JAW BONE GENETIC CHARACTERISTIC IN RELATION TO DENTAL IMPLANT

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ABSTRACT

The success of dental implant treatment is composed of multiple indications including quality of hard and soft tissues. However, there has been no available study on the association of gene expression parameters in bone remodeling and the success of dental implant treatment. The current study aims to evaluate the expression of bone remodeling-related genes, such as BMP2, FGF23, and RUNX2 to dental implant osteotomies sites according to the FDI (Federation Dentaries Internationale) world dental federation of tooth notation and reveal the relationship between molecular parameters with bone type according to Cone Beam Computer tomography, Surgeon tactile sensation, and wound healing.

The expression of BMP2, FGF23, and RUNX2 genes was not found to correlate with CBCT, Panoramic X-ray and Surgeon tactile, the majority of bone type (bone type 3). In wound healing status (good and very good), there was no relationship between wound healing status and the expression of 3 genes. In contrast, Bucco-lingual width at the area to place implant is found to have a significant difference in BMP2, FGF23. That seems to demonstrate that bone quantity affects to some gene expression. Relation of crestal thickness at osteotomy site and BMP2 was significantly different at 0.006 ($p < 0.05$) but for FGF23 and RUNX2 gene expression was not significant. In conclusion, bone remodeling-related genes such as BMP2 and FGF23 were correlated with bone quantity and quantity examination. This study suggested a positive correlation between osteogenic gene profile matching and the human jawbone microstructure.

KEY WORDS: BONE FORMATION / BONE RESORPTION /
ALVEOLAR REGENERATION / TISSUE HEALING /
HUMAN JAW BONE / SYSTEMATIC DISEASE

58 pages

การทำนายทางพันธุกรรมของการฟื้นฟูสภาพกระดูกขากรรไกรมนุษย์หลังจากการฝังรากฟันเทียม
JAW BONE GENETIC CHARACTERISTIC IN RELATION TO DENTAL IMPLANT

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บทคัดย่อ

ความสัมพันธ์ของลักษณะพันธุกรรมของกระดูกกับการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์อัตราความสำเร็จของรากเทียมประกอบด้วยหลายปัจจัยรวมถึงคุณภาพของกระดูกและเหงือกบริเวณรากฟันเทียม วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาการแสดงออกของยีนส์ที่ควบคุมการสร้างและสลายกระดูก เช่น BMP2, FGF23, RUNX2 ที่ตำแหน่งต่างๆ ของขากรรไกรในตำแหน่งที่ฝังรากเทียมโดยแบ่งตามโครงสร้างของกระดูกขากรรไกรในตำแหน่งบนขวา บนซ้าย ล่างขวาและล่างซ้าย(Federation Dentaries Internationale) และหาความสัมพันธ์ของตัวแปรในการแบ่งคุณภาพของกระดูกโดยใช้ภาพถ่ายรังสีคอมพิวเตอร์, ความรู้สึกด้านมือของศัลยแพทย์ขณะเจาะกระดูกเพื่อฝังรากเทียม และวัดความสอดคล้องกับผลการหายของแผลผ่าตัด

วัสดุและวิธีการโดยการศึกษานี้เป็นการศึกษาแบบภาคตัดขวางกึ่งการทดลองโดยก่อนทำการฝังรากเทียมวัดคุณภาพของกระดูกจากภาพถ่ายรังสีคอมพิวเตอร์ และขณะทำการฝังรากเทียมวัดคุณภาพกระดูกจากความรู้สึกด้านมือของศัลยแพทย์ และนำตัวอย่างกระดูกในแต่ละตำแหน่งที่ได้จากการเจาะมาวัดการแสดงออกของยีนส์ เพื่อเปรียบเทียบกับการหายของแผลผ่าตัดหลังการฝังรากเทียม 1 อาทิตย์ กระดูกที่ได้นำมาวิเคราะห์การแสดงออกของยีนส์ด้วยวิธี TaqMan RT-PCR โดยใช้สถิติเชิงพรรณนา และ เพียรสันไคสแควร์ในโปรแกรม SPSS 18 การแสดงออกของยีนส์ BMP2, FGF23 และ RUNX2 ไม่มีความสัมพันธ์กับภาพถ่ายรังสีคอมพิวเตอร์ ภาพถ่ายรังสีแพนอรามิก และความด้านมือของศัลยแพทย์ ในส่วนการหายของแผลผ่าตัดก็ไม่มีความสัมพันธ์กับยีนทั้ง 3 กระดูกส่วนใหญ่จัดอยู่ในระดับ 3 ในทางกลับกัน ความกว้างในแนวใกล้แก้มใกล้ลิ้นในตำแหน่งที่ทำการใส่รากเทียมมีความสัมพันธ์กับยีนส์ BMP2, FGF23 อาจกล่าวได้ว่าปริมาณกระดูกมีความสัมพันธ์กับยีนส์บางตัว และพบว่า ความหนาของสันกระดูกมีผลต่อยีนส์ BMP2 แต่ไม่มีผลต่อ FGF23 และ RUNX2 สรุปได้ว่ายีนส์บางตัว เช่น BMP2 และ FGF23 มีความสัมพันธ์กับคุณภาพและปริมาณของกระดูก การศึกษานี้พบความสัมพันธ์ของการแสดงออกทางพันธุกรรมกับกระดูกขากรรไกรมนุษย์ในตำแหน่งต่างๆ

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LIST OF ABBREVIATIONS

BMD	Bone mineral density
BMU	Bone multicelular unite
BMP2	Bone morphologic protien 2
BQI	Bone quality index
CBCT	Cone beam computer tomography
FDI	Federation Dentaries Internationale
FOV	Field of view
PBS	Phosphate buffer solution
FGF23	Fibroblast growth factor
GH	Growth hormone
GHR	Growth hormone receptor
HU	Hounsfield unite
IGH	Insulin like growth factor
MCI	Mandibular cortical index
MCW	Mandibular cortical width
M-CSF	Macrophage colony stimulating factor
MI	Mental index
OPG	Osteoprotegerin
PMI	Panoramic mandibular index
PTH	Parathyroid hormone
RANK	Receptor activator nuclear factor Kb
RANKL	Receptor activator nuclear factor Kb Ligand
TNF-α	Tumors necrosis factor-α
TRAP	Tartrate-resistant acid phosphate

CHAPTER I
INTRODUCTION

1.1 Background

Dental treatment with osseointegrated dental implants has been performed extensively worldwide in various clinical situations, with high success rates and excellent predictability (Evans and Chen, 2008; Jung *et al.*, 2008). Successful treatment outcome with dental implants depends on patient as well as procedure dependent parameters, which includes general health conditions, biocompatibility of the implant material, implant topography, surgical procedure, and quality and quantity of the local bone (Turkyilmaz *et al.*, 2007; Turkyilmaz *et al.*, 2008).

However, the importance of various parameters of bone quality in implant treatment is not clearly understood (Ericsson and Nilner, 2002; Lindh *et al.*, 2004) . Factor related to bone healing plays a fundamental role in osseointegration (Albrektsson *et al.*, 1981). Excessive trauma during surgery may negatively affect tissue maturation at the bone-to-implant interface, diminishing the predictability of osseointegration (Ercoli *et al.*, 2004). Therefore, to preserve tissue viability at the time of implant placement, it is necessary to perform adequate preparation of the surgical bed (Benington *et al.*, 2002; Yacker and Klein, 1996). Misch recommended using external and/or internal irrigation, as well as cool saline irrigation, intermittent pressure on the drills, pausing every 3 to 5 seconds, using new drills, and an incremental drill sequence. Generating less heat by preparing implant sites at 2500 rpm may also decrease the osseous damage (Sharawy *et al.*, 2002).

Several characteristics of bone tissue have been identified as important factors for the outcome of dental implant treatment. The term “bone quality” is complex, and it includes microscopic, morphological, and molecular parameters (Lindh *et al.*, 2004). Thus, no consensus definition of bone quality has been reached in the literature or applied in the clinical setting (Ribeiro-Rotta *et al.*, 2007; Ribeiro-Rotta *et al.*, 2010; Ribeiro-Rotta *et al.*, 2011).

The most widely used classification system is that proposed by Lekholm & Zarb (L&Z., 1985), although it has never been validated (Ribeiro-Rotta *et al.*, 2007; Ribeiro-Rotta *et al.*, 2010; Ribeiro-Rotta *et al.*, 2011). L&Z's system is based on radiographic images coupled with the surgeon's tactile perception.

Accurate and detailed analysis of the jawbone could be beneficial to the dental practitioners to make decisions regarding patient selection, implant surface type, and surgical techniques. Several classification systems and procedures were proposed for assessing bone quality and predicting prognosis as mechanical behavior of bone are fundamental factors in the attainment and maintenance of osteointegration (Friberg *et al.*, 1999).

1.2 Hypothesis

Molecular biology can predict the bone microarchitecture or quality of the jawbone, which, in turn, might be associated with bone-type classification system.

1.3 Objective

To evaluate molecular parameters of bone cells from dental implant sites at 4 different anatomical area of jawbone (anterior mandible, posterior mandible, anterior maxilla, and posterior maxilla) in humans.

Specific objectives:

1. To study the relationships of gene expression between the molecular parameters and CBCT classifications as compared with the surgeon tactile sensation.
2. To find relationship between gene expression and wound healing.

CHAPTER II
LITERATURE REVIEWS

2.1 Principles of bone biology and regeneration

Bone is a dynamic tissue sensitive to factors variable with an inherent capacity that allows the translation of mechanical stimuli into biochemical. signals, which therefore ability to affect osseous structure (Bonewald and Johnson, 2008; Burger *et al.*, 1995; Duncan and Turner, 1995).

The skeleton forms by either a direct or indirect ossification process during embryogenesis. In case of the mandible and maxilla mesenchymal progenitor cells condensate and undergo direct differentiation into osteoblasts by intramembranous osteogenesis. In mandibular condyle, long bones and vertebrae growth by endochondral osteogenesis (Rodan and Martin, 2000).

2.1.1 Bone cells

Osteogenic precursor cells are osteoblasts, osteoclasts, osteocytes, and hematopoietic elements of bone marrow .There are two major families of cells control bone remodeling. First the osteoclasts originate from cells in the hematopoetic line from precursors of macrophages. Second the Osteoblasts are derived from stem cells in the marrow stromal that are also precursors to adipocytes and chondrocytes. The cells differentiated into pre-osteoblasts, bone marrow, Osteoblasts new bone, mineralized bone, osteoid, osteoclasts, Lining cells

2.1.1.1 Osteoblast as cell round nucleus at base with strong basophilic cytoplasm and prominent and Golgi complex between nucleus and apex. Final stage transformed into flat lining cells or osteocytes embedded in bone matrix or undergo apoptosis. Osteoid later becomes calcified and maturation approximate 10 days (Boyce *et al.*, 2007; Datta *et al.*, 2008).

