augmented graft

	Width (mm)	Length (mm)	Height (mm)	Volume (mm ³)
3D-Printed HA	4.64 ± 0.8	11.99 ± 4.29	10.25 ± 1.23	202.8 ± 65.88
Bone after augmentation (CBCT)	4.5 ± 1.11	11.7 ± 4.54	10.9 ± 4.65	223.8 ± 80.46
P-value	0.7715	0.4431	0.6326	0.0994

Note: Statistically significant differences when compared between 3D-Printed HA and bone after augmentation ($p < .05$).

4.6 Histological Findings and Histomorphometric Analysis

The histological finding revealed the interface of the augmented bone layer and native bone layer. For higher magnification, new bone formation around graft particles was seen in all harvested bone cores. Blood vessels within the connective tissue were also found. No evidence of acute or chronic inflammation infiltrate was observed (Figure 4.8). The histomorphometric analysis revealed bone tissue, new bone formation, residual graft, and connective tissue as shown in Table 4.4. The percentage of bone tissue area, newly formed bone, connective tissue and residual bone graft were ($30.48 \pm 4.81\%$), ($28.6 \pm 1.88\%$), ($20.81 \pm 4.41\%$), and ($19.82 \pm 4.07\%$) respectively. Compared to the connective tissue and residual bone graft the percentage of newly formed bone was significantly different.

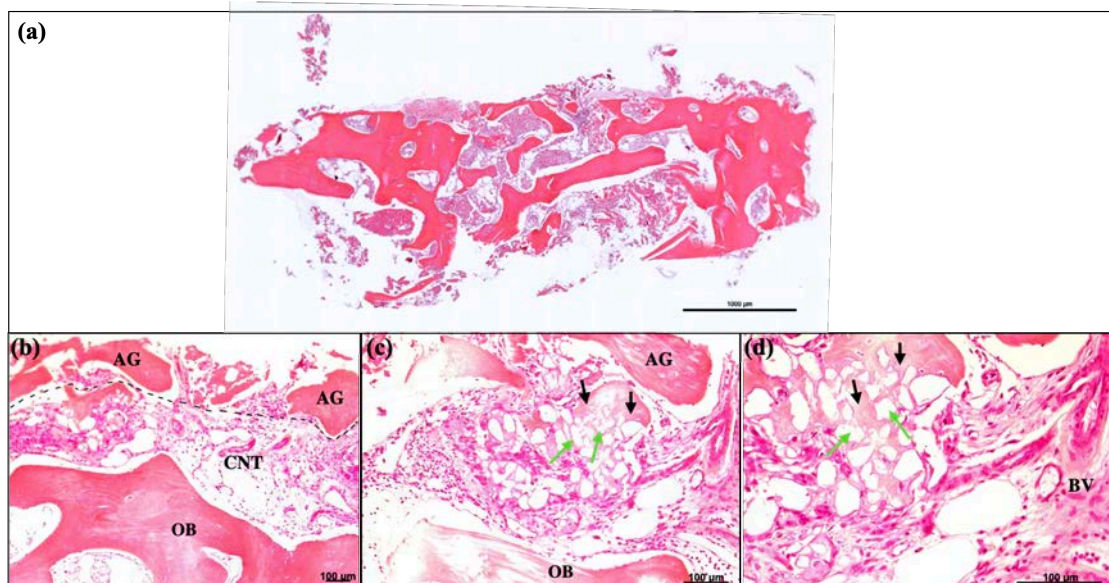


Figure 4.8 Histological images of a bone core sample with h&e staining : (a) in longitudinal section; (b) magnification x4 objectives.; (c) magnification x10 objectives.; (d) magnification x20 objectives. (OB = Old bone, CNT = Connective tissue, AG = Augmented graft area, BV = Blood vessel, FT= Fatty tissues, above dot line = augmented bone at buccal side, green arrow = graft particles spaces, black arrow = newly formed bone)

Table 4.4 Histomorphometric analysis presented in percentage of bone tissue, new bone formation, residual graft and connective tissue

Area of measurement	%,Mean $\pm$ SD
Bone tissue	30.48 $\pm$ 4.81 ^a
New bone formation	28.6 $\pm$ 1.88 ^b
Residual graft	19.82 $\pm$ 4.07 ^c
Connective tissue	20.81 $\pm$ 4.41 ^c

Note: Different letters (a,b,c) indicate significant differences between area of measurement ($p < .05$).

