The Use of Orthodontic Micro-Implant as Anchorage for Rapid Sutural Expansion in Rabbit

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ABSTRACT

    Maxillary constriction is an important problem in orthodontic treatment. Anchorage used for correcting transverse discrepancies was teeth and supporting palatal tissue but many complications occurred. The orthodontic micro-implant emerged as a stable bony anchorage without serious complications for orthodontic tooth movement. Uncommon used of orthopedics aspect is opportunity for using as anchorage for maxillary expansion. The purpose of this study was to evaluate an efficiency of the orthodontic micro-implant and tissue regenerated between gaps of expansion in cranial sutures in rabbit. **Material and methods**; Eighteen 30-day old, 1-1.5 kg inbreeding, male, New Zealand White rabbits used in this study. All animals, two orthodontic micro-implants and gutta percha markers were placed each sides of interfrontal suture. The animals were divided into 4 groups of 4 animals each and sham group. The expansion devices were activated after 3 days of operation twice time daily in all experiment groups for consecutive 7 days period. The variation in retention period (after complete activated, 2, 4 and 8 weeks retention period -- were group A, B, C and D respectively) for regenerated tissue examined. **Results**; The animals tolerated well to the surgical operation and the distraction procedure without any complication and neurological morbidity encountered. The distance of the suture expansion gained according to bone markers in each group was 3.72 mm ± 0.63 (group A), 4.35 mm ± 0.36 (group B), 4.45 mm ± 0.35 (group C) and 4.29 mm ± 1.49 (group D) respectively when comparing to the expected distance of 5.6 mm provided by distraction device. The obtained expanded gap was significantly more than the normal physiologic growth of the suture in sham group (0.48 mm ± 0.09). Gross morphological appearance after complete distaction (group A), the interfrontal suture was marked separated from each other in the elliptical shape pattern which

extended to adjacent mid-sagittal suture through occipital and nasal bone. The yellowish-red color with fibrous connective tissue-like consistency was filled in expanded gap by soft irregular surface texture. The regenerated tissue was gradually changed in coloration and firm to hard consistency similar to callus formation of bony healing process. And anatomical normal cranial suture was seemed to be re-established finally. The mean optical density of distraction gap was examined. The radiopacity of expanded gap increasing rapidly in group A and gradually increased afterward which almost the same level, when compared to the sham group, in group D. Biomechanical property was test by Vicker's hardness, not applicable in group A because it's soft texture, the surface hardness were increased from 3.45 ± 0.058, 8.98 ± 0.171 and 10.03 ± 0.025 in group B, C and D respectively which mostly normal when compared with sham group (10.05 ± 0.289). Histologic exam were found plenty of fibroblastic cells with seem align themselves palisades to the vector of distraction, islands of new bone spicules already seen throughout the gap and noted that new bone formation extended from both host bone surface in group A. The distracted gap was filled with newly formed bone which bone maturation cascades occurred and the last re-established cranial suture structure were nearly similar to that found in sham group. **Conclusion**; Distraction ostegenesis without an osteotomy could be used in cranial suture expansion in growing rabbit with satisfactory outcome. Newly formed bone in the distraction gap started rapidly since the completion distraction phase. The new bone formation kept increase gradually until achieved the normal bone level in 8 weeks groups. The re-established cranial sutures possessed the similar clinical, radiographic and histologic feature as found in normal cranial suture. Cranial suture expansion using distraction osteogenesis without an osteotomy appeared to be the promising procedure to increase the dimension between cranial and facial suture sepicially in craniofacial deformity or craniosynostoses. The clinical application should be the further step to study and evaluate.

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CHAPTER 1

INTRODUCTION

Introduction

Craniofacial complex consist of four areas which grow differently: (1) the cranial vault, (2) the cranial base, (3) nasomaxillary complax, (4) mandible. Cranial vault and nasomaxillary complex bone grow as intramenbranous bone generally which remodeling and growth achieved through bone formation within a periosteum or by bone formation at sutures, the periosteum-lined contact between adjacent bones.

At birth, flat craniofacial bones are rather widely separate by relatively loose connective tissue. Sutures, the fibrous tissue uniting the flat bone, are the major site of bone growth along the leading margins of the craniofacial bones during craniofacial development especially during rapid expansion of neurocranium. Sutures are formed during embryonic development at the sites of approximation of the membranous bones of the craniofacial skeleton. They serve as the major sites of bone expansion during postnatal craniofacial growth. For sutures to function as intramembranous bone growth sites, they need to remain in an unossified state, yet allow new bone to be formed at the edges of the overlapping bone fronts. This process relies on the production of sufficient new bone cells to be recruited into the bone fronts, while ensuring that the cells within the suture remain undifferentiated. Unlike endochondral growth plates, which expand through chondrocyte hypertrophy, sutures do not have intrinsic growth potential. Rather, they produce new bone at the sutural edges of the bone fronts in response to external stimuli, such as signals arising from the expanding neurocranium. This process allows growth of the cranial vault to be coordinated with growth of the neurocranium. Too little or delayed bone growth will result in wide-open fontanels and suture agenesis, whereas too much or accelerated bone growth will result in osseous obliteration of the sutures or craniosynostosis.

Abnormal sutural growth leads to abnormal shape and form of skeleton such as craniosynostosis of clavarium, maxillary constriction of nasomaxillary complex. Morphogenesis and phenotypic maintenance of the cranial sutures are regulated by tissue interactions, especially

those with the underlying dura mater. Removal of the dura mater in fetus causes abnormal suture development and premature suture obliteration. The dura mater interacts with overlying tissues of the cranial vault by providing: (1) intercellular signals, (2) mechanical signals and (3) cells, which undergo transformation and migrate to the suture. Skull growth after premature fusion of a single suture predicted by the following observations: (1) cranial vault bones that are prematurely fused act as a single bone plate with decreased growth potential; (2) asymmetrical bone deposition occurs mainly at perimeter sutures, with increased bone deposition directed away from the bone plate; (3) sutures adjacent to the stenotic suture compensate in growth more than those sutures not contiguous with the closed suture; and (4) enhanced bone deposition occurs along both sides of a nonperimeter suture that is a continuation of the prematurely closed suture. To prevent these consequently and potentially avoiding brain maldevelopment. Brain injury is presumed to be related to local or regional increases in intracranial pressure. A broad range of surgical options to treat craniosynostosis exist, from strip craniectomy to comprehensive or whole vault, cranioplasty. The optimal surgical timing for these approaches must balance both the desire for early intervention to reduce the effects of bone restriction on brain growth and the ability of a child to withstand the rigors of surgery.

For maxillary constriction, result in transverse discrepancy of dental arch relationship, a wide variety of modalities for orthodontic treatment in transverse dimension reported in the literature includes banded, bonded, and removable appliances, as well as appliances not typically used for expansion, such as headgear and functional appliances. The methods used for corrected are slow orthodontic expansion (**SOE**), rapid maxillary expansion (**RPE**), surgically assisted rapid palatal expansion (**SA-RPE**) or a two-segmented Le Fort I-type osteotomy with expansion.[1]

SOE indicates for very mild lateral discrepancies. Currently devices are the Coffin palatal arch, the Arnold expander and the quad-helix appliance. The expansion of dental arch occurs as a combination of bodily tooth movement and tipping.

RPE indicates in patients younger than 12 years, who have lateral discrepancy involving several teeth, whether the constriction is skeletal, dental or combination of both. The devices are all tooth-borne, but one type has palatal flanges. Most commonly used is the 'hygienic

appliance' (Hyrax), an all-wire frame soldered to bands cemented on the abutment teeth. The fixed split acrylic appliance which is tissue-borne with bands on the first molars and premolars and provided by a jackscrew was advocated by Haas[2] because it would resist the post-expansion forces that tend to collapse the maxilla whilst the teeth remain in their expanded state. The Howe acrylic-lined bondable expander with a midpalatal jackscrew[3] and the Minne expander, which consists of a heavy calibre coil spring with two metal flanges soldered to the bands, are less frequently used.

From 14 years on, RPE is accompanied by corticotomies that release the areas of bony resistance, which is **SA-RPE,** out of fear for alveolar bending, tooth tipping and extrusion[8], periodontal membrane compression and buccal root resorption[4], fenestration of the buccal cortex and instability-relapse with the necessity for overcorrection[2]. The same devices as for RPE are used. Although surgical release of the areas of maxillary support, undesired movements of the abutment teeth are noticed during expansion and retention. Prolonged retention and overcorrection are advisable to counteract skeletal relapse[5].

Many methods are available for achieving maxillary expansion. Dental expansion can be accomplished using a variety of appliances depending on the amount of expansion desired and the age of the patient.

Jackscrew appliances

Two general types of jackscrew appliances are most often used to expand the maxilla, tooth borne and tissue borne appliance. In patients in whom growth has not ceased, skeletal expansion is achieved along with dental expansion. Tooth-borne, or Hyrax, appliances fixed to the teeth only, either by orthodontic bands, or bondable acrylic pads that cover the occlusal surfaces and extend over the buccal and lingual surfaces of the teeth. Tissue-borne appliances, particularly the Haas-type appliance, include an acrylic button molded to the palate, in which the jackscrew is embedded and the bands are attached. Proponents of the tissue borne appliance claim that skeletal expansion achieved greater than the tooth-borne expander, because force is transmitted more directly to the palatal shelves.

The fixed jackscrew appliance can produce a significant molar expansion with ranging from a mean of 4.69 mm[6] to 7.9 mm[7]. Skeletal expansion ranged from 46%[8] to 58%[2].

Removable Expanders

Several investigators used removable jackscrew appliances to expand the maxilla, Boysen[9] placed in 6 years 4 months to 10 years 9 months children (mean 8 years 6 months), an appliance composed with acrylic covering the posterior maxillary occlusal surfaces to disarticulate the occlusion. The screw was activated twice per week for a total weekly expansion of 0.5 mm. Basal expansion was found less than resulting from the quad-helix. Sandikcioglu and Hazar[10] reported that the molar expansion with this appliance was 4.0 mm, and the skeletal expansion was 1.5 mm. No relapse was measured. Brin et al[11] showed that dental expansion of 3.3 mm and skeletal expansion of 6.0 mm, which is very unusual. No amounts of relapse were reported.

Nonscrew Expanders

Another type of appliance widely used for maxillary expansion is the palatal arch, made of 0.036- or 0.038-inch wire attached to the first molar bands, and is activated by expansion before cementation. Lateral forces delivered by the wires against the teeth serve to expand the dental arch. The quad-helix incorporates four helices in the palatal arch, and is used primarily for younger children for dental expansion.

Slow expansion

Slow expansion techniques use lower orthopedic forces and take longer time more than traditional rapid palatal expansion for the same amount of expansion. Its proponents consider that with lower forces, there is less suture trauma and less dental tipping. A Minne expander (Ormco Corp., Glendora, CA) , consists of a spring-loaded jackscrew attached to four orthodontic bands, usually used for slow expansion, although a traditional jackscrew appliance can also be used and turned less frequently.

Functional Appliances

Several studies have reported that significant dental expansion can be achieved with various functional appliances, and in several instances, significant skeletal expansion was achieved as well[9, 10, 12]. Although relapse data were incomplete at best, it appeared that dental

relapse could be significant, ranging from 19% to 100%. In fact, noted that while transverse increases gained with the Frankel appliance could alleviate arch‑length deficiencies, it could not correct crossbite.[13]

BeGole et al[14] reported on the amount of molar expansion normally occurring during fixed edgewise therapy. They found that in nonextraction cases, the maxillary molar width increased by 2.96 mm, and in extraction therapy, the molars narrowed by 0.22 mm. After treatment, the nonextraction patients demonstrated 0.52 mm of relapse, while extraction patients showed an additional expansion of 0.67 mm. Kirjavainen et al[15] reported on dental expansion achieved with a Kloehn‑type cervical headgear ranging from 2.8 mm to 5.1 mm was reported, but no skeletal expansion or an amount of relapse was reported.

Distraction osteogenesis

Ilizarov[16] worked largely in isolation used orthopedic devices to lengthen limp bones in process later called distraction osteogenesis. Distraction osteogenesis is usually involved an osteotomy and subsequent separation of osteotomy site by distractors. Bone ends are laterally apart, leaving it to nature to fill the gap with bone regeneration over time. Distraction osteogenesis provides an interesting model of in vivo mechanical interactions with suture growth. Forces generated by distraction devices in the maxilla or other cranial bones are likely transmitted as suture strain, which in turn may induce suture osteogenic response[17].

Type of appliance

Appliances use to correct transverse discrepancies could be classified due to anchorage into 3 groups, **1.Tooth‑borne appliances 2.Tooth‑tissue borne appliances and 3.Bone‑borne appliance.** The most popular tooth‑borne palatal expanders, Haas and Hyrax, are fixed appliances with a jackscrew incorporated at their center. Both designs usually involve banding the first premolars and first molars. However, the Haas appliance incorporates acrylic coverage against the palate, which makes it a tissue‑borne device, whereas the Hyrax consists of a metal framework only that stands at a distance from the palate and is entirely tooth‑borne. Hyrax expanders are popular because they are easy to clean and fabricate, and they interfere minimally with speech[1].