2.1.1.2 Osteoclast originates from hematopoietic stem cells. Found in contact with calcified bone surface and resorp to Howship’s lacuna. The

mechanism of bone resorption is associated with lysosomal pathway. Tartrate resistant acid phosphatise(TRAP+) and cathepsin K are actively synthesized by osteoclast and are found in the endoplasmic reticulum and transport vesicles.

2.1.1.3 Osteocyte is the terminal differentiation stage of the osteoblasts. Osteocytes embedded deep within the calcified bone matrix have numerous and long cell processes.

The osteocytes can initiate a BMU in response to micro damage and perhaps after mechanical loading. It is not clear if systemic hormones or local cytokines initiate new BMUs, or if they work by increasing activity or lifespan of existing BMUs.

The first sign of activity seen by standard microscopy is bone resorption, so early histologists thought that the first step in a remodelling cycle was performed by the osteoclasts. Now, however, it is known that osteoclasts must be activated by cells in the osteoblast lineage, a process that occurs on the molecular level (Ott, 2010) .

Although autogenous bone has been described as the gold standard for grafting autologous, homologous, and synthetic grafts have also been used in reconstructive procedures (Coradazzi *et al.*, 2007; Faria *et al.*, 2008; Sandor *et al.*, 2003; Cheong H, 2007; Nkenke *et al.*, 2002).

2.2 Bone remodeling (Bone turnover)

Bone remodelling happen according to processes of bone resorption and bone formation. The bone resorbed by osteoclasts and new bone is deposited by osteoblastic cells. The remodelling process take place in bone multicelular units (BMUs). Compose of BMUs are a front of osteoclasts residing on a surface of newly resorbed bone referred to as the resorbtion front a compartment containing vessels and pericytes and a layer of osteoblasts present on a newly fromed organic matrix known as the deposition front. The resorption front is clearly visualized by the cell stained for tartrate-resistant acid phosphatise(TRAP).Cell contact between cells expressing RANKL and osteoclast precursors expressing receptor activator of nuclear factor κB (RANK) induces osteoclast differentiation, fusion, and activation.

Modulation of this coupling mechanism occurs through a molecule known as osteoprotegerin(OPG) OPG binds RANK.

Osteocytes are multifunctional cells that actively participate in cell turnover, and they are very sensitive in regard to translating aggravations to the tissues into biochemical signals (Bonewald, 2002).

Autogenous bone can be used in block form or particulated. Particulated bone is indicated when the area to be reconstructed is protected by remaining osseous structures or by a reinforced membrane, which can avoid deformation or micromovement that might lead to graft failure (Coradazzi *et al.*, 2007). The remodelling coupling mechanism is the receptor activator of neuclear factor κB ligand(RANKL) mediated activation of osteoclasts. RANKL is a cytokine produced by osteoblasts. The cells produce RANKL in response to systemic hormones(e.g.,1,25-dihydroxyvitamin D3) and cytokines(e.g.,interleukin IL-6).

2.3 Regulation and Bone remodelling

Osteoblast regulate osteoclast formation through the receptor activator of nuclear factor-Kb (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) signaling system. Osteoblast expresses both RANKL and OPG wheres osteoclast express RANK. The RANK and RANKL interaction is responsible for osteoclast differentiation. In contrast, osteoclast differentiation is inhibited by OPG which specifically binds to RANKL and reduces its binding to RANK. Then, OPG/RANKL ratio increases osteoclastogenesis and bone resorption. On the other hand, high ratio of OPG/RANK predicts decrease in bone resorption (Boyce *et al.*, 2007; Yasuda *et al.*, 1998).

Bone was regulated through local factors and cytokines. Many cytokines and local factors are important regulators of osteoclast and osteoblasts function and some cytokines have been shown to regulate the expression of RANK and RANKL. The pro-inflammatory cytokines such as IL-1,IL-6,IL-11 and tumors necrosis factor-α (TNF-α) appear to promote osteoclasttogenesis and bone resorption function(Yasuda et al.,1998)Calcium hemostasis is necessary physiologic processes to maintain health (Bonewald, 2002).

The importance of osteoclastic bone resorption in the pathogenesis of these disease is reflected by the fact that the most successful drug treatments for bone disease work by inhibiting bone resorption (Rodan and Martin, 2000). Osteoclastic bone resorption is regulated by a complex interplay between circulating calciotropic hormones like parathyroid hormone, calcitriol and sex hormones; and local regulators of bone cell activity like receptor activator of nuclear factor kappa B ligand (RANKL), macrophage colony stimulating factor (M–CSF) and osteoprotegerin (Khosla 2001). Recent work has shown that neuroendocrine pathways and neurotransmitters also play a key role in the regulation of bone remodeling (Skerry *et al.*, 2001; Baldock *et al.*, 2002).

2.4 Anatomical sites of jaw bones

Maxillary and mandibular bone formation are formed by membranous ossification. The membranous ossification is cellular condensation and osseous matrix formation with out cartilaginous phases. There are not clear about morphology and activity. Although, the osteoblast differentiate will follow up to site-specificity functionality. That support in clinical with same procedure and same operator the response different in oral sector. The Maxilla and mandible do not merge but interlock by dentoalveolar complex .Finally tooth and alveolar bone considers by own growth site. That depends on inherited genetic factors up to muscular stain or occlusal force.

2.4.1 Bone anatomy in the maxilla

Maxillary basal bone relationships

The alveolar bone and basal ridge often diverge from each other. This inappropriateness will influence for socket preservation as the more divergent the alveolus becomes, the thinner the facial alveolar bone and the higher resorption following tooth loss (Nevins *et al.*, 2006; Schropp *et al.*, 2003).

2.4.1.1 Bone quality

The bone quality is not clearly defined in the literature. The maxillar has been described as less dense when compared with the mandible. They found that the anterior mandible had densest bone, followed by the posterior mandible,

anterior maxilla, and posterior maxilla (Lekholm U., 1985), base on the amount of cortical and trabecular.

2.4.1.2 Bone anatomy in the mandible

2.4.1.3 Tooth to alveolar relationship in the mandible Similar to the maxillar, position of mandibular teeth is commonly divergent to the position of the basal bone.

2.4.1.4 Mandibular symphysis

This area of mandible exhibits anatomical variations that influence the suitability of this region for harvesting autologous bone.

2.4.1.5 Mandibular rami and external oblique ridges

The anatomy of the ascending ramus is an important consideration both in the assessment of impacted third molars and as a donor site for autogenous bone grafting (Misch, 2008)

2.5 Classification of type of bone

(Misch, 2008) defined four bone density groups (D1 to D4) in both macroscopic cortical and trabecular bone types. The homogeneous, dense D1 bone type presents several advantages for implant dentistry. The cortical bone may heal with little interim woven bone formation, ensuring excellent bone strength while healing next to the implant

Definition of type I, II, III, & IV It is difficult to clearly defined. Bone quality is broken down into four groups according to the proportion and structure of compact and trabecular bone tissue (Ribeiro-Rotta *et al.*, 2010). Bone quality is categorized into four groups

Type I: homogeneous cortical bone;

Type II: thick cortical bone with marrow cavity;

Type III: thin cortical bone with dense trabecular bone of good strength;

Type IV: very thin cortical bone with low density trabecular bone of poor strength.

The cortical bone receives the outer one third of all its arterial and venous supply from the periosteum. This bone density is almost all cortical and the capacity of

regeneration is impaired because of the poor blood circulation. Also, greater heat is often generated at the apical portion of the D1 bone. D2 is a combination of dense-to-porous cortical bone on the crest and coarse trabecular bone on the inside. The D2 bone trabeculae are 40% to 60% stronger than D3.

The intrabony blood supply allows bleeding during the osteotomy, which helps control overheating during drilling and is most beneficial for bone-implant interface healing. D3 is composed of thinner porous cortical bone on the crest and fine trabecular bone within the ridge.

The most common locations for this type of bone are the posterior region of the maxilla. It is rarely observed in mandible. The bone trabeculae may be up to 10 times weaker than the cortical bone of D1. The bone-implant contact after initial loading is often less than 25%. Bone trabeculae are sparse and, as a result, initial fixation of any implant design presents a surgical challenge (Misch, 2008).

2.5.1 Bone mineral density measurements

The placed implant can be assessed from several different viewpoints as well as from three dimensional view. Moreover, once treatment planning is determined in the computer, it can be saved and applied to surgical sites by means of image-aided template production or image-aided navigation. It is important to note that although computer aided implant placement is a promising technique (Chan *et al.*, 2010; Ganz, 2008). The term bone quality is commonly used in implant treatment and in reports on implant success and failure. (Lindh *et al.*, 2004) emphasized that bone density (Bone Mineral Density, BMD) and bone quality are not same in term of bone metabolism, cell turnover and vasculality.

BMD is the amount of bone tissue in a certain volume of bone. Assessment of jaw BMD may be considered useful in implant planning (Gulsahi *et al.*, 2010). Several approaches have been introduced to measure jawbones and skeletal bones density.

Dental radiographs, especially panoramic images, have been used to predict low bone mineral density in patients. A number of mandibular cortical indices, including the mandibular cortical index (MCI), mandibular cortical width (MCW) and panoramic mandibular index (PMI), have been developed to assess and quantify the

quality of mandibular bone mass and to observe signs of osteopenia. The mental index (MI), which is the mean of the widths of the lower border cortex below the two mental foramina. Osteopenia can be identified by the thinning of the cortex at the lower border of the mandible. A thin mandibular cortical width has been shown to be correlated with reduced skeletal bone mineral density (Devlin and Horner, 2002; Devlin *et al.*, 2007; Dutra *et al.*, 2006; Yuzugullue *et al.*, 2009).

Qualitative and quantitative indexes, including the mandibular cortical index (MCI), mental index (MI) or panoramic mandibular index (PMI) have also been used for panoramic radiographs to assess the bone quality.

However in a study, there was no found such a correlation (Gulsahi *et al.*, 2010). Assessments have primarily been made of the bone tissue status of the entire jaw, and site specific variations have been ignored, as have the consequences of differences between the compact and trabecular parts of jawbone tissue. CT is the only method that allows the components of trabecular and compact bone tissue to be investigated separately (Lindh *et al.*, 2004). With CT, it is possible to measure bone density that its effect on the survival of the implant can be estimated. Norton & Gamble (2001) suggested an objective scale of bone density that was based on mean HU values taken from CT and could be used for bone tissue classification before implant treatment(Shapurian *et al.*, 2006). When considering all implant sites, the mean bone density was 887±180 HU in the other study (Turkyilmaz *et al.*, 2008), which is higher than those reported earlier (Norton and Gamble, 2001; Shapurian *et al.*, 2006). However, in the other study, variations in bone density between different regions of maxilla were found (Lindh *et al.*, 2004). Within individuals, both total BMD and trabecular BMD values were higher in the cuspid-frontal regions than in the posterior region of maxilla.

There are four types of bone in the human face and the length of treatment for placing and restoring implants with a "tooth" and crown depends on which type of bone the implant is placed in. Implants have to integrate with the surrounding bone before a tooth and crown is placed on it.

