Comparative Study of Clinical and Histological Changes of Orthodontic Tooth Movement in Recent and Healed Extraction Sites by Corticotomy - assisted Orthodontic Tooth Movement Technique in Rats

Kaviya Kanokpongsak

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Thesis Title Comparative Study of Clinical and Histological Changes of Orthodontic Tooth Movement in Recent and Healed Extraction Sites by Corticotomy - assisted Orthodontic Tooth Movement Technique in Rats

Author Miss Kaviya Kanokpongsak

Major Program Oral Health Sciences

Major Advisor :

…………….……………………………….

(Asst.Prof.Dr.Bancha Samruajbenjakun)

Co-advisor :

…………….……………………………….

(Asst.Prof.Dr.Kanokwan Panyayong)

Examining Committee :

……………….………….……….…Chairperson

(Prof.Smorntree Viteporn)

…………….……………………………….

(Asst.Prof.Dr.Bancha Samruajbenjakun)

…………….……………………………….

(Asst.Prof.Dr.Kanokwan Panyayong)

…………………….……………………….

(Asst.Prof.Dr.Srisurang Suttapreyasri)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Oral Health Sciences

…………………………………………

(Assoc.Prof.Dr.Teerapol Srichana)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

………………………………………Signature

(Asst.Prof.Dr.Bancha Samruajbenjakun)

Major Advisor

………………………………………Signature

(Miss Kaviya Kanokpongsak)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

…………………………………………Signature

(Miss Kaviya Kanokpongsak)

Candidate

ชื่อวิทยานิพนธ์ การศึกษาเปรียบเทียบการเปลี่ยนแปลงทางคลินิกและทางจุลพยาธิวิทยาของการเคลื่อนฟันทางทันตกรรมจัดฟันในแผลที่เพิ่งถอนฟันและแผลถอนฟันที่หายดีแล้วโดยใช้วิธีการผ่าตัดกระดูกทึบร่วมกับการเคลื่อนฟันทางทันตกรรมจัดฟันในหนูทดลอง

ผู้เขียน นางสาวกวิยา กนกพงศ์ศักดิ์

สาขาวิชา วิทยาศาสตร์สุขภาพช่องปาก

ปีการศึกษา 2556

บทคัดย่อ

บทนำ การกรอกระดูกทึบร่วมกับการเคลื่อนฟันคือการผ่าตัดเอาเฉพาะส่วนของกระดูกทึบทั้งด้านแก้มและด้านเพดานออกโดยยังคงเหลือกระดูกโปร่งอยู่ ซึ่งวิธีการนี้ทำขึ้นโดยมีหลักการเพื่อกระตุ้นการปรับเปลี่ยนโครงสร้างของกระดูกรองรับระหว่างการเคลื่อนฟันทางทันตกรรมจัดฟันซึ่งเรียกว่า “Regional acceleratory phenomenon” หรือ RAP โดยจากการศึกษาทางคลินิกหลายงานพบว่าการกรอกระดูกทึบสามารถเร่งให้ฟันเคลื่อนได้เร็วขึ้นกว่าวิธีปกติถึง 3-4 เท่า นอกจากนี้การศึกษาทางจุลพยาธิวิทยายังสนับสนุนผลที่พบทางคลินิกและพิสูจน์ขบวนการ RAP ที่เกิดขึ้น อย่างไรก็ตามจากการศึกษาที่ผ่านมามุ่งเน้นที่จะเปรียบเทียบการเคลื่อนฟันเข้าลักษณะของกระดูกรองรับที่เหมือนกัน คือ เป็นกระดูกที่ไม่ได้ถูกถอนฟันหรือแผลที่เพิ่งถอนฟัน ยังไม่มีการศึกษาใดที่สนใจศึกษาการเคลื่อนฟันร่วมกับการกรอกระดูกทึบเข้าสู่แผลถอนฟันที่มีลักษณะแตกต่างกัน ถึงแม้ว่าการเคลื่อนฟันโดยปกติเข้าสู่แผลถอนฟันที่เพิ่งถอนฟันกับแผลถอนฟันที่หายดีแล้วจะยังไม่มีข้อสรุปแน่ชัดว่าการเคลื่อนฟันเข้าสู่แผลถอนฟันแบบใดเคลื่อนได้เร็วกว่า แต่การศึกษาส่วนมากจะกล่าวว่าเป็นการเคลื่อนเข้าสู่แผลที่เพิ่งถอนฟัน **วัตถุประสงค์** เพื่อเปรียบเทียบการเคลื่อนฟันร่วมกับการกรอกระดูกทึบเข้าสู่แผลที่เพิ่งถอนฟันและแผลถอนฟันที่หายดีแล้วทั้งในทางคลินิกและทางจุลพยาธิวิทยา **วัสดุและวิธีการ** หนูทดลองพันธุ์ Wistar rat จำนวน 32 ตัวเพศผู้ถูกนำมาใช้ในการทดลองปากเดียวกันในรูปแบบสปลิทเมาท์ (split mouth) จากนั้นจึงแบ่งกลุ่มเป็นสี่กลุ่มย่อยตามช่วงเวลาที่ใช้ในการเคลื่อนฟันเป็น 0, 7, 21 และ 60 วัน หลังจากกรอกระดูกทึบกึ่งกลางสันเหงือกที่ถอนฟันกรามบนซี่ที่หนึ่งและให้แรงเคลื่อนฟันกรามบนซี่ที่สองมาทางด้านหน้าด้วยสปริงแบบปิดด้วยแรง 10 กรัม อัตราการเคลื่อนฟันจะคำนวณหาจากระยะทางระหว่างฟันกรามบนซี่ที่สองและสามหารด้วยระยะเวลาในการเคลื่อนฟันมีหน่วยเป็นวัน เมื่อครบกำหนดในแต่ละช่วงเวลาแล้ว จะเก็บตัวอย่างกระดูกขากรรไกรบน จากนั้นจึงคงสภาพและละลายส่วนแข็งของชิ้นตัวอย่าง จากนั้นจึงยึดกับพาราฟินและตัดชิ้นตัวอย่างและย้อมด้วยสี Hematoxylin และ eosin เพื่อศึกษาบริเวณฟันกรามซี่ที่สอง รวมทั้งใช้โปรแกรมเพื่อนับจำนวนพิกเซล