Traditionally, the devices used to correct transverse maxillary discrepancy are tooth borne appliance or tooth-tissue borne appliances i.e. Hyrax appliances, Hass appliance or other jack screw appliances, for slow orthodontic expansion and rapid maxillary expansion are transferred forced through teeth then resulted in activation on circummaxillary sutures for increasing maxillary width. Many study shown several complications occur to teeth attach to devices such as periodontal membrane compression, buccal root resorption[4] fenestration of buccal cortex,[2] buccal tipping of teeth, extrusion, root resorption, and fenestration of the alveolar process which lead to periodontal side effects.[18] . Due to Newton's third law, for every action there is an equal and opposite reaction, there are limitations in our ability to completely control all aspects of tooth movement.

In contrast, with bone-borne distractors applied at a higher level in the palatal vault, most of the maxillary expansion is orthopedic and at a more mechanically desired level[1, 5, 19]. In addition the forces are directly on the bone and no tooth tipping and other unwelcome side effects are to be expected. The commercially available bone-borne distractors like the Transpalatal Distractor (TPD™)[1] have to be fixed with screws on the palatal bone and have proven to be useful in acquired deformation patients. The MDO-R device (Orthognathics Ltd.) has no screw fixation; however it has a minimal width of 1.5 cm. In congenital patients with extreme narrow maxillas these devices seem to be impractible due to difficulties with screw fixation and the devices are often too large to be placed.

The Rotterdam palatal distractor(RPD; KLS Marti, Postfach 60, D78501 Tuttlongen, Germany), bone-borne palatal distractor, has been developed based on mechanical properties of a car jack, no screw fixation and stabilized with nails of the abutments plates. There is a relative contraindication in cases with class II deep bite, the activation rod on the palate may interfere the lower teeth. Absolute contra-indication is in case of a low palate, the nails of the abutments plates will loose and the distractor is not stable[20].

Evaluating three-dimensional morphologic changes induced by palatal expansion, Haas appliances demonstrated a greater orthopedic movement, and Hyrax appliances demonstrated dentoalveolar expansion by increasing the palatal angulation of the alveolus.[21] Comparing rapid maxillary expander and tandem-loop nickel titanium temperature-activated palatal expansion appliance showed that the rapid palatal expander widened the palate more

reliably, whereas the nickel titanium expander tipped the molars buccally to a greater extent and caused more distal molar rotation[22].

With quad-helix, maxillary basal bone expansion was found more than with a removable jackscrew appliance.[9] However, unknown how much dental expansion was attempted, because each type of appliance was activated merely until the crossbite, and lateral shifts were corrected. Adkins et al[12] reported that buccal teeth tipped an average of 7.3° as the mean expansion of 6.5 mm was achieved. Most of the patients who treated with quad-helix appliance were in the deciduous or mixed dentition, on average approximately 12 years. Several studies did report skeletal change, suture opening or increased maxillary width, with a little in the way of post-treatment follow-up, so it is not possible to determine how much of the skeletal expansion produced by this appliance was maintained in the long term.[23]

Ingervall et al[24] used transpalatal arch to correct unilateral crossbites. The group that had one molar with buccal root torque demonstrated more sutural opening, although both values were less than 1 mm, and the torqued molar did not move significantly but crossbite correction occurred in both group. Although palatal arches can minimal open maxillary suture but best used for dental expansion in children with primary or mixed dentition.

Hicks[25] evaluated the stability of slow expansion in 5 subjects, aged 10 to 15 years. Dental expansion ranged from 3.8 to 8.7 mm, with skeletal expansion comprising 24% to 30% of the dental expansion in the 10- to 11-years olds, but only 16% in the 15-year-old. Mossaz-Joelson and Mossaz[26] compared bonded and banded Minne expanders and found no difference in the amount of dental and skeletal expansion or relapse. Skeletal expansion comprised about half of the dental expansion. Finally, Akkaya et al[27] compared arch changes in a bonded Hyrax appliance group and a bonded Minne expander group. It was found that molar expansion and skeletal changes had no significantly different between the 2 groups.

Timing of Expansion

Bjork and Skieller[28] found that the transverse growth of the maxilla followed distance and velocity curves similar to those for body height, with similar times of growth spurt and growth completion. In addition, they found that while posterior growth was three times that of the anterior maxilla, the dental arch width showed only one quarter the increase of that of the

basal maxilla. In 1990, Korn and Baumrind[29] used a similar technique to study the growth of 31 children from 8.5 to 15.5 years of age. They found an average annual rate of transverse growth of 0.43 to + 0.18 mm per year, and confirmed that posterior growth was greater than anterior growth.

Snodell[30] et al found significant gender differences at 6 years of age that increased at 12 and 18 years. At 6 years of age, only cranial width, facial width, and maxillary width were significantly different between males and females. At 18 years of age, only mandibular first-molar width was not significantly different. From the study, At 6 years of age, females had reached a higher percentage of adult size than males for all parameters, with values ranging from 80% for adult nasal width to 103% for adult lower second-molar width. Male values ranged from 75% to 109% for the same measurements being represented at the extremes. In contrast, only 71% to 84% of the adult value was reached for vertical parameters by age 6-years old. Once again, females had reached a higher percentage of adult values than males. Females were similarly quicker to complete growth, with all growth ceasing by age 17, while males showed continued growth beyond 18 years for all parameters except maxillary width.

The growth and maturation of the intermaxillary suture are another source of information related to the optimal time to expand the maxilla. Melsen[31] divided suture maturation into three stages based on its morphology. In the infantile stage, the suture was broad and smooth, but by approximately 10 years of age had developed into a more typical squamous suture with overlapping sections. Melsen called this stage the 'juvenile" stage. Finally, the "adolescent" phase was seen at ages 13 to 14 years, where the suture was wavier with increasing interdigitation. These interdigitations could not be separated without fracturing them.

In another study, Persson and Thilander quantified suture closure by evaluating the degree of obliteration in the suture[31]. From these studies, the patients have passed their pubertal growth spurt may have difficulty in undergoing traditional orthopedic maxillary expansion. The increased interdigitation of the suture may require excessive force to separate.

Mao, Wang and Kopher summarized about force from craniofacial orthopedic devices likely transmitted as bone strain and suture strain[32]. Sutural growth is likely a function of certain optimal parameters of mechanical stimuli that remain to be determined instead of a particular type of orthopedic appliance.

Temporary Anchorage devices

Traditionally, orthodontists have used teeth, intraoral appliances, and extraoral appliances, to control anchorage – minimizing the movement of certain teeth, while completing the desired movement of other teeth. However, because of Newton's third law, for every action there is an equal and opposite reaction, there are limitations in our ability to completely control all aspects of tooth movement.[33]

Temporary anchorage devices (TADs) are temporarily fixed to bone for the purpose of enhancing orthodontic anchorage either by supporting the teeth of the reactive unit or by obviating the need for the reactive unit altogether, and which is subsequently removed after used. They can be located transosteally, superiosteally, or endosteally; and they can be fixed to bone either mechanically (cortical stabilized) or biomechanically (osteointegrated). The first clinical report in the literature of the use of TADs appeared in 1983 when Creekmore and Eklund[34] used vitalium bone screw to treat a patient with a deep impinging overbite. Even though the successful application of TADs, this technique did not gain immediate acceptance because lack of wide spread acceptance of surgical procedures, unaccepted field of implant dentistry, the lack of scientific data on the use of implantable materials, and fear of complications. Instead, traditional anchorage mechanics remained the principle treatment modality for managing orthodontic problems.[33]

The first report about the use of osseointegrated implanted for both restorative and orthodontic purpose by Linkow[35]. Class II elastics were worn from the implant-supported bridge to upper arch to facilitate tooth movement. After that Kokich[36] has developed protocols for determining how to accuracy place the dental implants in the final desired location for both orthodontic anchorage as well as the subsequent restorative therapy.

Although osseointegrated implants have been used successfully for orthodontic anchorage, the clinical applications are still limited in edentulous or retromolar areas because of their size and complicated fixture designs. Other disadvantages include a long waiting period (2 to 6 months) for bone healing and osseointegration[37], comprehensive clinical and laboratory work, difficult removal after treatment, and high cost. Miniplates[38, 39] and miniscrews[40, 41] have recently

been introduced as simpler alternatives to endosseous implants and onplants in orthodontics. Their advantages include smaller size, greater number of implant sites and indications, simpler surgical placement and orthodontic connection, shorter (or even no) waiting period, no need for laboratory work, easier removal after treatment, and lower cost.

Endosseous implants and palatal onplants are thought to provide absolute or rigid anchorage[37]. They integrated with the surrounding bone and thus remain absolutely stationary under orthodontic loading[42, 43]. For the miniscrew, it is suggested that a waiting period for bone healing and osseointegration before loading is unnecessary because the primary stability (mechanical retention) of the miniscrew is sufficient to sustain a regular orthodontic loading.[35, 44]

The currently available temporary anchorage devices can be classified as either biocompatible (Figure 1) or biological in nature (Figure 2). Both groups can be subclassified based on the manner in which their attached to bone, either biochemical (osseointegrated) or mechanical. For instance, an ankylosed tooth temporarily used for orthodontic anchorage and subsequently replaced would be considered a biological TAD that is fixed to bone biochemically. Likewise, a significantly dilacerated tooth can be used as a biological TAD that is essentially fixed to bone mechanically.[33]
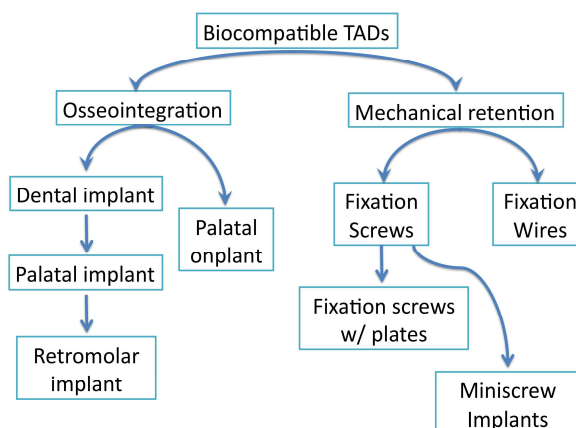
Figure 1.   Biocompatible temporary anchorage devices.[33]

The biocompatible TADs are either 1) a modification of a dental implant, or 2) a surgical fixation method. For example, a palatal implant is a miniaturized dental implant placed in the palate with the intention of osseointegration and subsequent use for orthodontic anchorage. On the other hand, a miniscrew is a fixation device placed in many locations for anchorage control without the intention of osseointegration but only for mechanical stability.[35]
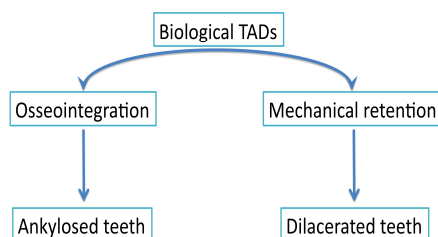
Figure 2.   Biologic temporary anchorage devices.[33]

It is important for orthodontists to be able to communicate with each other clearly and concisely. Previously, several different terms have been used to refer to the same entity. To explain, what has been referred to as a miniscrew implant has been referred to, in the literature, as a microimplant[45], microscrew implant[46], mini-implant[47], miniscrew[48], and screw-type implant.[49]

The difference between a screw and an implant can also be debated. Both can be defined based on function or design. For instance, the screw's original function was to utilize the mechanical advantage of the inclined plane wrapped around a central body to lift objects. It was later used to join two objects together. Its design is defined by its length, diameter, thread width, thread pitch, and head/end configuration. The implant's original function was to replace or augment a body part. It was later used as a modification of a screw for initial mechanical stability with anticipated osseointegration. Its design is also defined by its length, diameter, thread width, thread pitch, and head/end configuration. An implant, however, is usually shorter in relation to its diameter, whereas a screw is usually longer in relation to its diameter. Because there does not appear to be a clear-cut defining distinction, the term miniscrew implant will be used. Furthermore, "miniscrew implant" will be defined as having a diameter of less than 2.5 mm. Simple, yet distinct, acronyms for all of the currently available TADs are listed (TAD - Temporary Anchorage Device, PI - Palatal Implant, RMI - RetroMolar Implant, PO - Palatal Onplant, MSI - Mini Screw Implant, MBP - Mini Bone Plate, FW - Fixation Wire)
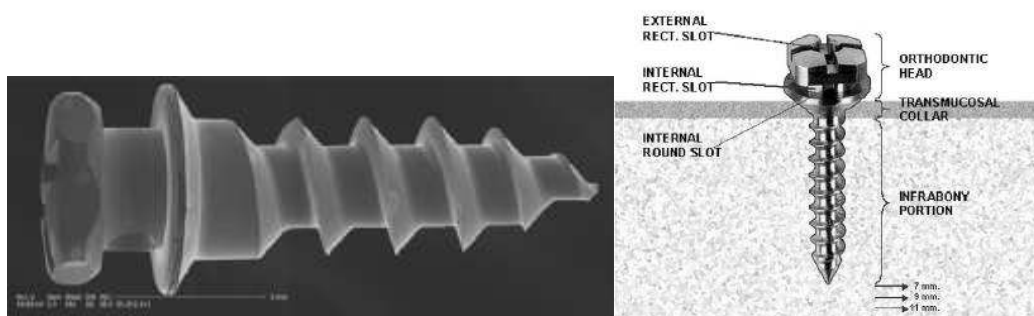
Figure 3. Components of micro-implant.