DISCUSSION

The physiological ridge resorption is a critical consequence following tooth loss, from diseases, trauma, or tumor resection(6). This event may result in an insufficient alveolar bone volume for implant placement. Therefore, additional bone augmentation procedures are often performed to provide the optimal width and height of the alveolar ridge prior to implant placement, especially for prosthetically driven positions. Several bone grafting techniques have been described such as autogenous block graft, expansion of the alveolar crest, and guided bone regeneration (GBR) (39, 119). Among these techniques, GBR by the use of particulate bone substitute in combination with barrier membrane has the benefit of reducing morbidity, discomfort and operation time. However, the disadvantages of this technique are mechanical instability and poor resistance to soft tissue pressure during repositioning flaps, and risk for graft collapse(2). Bone substitute material in block form represents an effective alternative to particulate grafting materials for the augmentation of ridge contours in case of severe bone resorption(2). In clinical situation, the use of block form of bone graft is time consuming and requires the skills of the surgeon to prepare and fit the graft to the recipient site. Immobilization of bone block graft can be determinant for the successful integration of the graft(120). Xenogenic block graft was also reported, but one major disadvantage is the brittleness of graft and high failure rate(121). Recontouring or shaping of xenogenic block graft to match the defect site is also difficult to perform at the chairside(81).

The emergence of biomaterials engineering and computer-aided design and manufacturing (CAD-CAM) technology has led to a new way to develop autograft alternatives. In a recent study, allogenic bone block graft was custom designed and fabricated by milling technique, however, there was a limitation in graft design for complicated defects and still pose a risk of disease transmission(122). In the past few years, various alloplastic biomaterials have been proposed for bone augmentation (25, 123). One of these is hydroxyapatite (HA), which exhibits similar chemical structure as natural bone with osteoconductive properties (124). HA is the least soluble form of the naturally occurring calcium phosphate salts, that provides an osteoconductive

scaffolding function, being highly resistant to physiologic resorption (125). Recently, a combination of 3-dimensional powder printing process and low temperature phase transformation were able to produce a novel low crystalline nano-hydroxyapatite structure (3DHA) (35). This low temperature technique allows to create the bone graft material with both osteoconductivity and osteoclastic resorbability in vivo due to their low crystallinity in comparison to typical high temperature sintering route (35, 126-128). The best feature of this 3DHA block graft was the custom-designed graft to match the defect site. Without the need of shaping the graft to fit the recipient bed make the surgical process faster and easier. Therefore, when compared with autogenous bone block graft from the previous studies (18, 129), patients reported less pain and fewer postoperative complications with, mean pain VAS score of ramus block graft was 3.96 (129) while mean VAS in our study was only 1.42 ± 0.51 . To evaluate the oral health-related quality of life of patients treated with 3DHA block graft. The total mean scores of OHIP-14 before and after treatment were 2.41 ± 1.03 and 2.00 ± 0.91 respectively. Mean score of OHIP-14 after treatment was significantly lower than before treatment may imply improvement in patients' quality of life following bone block graft and restoration with dental implant.

A proper adaptation of 3DHA block graft to the recipient site can improve graft stability. No screw for the rigid fixation of the block graft was needed, but titanium pins were used as membrane fixation. The stability of the graft is also enhanced when using bone blocks instead of particulate grafts (130).

CGFs were incorporated into the 3DHA graft for enhancing biological activity for bone regeneration besides its osteoconductive properties(131). PRF membrane was intentionally placed above the collagen membrane to facilitate soft tissue healing and minimized the chance of membrane exposure(34, 132). Ortiz-Vigón et al reported that patients suffered from intense pain and having soft tissue dehiscence in 3 days after xenogenic bone block graft and have to remove graft immediately (133). In this study, early graft failures were occurred in the first 2 out of 3 patients (66.67%) but when combined with CGFs and PRF in the rest of 9 patients, these reduced graft failures down to one case (11.11%) with only graft exposure after 2 months. CGFs and PRF

were also increasing soft tissue healing as seen from increasing healing index in early two weeks follow-up (HI = 2.75).