2.5.2 Bone Types (Lindh *et al.*, 2004; Drage *et al.*, 2007)

Type I bone is comparable to oak wood, which is very hard and dense. This type of bone has less blood supply than all of the rest of the types of bone. The blood supply is required for the bone to harden or calcify the bone next to the implant. Therefore, it takes approximately 5 months for this type to integrate with an implant as opposed to 4 months for type II bone.

Type II bone is comparable to pine wood, which isn't as hard as type I. This type of bone usually takes 4 months to integrate with an implant.

Type III bone is like balsa wood, which isn't as dense as type II. Since the density isn't as great as type II, it takes more time to "fill in" and integrate with an implant. The 6 months time is suggested before loading implant placed in this type of bone. Extended gradual loading of the implant can, however, improve the bone density

Type I, bone is comparable to styrofoam, which is the least dense of all of the bone types. This type takes the longest length of time to integrate with the implant after placement, which is usually 8 months. Additional implants should be placed to improve implant/bone loading distribution. Incremental loading of the implants over time will improve bone density. Bone grafting or augmentation of bone is often required. Bone expansion and or bone manipulation can improve initial implant fixation. Additional implants should be placed to improve implant/bone loading distribution. Incremental loading of the implants over time will improve bone density.

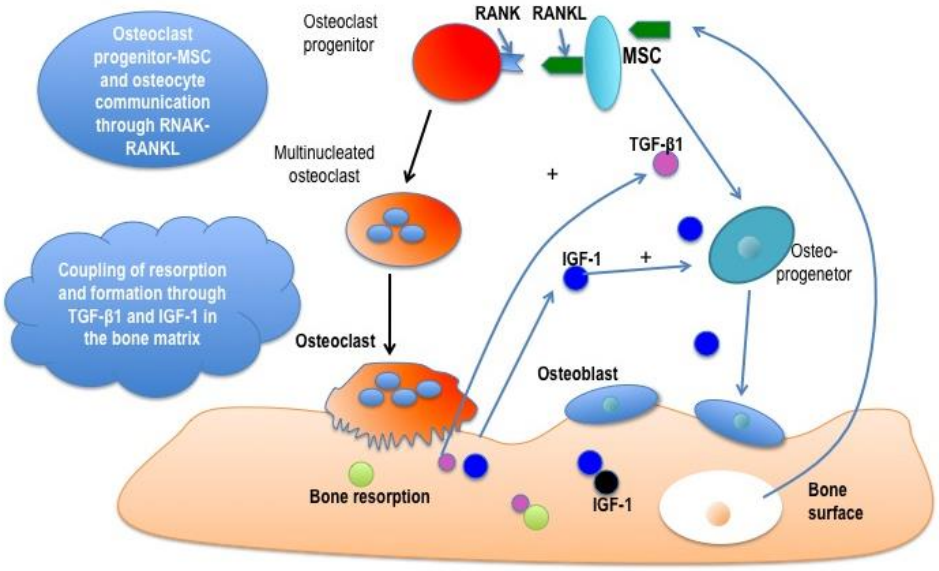
Bone grafting or augmentation of bone are often required. Bone expansion and or bone manipulation can improve initial implant fixation**.** There are four types of bone in the human face and the length of treatment for placing and restoring implants with a "tooth" and crown depends on which type of bone the implant is placed in. Implants have to integrate with the surrounding bone before a tooth and crown is placed on it.

Bone quality but also of structure has been shown that the quality and quantity of bone available at the implant site are very important local patient factors in determining the success of dental implants.

2.6 Histomorphometrical analysis

The gold standard method for evaluation of bone microarchitecture was histomorphometrical (Muller *et al.*, 1998) that allows two dimensional analysis, where structural parameters are either inspected visually or measured from sections.

The biological or molecular events involved in bone metabolism may influence the bone microarchitecture (Kearns *et al.*, 2008). In this context, bone remodelling process occurs at discrete sites on cortical and trabecular bone surfaces, and involves the integrated and sequential actions of osteoclasts and osteoblasts (Kearns *et al.*, 2008).On a cellular and molecular level, the receptor activator of nuclear factor-B (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) have been implicated in osteoblast–osteoclast cross-talk, which is crucial during bone remodeling



Up-regulation of RANKL and down-regulation of OPG have been implicated in a variety of human diseases, including osteoporosis, rheumatoid arthritis and Odontogenic tumors (Andrade *et al.*, 2008; Kearns *et al.*, 2008). Although a recent study demonstrated that the RANK-RANKL-OPG system is imbalanced in fibro-osseous lesions (Elias *et al.*, 2010). The expression of these molecules in different "normal" bone patterns remains to be elucidated. The posterior maxilla has a lower trabecular volume, with a reduction in the thickness and number of trabeculae (Drage *et al.*, 2007).

Many studies suggested that the particle size of the grafting material could effect on the osteogenic ability(Lee *et al.*, 2008). The appropriate particle size of graft material for periodontal surgery was 300 to 500 μm (Zaner and Yukna, 1984). The particle sizes between 250 and 1,000 μm are significantly better than particle sizes of ≤ 250 μm for bone formation (Mellonig, 1984). (Shapoff *et al.*, 1980) studied that particle sizes between 125 μm and 1,000 μm had increased surface area, which stimulates osteogenesis by increasing the number of pores.

The particle surface-to-volume ratio increases bone morphogenic protein levels. The percentage of particle >250 μm was higher in 800rpm than in 1500 rpm significantly (Sampath and Reddi, 1983).

2.7 Cone-beam computed tomography

CBCT was developed because of higher radiation exposure, higher cost, huge footprint, and difficulty in accessibility associated with CT. (Arai *et al.*, 1999; Chan *et al.*, 2010; Scarfe and Farman, 2008). However, its main disadvantage, especially with larger FOVs, is a limitation in image quality related to contrast resolution because of the detection of large amounts of scattered radiation (Scarfe and Farman, 2008).

The resolution and therefore detail of CBCT imaging is determined by the individual volume elements or voxels produced from the volumetric data set. In CBCT imaging, voxel dimensions primarily depend on the pixel size on the area detector, unlike those in CT, which the resolution of the area detector is submillimeter.

Bone Quality Assessment for Dental Implants. Therefore, the theoretical resolution of CBCT is higher than CT (Scarfe and Farman, 2008).

In the literature, the accuracy of CT and CBCT in the assessment of implant site dimensions were compared and CBCT measurements found more accurate than CT measurements (Al-Ekrish and Ekram, 2011; Kobayashi *et al.*, 2004; Loubele *et al.*, 2008; Suomalainen *et al.*, 2008).

The reformatted images of CBCT data result in three basic image types; axial images with a computer generated superimposed curve of the alveolar process and the associated reformatted alveolar cross-sectional images and panoramic-like

images. Such reformatted images provide to clinician that accurate two dimensional diagnostic information in all three dimensions. Both CT and CBCT images provide information on the continuity of the cortical bone plates, residual bone in the mandible and maxilla, the relative location of adjoining vital structures and the contour of soft tissues covering the osseous structures (Scarfe and Farman, 2008; Benson and Shetty, 2009).

Voxel values obtained from CBCT images are not absolute values, like HU values obtained using CT, various methods have been proposed to evaluate the bone density (Naitoh *et al.*, 2009, 2010; Mah *et al.*, 2010). HU provide a quantitative assessment of bone density as measured by its ability to attenuate an x-ray beam. To date, there was not any standard system for scaling the grey levels representing the reconstructed values (Katsumata *et al.*, 2007).

Calculated HU on a CBCT scan varied widely from a range of -1500 to over +3000 then after a correction has been applied to grey levels with the CBCT, the HU values are much similar to those one would expect in a medical CT device than to the original grey levels obtained from the CBCT scanners (Naitoh *et al.*, 2009; Mah *et al.*, 2010).

The clinical utility of preoperative implant planning by use of in imaging stent that helps relate the radiographic image and its information to a precise anatomic location or a potential implant site. The intended implant sites are identified by radiopaque markers retained within an acrylic stent which the patient wears during the imaging procedure so that images of the markers will be created in the diagnostic images.

The imaging stent subsequently may be used as a surgical guide to Orient the insertion angle of the guide bur and hence the angle of the implant. Generally, nonmetallic radiopaque markers are used in CT and CBCT imaging (Benson and Shetty, 2009).

The availability of CBCT is also expanding the use of additional diagnostic and treatment software applications. CBCT permits more than diagnosis, it facilitates image-guided surgery. Diagnostic and planning software are available to assist in implant planning to fabricate surgical models (eg, Biomedical Modeling Inc., USA); to facilitate virtual implant placement, to create diagnostic and surgical implant

guidance stents (eg, Virtual Implant Placement, Implant Logic Systems, Cedarhurst, USA; Simplant, Materialise, Belgium; Easy Guide, Keystone Dental, USA) and even to assist in the computer-aided design and manufacture of implant prosthetics (NobelGuide/Procera software, Nobel Care AG, Sweden) (Scarfe and Farman, 2008).

2.8 Bone healing and treatment failure

The healing of bone tissue includes both regeneration and repair phenomena depending on the character of the injury such as failure of vessels to proliferate into the wound, Improper stabilization of the coagulum and ganulation tissue in the defect, In growth of nonosseous tissues with a high proliferative activity, Bacterial contamination. Bone tissue formation following injury such as;

- Failure of vessels to proliferate into the wound
- Improper stabilization of the coagulum and granulation tissue in the defect
- Ingrowth of nonosseous tissues with high proliferative activity
- Bacterial contamination

Healthy bone is a dynamic tissue, continually resorbing bone and replacing it with new bone in discrete areas know as basic multicellular units, also called bone metabolic units (BMU).

A BMU is not a permanent structure. It forms in response to a signal, performs its function, and disbands, leaving residual lining cells and osteocytes. Each BMU undergoes its functions in the same sequence: origination and organisation of the BMU, activation of osteoclasts, resorption of old bone, recruitment of osteoblasts, formation of new bone matrix, and mineralisation. On the cancellous surfaces, a BMU does not just dissolve a pit on the surface, but it spreads across the surface leaving behind an area filled with new bone.

In the cortex the osteoclasts form a cutting edge and bore through the solid bone, and osteoblasts follow, filling in the tunnels and leaving behind a small vascular channel. Some BMUs originate when the bone has been damaged; others may originate at random surfaces on the bone.

Preoperative evaluation of bone density is essential to assist the clinician with the treatment planning of implant therapy. Detailed information on bone density will help surgeon to identify suitable implant sites, thereby improving the success rate of the procedures.

Precise and quantitative radiolograph examination is required to obtain this pre-operative information (Trisi and Rao, 1999).

Bone implant interface is biomechanically challenged in rotational, axial and lateral directions during healing, the prosthetic phase and clinical function. Ability to withstand loading is decisive for the clinical outcome and factors of importance are (i) type and magnitude of loading, (ii) the quality of the bone-implant integration, (iii) the mechanical properties of the surrounding bone.