The micro-implant generally make from titanium alloy. It has four components: head, neck, platform and screw body (Figure 3). The head has access to hold an orthodontic archwire, ligature wire or elastic chain. The neck area (isthmus) between head and platform may have a round perforation to hold additional ligatures or archwires. The smooth platform surface enhances peri-implant wound healing and prevents the screw head from protruding into the soft tissue. As the screw body has a self-tapping design or self-drilling design.[50] For self-drill method,the microimplant is driven into the tunnel of bone formed by drilling, making it tap during implant driving. This method is used when using small diameter microimplants. The other, self-tapping method, the micro-implant is driven directly into bone without drilling. This method can be use when using larger diameter (more than 1.5 mm) microimplants.[51]

Motoyoshi et al[50] reported that the maximum effective stress decreased as screw pitch decreased gradually. A thread pitch of 0.5mm may be recommended to decrease the stress concentration in these experimental conditions. However, considering the patterns of stress distribution found no remarkable difference of the effect derived from thread pitch.

The diameter of Micro-implant from 1.2 – 1.8 mm. are available. The diameter 1.2 -1.3 mm. can all withstand up to 450g of orthodontic force when patient has good quality of cortical bone. However maximum of the intraoral orthodontic forces ever needed are often less than 300g. When using forces greater than 300g, clinicians should select 1.4 – 1.6 mm in diameter. When there is no initial tightness with diameter 1.2 – 1.3 mm. micro-implants, clinicians should select the next larger sizes until there is a close fit between screw and bone. The 1.7 -1.8 mm. diameter mini-implant are designed specially for intermaxillary fixation during orthognathic surgery.[52]

In the mandible, the buccal surfaces and retromolar areas offer adequate thickness and high quality cortex for the acceptance of microimplants. Usually, those of 4 - 5mm in length with 1.2 - 1.3mm in diameter provide adequate retention. A micro-implant with 1.4 - 1.6 mm in diameter might improve retention when cortical bone is less dense or greater force is needed; e.g., when moving the entire mandibular dentition distally. Occasionally the mandibular lingual micro-implants are needed, and tori offer excellent implant sites.[12]

Kyung et al[63] recommend sizes more than 6 mm in maxilla, and 5 mm in mandible of micro-implants for insertion. The cortical surfaces of the maxilla are thinner and less compact than those of the mandible and accordingly will require longer micro-implants. A general rule of thumb should be, to use the longest possible micro-implant, without jeopardizing the health of adjacent tissues. The proper length of micro-implant is best selected during the pilot drilling. Further, one has to consider the path of insertion of micro-implant, while choosing the right one. It's better and quite easy to place microimplant in a perpendicular direction to the bony surface. However, there are many situations when the microimplant has to be placed in diagonal direction, to avoid injury to adjacent roots. When you choose to place the microimplant diagonally instead of perpendicular path, then it is prudent to use a little longer microimplant. Clinically in order to get better mechanical retention, it's good to choose a longer and thicker microimplant, rather than shorter and smaller one. However, there are limitations in choosing the same sizes of microimplant in different places. We always have to review the soft tissue thickness as well as the quality of bone at the sites where we choose to place them.

Schnelle et al. studied panoramic radiograph for the most coronal interradicular sites for placement microimplant in orthodontics patients[64]. They concluded that the adequate bone was located more than halfway down the root length which likely to be covered by movable mucosa, the most frequently cited clinical complication of soft tissue irritation. Costa et al. investigated the depth of hard and soft tissues in oral cavity in 20 patients for ideal microimplant placement sites[65]. The result showed that the bone thickness will allow 10 mm length microimplant only in the symphysis, retromolar, and palatal premaxillary regions. Microimplant 6 to 8 mm. in length can be placed in the incisive fossa, in the upper and lower canine fossae. These microimplants (4-5 mm.) only engage monocortically, whereas the others have ability to engage bicortically.

Stability of orthodontic micro-implant

Orthodontic micro-implant anchors such as titanium screws have been used for absolute anchorage during edgewise treatment. Miyawaki et al reporting the stability of implant anchors placed in the posterior region, human studies. The success rates and factors associated with the stability of titanium screws were examined in relation to clinical characteristics[53]. The 1-year success rate of screws with 1.0-mm diameter was significantly less than that of other screws with 1.5-mm or 2.3-mm diameter or than that miniplates. Flap surgery was associated with the patient's discomfort. A high mandibular plane angle and inflammation of peri-implant tissue after implantation were risk factors for mobility of screws. But they did not detect a significant association between the success rate and the following variables: screw length, kind of placement surgery, immediate loading, location of implantation, age, gender, crowding of teeth, anteroposterior jaw base relationship, controlled periodontitis, and temporomandibular disorder symptoms[53].

Liou et al studied about stationary of microscrews, they concluded that miniscrews are a stable anchorage but do not remain absolutely stationary throughout orthodontic loading. They might move according to the orthodontic loading in some patients[44].

Deguchi et al studied about bone-implant interface of small titanium screws as an orthodontic anchorage for establishing an adequate healing period in dog. Overall, successful rigid osseous fixation and the "three-week unloaded" healing group were: increased labeling incidence, higher woven-to-lamellar-bone ratio, and increased osseous contact. All of the loaded implants remained integrated. Mandibular implants had significantly higher bone-implant contact than maxillary implants. The data indicated that small titanium screws were also able to function as rigid osseous anchorage against orthodontic load for 3 months with a minimal (under 3 weeks) healing period.[54]

Garib et al. showed that tooth borne (Hyrax) and tooth-tissue borne (Hass-type) expanders tended to produce similar orthopedics effects. In both methods, RME led to buccal movement of the maxillary posterior teeth, by tipping and bodily translation[55]. If we use the microimplant as the bony anchors for suture expansion, it could be reduce unwanted tooth movement and alveolar bone bending from RME[55].

Cranial vault sutures

Cranial vault sutures, the fibrous tissues uniting the bones of the skull, are the major sites of bone growth along the leading margins of the cranial bones during craniofacial development, especially during rapid expansion of the neurocranium. To function as bone growth sites, sutures need to remain patent, while allowing rapid bone formation at the edges of the bone fronts. To begin understanding the role of cranial sutures as intramembranous bone growth sites, it is necessary to establish where sutures occur, and what regulates their formation and maintenance.

In humans, cranial vault sutures typically form with the interfrontal suture between the frontal bones, the sagittal suture between the parietal bones, the paired coronal sutures between the two frontal and two parietal bones, the paired lambdoid sutures between the supraoccipital and parietal bones, and the squamosal sutures between the parietal, temporal, and sphenoid bones. This arrangement is very similar to the arrangement seen in other species such as rabbits, mice, and rats (Figure 4), which have been used as research tools to examine suture biology and pathology.
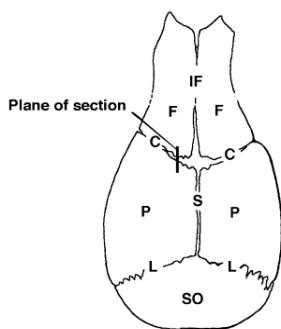
Figure 4.   Diagramatic representation of rabbit skull showing the sutures (C: coronal, IF:interfrontal suture, S: sagittal, L: Lamboid)   and membranes bones (F: frontal bone, P: parietal bone, SO: supraoccipital bone) of the cranial vault[56]

Regulation of suture morphogenesis

After induction of osteogenic potential, initiation of intramembranous bone formation proceeds through development of mesenchymal blastemas, the precursors of each of

the bones of the cranial vault. During this process, mesenchymal cells begin to differentiate and deposit extracellular matrix consisting primarily of type I and other collagens as well as other bone-related proteins and proteoglycans, which are then mineralized. Intramembranous ossification proceeds radially from each of these foci. The borders of each cranial bone are initially widely separated due to rapid expansion of the neurocranium. However, as ossification proceeds and neural growth abates, the bone fronts approximate one another and suture formation is initiated as the bone fronts abut or overlap one another, with fontanels representing the unossified regions of confluence of more than two cranial vault bones.

During morphogenesis of the rat coronal suture, the approaching frontal and parietal bone fronts of E19 calvaria are separated by presumptive suture (ps) matrix. The tips of the two bone fronts contain large numbers of osteoprogenitor cells and large cuboidal osteoblasts. By P1, 72 hours later, the two bone fronts overlap one another and a highly cellular suture matrix is seen separating the bone fronts. Although a distinct fibrous periosteal layer is seen around the approximating bone fronts of facial bones, no such intervening layers are seen in the developing rat coronal suture or in the developing mouse suture.

During rapid expansion of the neurocranium, the suture remains highly cellular, but as cranial expansion slows by P21, the number of cells lining the bone fronts declines and the suture narrows. Histologically, these events are remarkably similar to those occurring during development of the human cranial vault. During development of the sutures, the growing and expanding bone fronts both invade and recruit the intervening mesenchymal tissue into the advancing edges of the bone fronts. During this process, the mesenchyme becomes separated by the intervening bones into an outer ectoperiosteal layer and an inner dura mater. It is currently unclear which tissues and signaling factors are responsible for induction of suture formation. Although the dura mater is not necessary to induce initial overlap of the bone fronts during coronal suture development, its presence is required for initial stabilization of the suture.

The midline sutures (sagittal, interfrontal) are butt sutures, which do not overlap, whereas the transversely situated sutures (lambdoid, coronal) do overlap. It is currently believed that the approximating bone fronts set up a gradient of growth factor signaling between them, which initiate suture formation. However, it is currently unknown how this occurs or whether this signaling regulates the type of suture that will appear.

Regulation of suture patency

In the rat, all cranial vault sutures with the exception of the posterior interfrontal suture remain patent for the life of the animal. In humans, the interfrontal suture fuses between the second and fifth year after birth, with approximately 10% of the population having metopic sutures remaining patent. Early attempts to culture sutures to examine factors regulating suture patency failed, probably because calvaria were dissected at 37°C and the resulting hypoxia produced cartilage at the suture sites. However, in later attempts, transplants of E19 calvaria into parietal bone defects in adult rats resulted in normal coronal suture development. Removal of fetal dura mater before transplant initially resulted in normal overlap of the bone fronts. In the absence of dura mater, however, the newly formed sutures were unable to sustain themselves and became obliterated by bone. When these experiments were repeated by using an in vitro organ culture system, similar results were obtained. Furthermore, the more fully developed coronal sutures of P1 clavaria were found to be able to sustain themselves in culture even in the absence of dura mater. These results indicated that dura mater is permissive for suture formation, but that an inductive stimulus from dura mater is required during suture formation before the suture is able to maintain itself. A similar inductive event was noted to be required for mouse suture development, which also showed postnatal independence from continued presence of dura mater. In experiments where the ectoperiosteal layer was removed, it was found that the periosteum was not required for maintenance of suture patency. The role of these tissues is different due to two alternate possibilities, depending on the source of the mesenchyme originating the tissue. One possibility is that the dura mater is strictly neural crest derived and that periosteum has some contribution from paraxial mesoderm; hence, their role in regulating suture morphogenesis is different. The other possibility is that all subepidermal cranial vault tissues are neural crest in origin and the role of the tissues becomes altered as their association with one another changes, i.e., ectoperiosteum becomes associated with forming bone and dermis, whereas dura becomes associated with forming bone and brain. It should be noted that the facial sutures, which appear very similar to cranial vault sutures in both morphology and function, do not have contact with an underlying dura mater. It is likely that tissues surrounding the facial sutures regulate the sutures in a similar manner to the dura, but which tissues provide the signals have not been identified. Studies on the normally fusing posterior interfrontal suture in postnatal animals demonstrated that inhibiting contact between the suture and underlying dura mater led to delayed fusion of this

suture. When posterior interfrontal sutures were cultured in the presence of duramater, they fused as seen in vivo[57].