After 6 months, CBCT x-rays demonstrated an adequate bone volume for placing the implant in all patients. No significant resorption or non-fixation of the graft was observed. Using the same method of measurement by the author's previous studies (134, 135). The mean maximum horizontal bone gain was 3.45 ± 0.41 mm. This was comparable with the results from Mendoza-Azpur et al. reported that the maximum horizontal bone gain was 4.8 ± 0.79 mm in autologous bone block graft with particulate xenograft covered with resorbable membrane and 5.6 ± 1.35 mm in GBR site without autogenous block graft (136). Kloss FR et al. reported a horizontal bone gain of 2.6 ± 2.5 mm for allogeneic blocks (137). The result has also been the same in the current systematic review, Naenni N et al, reported horizontal bone gain from primary lateral bone augmentation of 2.90 ± 0.83 mm (138).

For volume gain, after 6 months was 223.8 ± 80.46 mm³ compared to the volume of 3D printed HA (202.8 ± 65.88 mm³) were slightly higher due to the addition of particulate bone graft but not statistically significantly different. Moreover, the second layer of xenogenic particulate bone graft may slow the resorption of 3DHA block graft (134, 139) and increase the volume of grafted area. These findings agree with the hypothesis that xenograft added to autogenous block graft led to less resorption compared to autogenous block graft alone (138). In the previous study of allogenic block graft, volume gain after 6 months was 411.1 ± 159.4 mm³ was higher than in our study due to their larger defects (137). However, volume changes after 6 months were comparable to lateral GBR using a freeze-dried bone allograft (FDBA) and collagen membrane showed 247.0 ± 114.1 mm³ but volumetric analysis based on polyvinylsiloxane dental impression (140). The 3D volumetric changes in our study were calculated from CBCT of baseline and 6 months of bone which has no bias from soft tissue thickness.

At the re-entry stage, the sample bone core was intact during the biopsy procedure without any soft tissue infiltration. From histological findings in the H&E staining section, found interface area between native bone and augmented area. In higher magnification a newly formed bone around graft particles and blood vessels.

Histomorphometric analysis showed the percentage of bone tissue, new bone formation, residual graft, and connective tissue were comparable to results from a previous study of 3DHA using alveolar ridge preservation(134). The behavior of 3DHA material from histological findings founded that the 3DHA crystal in the grounded sections has a larger surface area and a smaller crystal size compared to sintered HA xenograft (Cerabone). This difference may associate with higher resorption rates of 3DHA from low temperature technique manufacturing. The limitations of patient numbers in this study could not clarify superior bone formation in the PRF group. A systematic review by Miron et al. supported the clinical use of PRF for soft tissue regeneration and wound healing for a variety of procedures in medicine and dentistry(141). In vivo study of Engler-Pinto A et al, found the combination of PRF and xenograft presented a greater amount of new bone formation when compared with xenograft group, as well as higher expression of vascular endothelial growth factor (VEGF), osteocalcin (OCN), and bone morphogenetic protein (BMP2,4) (142). Amaral Valladão et al. reported the use of PRF combined with bone graft and PRF membrane can improve the outcomes of GBR for vertical and horizontal bone gain (143). However, there is no evidence of the effect of a PRF membrane on osteogenic properties (144).



CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study demonstrated customized 3D printed hydroxyapatite design and fabrication by additional manufacturing technique or 3D printing. In clinical performance, this graft can be used as a bone block graft and fitted to the required defect. From histological investigation found newly formed bone and blood vessels at the interface of graft and native bone area. The volumetric and linear dimensional changes of augmented area had shown optimal bone gain for implant placement and all implants can be placed with good primary stability. Moreover, the patients were satisfied with their oral health-related quality of life when compared to before treatment. Although the study conducted on the use of 3D printed hydroxyapatite block graft has shown promising results.

5.2 Recommendations

From the perception of the clinician, the osteotomies for implant placement were performed with looser bone density in 3DHA grafted site compared to the autogenous bone grafted site. The under-preparation drilling technique was recommended to improve the primary stability of the implants.

Since the number of patients who participated in the study was limited, a randomized controlled trial should be conducted to compare the effectiveness of the 3D printed graft to the standard autogenous graft.

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The seal of Thammasat University is a circular emblem. It features a central five-petaled lotus flower. Above the lotus is a horizontal bar with five lines, and above that is a crown-like structure. The lotus is flanked by two crossed swords. The entire emblem is enclosed in a circular border. The top half of the border contains the university's name in Thai script, and the bottom half contains the name in English, "THAMMASAT UNIVERSITY".