เพื่อประเมิน periodontal space รอบรากฟัน mesiopalatal root ของฟันกรามซี่ที่สอง **ผลการศึกษา** อัตราการเคลื่อนฟันร่วมกับการกรอกระดูกทึบเข้าสู่แผลที่เพิ่งถอนและแผลถอนฟันที่หายดีแล้ว มีอัตราการเคลื่อนฟันที่ไม่แตกต่างกันในทุกๆช่วงเวลา เช่นเดียวกับลักษณะทางจุลพยาธิวิทยาโดยรวมและร้อยละของ periodontal space รอบรากฟัน mesiopalatal root ของฟันกรามซี่ที่สองไม่มีความแตกต่างกันในทุกๆช่วงเวลา แต่เมื่อเปรียบเทียบภายในกลุ่มพบว่าเกิดการละลายของกระดูกเพิ่มขึ้นในช่วงวันที่ 7-21 และในวันที่ 60 กลับมามีลักษณะใกล้เคียงกับวันแรก **สรุปผลการศึกษา** การกรอกระดูกทึบสามารถกระตุ้นการเคลื่อนฟันเข้าสู่แผลถอนฟันที่ต่างชนิดกันได้ด้วยอัตราเร็วที่ไม่ต่างกัน รวมทั้งลักษณะทางจุลพยาธิวิทยาก็พิสูจน์ว่าเกิดขบวนการ regional acceleratory phenomenon ไม่ได้แตกต่างกันระหว่างทั้ง 2 กลุ่ม ดังนั้นในการตัดสินใจแผนการรักษาในผู้ป่วยที่จำเป็นต้องได้รับการถอนฟันในการจัดฟันจึงสามารถทำก่อนหรือพร้อมกับการกรอกระดูกทึบได้