Implant integration is time dependent and the biomechanical properties of the bone-implant interface improve with time (Friberg *et al.*, 1999; Sennerby *et al.*, 1993). Therefore, the use of a two-stage procedure with three to six months of healing usually ensures a mature bone-implant interface and good clinical results. However, the trend today is to use immediate/early loading protocols, which make great demands on the bone-implant interface since the implants will be loaded during initial healing.

A risk of successful treatment can be considered in extraction sites with a history of failed endodontic treatment or adjacent teeth with endodontic pathology (Quirynen *et al.*, 2003).

Esposito et al , found that surgical trauma and anatomical conditions both were the most significant etiologic factors for early implant failures in Branemark implants (3.63%).Early implant failures are due to excessive surgical trauma along with impaired wound healing, premature loading and infection (Esposito *et al.*, 1998).

Ercoli et al, showed that different drill designs, the materials of which the drills are made, and the drills' mechanical properties affected their cutting efficiency and durability. However, bone temperature during drilling is more influenced by coolant availability and temperature, than by drill design. In the present study, the experimental protocol used external irrigation and intermittent drilling, which contributed to maintaining cell viability after the repeated use of drills (Ercoli *et al.*, 2004). Watcher and Stoll reported that these findings are probably related to a

reduction in bone-tissue temperature, because the intermittence of the movements allowed bone chips removed during drilling to escape, and allowed for access by the coolant (Wachter and Stoll, 1991). They also reported that the application of intermittent force led to a decrease in the mean recorded temperatures obtained.

Considering the complexity and the multifactorial nature of bone-heating after implant osteotomy, it is possible to affirm that an appropriate surgical protocol should be adopted during drilling procedures, with emphasis on the control of biological and clinical factors, to promote the preservation of cell viability and consequent increase in the success rate of osseointegration. Bone usually varies in density from person to person, bone to bone in the skeleton, and from site to site in the same bone. Regarding the effect of density on the temperature generated, researchers reported that bone density is a far greater indicator of bur temperature than depth of the osteotomy.

However, further studies are necessary to resolve by use cool saline and new drill (Yacker and Klein, 1996). This may be due to bone density and bur temperature more than depth of the osteotomy.

This may be due to the time gap that is being allowed while changing the drills that allows the material to cool down and the new drill, which is being used, for drilling will be cooler to start drilling again.

CHAPTER III
MATERIALS AND METHODS

3.1 Study design

Study design: This study was a pilot, descriptive cross sectional study. The ethical approval was obtained from the Mahidol University Ethics Review Board, Mahidol University, Bangkok (MU-DT/PY-IRB 2013/033.0807)

Study site: Department of Dental Implant, Faculty of Dentistry, Mahidol University, Bangkok, Thailand.

Study period: March 2014 to August 2014

3.2 Sample size

The study sample consisted of 30 dental implant sites. Subjects were selected based on following inclusion and exclusion criteria.

Inclusion Criteria

- Male or female
- Age more than 18 years
- Reasonable amounts of alveolar bone and no complex oral rehabilitation needs
- Willing and able to accept the protocol and to give a written informed consent for the surgical procedure
- Absence of soft or hard tissue inflammation
- Adequate oral hygiene, assessed by the plaque index, sulcus bleeding index, periodontal severity index (PSI)

- Good general health or controlled systemic disease

Exclusion Criteria

- Immediate implant placement
- Neurologic disease that contraindicates implant therapy
- Previous or current radiotherapy or chemotherapy
- Psychological or psychiatric conditions that could influence the treatment
- Blood dyscrasias and liver failure
- Poor metabolic control (Hb a 1c glycosylated hemoglobin > 13.0% or creatinine > 1.7 ml/dl)
- Smoking of >1 pack of cigarettes/day

3.3 Protocol of dental implant

Visit 1

1. After history taking and clinical examination, the participants were selected based on inclusion and exclusion criteria.

2. General information (age, sex, smoking history, concomitant systemic diseases, drug allergy), position of implant, posterior support, duration of edentulism, remaining teeth, periodontal disease, and length and width of edentulous space (mm) were assessed.

3. Radiographic images: periapical and panoramic radiographs and CBCT (Cone Beam Computed Tomography) were performed to evaluate if the bone sites had the minimal volume necessary to receive an implant (4.0 mm*11.5 mm; Intralock)

4. Classification of bone types was done by a radiologist and experienced surgeon/s according to the original classification system proposed by Lekholm & Zarb (1985), based on radiography and surgeon's tactile perception during drilling. These classification methods was categorized bone types into four groups: I, II, III, & IV, according to the distribution of cortical and trabecular bone.

Visit 2

1. Local anesthesia was injected and a flap was opened at the implant site. Then, the gingival thickness was measure by a probe.

2. A guild pin 2.2 mm was used to drilling at the implant site and bone was collected bone by autogenous bone harvester (Mega Gen Implant. Co., Ltd.) to obtain specimen from each site. Surgeon's tactile perception was noted during drilling and the bone was categorized into one of the four groups: I, II, III, & IV. Specimens were transferred into 2 ml Eppendorf tube and place on ice and then centrifuged and cleaned by normal saline, following which the specimen was placed in refrigerator at -80°C.

3. The implant (Intralock Co., Ltd.) was then placed and the healing abutment was connected by an experienced surgeon.

4. The surgical site was sutured and medication were prescribed; Amoxicillin 500 mg (1*3) for 3 days, Paracetamol 500 mg (2*4) for 3 days, and Ibuprofen 400 mg (1*3) for 3 days.

Visit 3 Stitch off on Day 14

1. The wound healing was assessed and scored according to Mombelli index (Mpi).

2. Any pain or complication was noted.

Visit 4 After 3 month

1. Periapical radiograph of the implant site was taken.

3.4 Molecular Method

Sample preparation and surgical procedure

Bone Specimens from patients were collected by autologous bone harvester (Mega Gen company) and transferred into 2ml Eppendorf tube and placed on ice and then centrifuged and cleaned by Phosphate normal saline. After that specimens were placed in refrigerator at -80°C. Primary closure was obtained for all the surgical

sites and the patient was instructed to maintain hygiene around the surgical site using a soft-bristle toothbrush. Patients were prescribed with analgesics, Paracetamol 500 mg 4 times a day for 3 days and antibiotics, Amoxicillin 500 mg 3 times a day for 5 days. Recall appointments were made and wound healing was measured at one week following the dental procedure. During the visit, the sutures were removed and the site was irrigated with normal saline solution. In case wound healing were not satisfactory, the sutures were removed after 14 days. The healing of the soft tissues around the surgical area was visually evaluated and classified into "good" or "very good" according to a modified soft tissue healing index used for classifying healing of skin following trauma in patients with diabetes mellitus 15(IDS A guidelines).

The modified RNA extraction described by Seriwatanachai et al. 2008 was used in this study. Total bone RNA was extracted from bone tissue by using TRIzol Reagent (Invitrogen) according to the manufacturer's instruction. Briefly, approximately 20 mg of bone tissue was grinded in liquid nitrogen by using a baked mortar and pestle, and then the powder was transferred to 1 ml of TRIzol and centrifuged at 12,000 g for 10 min. Supernatant was transferred to a new tube and 200 µl of chloroform was added followed by 15 min of centrifugation at 12,000 g. The upper part was transferred to a new tube. Isopropanol 500 µl per 1 ml of TRIzol was added for RNA sedimentation (incubate 10 min at RT), then, centrifuged at 12,000 g for 10 min. Supernatant was discarded and 1 ml of 75% ethanol per 1 ml of TRIzol was added, then, centrifuged at 7,500 g for 5 min (2 times). Eventually, RNA pellet was dried at room temperature for 10-15 min before dissolve in 0.1% DEPC–treated water. RNA concentrations were determined using spectrophotometry (A260) and the purity was assessed from the A260:A280 ratio. The RNA sample was stored at -80°C until used. The quality of RNA was checked on a 1% agarose gel containing 0.5 µg of ethidium bromide (EtBr)/ml.

Reverse transcription

Total RNA from bone tissue was subjected for cDNA synthesis. To remove genomic DNA, RNA sample was incubated with 1 U of deoxyribonuclease I (DNase I) per ug RNA at 25°C for 15 min. The reverse transcription was conducted by using cDNA synthesis kit according to manufacturer's protocol. Twenty-microliter

reactions contained: 1 μg of total RNA sample (variable), 2 μl Oligo(dT) 20 primer, nuclease-free water (variable), 4 μl 5x iScript select reaction mix and 1 μl iScript reverse transcriptase (iScript™ cDNA Synthesis; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The reverse transcription reaction was 60-90 min at 42°C, and then at 85°C for 5 min to heat-inactivate the reverse transcriptase activity. The cDNA was treated with ribonuclease H (RNase H; 5 unit) at 37°C for 40 min to remove RNA: DNA hybrids. The sample containing RNA without reverse transcriptase (minusRT) was included in the reaction and considered as the negative control. Finally, the cDNA product was stored at -20°C to 4°C until used for RT-PCR reaction analysis.

Realtime-PCR

TaqMan real-time PCR primers were obtained from Applied Biosystems (ABI), and the real-time PCR reaction was performed on the ABI 7500 Sequence Detection System (Applied Biosystems, Foster, CA, USA). The reagent used in realtime PCR was TaqMan Universal PCR Master Mix (Applied Biosystems, USA). Expression of target genes was normalized with *GAPDH* expression. The forward and reverse oligonucleotides primers of each gene were shown in Table 4.1.

Realtime-PCR

TaqMan real-time PCR primers were obtained from Applied Biosystems (ABI), and the real-time PCR reaction was performed on the ABI 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Expression of the target genes was normalized to Gapdh.

3.5 Data Analysis

All the analyses were calculated using a Pearson’s chi-square or Fisher's Exact Test, Exact 2-tailed, p-value < 0.05 as statistically significant cut off point. Descriptive analyses performed for molecular parameters were expressed as minimum and maximum values, along with the mean and standard deviation. SSPS 18.0 for Windows (Chicago, IL, USA) was used for the data analysis.

CHAPTER IV
RESULTS

Table 4.1 Forward and reverse primers used for human *BMP2, RUNX2, FGF23,* and *GAPDH* genes analysis

Gene	Forward Primer	Reverse Primer
BMP2	ACCCGCTGTCTTCTAGCGT	TTTCAGGCCGAACATGCTGAG
RUNX2	GAACCCAGAAGGCACAGACA	GGCTCAGGTAGGAGGGGTAA
FGF23	ACCACATGGTCAGGCTCTTG	TCCAAGGGGATTGAGACCCA
GAPDH	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT

General information of participants and site of implants

The description of mean and standard deviation regarding 30 samples were shown in table 4.2. The average age of the subjects was 53.57±11.425 and bucco-lingual space (mm) was 8.78±0.962. The mean and standard deviation of crestal bone thickness at left mandible, right mandible, left maxillar, and right maxillar were 1.62±0.192, 1.66±0.213, 0.61±0.175, and 0.63±0.173, respectively. The average bucco-lingual width and interocclusal height were 8.04±1.345 and 10.04±1.833, respectively. The gingival thickness (mm) and crestal thickness (mm), were 3.15±0.987 and 1.14±0.609, respectively.