APPENDICES

ScF 03_01 (Eng)

The approved investigator must comply with the following conditions:

1. Researcher fully understand that it is unethical to collect studied data before the protocol has been approved by The Ethical Review Sub-Committee Board for Human Research Involving Sciences, Thammasat University, No. 3 (ECScTU).
 2. The research protocol activities must be ended on the approval expired date. If require to extend the approval, application should be done along with research progress report not less than one month prior to expiry date.
 3. The researcher(s) must strictly conduct the research activities as mentioned in the proposal.
 4. The researcher(s) must submit the progress report according to schedule (ScF 09_01 Progress Report Form).
 5. Use only the participant information sheet, consent form, research tools, advertising leaflet (If any) that have been approved and stamped by the ECScTU's seal of approval.
 6. Report to ECScTU for any serious adverse events within 5 working days (ScF 10_01)
 7. Report to ECScTU if any changes of the protocol for approval prior to continue the activities (ScF 08_01 Protocol Amendment Form).
 8. Submit the final report (ScF 11_01 Protocol Final Report Form) within 30 days after the completion of the research.
-

APPENDIX B

Informed consent form

ScF 05_02 (หน้า 1 จาก 2)

หนังสือแสดงความยินยอมเข้าร่วมการวิจัยของอาสาสมัครวิจัย

Informed Consent Form

ทำที่.....

วันที่.....เดือน.....พ.ศ.

เลขที่ อาสาสมัครวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย การปลูกถ่ายกระดูกก่อนทำการฝังรากเทียมจากชิ้นกระดูกไฮดรอกซีอะพาไทต์
ด้วยวิธีการขึ้นรูปแบบสามมิติ : การศึกษานำร่อง.....

ชื่อผู้วิจัย นายบวรวุฒิ บุรณวัฒน์.....

ที่อยู่ติดต่อ คณะทันตแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์.....

โทรศัพท์ 085-9977669.....

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงอาสาสมัครวิจัยโดยตลอด และได้รับคำอธิบายจากผู้วิจัยจนเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงอาสาสมัครวิจัย โดยข้าพเจ้ายินยอมสละเวลามาทำการรักษาโดยการปลูกกระดูกร่วมกับการเจาะเลือด และเข้ารับการติดตามผลหลังการรักษา ในช่วงวันที่ 14, 30, 90 และ 180 วัน หลังการผ่าตัด รวมถึงถ่ายภาพในช่องปาก และถ่ายภาพรังสีก่อนรักษา และในช่วง 6 เดือนหลังการรักษา และการเก็บชิ้นเนื้อภายหลังการรักษา 6 เดือน เมื่อเสร็จสิ้นการวิจัยแล้วข้อมูลที่เกี่ยวข้องกับอาสาสมัครวิจัย จะถูกทำลายทั้งหมด (แบบฟอร์มเก็บข้อมูล ขึ้นเนื้อที่ส่งตรวจ)

ข้าพเจ้ามีสิทธิถอนตัวออกจากการศึกษาเมื่อใดก็ได้ตามความประสงค์ โดยไม่ต้องแจ้งเหตุผล ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบต่อการศึกษา/ ผลต่อการรักษา/ ผลต่อการเรียน และไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติตามข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงอาสาสมัครวิจัยและข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็นความลับ โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

ScF 05_02 (หน้า 2 จาก 2)

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงอาสาศัมครวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่: คณะอนุกรรมการจริยธรรมการวิจัยในคน มหาวิทยาลัยธรรมศาสตร์ ชุดที่ 3 ห้อง 110 ชั้น 1 อาคารปิยชาติ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต โทรศัพท์ 02-986-9213 ต่อ 7358

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารข้อมูลสำหรับอาสาศัมครวิจัย และสำเนาหนังสือแสดงความยินยอมเข้าร่วมการวิจัยของอาสาศัมครวิจัยไว้แล้ว

ลงชื่อ.....

(.....)

ผู้วิจัย

วันที่...../...../.....

ลงชื่อ.....

(.....)

อาสาศัมครวิจัย

วันที่...../...../.....

ลงชื่อ.....

(.....)

พยาน

วันที่...../...../.....

ลงชื่อ.....

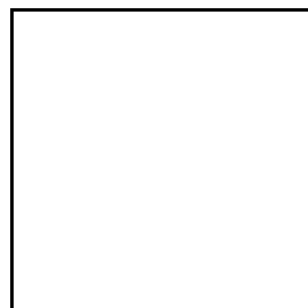
(.....)