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ABSTRACT

Introduction: Corticotomy-assisted orthodontic tooth movement is surgical buccal and lingual cortical plate penetration with intact spongy bone. This technique has been based on the concept to stimulate remodeling process as conventional orthodontic tooth movement, which called "regional acceleratory phenomenon (RAP)". There are many clinical case reports of decortications which accelerating tooth movement 3-4 times faster than conventional technique. Also, many histological studies confirmed clinical results and RAP process. However most of them concentrate on recent extraction socket or non-extraction bone. There was no any research that focuses on corticotomy-assisted tooth movement toward different socket bone. Even though the orthodontic tooth movement into extraction site has still currently been controversy, most studies found that recent socket is more suitable for faster rate of tooth movement. **Objectives:** To clinically and histologically compare the rate of tooth movement between recent and healed extraction sockets with corticotomy-assisted orthodontic tooth movement in rats. **Materials and methods:** thirty two adult male Wistar rats were split mouth into corticotomy-assisted tooth movement toward recent and healed maxillary first molar extraction sites which then randomly divided into 4 subgroups depend on tooth movement period which consisted of 0,7,21 and 60 days. The mid alveolar two-point decortications mesial to second maxillary molar were done and molar protraction was generated with 10 grams nickel-titanium close coil spring. The rate of tooth movement was calculated by dividing distance between second and third maxillary molar with protraction day. After that the maxilla were fixed, decalcified, paraffin embedded and sectioned through molar area. Hematoxylin and eosin staining was done to examine overall and catabolic morphology. The pixel counting program was used to evaluate the periodontal space around mesiopalatal root of second maxillary molar. **Results:** The mean rate of tooth movement after the corticotomies in every time point was not significantly faster on the recent side than on the healed side. In addition the overall and catabolic morphology of second molar was not different between two groups. The periodontal space of second molar's mesiopalatal root as well as presented the same tendency. However the width of periodontal space was significantly different among the timing of tooth movement. **Conclusions:** corticotomy-assisted tooth movement could stimulate tooth movement toward different socket types. The histological basic of tooth movement of this study presented regional acceleratory phenomenon in every period of tooth movement but there were no differences between opposition sites. Moreover, the amount of nonmineralized periodontal space was not depended on socket type difference but it is based on period of tooth movement as process of bone healing. These findings might be advantage to consider appropriate timing of extraction and decortication which would be done together or separately.

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CHAPTER 1

INTRODUCTION

Background and rationale

Orthodontic tooth movement is critically impacted by increased alveolar bone metabolism and reduced alveolar bone density which is remodeling process.[1] Therefore, bone turnover rate is an important index that indicates quality and quantity of controlled tooth movement.[2]To decrease the treatment time, there are many methods to increase the rate of tooth movement. Mechanical, chemical and surgical methods are mainly consideration to achieve those goals.[3-11]Even though the chemical agents such as prostaglandin E2 or parathyroid hormone increases rate of tooth movement, the critical side effects are root resorption and local pain when local injection of PGE2.[6-8]In addition, the undesired bone resorption in weight –bearing bone is probably generated when applying continuous infusion of parathyroid hormone.[9]The electromagnetic fields or mechanical stimuli was not absolutely proved to be the effective method to accelerate tooth movement[10]and the instrument was not comfortable to be the general application in the dental clinic.[11]

Corticotomy-assisted orthodontic tooth movement is surgical buccal and lingual cortical plate penetration with intact spongy bone.[2]This technique is based on the concept to stimulate remodeling process as conventional orthodontic tooth movement, which called "regional acceleratory phenomenon (RAP)". This process activates temporary physiologic bone healing of injured tissues, buccal and lingual plate, and surrounding surgically treated tissues, bone marrow and periodontal ligament space, that cause the reduction of bone density and remained bone matrix.[12-13]It is the temporary stage of localized soft and hard-tissue remodeling that resulted in rebuilding of the injured sites to a normal state through recruitment of osteoclasts and osteoblasts via local intercellular mediator mechanisms involving precursors, supporting cells, blood capillaries and lymph.[14]There are many clinical case reports of decortications which accelerating tooth movement 3-4 times faster than conventional technique.[15-17]

Due to the satisfactory reports of corticotomy-assisted tooth movement's satisfactory results, histological backgrounds that describe and confirm this phenomenon have been examined. Sebaoun et al[18] reported that the surgical injury to alveolus in the rat induced a three-fold increase in anabolic and catabolic remodeling by the third week after the corticotomy, while Wang et al indicated that bone resorption production around moving teeth was raised up in 21 days after corticotomy.[19]To measure rate of tooth movement, Mostafa et al showed a double rate of tooth movement in dogs and observed increase of bone turnover and RAP phenomenon.[20]In another animal study by Iino et al, the third molars were mesialized significantly faster than the control side in 12 dogs.[21]In conclusion, the regional acceleratory phenomenon as discovering by Frost had been proved to be explanation.