Table 4.2 General information of the participants and site of implants

General information of the participants and site of implants (30 samples)	
Age	M±SD 53.57±11.425 mini-maxi (27-79)
Bucco-lingual Space mm	M±SD 8.78±0.962 mini-maxi (6-10)
Crestal bone thickness at left mandible (mm)	M±SD 1.62±0.192 mini-maxi (1.2-2)
Crestal bone thickness at right mandible (mm)	M±SD 1.66±0.213 mini-maxi (1.2-2)
Crestal bone thickness at left maxillar (mm)	M±SD 0.61±0.175 mini-maxi (0.3-1.1)
Crestal bone thickness at right maxillar (mm)	M±SD 0.63±0.173 mini-maxi (0.2-1.2)
Bucco lingual width	M±SD 8.04±1.345 mini-maxi (5.3-10.7)
Interocclusal height	M±SD 10.04±1.833 mini-maxi (8-15)
Gingival thickness (mm)	M±SD 3.15±0.987 mini-maxi (1.84-5.23)
Crestal thickness (mm)	M±SD 1.14±0.609 mini-maxi (0-2)

Among 30 implant sites, 12 (40%) were from female and 18 (60%) from male. Regarding associated systemic diseases, 23 (76.7%) samples were from normal persons, 1 (3.3%) from a diabetic, 3 (10%) from a hypertensive, and 3 (10%) from hypertensive patients with hypothyroidism patients. All of the participants had no periodontal disease. Five out of 30 samples (16.7%) were collected from smoking patients.

Regarding bone types according to CBCT, panoramic X-ray, and surgeon tactile sensation, the most common bone type was bone Type 3, 24 (80%), 21 (70%), and 14 (46.7%), respectively. Regarding gingival thickness, thick biotype was 27 (90%) and thin biotype was 3 (10%). (Table 4.3)

Table 4.3 Information regarding site of implants

Information regarding site of implants (30 samples)		
		n (%)
CBCT result	Bone Type 2	5 (16.7)
	Bone Type 3	24 (80)
	Bone Type 4	1 (3.3)
Panoramic X-ray result	Bone Type 2	6 (20)
	Bone Type 3	21 (70)
	Bone Type 4	3 (10)
Type of bone	I	2 (6.7)
(Surgeon tactile sensation)	II	11 (36.7)
	III	14 (46.7)
	IV	3 (10)
Gimgival biotype	Thick	27 (90)
	Thin	3 (10)

In Table 4.4, detail position of implants, size of implants used, duration of implant procedure, and wound healing were mentioned. Regarding position of implants; 1 (Right maxilla), 2 (Left maxilla), 3 (Right mandibular), and 4 (Left mandibular), the numbers were 6 (20%), 4 (13.3%), 10 (33.3%), 10 (33.3%), respectively. The average duration of operation was 26.03±7.707 minutes, and minimum 20 minutes for 13 sites and maximum 45 minutes for 2 sites. After operation per oral Amoxacillin 500 mg TID and Paracetamol 500 mg QID for 3 days were given. Regarding wound healing, good status wound healing was 15 (50%) and very good status of wound healing was 15 (50%).

Table 4.4 Information regarding site of implants and wound healing

Information regarding site of implants and wound healing		
		n 30 (100%)
Position of implant	14	3 (10)
	15	1 (3.3)
	16	1 (3.3)
	17	1 (3.3)
	26	4 (13.3)
	34	1 (3.3)
	35	1 (3.3)
	36	6 (20)
	37	2 (6.7)
	44	1 (3.3)
	45	1 (3.3)
	46	7 (23.3)
	47	1 (3.3)
Position of implant (Recode)	1	6 (20)
	2	4 (13.3)
	3	10 (33.3)
	4	10 (33.3)
Size of implant	4.00	21 (70)
	4.75	9 (30)
Duration of operation (min)	20	13 (43.3)
	25	9 (30)
	30	2 (6.7)
	32	1 (3.3)
	36	2 (6.7)

Table 4.4 Information regarding site of implants and wound healing (cont.)

Information regarding site of implants and wound healing		
		n 30 (100%)
	42	1 (3.3)
	45	2 (6.7)
	M±SD (26.03±7.707)	
	mini-maxi (20-45)	
Wond healing	Good	15 (50)
	Very Good	15 (50)

Regarding gene expression, undetermined and determined gene expression of BMP2, FGF23, and RUNX2 were; 16 (53.3%) and 14 (46.7%), 23 (76.7%) and 7 (23.3%), 16 (53.3%) and 14 (46.7%), respectively. (Table 4.4)

Table 4.5 Gene expression of *BMP2, FGF23,* and *RUNX2*

Gene expression of *BMP2, FGF23,* and *RUNX2*		
		n (%)
BMP2	Undetermined gene expresion	16 (53.3)
	Determined gene expression	14 (46.7)
FGF23	Undetermined gene expresion	23 (76.7)
	Determined gene expression	7 (23.3)
RUNX2	Undetermined gene expresion	16 (53.3)
	Determined gene expression	14 (46.7)

Relation of gene expression and wound healing were mentioned in Table 4.6. However, we could not find any significant relationship between all 3 gene expressions and wound healing ($p > 0.05$).

Table 4.6 Relationship between wound healing and *BMP2, FGF2, and RUNX2* gene expressions

Gene	Expression Yes/No	Wound Healing			
		Good	Very Good	Total	
BMP2	Undetermined gene expresion	10	6	16	0.14*
	Gene expression	5	9	14	
	Total	15	15	30	
FGF23	Undetermined gene expresion	15	8	23	0.06**
	Gene expression	0	7	7	
	Total	15	15	30	
RUNX2	Undetermined gene expresion	9	7	16	0.46*
	Gene expression	6	8	14	
	Total	15	15	30	

* Pearson Chi-Square, Asymp.Sig (2-sided)

** Fisher's Exact Test, Exact Sig (2-sided)

The expression of the *BMP2, FGF2, and RUNX2* was not related with wound healing (p-value 0.14, 0.06, and 0.46, respectively). The correlation of FGF23 in condition gene expression and good wound healing cell was 0 as determine by Fisher's Exact Test, which was significant but in a negative way.

Relationship between gene expression and position of implants were mentioned in Table 4.7. But we could not find significant relationship between all 3 gene expressions between maxilla and mandibular bone ($p > 0.05$).

Table 4.7 Relationship bewtween position of implant and *BMP2, FGF23, and RUNX2* gene expressions

Gene	Expression Yes/No	Position of implant			
		Maxi	**Mandi**	**Total**	**p-value**
BMP2	Undetermined gene expresion	7	9	16	0.26**
	Gene expression	3	11	14	
	Total	10	20	30	
FGF23	Undetermined gene expresion	9	14	23	0.37**
	Gene expression	1	6	7	
	Total	10	20	30	
RUNX2	Undetermined gene expresion	5	11	16	0.79*
	Gene expression	5	9	14	
	Total	10	20	30	

* Pearson Chi-Square, Asymp.Sig (2-sided)

** Fisher's Exact Test, Exact Sig (2-sided)

Relationship between gene expressions and bone types categorized according to CBCT were mentioned in Table 4.8. No significant relationship could be found between all 3 gene expressions between 4 bone type ($p > 0.05$).

Table 4.8 Bone type catergorized according to CBCT and *BMP2, FGF2, and RUNX2* gene expression

Gene	Expression Yes/No	Bone type		
		2	3	4
BMP2	Undetermined gene expresion	2	13	1
	Gene expression	3	11	0
FGF23	Undetermined gene expresion	4	18	1
	Gene expression	1	6	0
RUNX2	Undetermined gene expresion	3	13	0
	Gene expression	2	11	1

Relationship between gene expressions and bone types categorized according to panoramic X-ray were mentioned in Table 4.9. No significant relationship could be found between all 3 gene expressions and 4 bone types ($p > 0.05$).

Table 4.9 Bone type according to panoramic X-ray and gene expressions

Gene	Expression Yes/No	Bone type		
		2	3	4
BMP2	Undetermined gene expresion	2	12	2
	Gene expression	4	9	1
FGF23	Undetermined gene expresion	5	16	2
	Gene expression	1	5	1
RUNX2	Undetermined gene expresion	4	11	1
	Gene expression	2	10	2

Relationship between gene expressions and bone type as categorized by surgeons' tactile sensation were mentioned in Table 4.10. Similarly, we also could not find any significant relationship between all 3 gene expressions and the 4 bone type ($p > 0.05$).

Table 4.10 Relationship between bone types categorized by surgeons' tactile sensation and *BMP2, FGF2, and RUNX2* gene expressions

Gene	**Expression Yes/No**	**Bone type according to Surgeons' tactile sensation**			
		1	**2**	**3**	**4**
BMP2	Undetermined gene expression	1	4	9	2
	Gene expression	1	6	5	2
FGF23	Undetermined gene expression	2	8	10	3
	Gene expression	0	2	4	1
RUNX2	Undetermined gene expression	2	6	8	0
	Gene expression	0	4	6	4

Relationship between gene expressions with bone types categorized according to CBCT, panoramic X-ray, and surgeons' tactile sensation were mentioned in Table 4.7, 4.8, and 4.9. But we also could not find any significant relationship between them.

Relation of gene expressions with duration of teeth loss was mentioned in Table 4.11. Mean duration of teeth loss in determined gene expression of BMP2 and FGE23 were higher than undermined gene expression, but there was no statistical significance (p >0.05).

Table 4.11 Relationship between duration of teeth loss and BMP2, FGF2, and RUNX2 gene expressions

	Duration of teeth loss	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
BMP2	Undetermined gene	16	7.31	5.27	-0.377	0.7
	Determined gene	14	8.042	5.32		
FGF23	Undetermined gene	23	7.52	4.98	-0.246	0.8
	Determined gene	7	8.08	6.33		
RUNX2	Undetermined gene	16	9.1	5.6	1.672	0.1
	Determined gene	14	6	4.36		

Independent t-test (Sig. 2-tailed)

Relationship between gene expressions and buccolingual width was mentioned in Table 4.12. Mean buccolingual width in determined gene expression of BMP2 and FGE23 was significantly higher statistically than undermined gene expression, p-value 0.025 and 0.001, respectively.