Distraction osteogenesis applied to the craniofacial skeleton revealed the effects of distraction osteogenesis on mandibular lengthening in dogs. Animal experiments and clinical studies were conducted then to examine the effects of this method on craniofacial morphology and the longitudinal growth[58]. These studies showed that distraction osteogenesis was also applicable to the maxillary complex[59] although it was more complicated in the anatomic structure than the mandible or other long bones. Furthermore, suture expansion has been established for the median palatal suture to accelerate lateral growth of the maxilla in clinical orthodontics. For the craniofacial sutures, Movassaghi et al[60] also showed that the frontonasal suture of rabbit was separated successfully without osteotomy (Table 1).

Table 1. Distraction protocol used in study of craniofacial suture in rabbit models.

Study	Number of rabbit	Site	Distraction rate	Retention	Length gained	Relapse
Movassaghi[60] 1995	9 (30 days old)	Frontonasal suture	Continuous force with spring, 5 wks	-	19.9 mm	-
Remmler[61] 1995	30 (22-week-old)	Coronal Suture	2.5 mm/wk, 5 wks	4 wks	10.44 mm.	NS
Parr[62] 1997	6 rabbits 6 rabbits	Midnasal suture	1 Newton, spring 3 Newton, spring	12 wks	5.2 mm. 6.8 mm.	-
Tung[63] 1999	12 juvenile 12 adult	Coronal Suture	0.5 mm/day, 15 days 0.75mm./day, 15 days	30 days 30days	6.6 mm 9.8 mm.	17 %(1st wk) $\pm$12.3%(2nd wk)

Objective:

The aim of this study was to investigate the ability of the modified expansion appliance to expand interfrontal suture in growing rabbits and evaluate the regenerated tissue after rapid sutural expansion both in morphological and histological pattern.

CHAPTER 2

MATERIALS & METHODS

This study was approved by the animal experiment ethical committee of Prince of Songkla University.

Materials

Eighteen 30‑day old, 1‑1.5 kg inbreeding, male, New Zealand White rabbits served as experimental subjects. Stock diet and water were provided ad libitum and kept in cages at the animal house 24ºC and 55% relative humidity in at least 12 hours light per day. Eighteen 30‑day old New Zealand White rabbits were divided into two groups, sham group (n= 2) and 4 experimental groups, which were A,B,C and D (n=16 in each group).

Expansion device

The modified expansion appliance consisted of two components: 1. the abutments part and 2. the acrylic expansion part. The abutments were 1.6‑mm. diameter, 10‑mm. long, self‑tapping titanium orthodontic micro‑implant, (Absoanchor, Dentos Inc., Daegu City, Korea) (Figure 5). The acrylic expansion part was made from acrylic component which hold the 10‑ mm. orthodontic palatal expansion screw (Dentarum Co.,Ltd) and 2 abutment holes on the ventral side of acrylic component in order to fix with abutment by orthodontic cement (Figure 6).
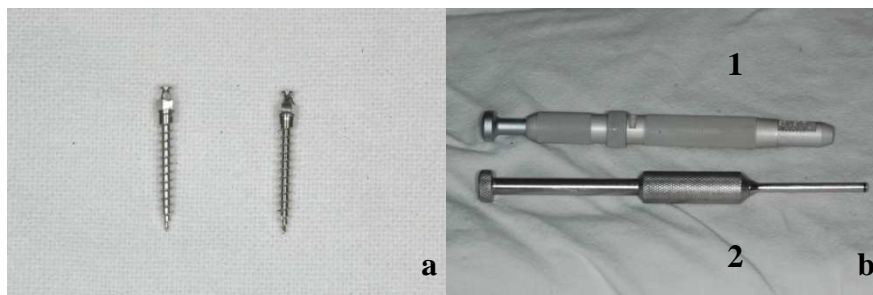
Figure 5.　**a.** 1.6-mm. diameter, 10-mm. long, self-tapping titanium orthodontic micro-implant (Absoanchor Dentos Inc., Daegu City, Korea). **b. 1** TG-screw driver, **2** non- torque gauge screw driver.
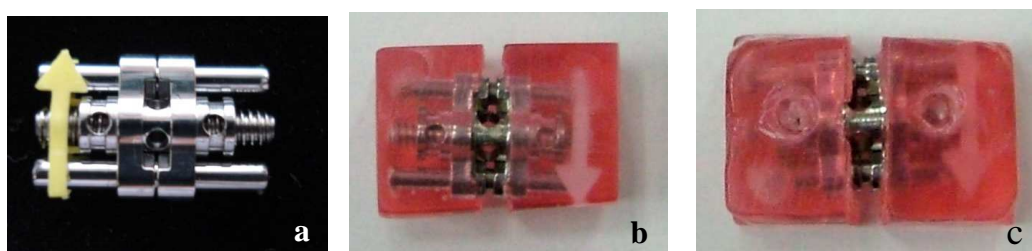
Figure 6.　**a.** Orthodontic palatal expansion screw **b.** the modified expansion appliances, top view **c.** the modified expansion appliances, ventral view.

Methods

Aseptic condition was prepared for surgical procedure. The animals were anesthetized with an intra muscular injection of ketamine hydrochloride (25mg/kg) and diazepam (5mg/kg) which were repeated if needed. The animal was observed to breathe spontaneously before operation started. Hair over the calvarium was shaved and disinfected with betadine solution(Figure 7). Then, the animal was draped to allow aseptic access in the operation field at clavarium. Penicillin G Sodium 0.5 million units were administered intramuscular preoperatively and each day postoperatively for a total of 3 consecutive days.

Figure 7.   The rabbit was anesthetized, shaved hair and draped to start the operation.

The skin in the operative area was injected with 1.8 ml of 2% lidocaine hydrochloride with 1:100,000 epinephrine solutions. A midline incision was cut through the skin of the calvaria, the periosteum was elevated to expose the calvarium and identified frontal suture (Figure 8a). Then the cranial skin flap was elevated (Figure 8b). The subcutaneous fascia was divided, periosteal flaps were reflected bilaterally and the cranial vertex was exposed.

a   b
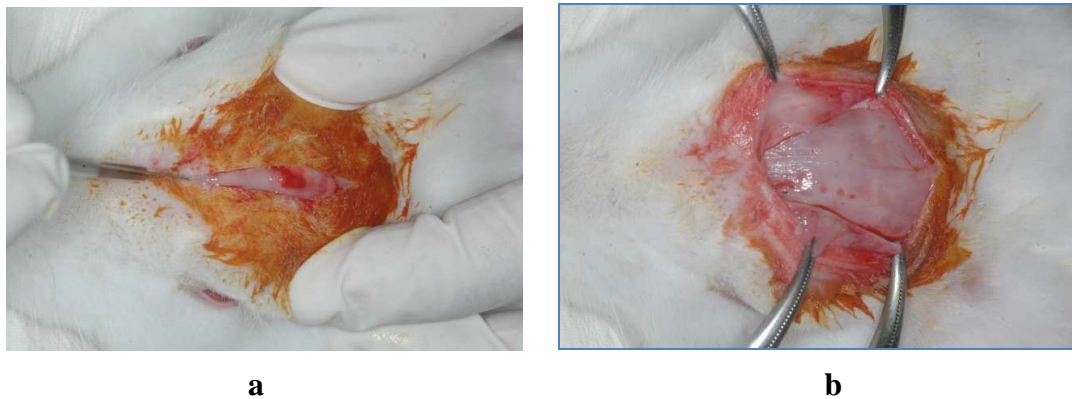
Figure 8.   **a.** The rabbit was disinfected with betadine solution and midline incision was made through the clavarium. **b.** the cranial skin flab was elevated.

An acrylic template was fabricated for reproducible placement of orthodontic micro-implant and gutta percha markers. The pilot holes for microimplants placement and gutta percha marker were drilled with 0.10-mm diameter perpendicular to sagittal plane of interfrontal suture about 4 mm on both sides of the suture, then micro-implants were placed (Figure 9a, 9b) in the pilot hole by self tapping control torque method within the range of 0.3 Ncm by torque

screwdriver, TG-screw driver (Absoanchor Dentos Inc., Daegu City, Korea). The gutta percha markers were placed in the holes parallel to the micro-implant bilaterally (Figure 10).After that thee distances between gutta percha markers and micro-implants were measured and recorded (Figure 11).

The flap was stabbed for emergence of orthodontic micro-implant. The excision was sutured with Vicryl® 4-0(Figure 12a), and then modified expansion appliance was fixed by orthodontic band cement (Figure 12b). The animals were observing until recovered then move to rest in the cage. After 3 days, the expansion screw was activated twice daily at 0.4 mm/time (0.8 mm per day) for 7 days. The activation was performed with gentle restraint of the rabbit but without causing any discomfort. After complete of the activation period. Group A, B, C and D were sacrificed and harvested cranium bone at the date of completed activation, 2weeks , 4 weeks and 8 weeks after activation respectively. The sham group was sacrificed at 8weeks after activation (Figure 13).
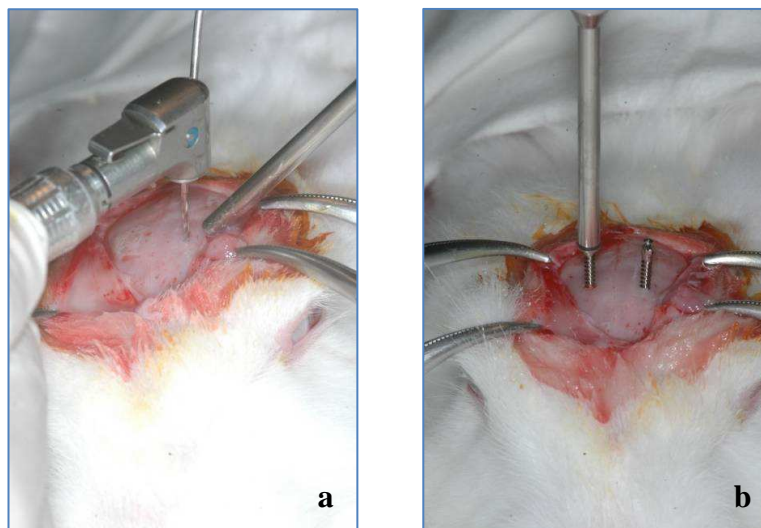
Figure 9.   **a.** The pilot drilled hole for placement micro-implants and gutta percha markers. **b.** The microimplants were placed.
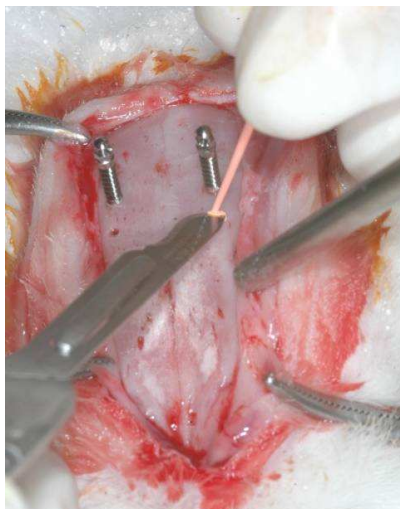
Figure 10.   The gutta percha markers were placed parallel to the micro-implant

bilaterally.

Figure 11.   The distances between gutta percha markers and micro-implants were

measured and recorded.
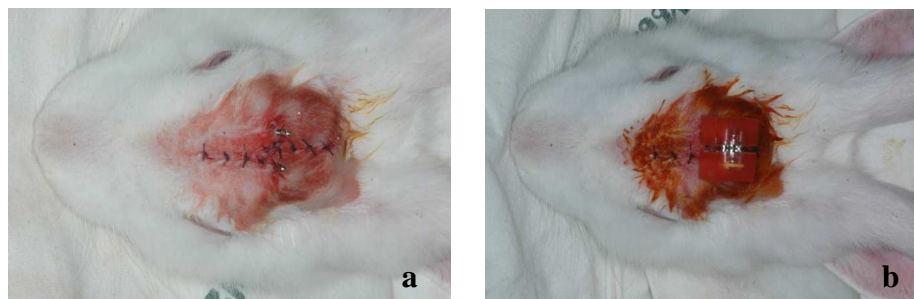
Figure 12. a.The skin flap covered the clavarium and sutured. b. The modified expansion device was fixed with head of micro-implants by orthodontic cement.
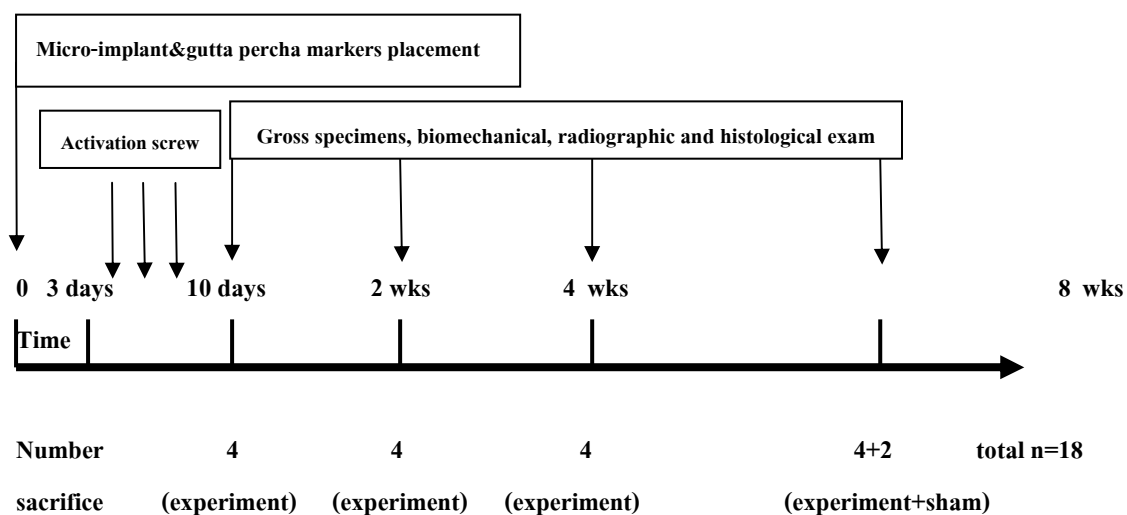
Figure 13. Scheme of the experiment.