Although there are many histological reports of corticotomy, most of them concentrate on recent extraction socket or non-extraction bone. In clinical application of extraction cases, which the main purposes of tooth extraction are to reduce anterior teeth protrusion and to facilitate crowding correction; timing of extraction is also an important consideration because of different socket bone feature and side effect of extraction timing. Even though tooth movement into recent extracted socket is advantageous on broader alveolar bone, decreased tendency of gingival invagination while in healed sockets have progressive horizontal atrophy of alveolar process.[22]The delayed extraction to obtain recent extraction wound would be affected the anterior teeth proclination before canine retraction phase.

The concept of speed of orthodontic tooth movement into extraction site has currently been controversial. A more accelerated tooth movement into recent site than into healed site has been reported. Tooth movement is increased from 1 mm/month to 6.5 mm/3 weeks in canine retraction into recent first premolar extraction socket that reported by Liou and Huang.[23]Hasler at al also found that, in the activation period, tooth movements were speedier into recent sites. However the total distance starting from the period of observation was not different in both groups.[24]On the contrary, Diedrich and Wehrbein found greater tooth retraction velocity into healed socket than into recent socket. The reason why faster tooth movement toward healed socket was histological finding of healed socket which was low bone density, mature lamellar bone and gingival invagination. While on the recent side, the bone density was higher and lesser mature lamellar bone.[22]In animal study, Murphey observed the compression and tension area of 6 weeks healed and fresh sockets in monkeys. He concluded that there were more osteoclastic activities on compression site and new bone formation in healed sites. Thus, it could imply that tooth movement were more refrained into healed sockets than in recent sockets.[25]

To compare both rate of tooth movement and histological characteristic of corticotomy-assisted tooth movement into recent and healed socket, the animal study would be preferred. However the important consideration when operating animal experiments is whether the finding could represent human situation. Biologically, even though the rat alveolar bone is generally denser than that in human,[26] the tissue response to orthodontic tooth movement and extracted wound healing process in rat is similar to human in faster rate.[27]The healing process of the molar extraction sockets in rats has been divided into three phases, which were blood clotting, bone formation and bone remodeling phase.[28-29]

This study was generated to compare the rate of corticotomy-assisted orthodontic tooth movement of maxillary second molar which moved toward recent and healed extracted sockets of maxillary first molar and to compare the overall histological appearance of maxillary second molar area at 0,7,21 and 60 days after orthodontic force application.

Review of literatures

<u>Alveolar socket healing process and orthodontic tooth movement toward recent and healed extracted socket</u>

Many researchers interested in the process of an alveolar socket healing after tooth extraction. The sequences of extracted wound healing consisted of blood clot formation, replacement of the blood clot by granulation tissue, substitution granulation tissue by connective tissue, complete epithelization, appearance of osteoid tissue in the base of socket and filling the apical two thirds of the socket by trabecular bone.[30]

Amler and colleagues[31]also studied this process histologically in single root teeth. They found that after extraction, a blood clot filled the socket. In the next 7 days, the clot was replaced with granulation tissue. After that in 20 days, the granulation tissue was replaced by collagen and bone began forming at the base and the periphery of the extraction socket. Therefore, at 5 weeks, it was estimated that on average two-thirds of the extraction socket had filled with bone. Epithelium was found to require a minimum of 24 days to completely cover the extraction socket, with some extraction sites requiring up to 35 days to completely covering the socket. The epithelium was found to grow progressively, enveloping islands of granulation tissue, debris, and bone splinters. They noted that all stages of bone regeneration progressed from the apex and periphery and proceeded finally to the center and crest of the extraction socket.

Tennenbaum et al[32] also found that after extraction in the first week, there was no bone formation. The new bone formation was noted at 8th day, particularly under the wall but not on the surface of the extraction socket. At 10 days, bone formation was noted on the surface of the socket wall. At 12 days, new bone formation continued along the socket wall and in the trabecular spaces surrounding the extraction site.