Table 4.12 Relationship bewtween buccolingual width and BMP2, FGF2, and RUNX2 gene expressions

	Buccolingual width	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
BMP2	Undetermined gene	16	7.53	1.2	-2.369	**0.025**
	Determined gene	14	8.61	1.3		
FGF23	Undetermined gene	23	7.62	1.16	-3.659	**0.001**
	Determined gene	7	9.4	0.96		
RUNX2	Undetermined gene	16	7.75625	1.28	-1.231	0.22
	Determined gene	14	8.35	1.38		

Independent t-test (Sig. 2-tailed)

Relationship between gene expressions and interocclusal height was mentioned in Table 4.13. Mean interocclusal height in determined gene expression of BMP2 and FGE23 were significantly higher statistically than undermined gene expression, p-value 0.06 and 0.033, respectively.

Table 4.13 Relationship between interocclusal height and BMP2, FGF2, and RUNX2 gene expressions

	Interocclusal height	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
BMP2	Undetermined gene	16	9.42	1.14	-2.085	**0.06**
	Determined gene	14	10.75	2.23		
FGF23	Undetermined gene	23	9.65	1.7	-2.234	**0.033**
	Determined gene	7	11.31	1.75		
RUNX2	Undetermined gene	16	10.09	2.11	0.158	0.87
	Determined gene	14	9.98	1.52		

Independent t-test (Sig. 2-tailed)

Table 4.14 Relationship between gingival thickness and BMP2, FGF2, and RUNX2 gene expression

	Gingival thickness	N	Mean	Std. Deviation	t	p-value
BMP2	Undetermined gene	16	2.8	0.6	-2.12	0.055
	Determined gene	14	3.53	1.2		
FGF23	Undetermined gene	23	3.08	0.98	-0.655	0.52
	Determined gene	7	3.36	1.03		
RUNX2	Undetermined gene	16	3.57	1.1	2.81	**0.007**
	Determined gene	14	2.66	0.52		

Independent t-test (Sig. 2-tailed)

Relationship between gene expressions and gingival thickness was mentioned in Table 4.14. Mean gingival thickness in determined gene expressions of BMP2 and FGE23 were higher than undermined gene expression, but there were not statistically significant ($p > 0.05$). On the other hand, mean gingival thickness in determined gene expression of RUNX2 was significantly lower statistically than undermined gene expression (p-value 0.007).

Relationship between gene expressions and crestal bone thickness at ossteotomy site was mentioned in Table 4.15. Mean crestal thickness at ossteotomy site in determined gene expression of BMP2 and FGE23 were significantly higher statistically than undermined gene expression, p-value 0.005 and 0.04, respectively.

Table 4.15 Relationship between crestal bone thickness at ossteotomy site and BMP2, FGF2, and RUNX2 gene expressions

	Gingival thickness	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
BMP2	Undetermined gene	16	0.861875	0.532769	-3.058	**0.005**
	Determined gene	14	1.462857	0.541912		
FGF23	Undetermined gene	23	1.017826	0.571019	-2.151	**0.04**
	Determined gene	7	1.551429	0.587464		
RUNX2	Undetermined gene	16	1.073125	0.561566	-0.658	0.516
	Determined gene	14	1.221429	0.672399		

Independent t-test (Sig. 2-tailed)

Relationship between BMP2 gene expression with crestal bone thickness in left and right mandible & left and right mandible were shown in Table 4.16. The mean crestal bone thickness in left mandible in determined gene expression was significantly higher statistically than undermined gene expression, p-value 0.06.

Table 4.16 Relationship between crestal bone thickness and *BMP2* gene expression

	BMP 2	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
Crestal thickness (Left mandible)	Undetermined gene	16	1.56	0.17	-1.93	0.06
	Determined gene	14	1.69	0.18		
Crestal thickness (Right mandible)	Undetermined gene	16	1.64	0.19	-0.530	0.59
	Determined gene	14	1.68	0.23		
Crestal thickness (Left maxilla)	Undetermined gene	16	0.58	0.15	-1.09	0.28
	Determined gene	14	0.65	0.19		
Crestal thickness (Right maxilla)	Undetermined gene	16	0.62	0.1	-0.34	0.73
	Determined gene	14	0.64	0.23		

Independent t-test (Sig. 2-tailed)

Relationship between FGF23 and RUNX2 gene expressions and crestal bone thickness in left and right mandible & left and right mandible were shown in Table 4.17. There was no significant difference in FGF23 and RUNX2 gene expressions at the 4 different sites of jawbones.

Table 4.17 Relationship between crestal bone thickness obtained from panoramic X-ray (Benson *et al.*, 1991) and *FGF23* gene expression

	FGF23	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
Crestal thickness (Left mandible)	Undetermined gene	23	1.6	0.18	-1.21	0.23
	Determined gene	7	1.7	0.2		
Crestal thickness (Right mandible)	Undetermined gene	23	1.69	0.18	1.320	0.19
	Determined gene	7	1.57	0.28		
Crestal thickness (Left maxilla)	Undetermined gene	23	0.61	0.14	0.12	0.9
	Determined gene	7	0.6	0.26		
Crestal thickness (Right maxilla)	Undetermined gene	23	0.63	0.1	0.15	0.88
	Determined gene	7	0.61	0.31		

Independent t-test (Sig. 2-tailed)

Table 4.18 Relationdhip between of crestal bone thickness and *RUNX2* gene expression

	RUNX2	**N**	**Mean**	**Std. Deviation**	**t**	**p-value**
Crestal thickness (Left mandible)	Undetermined gene	16	1.58	0.15	-1.29	0.2
	Determined gene	14	1.67	0.22		
Crestal thickness (Right mandible)	Undetermined gene	16	1.71	0.19	1.560	0.12
	Determined gene	14	1.6	0.22		
Crestal thickness (Left maxilla)	Undetermined gene	16	0.61	0.14	0.02	0.98
	Determined gene	14	0.61	0.21		
Crestal thickness (Right maxilla)	Undetermined gene	16	0.64	0.09	0.48	0.62
	Determined gene	14	0.61	0.23		

Independent t-test (Sig. 2-tailed)

We could not find any significant relationship between crestal bone thickness and gingival thickness with surgeons' tactile sensation.

CHAPTER V
DISCUSSION

The gold standard of bone graft is autogenous bone**.** To understand the functional role of bone and soft tissue cell during healing we examined the effect of gene expression in bone tissue cells. To direct evaluate function and vitality of bone cell might be difficult. Relative to The RNA and cDNA will represent bone function. Bone morphogenetic proteins play an important role in the regulation in the bone induction and maintenance for treating periodontal defects.(Rao *et al.,* 2013) Bone cell and growth factors are effect to cell proliferation and differentiation. Bone morphogenetic protein 2 (BMP2) is the most powerful cytokine that promotes differentiation of mesenchymal cells into osteoblasts in vitro and induce bone formation in vivo. BMP2 exhibits this osteogenic action by activating Smad signalling and by regulating transcription of osteogenic genes such as alkaline phosphatase (ALP), Type I collagen, osteocalcin, and bone sialoprotein (Bsp). BMP2 is known to control the expression and functions of Runt-related gene 2 (RUNX2) through Smad signalling. It was noted that BMP2 regulated Osterix expression independently through two distinct transcription factors, Runx2 and Msx2 were essential for osteoblast differentiation. RUNX2/Core-binding factor 1 (Cbfa1), is an essential transcription factor for osteoblast differentiation and bone.

RUNX2 protein was first detected in preosteoblasts during the early stages of osteoblast differentiation. RUNX2 is reduced during osteoblast maturation and bone development. Smad signaling is required for induction of Osterix, and that Osterix expression is regulated via both Runx2-dependent and –independent mechanisms by BMP2 signaling. Furthermore, Osterix promotes osteoblast differentiation of Runx2-deficient mesenchymal cells in association with up-regulation of several genes which are not up-regulated by Runx212. The Fibroblast Growth Factor 23 (FGF23) produced by osteoblastic cells. Immunohistochemical analysis showed FGF23 production of osteoblasts and granulation tissue in the fracture callus during bone healing. FGF23 is

involved in bone healing and is a promising candidate as an indicator for healing processes prone to reunion versus nonunion. We therefore used a relative to RNA and cDNA to represent bone function. The reduced matrix mineralization, reduced in trabecular bone volume, increased cortical porosity, reduced bone strength and reduced trabecular connectivity, is associated with an increase of many resorping genes expression In addition, to improve osseointegration in the elderly patients by vitamin D supplementation were similar to osteoporotic patients(Mengatto *et al.*, 2011). These data emphasized a contribution of growth factors and molecular regulators in bone osteointegration processes and hard tissue healing. In our study the *BMP2, RUNX2* and *FGF23* expression was not statistically significant difference between types of bones according to Cone-beam computer tomography panoramic radiography and surgeon tactile senses. However, we did not find relationship between better wound healing among our gene expressions. Also history of periodontal disease, smoking, and metabolic disorders can affect gene expression but in our study we cannot find significant difference. In clinical apply the genome microarray analyses at the osteotomy site to identify critical gene networks involved in osseointegration found that the circadian regularity system and cartilage extracellular matrix may be encourage the osseointegration by vitamin D(Alvim-Pereira *et al.*, 2008). In some study found dexamethasone can promote would healing(Advani *et al.*, 1997). Our limitations were small sample sizes. Therefore only 30 bone extracted samples. However, the results of the study showed that the method of gene extraction and observation of gene expression was valid and repeatable. To move to definite results, in some variable numbers be 0 in future study we should be increased sample size Furthermore, our finding indicated that *BMP2* and *FGF23* were expressed statistical differently in difference crestal thickness at osteotomy site. Our results in jaws bone area according to animal research *BMP-2* levels in mandibular extraction sockets were smaller than maxillary sockets but we cannot find significant different between gene expression and maxilla or mandible. In vitro study, *BMP2* stimulation PDL cells and osteoblasts will up to dose-dependent. Relative to osteoblasts, PDL cells were susceptible to apoptosis and cytotoxicity with 10 times lower concentration of *BMP2*(Muthukuru, 2013). In oral cavity, *FGF23* presents a unique opportunity to simultaneously observe four different types of mineralized tissue such as bone,

cementum, dentin, and enamel. *RUNX2* in osteoblasts reduces during bone development. Our results we cannot found the relationship significant differences gene expression increase in periodontitis and smoking because in some cells were 0. Higher *FGF23* associated with and alcohol intake induce bone resorption(Kendrick *et al.*, 2011) same with our study. Smoking has an adverse effect on fracture healing and bone regeneration. In smokers, *BMP2* gene expression of human periosteum was reduced(Chassanidis *et al.*, 2012) but our study smoker was 5 we cannot find the relationship for 3 gene expression. The molecular evaluation at osteotomies sites were not found relationship significant difference with CBCT surgeon tactile sense and radiographic aspects(Pereira *et al.*, 2013).

Conclusion

BMP2, FGF2, RUNX2 gene expressions were difference in jaw bones position. The molecular biology can contribute to the validation of protein and growth factor. Therefore these analyses will be useful to gather data for bone quality or stage of healing of the jaw bone.