The animals were sacrificed with an intraperitoneal overdose of thiopental sodium (100-150 mg/kg.). After that gross morphological were evaluated, then the cranium specimens were harvested en bloc, at 10 mm either side from the expanded suture and micro-implant sites, about 1x2 inch. The radiographs were taken from the specimens then the specimens were divided into 2 parts for biomechanical and histological studies.

a   b

c   d
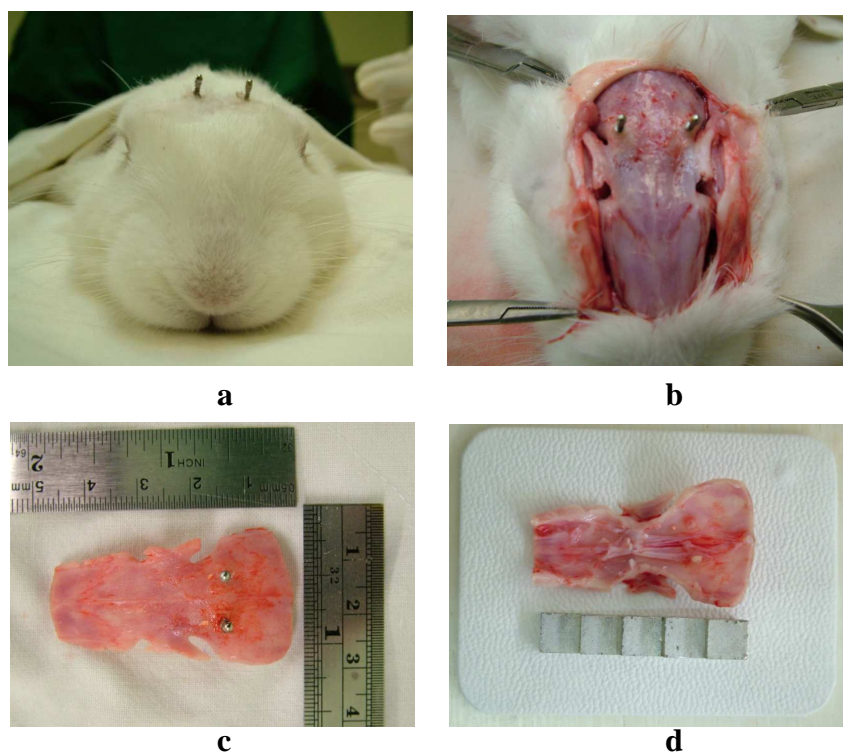
Figure 14. After complete activation and retention phase, the expansion device was removed **a.** The skin flaps was elevated and then observed the morphological of expansion area **b.** The clavarium was collected and prepared for x-ray examination **c.**The dimension of the specimen **d.** The specimen was placed with aluminum step wedges
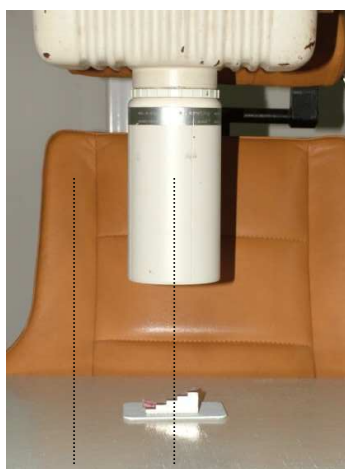
Figure 15. Radiographic method and step wedge included to calibrate optical density
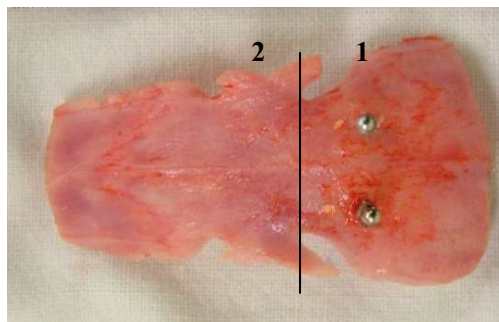
Figure 16.   Specimens were divided in to 2 part, 1st part for histological study and 2nd part for biomechanical study.

Quantitative distracted distance study

The dimensions between the centers of the gutta-percha marker were measured by the digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan) 2 times: during micro-implant placement and at the time that the experimental animal were sacrificed. At each time, the measurements were repeated three times under identical experimental conditions. The measurement was performed by one operator (Klytong.E).

Quantitative Biomechanical study

After observation and radiographic taking, the specimens were divided into 2 parts, for histologic and surface hardness studies. The 2nd part would be trimmed to 5x15 mm which included the expanded gap then embedded in acrylic block size about 15x40 mm. After acrylic was set, the excess acrylic covered specimens surface would be removed and polished with sand paper for smooth surface which required for Vicker's hardness testing Beuhler Micromed II, Digital micro-hardness Tester, Beuhler Co., England).

In condition of testing used 10 gram loaded force and 10 sec time duration for indentation test. Then the corners were identify by inspect through eye pieces and the testing machine automated calculated the surface hardness value. Each specimen blocks were random selected 3 areas testing then means were calculated for each specimen.

Figure 17.   Vicker's surface hardness testing machine (Micromet II ,Buehler Ltd.,

Illinois, USA.)

Quantitative Radiodensitometry

Radiographs of all specimens were taken using Gendex X-ray machine (Gendex Corporation, Illinois, USA.) with 75 kvp, 10 mA, 0.26 sec and the Super Polysoft Insight Kodak X-ray film (Kodak Ultra‑speed, Eastman Kodak company , Rochester, NY). The distance between the film and focal spot were kept on 50 cm in every subject. An aluminum 5 steps wedge was used for film calibration. The films were automatically processed using by Dent‑X9000 processor. The radiographs were scanned using Bio‑Rad[®] Model GS‑700 Imaging Densitometer (BIO-RAD Laboratories Ltd, Hemel Hempstead, UK) to obtain digital radiographic images of the specimens and analysed with Molecular Analyst[®] software (Figure 18). The average radiographic optical density (Mean OD) was measured and calculated for comparing the amount of mineralized tissue regenerated in expanded gap which subsequently by retention period.

Figure 18.   Bio-Rad® Model GS-700 Imaging Densitometer (BIO-RAD Laboratories Ltd, Hemel Hempstead, UK)

Qualitative Evaluation

After bony specimens were visually examined for gross morphological appearance then divided to 2 parts for biomechanical examination and histological examination. The 1st part of specimens were fixed with 10% buffered formalin solution and decalcified with 12.5% Ethylenediamine Tetraacetic Acid (EDTA). After completion of decalcification, each bone specimen was trimmed into size 3.5x25 mm involved expanded gap and mini-implant holes in order to examine regeneration of new tissue which regenerate between expanded gaps as shown in figure 19.
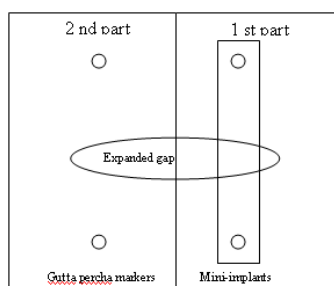
Figure 19.   The specimen was trimmed to appropriate size to make histologic slide. The preparation of bone specimens were shown as in figure.

All bone specimens were embedded in paraffin and cut in 5 μm thick. Each histologic section was stained with haematoxylin and eosin (H&E) stain. These slides were

studied under light microscope for observed of new tissue formation, soft tissue reaction and the degree of inflammation.

Data analysis

   The analyses were performed by using Nonparametric ANOVA. Calculations of mean numbers for each specimen, follow by computation of group mean number and standard deviation, were carried out in all measurements.

CHAPTER 3

RESULTS

Clinical observation

   The animals tolerated well with the surgical procedure and anesthesia. No rabbit was excluded from the study due to postoperative complication, wound infection or accidental death. The animals recovered well from anesthesia and they were able to eat normally after one day. The expansion devices were not disturbed daily activities and stilled stable during experiment period. The surgical wound, treated by wound dressing and chloramphenicol ointment application, healed with no sign of infection. Three-days after the operation, expansion device was activated twice a day without any restraint. After activated for 7 consecutive days, the wound was healed without any complication.

   After complete activation, the animals were healthy and able to eat normally. All animals were healthy during the observation and retention period. No failure of expansion devices was found in any period and groups.

Gross Examination

   The orthodontic palatal expansion screw (Dentarum Co.,Ltd) was activated twice daily at 0.4 mm/time (0.8 mm per day) for 7 consecutive days ,totally the increased distance was 5.6 mm. The distance between gutta percha markers in group sham, A, B, C and D were 0.48 ± 0.06, 3.81 ± 0.27, 4.35 ± 0.18, 4.45 ± 0.17, and 4.30 ± 0.74 mm. respectively. Clinical observation after elevated skin flap showed the expanded gap was filled with new rough surface elliptical shape generated tissue which widest between mini-implant. The hardness of new generated tissue was gradually increased in hardness consequently retention period which soft in group A (no retention period) and hardest in group D (8 weeks retention period). The gutta-percha markers were a biocompatible material because there were no sign of infection at the markers. These markers were helpful for located the edge of bone margin.

Gross morphological appearance of Sham group. By normal development of bony and suture structure, gross morphological structure of tissue between micro-implants or gutta-percha

markers, was yellowish-white, smooth texture, surface hardness nearly similar normal clavarium bone nearby (Figure 20).

Figure 20.   The gross specimen from sham group.

 At complete activated expansion devices (Group A); the regenerated tissue in expanded gap had grayish-red color and softly which distinct from normal bone adjacent to the expanded gap. The tissue presented with the consistency similar to fibrous connective tissue. The shape and size were corresponded with gap space which expanded in elliptical widest between mini-implants.The texture of new generated tissue was irregular, rough surface. There was fibrous tissue linked with covered periosteum. The hardness was very soft compared with adjacent normal bone (Figure 21).

Figure 21.   The gross specimen from group A

At 2 weeks after complete activated expansion devices (Group B). By 2 weeks after completed activated expansion device, the grayish-pink tissue in the expanded gap was decreased in width and length than **group A** due to maturation of new regenerated tissue which more harden likely bone tissue. The area of expanded gap could be distinguished from the adjacent normal bone. The

regenerated tissue was firm consistency than at complete activated expansion device but stilled less hardness than adjacent normal bone (Figure 22).
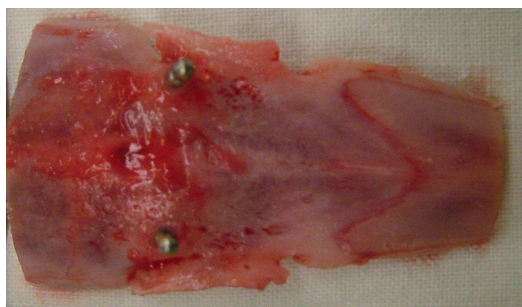
Figure 22.   The gross specimen from group B.

At 4 weeks after complete activated expansion devices (Group C). By 4 weeks after completed activated expansion device, the regenerated tissue became more likely adjacent tissue which looked like callus bone formation than fibrous connective tissue as at complete activation period. This stage was more difficult to indicate new tissue area from old normal bone. Consistency was more hardness than 2 weeks retention period but not equal as normal adjacent bone. The dense bone-like tissue also extended over the edge of expanded gap. The color changed to more pale-yellowish but less pink-color tissue than early this stage (Figure 23).
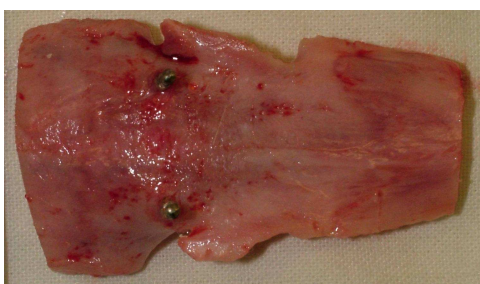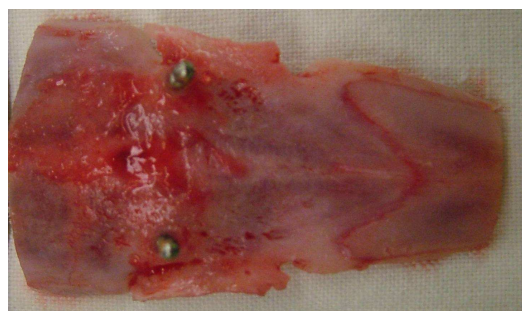

Figure 23.   The gross specimen from group C.