พยาน

วันที่...../...../.....

กรณี อาสาศัมครไม่สามารถเขียนได้

ลายพิมพ์นิ้วมือ



อาสาศัมครวิจัย

วันที่...../...../.....

APPENDIX C

Patient examination record form

Dental Implantology Clinic
Faculty of Dentistry, Thammasat University Hospital

Code number.....

Chief complaint

.....

1. Medical history

a. Systemic disease:

☐ Poor controlled diabetes mellitus	☐ Rheumatoid arthritis
☐ Under high steroid therapy	☐ Osteoporosis
☐ Menopause	☐ Uncontrolled hypertension
☐ Others.....	

b. Current medications:

.....

.....

.....

c. Drug allergy:

.....

.....

2. Cause of extraction (Implantation area)

.....

.....

3. Habit

☐ Bruxism	☐ Clenching	☐ Non-smoking
☐ Smoking..... cig. /day		

4. Extraoral examination

☐ Head	
☐ Face	
☐ Nose	
☐ Lip	
☐ High lip line	☐ Gummy smile ☐ Low lip line

1

5. Intraoral examination

Tooth number (edentulous area):

Alveolar ridge width:

B-L mm

M-D mm (at gingival margin)

Approximate tissue thickness mm

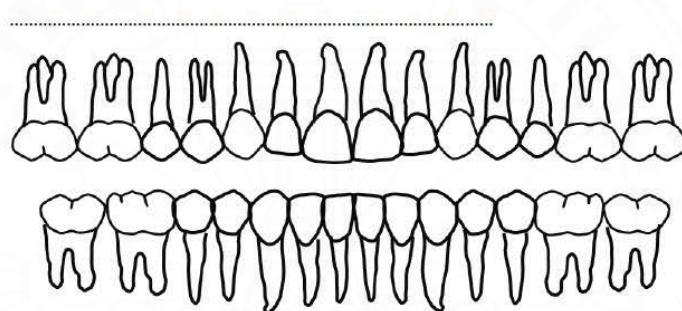
Keratinized tissue (AGW1) mm

Angle's classification: ☐ Class I ☐ Class II ☐ Class III

Interocclusal space mm

Lateral movement: ☐ Canine guidance ☐ Group function

Type of antagonist tooth: ☐ natural tooth ☐ Prosthesis



6. Oral hygiene status

Last visit to the dentist for.....

Plaque index (O'Leary)

☐ good (<20%) ☐ fair (20 – 50 %) ☐ Poor (>50%)

Bleeding index

Periodontal Diagnosis:

History of Periodontal treatment:

☐ when

☐ how

7. Radiographic finding

Technique: ☐ Parallel ☐ Bisecting ☐ OPG ☐ CT/CBCT

Bone available: From sinus floor to alv. crest mm

From inf. alv. canal to alv. crest mm

M-D width of neighboring tooth at CEJ mm

CEJ – alv. crest..... mm

8. Cerec scanner result:

.....

.....

9. Operation (Surgical time)

a. Preparation & Operative finding:

Local anesthesia with.....

b. Pre-medication

.....

.....

c. Complication

☐ Intra – operation

.....

.....

☐ Post – operation

.....

.....

d. Measurements:

Alveolar ridge width:B-L mm

M-D mm

Keratinized tissue mm

e. Post-medication

.....

.....

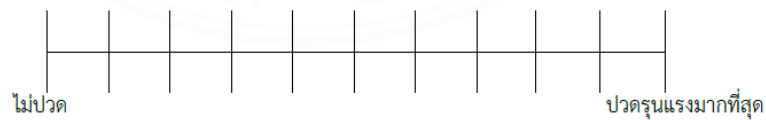
f. Radiographic finding (CT/CBCT after extraction)

Vertical buccal bone height (VBBH1) mm

Horizontal bone width (HBW1) mm

g. VAS score at time of surgery

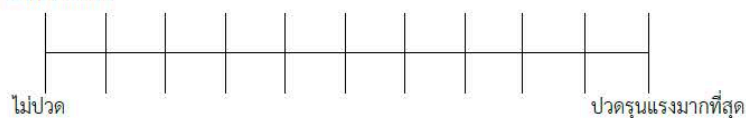
☐ VAS Score



10. Follow up time

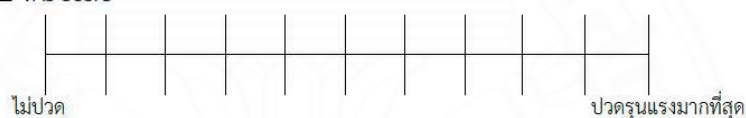
Follow up day 14: (Stitches off):