To investigate the formation of new bone, Devlin et al[33] investigated the 2nd week healed sockets with immunostaining to identify new bone growth. In their histological samples, the woven bone trabeculae presented at the periphery of the socket surrounding by osteoprogenitor cells, preosteoblasts, and osteoblasts. They also noted that the periodontal ligament was displaced to the center of the extraction socket and was not attached to the socket wall. Amler and colleagues[31], on the contrary, noted that bone fragments being exfoliated from the healing extraction socket. While Huebasch et al.[34] found that the bone formation appeared under the socket wall after one week extraction. These findings indicated that, in humans, the first phase of extracted socket healing was most likely osteoclastic undermining and rejection of the original socket wall into the healing socket.

The healing process in rats is basically similar to that in humans in the sequence of events, but occurs more rapidly. The healing process of the molar extraction sockets in rats has been divided into three phases. An early phase, within 5 days, found that organization of the blood clot is completed and the socket is partially covered by epithelium. Then the bone formation phase, 5-20 days, and the complete bone remodeling phase in 60 days. Quantitative assessment of the healing of the extraction socket of a single mandibular molar in rats revealed that healing was completed in 21 days while that of three mandibular molars was completed in 60 days.[28-29]

Although, basically the wound healing process in rat and human was similar, the timing of each process was slightly different. For instance, Bodner[35] could detected the bone formation radio graphically on day 7th, which coordinated with Smith [36] and Tennebaum[32] who reported new bone formation in the apical area of the socket on 8th and 7th day post-extraction, respectively. While Huebsch[34]reported that whereas initial bone formation was observed histologically on day 5th , bone formation was first visible radio graphically on day 16th .

When considering the tooth movement toward extracted wound, most results demonstrated faster tooth movement toward recent extracted site. For example, Hasler et al[24]measured rate of canine retraction into first premolar extraction sites in 22 patients aged 10-27 years using Gjessing canine retraction spring. The experiment compared the canine distalization rate between recent extraction premolar and a median time of 86 days after extraction. The study was ended when one of the two canines had been distalized. During the active retraction period, the canine on the recent extraction side was distalized significantly more than on the healed side (median difference 1.14 mm, range -0.22 to 2.84 mm). However when comparing the median different in total time span after extraction, it was 0.75 mm which didn't significantly different.

In animal study, Yuan et al[37]founded that the tooth movement toward recent extraction side moved faster than that on the healed side. The study performed on 36 male Sprague-Dawley rats for maxillary second molars mesialization into maxillary first molars extraction sites. Tooth movement was measured with cephalometric films by Image Analysis Technique before appliance activation and after 1, 3, 4, 7, 10 and 14 days since application activation.

Diedrich and Wehrbein[22] assessed the advantage to begin treatment early or delayed after tooth extraction. The results based on the basis of hard tissue finding, which were density, maturity, osteodynamics, and soft-tissue responses at the extraction sites following bilateral extraction of the second incisors in 3 foxhounds. After an 8-week bodily tooth movement period and 2-month retention period, evaluation was undertaken on the basis of clinical, radiologic and histologic criteria. Histological analysis yielded that in delayed group, low bone density with more mature lamellar bone, pronounced horizontal atrophy of the alveolar process with periosteal bone apposition in the direction of tooth movement, increased tendency toward gingival invagination. Recent extraction group, on the other hands, revealed higher bone density with less bundle bone at the extraction sites, broader alveolar process, and reduce tendency of gingival recession. As results of these finding, the orthodontic retraction into extraction sites were recommended to initiate at an early stage.

Even though there are many studies that supporting the early tooth movement into extraction socket, Murphey et al[25]observed the different results. The study objective was to histologically evaluate the effect of recent versus healed extraction sites on orthodontic retraction histologically by employing oxytetracycline vital staining. The sample of the study was six female Macaca rhesus monkeys which separated into healed and recent extracted socket group. The healed socket group was extracted mandibular left first molar site and allowed for wound healing 7 weeks. While the recent socket group was immediate mandibular right first molar extraction site. The second premolars on both side were distalize with three-tooth sectional orthodontic appliances which were placed immediately after recent site extraction. The fluorescent microscopic evaluation showed that in the first and third week, the osteoclastic widening of the periodontal space in area of compression was greater on the healed side. Moreover, the heal side showed an increased amount of new bone and spicule formation on tension area. So the tooth movement was greater on the healed side. This difference was probably caused indirectly by the previous reversal of second premolar mesial drift during the initial 7-week healing period. The simultaneous independent activation of both mandibular second premolars showed that no advantage resulted from immediate retraction into site of a recent extraction.