At 8 weeks after complete activated expansion devices (Group D). By 8 weeks after completed activated expansion device, the regenerated tissue showed both gross morphological

structure and hardness nearly similar to normal clavarium bone. In this stage, new generated tissue was more difficult to be distinguished from adjacent bone. The tissue appeared more likely adjacent normal bone include texture, hardness and color. Compare with sham group, gross morphological appearance were nearly similar normal clavarium bone (Figure 24).
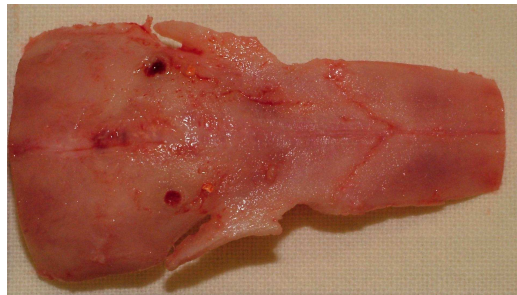
Figure 24.   The gross specimens from group D.

Distance gained from the modified expansion devices

The distance of the suture expansion gained according to gutta-percha markers in each group was showed in table 2. The obtained expanded gap was significantly more than the normal physiologic growth of the suture in the sham group but had no significant difference of variance among groups(p value = 0.1090, 0.1435, 0.1778). In addition, no significant difference of the expanded distance between the bone level and top level of the microimplant was found. In control group,the distance gained from the device was 0.48±0.06 mm while in experimental group, the dictance gained at top level and bone level were 0.35±0.12 and 0.57±0.05 mm respectively.

Table 2.   The distance gained at between gutta-percha markers, top level between micro-implants

   and bone level between micro-implants.

Distance	Sham	Completed DO	Retention 2 weeks	Retention 4 weeks	Retention 8 weeks
Gutta-percha markers	0.48±0.06	3.81±0.27	4.35±0.18	4.45±0.17	4.30±0.74
Top level of MIA	0.35±0.12	5.36±0.47	5.37±0.06	5.44±0.20	5.09±0.16
Bone level of MIA	0.57±0.05	4.58±0.42	5.20±0.25	5.35±0.22	5.20±0.28
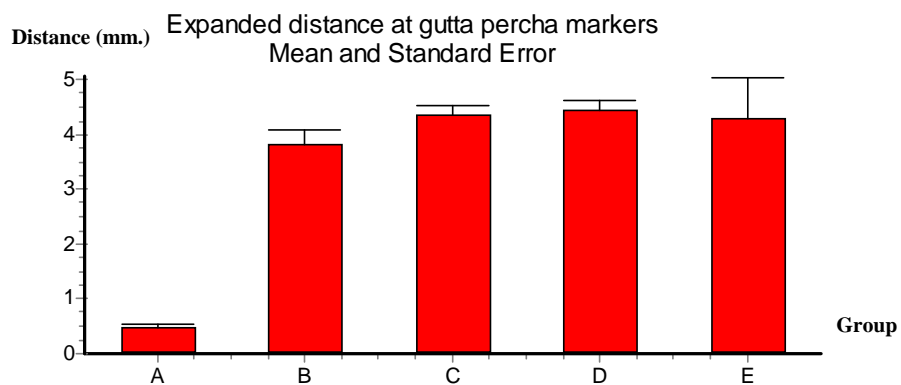
Figure 25.   Expanded distances at gutta-percha markers: A: group A (0 week),

   B: group B (2weeks), C: group C (4 weeks), D: group D (8 weeks)

   and E: sham control group.

Expanded distance at top level of orthodontic micro-implant
Mean and Standard Error
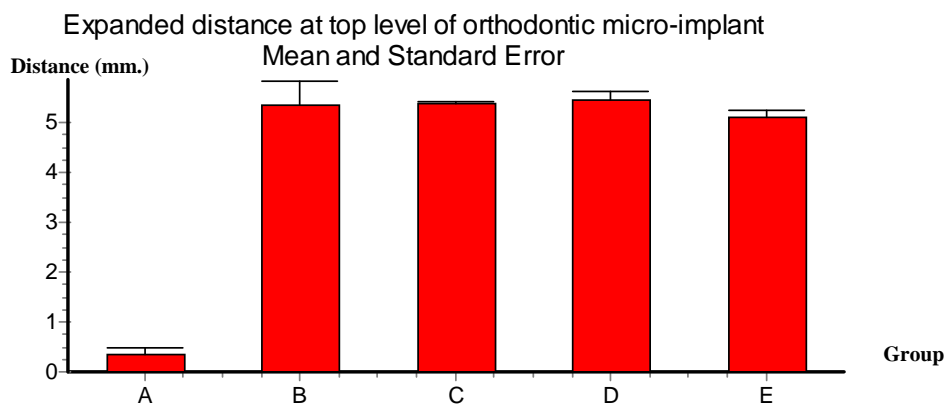
Distance (mm.)

Figure 26. Expanded distances at top level of orthodontic micro-implants A: group
A (0 week), B: group B (2 weeks), C: group C (4 weeks), D: group
D (8 weeks) and E: sham control group.

Expanded distance at bone level of orthodontic micro-implant
Mean and Standard Error
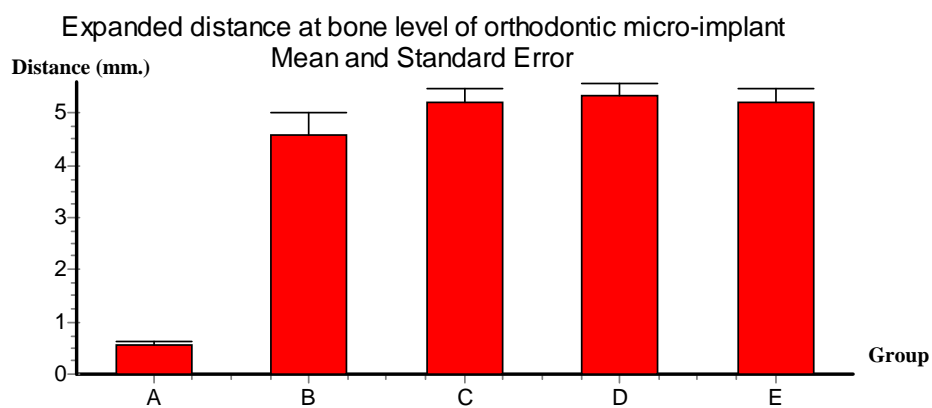
Distance (mm.)

Figure 27. Expanded distances at bone level of orthodontic micro-implant: A:
group A (0 week), B: group B (2 weeks), C: group C (4 weeks), D:
group D (8 weeks) and E: sham control group.

Radiographic Evaluation

        The radiographic examination was performed after expansion experiment and subsequently retention period 2, 4, 8 weeks. By using the intraoral cross-sectional occlusal film after harvested the bone specimens which soft tissue was stripped out.

The radiograph of sham group. The interfrontal suture which is the thin line could not be notified in some area was defined as a normal bone radiodensity (Figure 28).
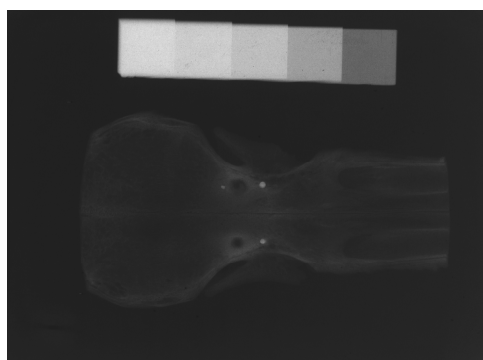
Figure 28.   The radiographic film of sham group.

At complete activated expansion devices (Group A). After activated expansion device for 7 days, there was radiolucent gap about 4-5 mm occurred. Radiopaque area was not shown in radiolucent gap. The shape of radiolucent gap lookalikes elliptical shapes which widest between the mini-implants. The radiolucent zone was clearly cut to the old normal bone margins along with interfrontal suture which corresponded with gross morphological appearance (Figure 29).
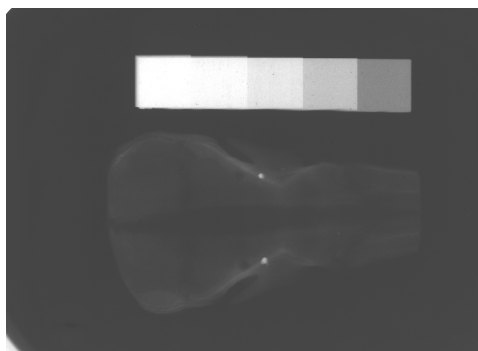
Figure 29.   The radiographic film of group A

At 2 weeks after complete activated expansion devices (Group B). The radiolucent zone was more radiodensity. There were three zones which were 2 thick radiopaque bands attached with lateral normal bone margins and single radiolucent band between those. For details, radiopaque band looked like fine radiopaque streaks extended form the lateral normal bone margin which tended to run parallel with the expansion vector (Figure 30).
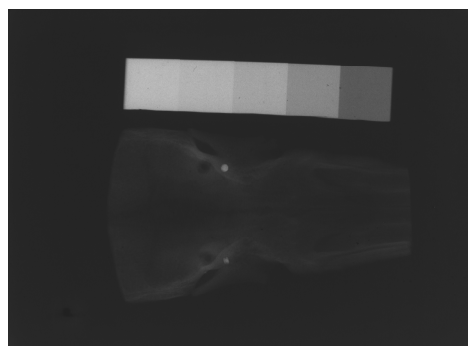
Figure 30.   The radiographic film of group B

At 4 weeks after complete activated expansion devices (Group C). This stage radiolucent band at the central of expanded gap reduced width but still clearly seen as radiolucent line. The radiopaque bands increased more radio-density than 2 weeks retention period but less than normal clavarium bone. The fine radiopaque streaks were still presented but not clearly indicate because maturation of decalcified tissue. The continuity with normal bone margins and new hard tissue were difficult to distinguish (Figure 31).
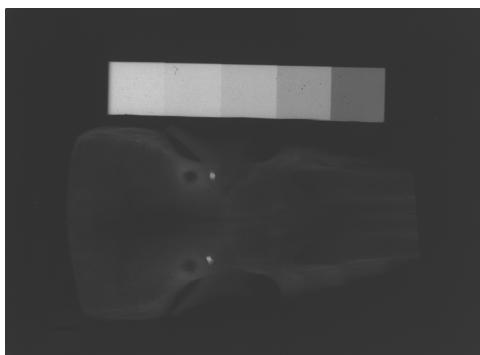
Figure 31. The radiographic film of group C

At 8 weeks after complete activated expansion devices (Group D). The radio-density of generated tissue in expanded gap had grayish-pink color and softly kept increasing until it achieved nearly the same level of density when compared with normal adjacent bone. This was difficult to indicate the old normal bone margin because the continuity with normal adjacent bone. The radiolucent line stilled presented at the central of expanded gap look like radiolucent of wide suture (Figure 32).
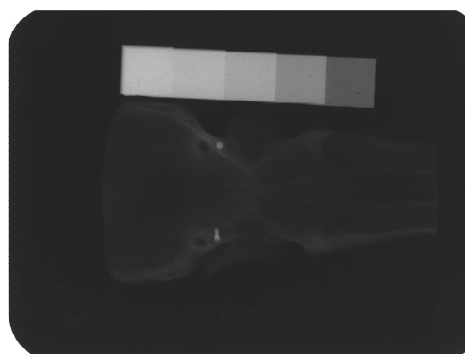
Figure 32. The radiographic film of group D

Bone density of regenerated tissue in expanded gap

The density established to characterize the amount of mineralized tissue produced which represented as the average radiographic optical density (Mean OD). The densitometry values in means gray level of expanded gap were shown in Table3 and Figure 33. The regenerated tissue was increased radiopacity gradually since complete activation period until

retention period 2 weeks, 4 weeks and 8 weeks. The results shown expanded gap bone density increased more from complete activation to nearly normal in 8 weeks after complete activation as found in sham group.