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APPENDICES

APPENDIX A

สำนักงานคณะกรรมการจริยธรรมการวิจัยในคน
ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์
มหาวิทยาลัยมหิดล โทร ๐๒-๒๐๐-๗๖๒๒

ที่ ศธ ๐๕๑๗.๐๓๑๕/จธ. ๕๐๐
วันที่ ๖ พฤศจิกายน ๒๕๕๖
เรื่อง แจ้งผลรับรองโครงการวิจัยเรื่อง "การทำนายทางพันธุกรรมของการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังจากการฝังรากฟันเทียม (Genetic prediction of human jaw bone in dental implant)"
เรียน ศ.ณัฐเมศร์ วงศ์สิริฉัตร

ตามที่ ทพญ.นวกมล สุริยันต์ นักศึกษาหลักสูตรปริญญาโท สาขาทันตกรรมรากเทียม นักศึกษาในกำกับดูแลของท่านได้ส่งโครงการวิจัยเรื่อง "การทำนายทางพันธุกรรมของการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังจากการฝังรากฟันเทียม (Genetic prediction of human jaw bone in dental implant)"รหัสโครงการ MU-DT/PY-IRB 2013/033.0807 มาเพื่อขอรับการพิจารณาจากคณะกรรมการจริยธรรมการวิจัยในคน ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดลนั้น

คณะกรรมการจริยธรรมการวิจัยในคนฯ ได้พิจารณาโครงการวิจัยที่แก้ไขตามข้อเสนอแนะของกรรมการฯ แล้ว **มีมติเห็นสมควรให้การรับรอง**

ทั้งนี้คณะกรรมการจริยธรรมการวิจัยในคนฯ ขอแจ้งให้ทราบถึงระเบียบและแนวทางการปฏิบัติภายหลังโครงการวิจัยได้รับการรับรองดังนี้

๑. ขอให้นำเอกสารชี้แจงผู้เข้าร่วมการวิจัยที่มีตราประทับรับรอง จากคณะกรรมการจริยธรรมการวิจัยในคน ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ไปสำเนาใช้กับผู้เข้าร่วมการวิจัยเท่านั้น
๒. หากต้องการจะปรับเปลี่ยนรายละเอียดบางส่วนของโครงร่างวิจัย ขอให้กรอกแบบฟอร์มการขอปรับเปลี่ยนโครงร่างวิจัย (Protocol Amendment) มายังคณะกรรมการจริยธรรมการวิจัยในคนฯ เพื่อขอรับการพิจารณารับรองก่อนเริ่มดำเนินการ เมื่อคณะกรรมการจริยธรรมการวิจัยในคนฯ พิจารณารับรองแล้วจึงจะสามารถดำเนินการต่อไปได้
๓. หากเกิดเหตุการณ์ไม่พึงประสงค์หรือเหตุการณ์ที่ไม่อาจคาดเดาได้ล่วงหน้าเกิดขึ้นกับผู้เข้าร่วมโครงการวิจัย ขอให้รายงานมายังคณะกรรมการจริยธรรมการวิจัยในคนฯ โดยกรอกแบบฟอร์ม "รายงานเหตุการณ์ไม่พึงประสงค์" มาให้คณะกรรมการจริยธรรมการวิจัยในคนฯ
๔. หากดำเนินการวิจัยเสร็จสิ้นใน ๑ ปี ขอให้แจ้งปิดโครงการวิจัยตามแบบฟอร์ม มายังคณะกรรมการจริยธรรมการวิจัยในคนฯ ในกรณีที่โครงการวิจัยมีระยะเวลานานกว่า ๑ ปี ขอให้ส่งรายงานความก้าวหน้าของโครงการวิจัย พร้อมขอต่ออายุการรับรองโครงการมายังคณะกรรมการจริยธรรมการวิจัยในคนฯ ก่อนหมดอายุโครงการอย่างน้อย ๓๐ วัน

จึงเรียนมาเพื่อทราบ และโปรดแจ้งนักศึกษาเพื่อดำเนินการตามระเบียบของคณะกรรมการจริยธรรมการวิจัยในคนฯ ต่อไปด้วย

(รศ.ดร.ชลธชา ห้านิรัติศัย)
ประธานคณะกรรมการจริยธรรมการวิจัยในคน
ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์มหาวิทยาลัยมหิดล

สำเนาเรียน คณบดีบัณฑิตวิทยาลัย

APPENDIX B

Certificate of Approval

COA.No.MU-DT/PY-IRB 2013/036.2910

Documentary Proof of Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board

Title of Project:	Genetic Prediction of Human Jaw Bone in Dental Implant.
Project Number:	MU-DT/PY-IRB 2013/033.0807
Principle Investigator:	Miss Nawakamon Suriyan
Coinvestigator:	Professor Natthamet Wongsirichat
	Dr. Dutmanee Seriwatanachai
	Associate Professor Somcha Sessirisombat
Name of Institution:	Faculty of Dentistry
Approval includes:	1. MU-DT/PY-IRB Submission form version3, October 29, 2013
	2. Proposal version2, August 13, 2013
	3. Participant information sheet version3, October 29, 2013
	4. Consent form version2, August 13, 2013
	5. Advertisement for recruitment version2, August 13, 2013
	6. Case record form version3. October 29, 2013
	7. CV version received date October 29, 2013

Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board is in full compliance with International Guidelines for Human Research Protection such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Date of Approval: October 29, 2013

Date of Expiration: October 28, 2014

Signature of Chair:

(Associate Professor Dr. Choltacha Harnirattisai)

Chair

Signature of Dean

(Associate Professor Passiri Nisalak)

Dean, Faculty of Dentistry

Office of Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board, Building 4, Fifth Floor, Faculty of Dentistry, Mahidol University, 6 Yothi Street, Rajthevi, Bangkok 10400, THAILAND Tel: (662)-200-7622

APPENDIX C

เอกสารชี้แจงผู้เข้าร่วมการวิจัย

(Participant Information Sheet)

ในเอกสารนี้อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามหัวหน้าโครงการวิจัย หรือผู้แทนให้ช่วยอธิบายจนกว่าจะเข้าใจดี ท่านจะได้รับเอกสารนี้ ๑ ฉบับ นำกลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อนสนิท แพทย์ประจำตัว ของท่าน หรือผู้อื่นที่ท่านต้องการปรึกษา เพื่อช่วยในการตัดสินใจเข้าร่วมการวิจัย

ชื่อโครงการ (ภาษาไทย)

ความสัมพันธ์ของลักษณะพันธุกรรมของกระดูกกับการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังการฝังรากฟันเทียม

ชื่อผู้วิจัย ทพญ.นวกมล สุริยันต์

สถานที่วิจัย สถานที่ทำงานและหมายเลขโทรศัพท์ที่ติดต่อได้ทั้งในและนอกเวลาราชการ

นักศึกษาหลักสูตรปริญญาโทสาขาทันตกรรมรากเทียม คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

เบอร์โทรศัพท์ ๐๘๑-๕๖๘๒๕๘๘

ผู้ให้ทุน ทุนหลักสูตรวท.ม ทันตกรรมรากเทียม และอยู่ระหว่างดำเนินการขอทุน สำนักงานคณะกรรมการวิจัยแห่งชาติ

โครงการวิจัยนี้ทำขึ้นเพื่อต้องการศึกษาการแสดงออกของลักษณะทางพันธุกรรมที่ควบคุมการสร้างและสลายกระดูกที่ตำแหน่งต่างๆของขากรรไกรในกลุ่มตัวอย่างที่มีสุขภาพดีหรืออาจมีโรคประจำตัว เช่น โรคเบาหวานและความดันโลหิตสูงที่ควบคุมได้โดยผู้เข้าร่วมวิจัยอาจไม่ได้รับประโยชน์โดยตรงจากงานวิจัยนี้แต่มีประโยชน์ที่คาดว่าจะได้รับเพื่อใช้เป็นข้อมูลประกอบเพิ่มเติมในการพิจารณาคุณภาพของกระดูกขากรรไกรในตำแหน่งต่างๆ เพื่อการวางแผนการรักษา

ท่านได้รับเชิญให้เข้าร่วมการวิจัยนี้เพราะ

◙ อายุมากกว่า ๑๘ ปีไม่มีโรคประจำตัวหรือมีโรคเบาหวานและความดันโลหิตที่ควบคุมได้

◙ มีปริมาณกระดูกเพียงพอในการฝังรากฟันเทียม

◙ ยินยอมเข้าร่วมในงานวิจัย

◙ ไม่มีการอักเสบของกระดูกและเนื้อเยื่ออ่อน

◙ มีสุขภาพช่องปากดีโดยพิจารณาจาก คะแนนดัชนีความรุนแรงของสภาวะโรคปริทันต์

โดยจะมีผู้เข้าร่วมการวิจัยนี้ทั้งสิ้นประมาณ ๓๐ คนซึ่งทั่วไปในการใส่รากเทียมท่านจะต้องมาพบทันตแพทย์ในการรักษาทั้งหมด ๓ ครั้งเป็นเวลา ๑๔ วัน ผู้วิจัยจะขอเก็บข้อมูลเพิ่มเติมจากท่านในวันที่ท่านมีนัดมาพบทันตแพทย์โดยท่านจะเสียเวลาเพิ่มขึ้นประมาณ ๕-๑๐ นาทีในแต่ละครั้ง **โดยในงานวิจัยนี้จะทำการศึกษาในระยะการฝังรากเทียมเท่านั้น**

หากท่านตัดสินใจเข้าร่วมการวิจัยแล้ว จะมีขั้นตอนการวิจัยดังต่อไปนี้คือ

ครั้งที่ ๑. ทันตแพทย์ของท่านจะนัดท่านมาตรวจสุขภาพช่องปากและภาพถ่ายรังสีคอมพิวเตอร์ซึ่งเป็นการรักษาตามขั้นตอนปกติ โดยผู้วิจัยจะขอบันทึกสภาวะสุขภาพช่องปากหลังจากนั้นจะนัดท่านมาเพื่อทำการฝังรากเทียม

ครั้งที่ ๒. ในระหว่างที่ทันตแพทย์ของท่านทำการผ่าตัดเพื่อฝังรากฟันเทียมผู้วิจัยจะขอบันทึกระดับความสูงของเหงือกก่อนการฝังรากฟันเทียม และสอบถามข้อมูลการประเมินความหนาแน่นของกระดูกจากทันตแพทย์ของท่าน และเก็บ

APPENDIX D

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัยโดยได้รับการบอกกล่าวและเต็มใจ

วันที่..... เดือน..... พ.ศ.....

ข้าพเจ้า.....อายุ.....ปี อาศัยอยู่บ้านเลขที่.....

ถนน.....ตำบล.....อำเภอ.....

จังหวัด.....รหัสไปรษณีย์.....โทรศัพท์ .....