Corticotomy-assisted tooth movement

As aforementioned, tooth movement mainly depends on alveolar bone structures which include bone density and maturity. Therefore there are many invented methods to increase the movement's velocity. The corticotomy procedure is a surgical technique in which a fissure is made through both the buccal and/or lingual cortical plates that surround the tooth, so that the tooth sits in a block of bone connected to other teeth and structures only through the medullary bone.[38]

In 1959, Kole[2] introduced the corticotomy procedure, which he considered a safer method. He believed that, by leaving the spongiosa intact with corticotomy, it would be able to preserve the blood supply to the tooth and to prevent many of the adverse effects associated with osteotomies. The original technique was a combination of interradicular corticotomies and supra-apical osteotomies. The major resistance to orthodontic forces came from the cortical bone, and this cortical layer was weakened by corticotomy. Once the resistance of the cortical bone was reduced by corticotomy, the tooth was then used as a handle embedded in a block of bone, which was readily moved through the less dense medullary bone.

After that, Suya[5] performed the treatments using corticotomy on more than three hundred post-adolescent and adult Japanese patients. However, he replaced the supra-apical osteotomy, which was used by Kole, with a corticotomy. Based on his clinical observations, Suya reported that 69% of the time, comprehensive orthodontic treatment was completed within 127 days. Like Kole, he insisted that this technique dramatically reduced treatment time because the resistance in the cortical bone was removed by the surgical procedure, thus allowing the band of less-dense medullary bone surrounding the teeth to be moved en block.

In 1990, the application of corticotomy was reintroduced to the orthodontic community when Wilcko and Ferguson[39] presented two case reports pertaining to one adult patient and one 14-year-old patient. Following the surgery, orthodontic adjustments were made approximately every 2 weeks. From bracketing to debracketing, the adult case was completed in 18 weeks; treatment in the 14-year-old patient required only 12 weeks. In an attempt to clarify the mode of tooth movement after the corticotomy, pre- and post-treatment computed tomography scans were compared. The cone beam computed tomograph also indicated that a demineralization-remineralization phenomenon had taken place rather than a bony block movement. [17]

The Wilckos[17] were convinced that corticotomy initiated the RAP response. According to the Wilckos, as the body attempts to heal the decorticated areas, there was a marked increase in regional bone turnover due to activation of new remodeling. This process released calcium from the alveolar bone, resulting in transient osteopenia, which was decrease bone mineralization. Therefore, corticotomy favors and facilitates tooth movement.

Even though there are many clinical reports for higher advantages of corticotomies, the tissue response backgrounds didn’t clarified. Thereby, varies researches tried to figure out the underlying cellular and immunological response. Sebaoun et al[18] investigated the alveolar response to corticotomy with injury of maxillary buccal and palatal cortical plates in 36 healthy adult rats adjacent to the upper left first molars. At 3 weeks, the surgical group had significantly less calcified spongiosa bone surface, greater periodontal ligament surface, higher osteoclast number and greater lamina dura apposition width. The catabolic (osteoclast count) and anabolic activity (apposition rate) were three-fold greater, calcified spongiosa decreased by two-fold and PDL surface increased by two-fold. Therefore, surgical injury to alveolar bone potentially induced a significant increase in tissue turnover by week 3 dissipated to a steady state by postoperative week 11th.

Cho et al[40]identified clinical effect of cortical activation by measuring the distance from the original position to the final position with orthodontic force for 8 weeks. The total amount of experimental group was approximately four times as great as the control group in the maxilla and two times in the mandible. Histologically, the cortical activation group showed higher cellular activity as an increasing number of osteoclasts with developed organelles and ruffled border were situated in the mesial alveolar bone surface. Furthermore, fibroblast, cementoblast also showed higher cellular activity in periodontal ligament on both the tooth and bone surface. In conclusion, the results displayed that the cortical activation increased the activity of numerous cellular components including formative and resorptive cells.

Iino et al[21]performed coritcotomies on the cortical bone of the mandibular left third premolar region in 12 male adult beagles with right sham side. The continuous force of 0.5 N was used to mesialize teeth. Tooth movement velocities form 0 to 1st week and from 1st to 2nd week after the corticotomies were significantly faster on the experimental side than on the sham sides. Hyalinization of the periodontal ligament appeared only at 1st week after the corticotomies on the experimental sides, whereas it was observed from 1st to 4th week after the corticotomies on the sham sides.