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

☐ VAS Score

☐ Healing index

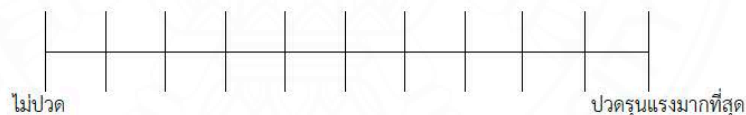
Follow up 1 months:

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

☐ VAS Score

☐ Healing index

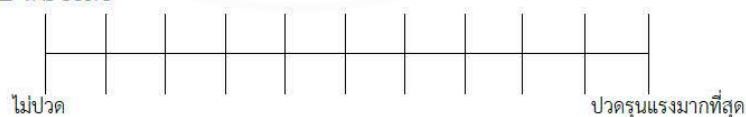
Follow up 3 months:

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

☐ VAS Score

☐ Healing index

Follow up 6 month: (Implant placement):

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

☐ VAS Score

☐ Healing index

11. Re-entry time and implant placement (6 months after surgery)

a. Before surgery:

Plaque index (O'Leary)

☐ good (<20%)

☐ fair (20 – 50 %)

☐ Poor (>50%)

Bleeding index

Alveolar ridge width: B-L mm

M-D mm

Keratinized tissue mm

Cerec scanner result.....

.....

.....

Radiographic finding (CT/CBCT 6 month before re-entry time)

Vertical buccal bone height mm

Horizontal bone width mm

b. Preparation & Operative finding:

Pre-medication

.....

.....

Local anesthesia with.....

Tissue thickness.....mm

Alveolar ridge width: B-L mm

M-D mm

Bone support: ☐ sufficiency

☐ insufficiency

☐ horizontal bone defect

☐ vertical bone defect

Bone shape: ☐ knife edge ☐ round

☐ other.....

c. Bone augmentation: ☐ yes (simultaneously with implant placement) ☐ no

☐ Primary augmentation needed before implant placement

How.....

d. Implant placement procedures:

Bone quality Type: ☐ 1 ☐ 2 ☐ 3 ☐ 4

Implant system:

Configuration: ☐ Bone level ☐ Straight ☐ Conical

Implant location#.....

Implant size: ☐ Ø..... mm. ☐ length mm

☐ Cover screw ☐ Healing abutment (Height mm Ø.....mm.)

Drilling speed rpm

Irrigation system internal / external

Implant insertion speed and torque.....Ncm

Primary stability obtains.....

Defect bone ☐ no bony defect ☐ dehiscence ☐ fenestration

GBR simultaneously with implant placement

How.....

Augmentation material

☐ Autogenous ☐ Xenograft ☐ Synthetics

☐ others.....

Membrane: ☐ Bio-Gide ☐ Biomend ☐ others.....

Pin:.....

Stage: ☐ one (transmucosal) ☐ two (submerged)

Loading: ☐ immediate ☐ early ☐ delayed

Post-medication:

.....

.....

Complication (intra-operation)

☐ no primary stability ☐ wrong direction

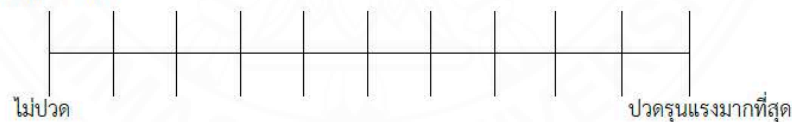
☐ others.....

e. Follow up time

Follow up 1 week:

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

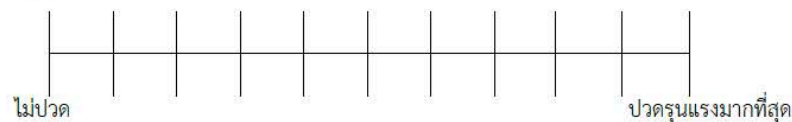
☐ VAS Score



Follow up 1 month:

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

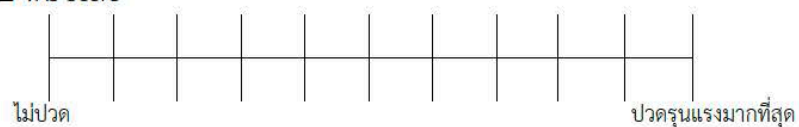
☐ VAS Score



Follow up 3 months:

☐ infection ☐ swelling ☐ pus / exudates ☐ others.....