Table 3.   The data of radiographic optical density

	Sham	Completed DO	Retention 2 weeks	Retention 4 weeks	Retention 8 weeks
Mean OD	1.20068	0.81256	1.05791	1.06048	1.09964
$\pm$ SE	$\pm$0.01407	$\pm$0.12530	$\pm$0.01917	$\pm$0.01589	$\pm$0.01407
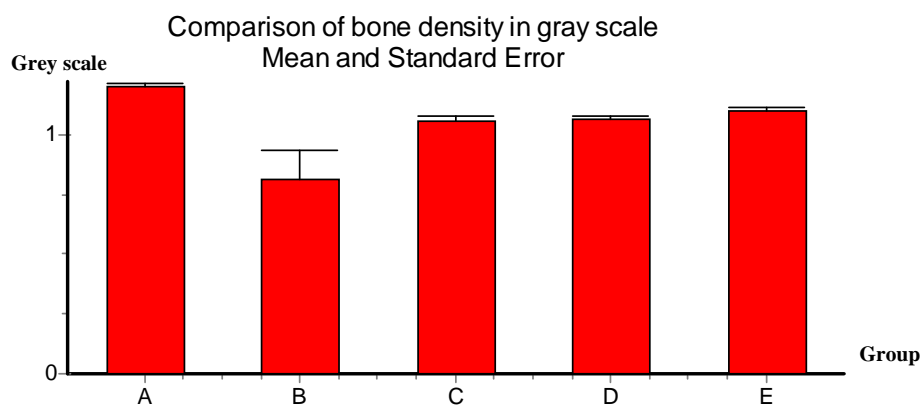
Figure 33.   Distribution of mean optical density of expanded gap in rabbit's clavarium specimens: A: group A (0 week), B: group B (2 weeks), C: group C (4 weeks), D: group D (8 weeks) and E: sham control group.

Table 4.   The Dunn's Multiple Comparisons Test for significant difference of radiographic optical density between groups.

Dunn's Multiple Comparisons Test

Comparison	Mean Rank Difference		P value
=================================	===========		========
Sham vs. Completed DO	15.000	*	P<0.05
Sham vs. 2 wks retention	8.250	ns	P>0.05
Sham vs. 4 wks retention	8.250	ns	P>0.05
Sham vs. 8 wks retention	4.500	ns	P>0.05
Completed DO vs. 2 wks retention	-6.750	ns	P>0.05
Completed DO vs. 4 wks retention	-6.750	ns	P>0.05
Completed DO vs. 8 wks retention	-10.500	ns	P>0.05
2 wks retention vs. 4 wks retention	0.000	ns	P>0.05
2 wks retention vs. 8 wks retention	-3.750	ns	P>0.05
4 wks retention vs. 8 wks retention	-3.750	ns	P>0.05

It was found, from Kruskal-Wallis Test (Nonparametric ANOVA), that the mean optical density had significant difference among groups (p≤0.05) and from Dunn's Multiple Comparisons Test, significant differences was found only between Sham group and Completed DO group.

Biomechanical evaluation

After the 2nd part of specimens were thawed to the room temperature, then were mouted an acrylic mold with the outer surface cortex facing up. The bone surface was smoothed by abrasive papers as needed to obtain the regular surface testing. Then surfaces hardness of the expanded gap was tested by the micro hardness tester (Beuhler Micromed II, Digital micro-hardness Tester, Beuhler Co., England), using the Vicker diamond indentor (Figure 34). The Vicker's hardness of the specimens was calculated by the following formula.

$$HV = \frac{\text{Test load (Kgf)}}{\text{Surface area of indentation (mm}^2)}$$

$$= \frac{2F \sin (\text{ø}/2) \times 1000}{d^2}$$

$$= 1854 \times F/d^2$$

HV = Vicker's hardness

F   = Test load (Kgf)

d   = Arithmetic mean of the two diagonals d1 and d2 (microns)

ø   = Angle between the opposite faces at the vertex of the pyramidal indentor

    (136 degree)
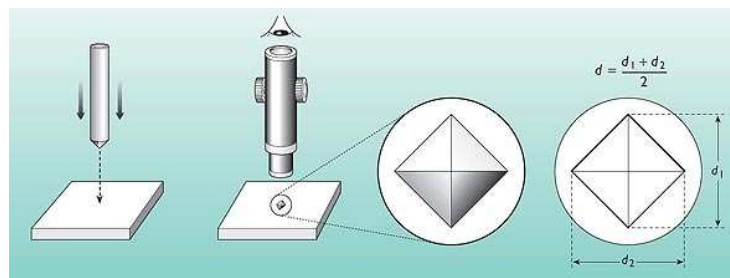
Figure 34.   Schematic of surface hardness testing, Vicker's hardness.

Three random areas of expanded gap surface served as the testing point for the surface hardness of the regenerated tissue. The distance of the testing area should be apart from each other for at least 2 times the length of the diagonal in order to prevent testing the same region then calculated and recorded. The result was showed in Table 5. Only group A (completed activated expansion device) couldn't be tested because of the soften of fibrous-like tissue characteristic.

Table 5.   Distribution of Vicker's hardness of each group.

Group	Retention period (wks)	Vicker's hardness (Mean± SD)
Sham	8	10.05 ± 0.289
A	0	N/A
B	2	3.45 ± 0.058
C	4	8.98 ± 0.171
D	8	10.03 ± 0.025

The Vicker's hardness increased gradually from group A ,B, C and highest in group D and the surface hardness of group D was nearly the same as sham group (10.03 ± 0.025 and 10.05 ± 0.289 respectively)(Fig 35). It can be stated that the new sutural regenerated tissue had a characteristic as general bone. Statistical significant was found among the experiment group($P<0.05$). From Dunn's Multiple Comparisons Test, significant differences (*$p<0.05$, ** $p<0.01$) were found between Sham group and group D and between group A and group D (Table 6).
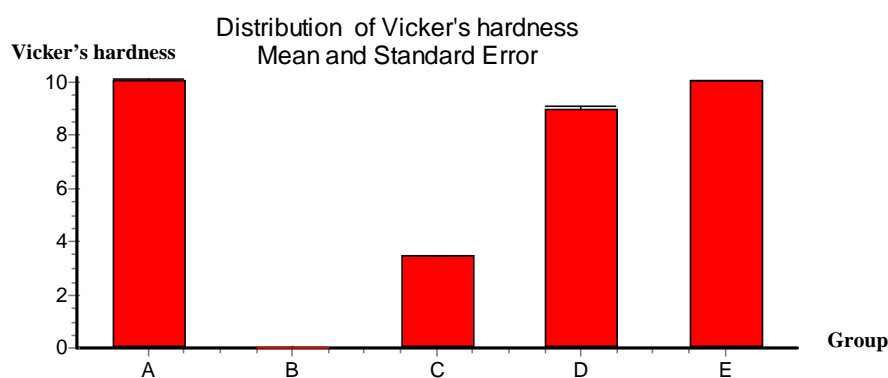
Figure 35.   Distribution of percentage of Vicker's hardness: A: group A (0 week),
B: group B (2 weeks), C: group C (4 weeks), D: group D (8 weeks)
and E: sham control group.

Table 6.   Dunn's Multiple Comparisons Test for Vicker's hardness. (* p<0.05, ** p<0.01).

Comparison	Mean Rank Difference		P value
Sham group vs. completed DO	13.500	*	P<0.05
Sham group vs. 2 wks retention	9.500	ns	P>0.05
Sham group vs. 4 wks retention	5.500	ns	P>0.05
Sham group vs. 8 wks retention	0.750	ns	P>0.05
Completed DO vs. 2 wks retention	-4.000	ns	P>0.05
Completed DO vs. 4 wks retention	-8.000	ns	P>0.05
Completed DO vs. 8 wks retention	-12.750	**	P<0.01
2 wks retention vs. 4 wks retention	-4.000	ns	P>0.05
2 wks retention vs. 8 wks retention	-8.750	ns	P>0.05
4 wks retention vs. 8 wks retention	-4.750	ns	P>0.05

Histological Evaluation

The 1st part of bone specimens included regenerated tissue in the expanded gap, the native bone edge margin and the microimplants' implantation site. The specimens were sectioned in the coronal plane and cut through the implantation site.

In group A, at complete activated expansion devices, there were plenty of spindle shape undifferentiated mesenchymal cells, which looked like fibroblast, were seen throughout the expanded gap. The undifferentiated mesenchymal cells also arranged as bud to led primitive microvascular development. There was dense collagen fibers production which arranged parallel to the expansion vector. In this stage, predominant histological appearance was a rich fibrillar matrix collagen and undifferentiated mesenchymal cells. Among the fibrillar matrix collagen, there were some clusters liked osteoid materials which surround with osteoblast-like cells. The osteoblastic cells which lied on surface of these new fine bone spicules eventually became enveloped. The pale pink material oriented in the expansion vector found behind the pink line which separated from native bone. The reversal line separated the old mature bone and new bone

as observed. There were some longitudinal new bone trabeculae composed with osteoid and osteoblast observed at the edge of normal bone margin which lied on the new bone surface.

Expanded gap

Figure 36.   Coronal histological sections through the calvaria showing expanded gap which pass through between micro-implants after completed activation of the modified expansion device. Note the regenerated tissue between expanded gap was rich of undifferentiated mesenshymal cells, fibrillar matrix collagen and blood supply (Specimens stained with Hematoxylin and Eosin).

In group B, at 2 weeks after complete of activated expansion devices. There were still more osteoprogeniter cells especially at the central of expanded gap disseminated in wavy collagenous fibers. The vascular channels were larger than the original bone nearby. Proliferated osteoblasts were gradually embedded in the bone matrix and became osteocytes with relatively larger osteocytic lacunae than those in the pre-existing bone. The primary bone trabeculae became more mature by gradual increasing mineralization of the osteoid. Honey comb-like newly formed woven bone was observed near the original bone stumps. The trabeculae were larger and elongated from the both edge of native bone margin. These longitudinal new trabeculae bone seemed try to bridge the expanded gap by extending from both old bone margins.

Expanded gap

Figure 37.   Coronal histological sections through the calvaria showing expanded gap which pass through between micro-implants 2 weeks after completed activation of the modified expansion device. There were primary bone trabeculae dispersed in fibrillar matrix collagen field. Note that regenerated tissue between expanded gaps was rich of undifferentiated mesenshymal cells, gradually increased of osteoid mineralization and structure were well organized, stilled rich of fibrillar matrix collagen and blood supply (Specimens stained with Hematoxylin and Eosin).

In group C, at 4 weeks after complete of activated expansion devices. The fibroblast-like cells stilled presented but loosely than early phase. There was a mixture of woven and maturing lamellar bone which located near original bone. These newly regenerated bones were shown to be immatured: active resorption of unfavorable structures and remodelling eventually for bone maturation changed woven bone to lamellar bone. The colored was pale pink, woven bone almost found in new trabeculae bone, not homogenous like normal bone. The lacunae were also reduced in size than previous stage.   The bone marrows, occupied with fibroblast- like cells and undifferentiated mesenchymal cells, were larger than original adjacent bones. The interface between original bone and new bone were more difficult to distinguish. The both ends of new trabeculae bones were joined by centers of expanded gap. At the junctions, some fibrous tissue formed like sutural pattern which abundance of fibroblast-like cells(Figure 38).
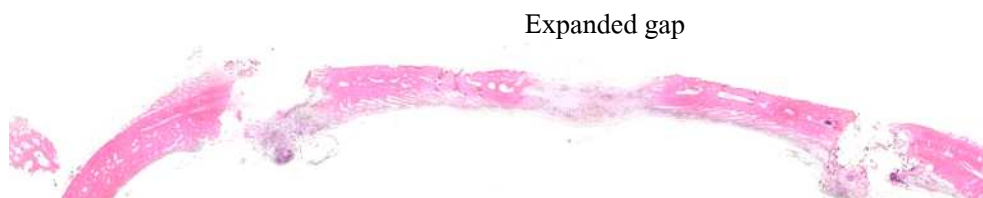
Fibroblast-like cells

Figure 38. Coronal histological sections through the calvaria showing expanded gap which pass through between micro-implants 4 weeks after completed activation of the modified expansion device. The bone trabeculae were joined at the center of expanded gap. The new bone tissue was shown to be immature and unwell-organized bony structure. The bone marrow stilled larger than original adjacent bone (Specimens stained with Hematoxylin and Eosin).

At 8 weeks after complete of activated expansion devices. The fibroblast-like cells reduced in numbers in all area but stilled plenty in suture-like zone at the central of expanded distance. The bone structure was observed to be more mature, more calcified matrix and lamellar bone structure. Then the zone of coarse woven bone that was very cellular showed a transition to mature lamellar bone through remodeling, forming tightly packed osteons. The colored was more homogenous with normal bone than previous group. Osteocytes in round bone lacunae looked more like what was found in the original bone. Newly formed Harversian systems were also found in the some fields of vision. The bone marrows also reduced in size and number of cells occupied. The interface between original bone and new bone were not separated(Figure 39).