ขอแสดงเจตนายินยอมเข้าร่วมโครงการวิจัย[1]เรื่องความสัมพันธ์ทางพันธุกรรมของการฟื้นฟูสภาพของกระดูกในงานทันตกรรมรากฟันเทียม

โดยข้าพเจ้าได้รับทราบรายละเอียดเกี่ยวกับที่มาและจุดมุ่งหมายในการทำวิจัยรายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ประโยชน์ที่คาดว่าจะได้รับของการวิจัยและความเสี่ยงที่อาจจะเกิดขึ้นจากการเข้าร่วมการวิจัย รวมทั้งแนวทางป้องกันและแก้ไขหากเกิดอันตรายขึ้น ค่าตอบแทนที่จะได้รับ ค่าใช้จ่ายที่ข้าพเจ้าจะต้องรับผิดชอบจ่ายเอง โดยได้อ่านข้อความที่มีรายละเอียดอยู่ในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด อีกทั้งยังได้รับคำอธิบายและตอบข้อสงสัยจากหัวหน้าโครงการวิจัยเป็นที่เรียบร้อยแล้ว โดยไม่มีสิ่งใดปิดบังซ่อนเร้น

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้[2] :

ข้าพเจ้าได้ทราบถึงสิทธิ์ที่ข้าพเจ้าจะได้รับข้อมูลเพิ่มเติมทั้งทางด้านประโยชน์และโทษจากการเข้าร่วมการวิจัย และสามารถถอนตัวหรือองคเข้าร่วมการวิจัยได้ทุกเมื่อ โดยจะไม่มีผลกระทบต่อการบริการและการรักษาพยาบาลที่ข้าพเจ้าจะได้รับต่อไปในอนาคต และยินยอมให้ผู้วิจัยใช้ข้อมูลส่วนตัวของข้าพเจ้าที่ได้รับจากการวิจัย แต่จะไม่เผยแพร่ต่อสาธารณะเป็นรายบุคคล โดยจะนำเสนอเป็นข้อมูลโดยรวมจากการวิจัยเท่านั้น

หากมีอาการผิดปกติ รู้สึกไม่สบายกาย หรือมีผลกระทบต่อจิตใจของข้าพเจ้าเกิดขึ้นระหว่างการวิจัย ข้าพเจ้าจะแจ้งผู้วิจัยโดยเร็วที่สุด

หากข้าพเจ้ามีข้อข้องใจเกี่ยวกับขั้นตอนของการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัยขึ้นกับข้าพเจ้า[*] ข้าพเจ้า จะสามารถติดต่อกับ(ระบุชื่อผู้รับผิดชอบที่โทรศัพท์/วิทยุติดตามตัวที่ติดต่อได้ 24 ชั่วโมง)

หากข้าพเจ้า[*] ได้รับการปฏิบัติไม่ตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าจะสามารถติดต่อกับประธานคณะกรรมการจริยธรรมการวิจัยในคนหรือผู้แทน ได้ที่สำนักงานคณะกรรมการจริยธรรมการวิจัยในคนประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล คณะทันตแพทยศาสตร์ อาคาร 4 ชั้น 5 เลขที่ 6 ถนนโยธี แขวงทุ่งพญาไท เขตราชเทวี จังหวัดกรุงเทพฯ 10400 หมายเลขโทรศัพท์ 02-200-7622 โทรสาร 02-200-76223

ข้าพเจ้าเข้าใจข้อความในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และหนังสือแสดงเจตนายินยอมนี้โดยตลอดแล้ว จึงลงลายมือชื่อไว้

ลงชื่อ.....ผู้เข้าร่วมการวิจัย/ผู้แทนโดยชอบธรรม/ วันที่.....

(.....)

ลงชื่อ.....ผู้ให้ข้อมูลและขอความยินยอม/หัวหน้าโครงการวิจัย/ วันที่.....

(.....)

ในกรณีผู้เข้าร่วมการวิจัยไม่สามารถอ่านหนังสือได้ผู้ที่อ่านข้อความทั้งหมดแทนผู้เข้าร่วมการวิจัยคือ..... จึงได้ลงลายมือชื่อไว้เป็นพยาน

ลงชื่อ..... พยาน/ วันที่.....

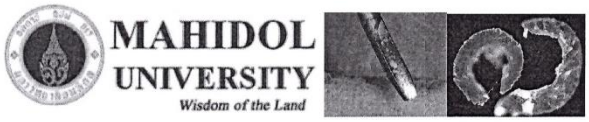
(.....)

หมายเหตุ : หากผู้เข้าร่วมการวิจัยเป็นผู้เยาว์ (อายุต่ำกว่า 18 ปี บริบูรณ์) ให้เปลี่ยนข้อความตรงที่ตำแหน่ง 1, 2 และสรรพนามข้าพเจ้าตรงเครื่องหมาย * ดังนี้

(1) ขอแสดงเจตนายินยอมให้เด็กในปกครองของข้าพเจ้าเข้าร่วมโครงการวิจัย

(2) ข้าพเจ้าจึงสมัครใจให้เด็กในปกครองของข้าพเจ้าเข้าร่วมโครงการวิจัยนี้

APPENDIX E



ขอเชิญเข้าร่วมโครงการวิจัยเรื่อง

ความสัมพันธ์ของลักษณะพันธุกรรมของกระดูก

กับการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังการฝังรากฟันเทียม

โครงการวิจัยนี้ต้องการศึกษาการแสดงออกของลักษณะทางพันธุกรรมที่ควบคุมการเมตาบอลิซึมของกระดูกที่ ตำแหน่งต่างๆของขา กรรไกรในกลุ่มตัวอย่างปกติ หรือมีโรคเบาหวานหรือความดันโลหิตสูงที่ควบคุมได้

โดยต้องการผู้มีคุณสมบัติดังนี้

- **มีอายุ 18 ปีขึ้นไปมีสุขภาพดีหรืออาจมีโรคประจำตัวคือโรคเบาหวานหรือโรคความดันโลหิตที่ควบคุมได้**
- **ต้องการฝังรากฟันเทียมและมีปริมาณกระดูกที่เหมาะสม**
- **ไม่เคยมีการติดเชื้อบริเวณเนื้อเยื่ออ่อนและกระดูก**
- **สามารถมาตรวจตามนัดและเข้าร่วมการวิจัยเป็นระยะเวลา 14วัน(3ครั้ง)**

หากท่านต้องการเข้าร่วมโครงการวิจัยกรุณาติดต่อที่

ทันตแพทย์หญิงนวกมล สุริยันต์

คลินิกทันตกรรมสาขารากฟันเทียม คณะทันตแพทยศาสตร์

มหาวิทยาลัยมหิดล หมายเลขโทรศัพท์ 081-5682588,085-8251017

APPENDIX F

สำนักงานคณะกรรมการจริยธรรมการวิจัยในคน
ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์
มหาวิทยาลัยมหิดล โทร ๐๒-๒๐๐-๗๖๒๒

ที่ ศธ ๐๕๑๗.๐๓๑๙/จธ. ๕๐๐
วันที่ ๖ พฤศจิกายน ๒๕๕๖
เรื่อง แจ้งผลรับรองโครงการวิจัยเรื่อง "การทำนายทางพันธุกรรมของการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังจากการฝังรากฟันเทียม (Genetic prediction of human jaw bone in dental implant)"
เรียน ศ.ณัฐเมศร์ วงศ์สิริฉัตร

ตามที่ ทพญ.นวกมล สุริยันต์ นักศึกษาหลักสูตรปริญญาโท สาขาทันตกรรมรากเทียม นักศึกษาในกำกับดูแลของท่านได้ส่งโครงการวิจัยเรื่อง "การทำนายทางพันธุกรรมของการฟื้นฟูสภาพของกระดูกขากรรไกรมนุษย์หลังจากการฝังรากฟันเทียม (Genetic prediction of human jaw bone in dental implant)"รหัสโครงการ MU-DT/PY-IRB 2013/033.0807 มาเพื่อขอรับการพิจารณาจากคณะกรรมการจริยธรรมการวิจัยในคน ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดลนั้น

คณะกรรมการจริยธรรมการวิจัยในคนฯ ได้พิจารณาโครงการวิจัยที่แก้ไขตามข้อเสนอแนะของกรรมการฯ แล้ว **มีมติเห็นสมควรให้การรับรอง**

ทั้งนี้คณะกรรมการจริยธรรมการวิจัยในคนฯ ขอแจ้งให้ทราบถึงระเบียบและแนวทางการปฏิบัติภายหลังโครงการวิจัยได้รับการรับรองดังนี้

๑. ขอให้นำเอกสารชี้แจงผู้เข้าร่วมการวิจัยที่มีตราประทับรับรอง จากคณะกรรมการจริยธรรมการวิจัยในคน ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ไปสำเนาใช้กับผู้เข้าร่วมการวิจัยเท่านั้น
๒. หากต้องการจะปรับเปลี่ยนรายละเอียดบางส่วนของโครงร่างวิจัย ขอให้กรอกแบบฟอร์มการขอปรับเปลี่ยนโครงร่างวิจัย (Protocol Amendment) มายังคณะกรรมการจริยธรรมการวิจัยในคนฯ เพื่อขอรับการพิจารณารับรองก่อนเริ่มดำเนินการ เมื่อคณะกรรมการจริยธรรมการวิจัยในคนฯ พิจารณารับรองแล้วจึงจะสามารถดำเนินการต่อไปได้
๓. หากเกิดเหตุการณ์ไม่พึงประสงค์หรือเหตุการณ์ที่ไม่อาจคาดเดาได้ล่วงหน้าเกิดขึ้นกับผู้เข้าร่วมโครงการวิจัย ขอให้รายงานมายังคณะกรรมการจริยธรรมการวิจัยในคนฯ โดยกรอกแบบฟอร์ม "รายงานเหตุการณ์ไม่พึงประสงค์" มาให้คณะกรรมการจริยธรรมการวิจัยในคนฯ
๔. หากดำเนินการวิจัยเสร็จสิ้นใน ๑ ปี ขอให้แจ้งปิดโครงการวิจัยตามแบบฟอร์ม มายังคณะกรรมการจริยธรรมการวิจัยในคนฯ ในกรณีที่โครงการวิจัยมีระยะเวลานานกว่า ๑ ปี ขอให้ส่งรายงานความก้าวหน้าของโครงการวิจัย พร้อมขอต่ออายุการรับรองโครงการมายังคณะกรรมการจริยธรรมการวิจัยในคนฯ ก่อนหมดอายุโครงการอย่างน้อย ๓๐ วัน

จึงเรียนมาเพื่อทราบ และโปรดแจ้งนักศึกษาเพื่อดำเนินการตามระเบียบของคณะกรรมการจริยธรรมการวิจัยในคนฯ ต่อไปด้วย

(รศ.ดร.ชลธชา ห้านิรัติศัย)
ประธานคณะกรรมการจริยธรรมการวิจัยในคน
ประจำคณะทันตแพทยศาสตร์และคณะเภสัชศาสตร์มหาวิทยาลัยมหิดล

สำเนาเรียน คณบดีบัณฑิตวิทยาลัย

BIOGRAPHY

NAME	Dr. Nawakamon Suriyan
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PLACE OF BIRTH	-
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PUBLICATION

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