☐ VAS Score



f. ISQ measurement

At implant placement

At 1 month follow up.....

At 3 month follow up (prosthetic loading)

g. Histology and Histomorphology analysis

Percentage of connective tissue.....

Percentage of new bone formation.....

Percentage of residual graft particle.....

Others

อ้างอิงจาก แบบตรวจคลินิกทันตกรรมรากเทียม คณะทันตแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

Oral Health Impacts Profile-14 (OHIP-14) form

แบบสอบถามคุณภาพชีวิตในมิติสุขภาพช่องปาก (Oral Health Impacts Profile-14, OHIP-14)

คำชี้แจง กรุณาตอบผลกระทบที่ท่านได้รับจากปัญหาเกี่ยวกับฟัน ช่องปาก หรือฟันปลอมของท่าน ตามคำถามทั้ง 14 ข้อ และโปรดทำเครื่องหมาย ✓ ลงในช่องที่ตรงกับประสบการณ์ของท่าน

โดย บ่อยมาก หมายถึง เหตุการณ์นั้นๆเกิดขึ้นทุกครั้งหรือเกือบทุกครั้ง
 ค่อนข้างบ่อย หมายถึง เหตุการณ์นั้นๆเกิดขึ้นเป็นประจำ
 เป็นบางครั้ง หมายถึง เหตุการณ์นั้นๆ เกิดขึ้นเพียงไม่กี่ครั้งในรอบปี
 ไม่เคย หมายถึง เหตุการณ์นั้นๆ ไม่เคยเกิดขึ้นเลย

ข้อ	คำถาม	บ่อย มาก (4)	ค่อนข้าง บ่อย (3)	เป็น บาง ครั้ง (2)	แทบ จะไม่ เคย (1)	ไม่ เคย (0)
1.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้ออกเสียง หรือพูดลำบาก					
2.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้รับประทานอาหารแฉะลง					
3.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้รู้สึกปวด หรือเจ็บฟัน หรือ ส่วนอื่นๆ ในช่องปาก					
4.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้กินอาหารลำบาก					
5.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้รู้สึกกังวลกับลักษณะของ ตัวเอง					
6.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้เกิดความเครียด					
7.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้ต้องกินอาหารที่ไม่อยากกิน หรือต้องอดอาหารที่อยากกิน					
8.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้เกิดปัญหาระหว่างการกิน อาหาร					
9.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้ไม่ได้พักผ่อน					
10.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้รู้สึกอาย ไม่กล้าออก สังคม					
11..	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้แสดงความรู้สึกหงุดหงิด รำคาญต่อผู้อื่น					
12.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้มีปัญหาในการทำงานตาม หน้าที่ตามปกติ					
13	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้รู้สึกว่าชีวิตโดยทั่วไปน่า พอใจน้อยลง					
14.	ปัญหาจากช่องปาก ฟัน หรือฟันปลอม ทำให้ไม่สามารถทำกิจกรรม หรือ ทำอะไรใดๆได้เลย					

อ้างอิงจาก ดัชนี Oral Health Impacts Profile-14 (OHIP-14) Regression short form ดัชนีฉบับและรูปแบบภาษาไทย และ Item-Impact short form ,
 สุดาตวง ฤกษ์ภูพานิช. คุณภาพชีวิตในมิติสุขภาพช่องปาก. กรุงเทพฯ : ภาควิชาทันตกรรมชุมชน คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, 2557.

APPENDIX E

Healing Index of Landry, Turnbull and Howley(145)

Healing index	Tissue color	Bleeding on palpation	Granulation tissue	Incision margin	Suppuration
1 - Very Poor: 2 or more signs are present	≥ 50% of red gingiva	yes	yes	not epithelialized, with loss of epithelium beyond incision margin	yes
2 - Poor	≥ 50% of red gingiva	yes	yes	not epithelialized, with exposed connective tissue	no
3 - Good	25 - 50% of red gingiva	no	no	no exposed connective tissue	no
4 - Very Good	< 25% of red gingiva	no	no	no exposed connective tissue	no
5 - Excellent	all pink tissues	no	no	no exposed connective tissue	no

APPENDIX F

The normality test of the data

All the data are normally distributed when using the Shapiro-Wilk test.