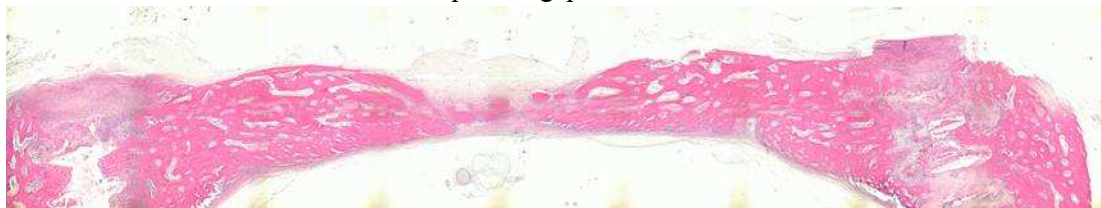Well-organized bony structure

Figure 39. Coronal histological sections through the calvaria showing expanded gap which pass through between micro-implants 8 weeks after completed activation of the modified expansion device. The bone trabeculae were joined at the center of expanded gap and fibrous tissue was form like sutural tissue as in sham group. The new bone tissue was shown well-organized bony structure than those previous stages. The bone marrow reduced in size like original adjacent bone (Specimens stained with Hematoxylin and Eosin).

At 8 weeks after complete of sham group: the bone structures were well organized as lamellar bone and some area showed remodeling process for reorganized structure. Noticing, there was some woven bone found in remodeling area and less mineralized than mature bone. Osteocytes were embedded in tiny, round and form Harversian systems. The sutural spaced filled with fibroblast- like cells and undifferentiated mesenchymal cells. It became irregular shape, not straight line or butt joint junction, interdigitation with another bone margin(Figure 40).

Harversian system found in some field
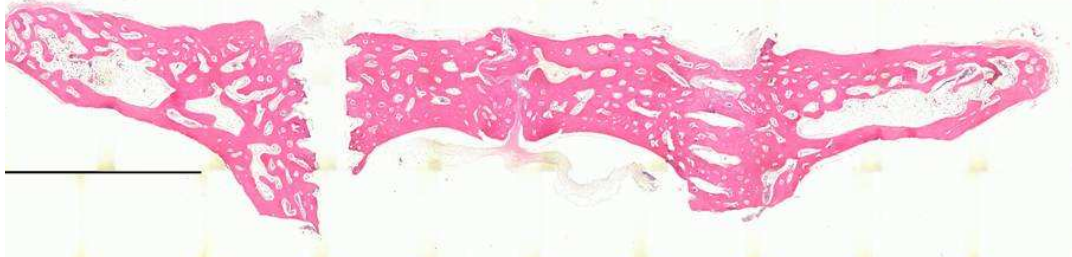
Figure 40.   Coronal histological sections through the calvaria between micro-implants of sham group. The bone trabeculae were well organized, lamellar bone and Harversian system found in some field (Specimens stained with Hematoxylin and Eosin).

In summary , the process of new bone formation, after completed activation of expanded devices, recruited and proliferate of undifferentiated mesechymal cells then generated bone materials and maturing of bony structure of new bone in 0, 2, 4 and 8 weeks ,was found. The maturation of new bone found in 8 weeks retention period was the same characteristic as normal sutural pattern as in sham group.

CHAPTER 4

DISCUSSION

In the past decade maxillary constriction, frequently observed and responsible for unilateral or bilateral posterior cross-bite and anterior crowding, were among the first dento-facial orthopedic procedures to be under taken. Clinical techniques for lateral expansion of the intermaxillary suture rely on the teeth as the handle through which forces are directed to bones. Ankylosed teeth were used as anchorage to expand maxillary suture in rhesus monkeys which skeletal expansion of maxilla was achieved without concomitant buccal tipping of ankylosed abutment teeth[64]. This anchorage situation of ankylosed teeth likes endosseous implant technique by histological aspects. The implant was successfully used for maximal anchorage preparation without patient compliance led to the expansion of implant technology in orthodontics. The micro-implant anchorage has been successfully as direct or indirect anchorage for molar protraction or uprighting, canine or anterior teeth retraction, molar distalization or other applications[33, 45, 48, 54].

Osseointegrated endodosseous implants are satisfactory abutment for sutural expansion studied in rabbit[62],as well as, microimplants which are widely used as bony anchorage. In this present study, orthodontic microimplants as bony anchorage for interfrontal suture expansion were used. Because there was no significant difference of expanded distances between gutta-percha bone markers, bone level and top level of the micro-implant was found, so it could be concluded that the bony fixed microimplants of the distraction device could resist the force from the suture structures and the surrounding soft tissue that was against the expanding vector, without bending outward during the distraction period in all group and microimplants were able to provide sufficiently stable sites as a bony anchorage for direct application of external force in the process of cranial suture expansion.

Experimental studies on different animal models have certainly leaded to a better understanding of the biological and biomechanical principles of cranio-facial distraction osteogenesis. In the present study, rabbits served as the models for the distraction process of the cranial suture. Rabbits, as an experimental animal, are cheap, easier to keep and care, having

primitive bone and soft tissue response as humans, and ethically better accepted for experiments than dogs or sheep. Moreover, because the bone used for the histological section is very small, it was very helpful to analyze a histological picture of the whole distraction area on a single histological section. Although performing the operation on such a small bone is technically demanding, the problem was overcome by using a tiny distraction device modified from microimplant and orthodontic expansion screws.

Animal was age selected as normally young human which less interdigitation between both palatine bones[65,66]. Growing cranial suture in juvenile rabbits were distracted by internal distraction device, produce by Synthes Maxillofacial, without osteotomy line (across cranial suture) compare with osteotomy cut[65]. It was showed that the degree of distraction and new bone formation were achieved in growing animal without invasive cranial osteotomy. In the present study, the distraction procedure could be successfully achieved, without any invasive osteomy, with acceptable result at 70-80% of expected distance.

The technique of distraction osteogenesis involves the creation of new bone by gradual separation of two or more bony fragments following their surgical division. This technique can provide unlimited amounts of regenerated bone in the skeleton that still has the potential fracture healing. Histological changing cascades were explained after completed distraction for five days in sheep maxilla[65], the hematoma was already replaced by a heterogeneous population of mesenchyme-like cells and spindle-shaped cells. Some capillaries traversed the regenerating tissue without any specific orientation. In the present study, distraction osteogenesis was applied to expand the growing cranial suture in rabbits. The result of the study demonstrated the feasibility of using the distraction osteogenesis technique to expand cranial sutures without an osteotomy of the growing rabbit calvarium and to successfully regenerate new bone regeneration in the distraction gap with a reestablished normal anatomical cranial suture structure. The regenerated tissue in the distraction gap changed from a soft fibrous like structure to bony hard consistency tissue with sutures in the midline region. The histological examination revealed normal mature lamellar bone and bone marrow structure in the distraction gap at 8 weeks after complete distraction, as seen in the adjacent native cranial bone. Suture like structures were also observed in the middle part of the distraction gap. The re-established cranial suture structures were nearly similar to that found in the sham group. These findings also were confirmed by a radiographical study and surface. The initial radiolucent expanded gap was

replaced with a normal bony radiographical appearance and re-established interfrontal suture was eventually observed in the last experimental group. According to the densitometry, the amount of new bone formation in the distracted cranial suture started rapidly from the completion of the distraction process and then gradually increased to achieve the normal level in the last group at the 8 week period.

Vicker's hardness used for surface hardness testing to examine the biomechanical property of new tissue regenerated. After complete activation, the regenerated tissue in the distraction gap showed fibrous-like tissue which can't be measured by the testing machine while others groups showed rapidly increasing hardness nearly normal surface hardness as sham group at 4 weeks retention period and equally normal at 8 weeks retention period. In previous study [68], the consolidation period found varies from 4 to 12 weeks which conformed with this present study that 4 weeks seems to be sufficient for complete bone maturation.

The rapid palatal expansion was created by force generated by expansion screw type which range in 3-10 pounds (133 – 444 cN)[66].The force created by the expansion device in this study was range 196 – 281 cN. Although it could separate the cranial suture in rabbit but the clinical application in human should be carefully considered and evaluated.

Base on the present study, it showed that effectiveness of the orthodontic micro-implant as bony anchorage for orthopedic sutural expansion had 70 – 80 % effectiveness of orthodontic palatal expansion screw. The applications of orthodontic micro-implants were vary in orthodontic and orthopedic situations which increase more in orthopedic aspects. The histological and biomechanical data also indicated that new regenerated tissue was maturation to be normal bone and suture characteristics.

The finding of the present study should be considered with caution due to the small sample size. In addition, direct extrapolation of data obtained from animal studies to humans should be interpreted cautiously.

CHAPTER 5

CONCLUSION

Distraction osteogenesis without an osteotomy could be used in cranial suture expansion with satisfactory outcomes in growing rabbits. Newly formed bone in the distraction gap started forming rapidly since the time of the completion of the distraction phase. The new bone formation kept increasing gradually until it achieved the normal level in the 8 weeks group. The re-established cranial sutures possessed the similar clinical, radiographic and histologic features as found in normal cranial suture. From histologg and surface hardness examinations, the modified expansion appliance was successful used as distraction osteogenesis device in growing cranial suture of the rabbits. Cranial suture expansion using the distraction osteogenesis technique without an osteotomy appeared to be a promising procedure to increase the cranial vault dimension, especially in craniofacial deformity or craniosynostoses patients. Clinical application should be the next step to study and evaluate.

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APPENDIX

APPENDIX

Histological study

    The Image-Pro Plus 5.0 program was used for the histological study. The most central histological section of each expanded suture was selected. Each section was initially inspected using a light microscope (×5 objective) and saved as a digital image. A composite digital image was then created by combining 3–4 smaller images because it was not possible to capture the entire defect in one image at the level of magnification that was used. (Figure 1)
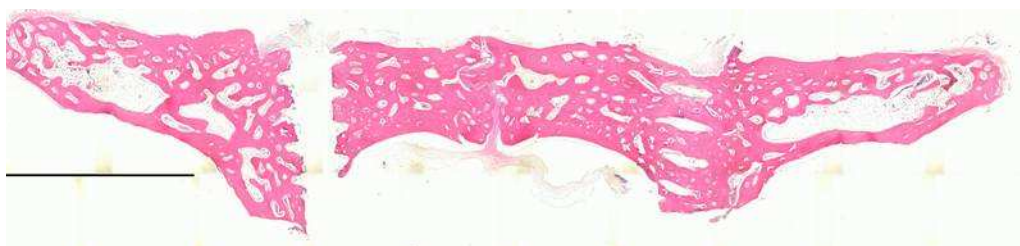

Figure 1. The capture images of the histological section merged to create a single composite image comprising the entire length of the surgical defect.

    The following criteria were used to standardize the histological study of the composite digital image that the captured images of each histological section were merged on the computer screen to create a single composite image comprising the entire length of the surgical defect.

Biomechanical study

    After gross morphological and radiological study, the 2nd part of expanded suture specimen 5x15 mm. were embedded to acrylic block about 15x40 mm (Figure 2). After specimen bone surface were polished with sand paper. Three randomize area of each specimen were selected to represented the surface hardness which selected at the center of distracted suture.
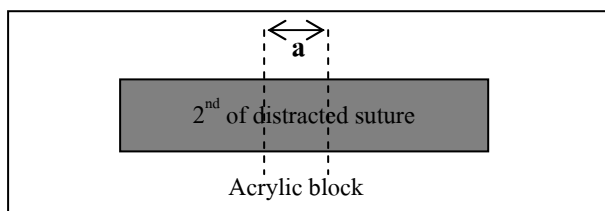
Figure 2 The schematic of an acrylic block that embedded with 2nd part of distracted

suture for surface hardness testing. a: area of distracted suture

Bone density

Optical density of distracted suture were examined by traditional radiographs which were scanned by Bio-Rad$^{®}$ Model GS-700 Imaging Densitometer (BIO-RAD Laboratories Ltd, Hemel Hempstead, UK) to obtain digital radiograph image. Theses digital radiograph images were analyzed by Image-Pro Plus 5.0 program which calculated the optical density in grey scale and calibrated with aluminum stepwedge.
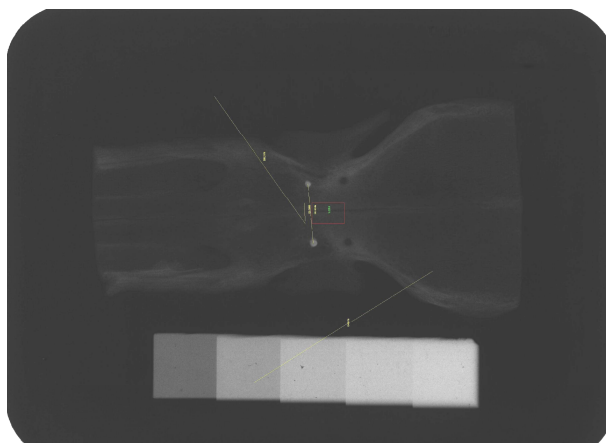

Figure 3   The digital image file were calculated by Image-Pro Plus program and

also calibrated by aluminum stepwedge.

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