1. Pain VAS score and Healing index (HI)

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
Pain VAS score	0.8029	0.258	Yes	ns
Healing index (HI)	0.9058	0.2536	Yes	ns

2. Oral Health Impact Profile (OHIP-14)

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
Before	0.8768	0.0523	Yes	ns
After	0.8942	0.1889	Yes	ns

3. Horizontal bone gain after 6 months

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
DICOMs	0.9346	0.4945	Yes	ns
STL	0.8788	0.1264	Yes	ns

4. Horizontal bone width at each level

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
0 mm	0.8469	0.0688	Yes	ns
1 mm	0.9223	0.4113	Yes	ns
2 mm	0.9104	0.3188	Yes	ns
3 mm	0.8355	0.0515	Yes	ns
4 mm	0.8552	0.0849	Yes	ns
5 mm	0.8888	0.194	Yes	ns
6 mm	0.927	0.4535	Yes	ns
7 mm	0.9648	0.8468	Yes	ns
8 mm	0.9023	0.2658	Yes	ns
9 mm	0.8497	0.074	Yes	ns
10 mm	0.9626	0.8251	Yes	ns
11 mm	0.9532	0.7252	Yes	ns
12 mm	0.841	0.0593	Yes	ns

5. The volumetric and dimensional changes of the augmented graft

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
3D-Printed HA	0.8274	0.1818	Yes	ns
Bone after augmentation (CBCT)	0.8639	0.2783	Yes	ns

6. Histomorphometry

Shapiro-Wilk test	W	P value	Passed normality test (alpha=0.05)?	P value summary
Bone tissue	0.888	0.161	Yes	ns
New bone formation	0.903	0.2363	Yes	ns
Residual graft	0.946	0.6211	Yes	ns
Connective tissue	0.9185	0.3445	Yes	ns

APPENDIX G

Score data of Oral Health Impact Profile (OHIP-14) in the individual patient.

Questionnaire		Patient number (no.)											
		1	2	3	4	5	6	7	8	9	10	11	12
Q1	Before	1	2	2	4	1	3	4	3	2	1	2	0
	After	1	1	0	2	1	3	2	3	2	1	1	0
Q2	Before	0	0	1	0	0	0	0	0	0	0	0	0
	After	0	0	1	0	0	0	0	0	0	0	0	0
Q3	Before	3	3	2	2	2	2	4	3	2	3	3	2
	After	3	3	2	2	2	2	3	3	2	3	3	2
Q4	Before	3	4	2	3	3	4	4	3	2	3	3	3
	After	3	3	2	2	2	2	2	3	2	2	3	2
Q5	Before	3	4	2	4	4	4	4	4	2	3	3	2
	After	3	3	2	3	3	3	4	3	2	3	3	1
Q6	Before	3	3	2	4	4	2	3	2	2	2	2	2
	After	3	3	2	3	3	2	3	2	2	2	2	2
Q7	Before	2	3	2	3	2	4	4	4	3	4	3	4
	After	2	2	2	2	2	2	3	3	2	3	3	3
Q8	Before	4	3	3	3	3	4	3	3	2	4	4	3
	After	3	3	2	3	3	4	3	3	3	4	3	3
Q9	Before	2	2	2	3	3	2	3	3	3	3	3	3
	After	1	2	2	2	2	2	1	3	2	3	2	3
Q10	Before	2	2	3	3	3	3	4	4	4	3	3	3
	After	2	2	2	2	2	2	3	3	3	3	2	2
Q11	Before	1	1	0	2	4	3	3	3	3	3	3	3
	After	1	1	0	2	2	2	1	2	2	2	3	3
Q12	Before	2	2	3	4	4	3	4	3	3	3	4	4
	After	2	2	2	3	4	3	4	3	3	3	3	4
Q13	Before	0	0	0	1	0	0	0	1	1	0	1	0
	After	0	0	0	0	0	0	0	1	1	0	1	0
Q14	Before	1	2	2	2	2	2	2	1	2	2	2	2
	After	1	2	1	2	1	2	2	2	2	1	2	1

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