Mostafa et al[20]compared extracted maxillary second premolars site with miniscrews placement to distalize first premolars in 6 dogs between corticotomy-faciliated group (CF group) and standard tooth movement group. The first premolars on the corticotomy side moved critically more rapidly. Histological findings founded more active and extensive bone remodeling on both the compressive and tension sides in the CF group. Because of greater benefit of corticotomies, the comparative studies between this technique and other surgical technique or immunological assistance had been published.

Otherwise, there were studied which compared the corticotomy technique with other surgical procedures. For example, Wang et al[19]examined underlying cellular responses to corticotomy- and osteotomy-assited tooth movements through 36 rats to measure activation for osteoclast and blood vessel count, and immunostaining with proliferating cell nuclear antigen (PCNA), transforming growth factor beta 1 (TGF beta1), vascular endothelial growth factor (VEGF) and osteocalcin. The CO + TM group had significantly more osteoclasts at 3 days (P <

0.005) compared with the OS + TM group. The alveolar bone surrounding the dental roots was replaced with multicellular tissue at 21 days in the CO + TM group but was intact in the OS 1 TM group with the exception of a distal distraction site. At day 21, immunostaining with PCNA, TGF beta 1, VEGF, and osteocalcin occurred at the mesial border of bone in the CO + TM group, whereas a diffuse pattern was observed in the distal distraction sites at 21 and 60 days in the OS+TM group.

Lee et al[41]compared the corticotomy-assisted and osteotomy-assisted tooth movement involved surgical incisions through alveolar bone to ascertain whether teeth move by distraction osteogenesis or by regional accelerated phenomenon (RAP).Randomly assigned 30 Sprague-Dawley rats to one of 5 experimental groups which consisted of corticotomy alone, corticotomy-assisted tooth movement, osteotomy alone, osteotomy-assisted tooth movement, or tooth movement alone. Each animal was imaged by microtomography immediately after surgery, after 21 days, and after 2 months. After 21 days, regional accelerated phenomenon was observed in the alveolar bone of the corticotomy-treated animals and distraction osteogenesis in the osteotomy-assisted tooth movement animals. The study indicated that osteotomies and corticotomies induce different alveolar bone reactions, which can be exploited for tooth movement.

While Sanjideh et al[39] intended to determine whether single or double times of decortication was more superior after 4 weeks on the rate of tooth movement. As expected, alveolar corticotomy significantly increases orthodontic tooth movement. In addition, performing a second corticotomy procedure after 4 weeks maintained higher rates of tooth movement over a longer duration and produced greater overall tooth movement than performing just one initial corticotomy, but the difference was small.

Baloul et al[42] tested if corticotomy-induced osteoclastogenesis and bone remodeling underlie orthodontic tooth movement and how selective alveolar decortication enhances the rate of tooth movement. Measurements were done with microcomputed tomography, Faxitron analyses, and quantitative real time polymerase chain reaction (q-PCR) of expressed mRNAs. The data suggested that the alveolar decortication enhances the rate of tooth movement during the initial tooth displacement phase. This result could explain by a coupled mechanism of bone resorption and bone formation during the earlier stages of treatment, and this mechanism underlies the rapid orthodontic tooth movement.

As previous evidences, there were strongly supports that decortication could activate faster tooth movement when compare with the sham side in similar alveolar bone condition. However the definite understanding of surgical-accelerated tooth movement into different extraction socket has not been studied. Thus, comparison between the rate of corticotomy-assisted orthodontic tooth movement toward recent and healed extracted sockets and the overall histological appearance in different wound healing process would be interesting issue.

Rat model and orthodontic force application

In 2004, Ren's model was designed to compensate the physiologic distal drift of the molars, growth of the snout and concomitant forward movement of the incisors, and the continuous eruption and possible distal tipping of the incisors, stainless steel ligature wires with a diameter of 0.2 millimeters were bent to enclose all three maxillary molars as single unit. Sentalloy closed coil spring was attached to deliver reproducible force of 10 ± 2 grams over a range of 3-15 millimeters of activation. A transverse hole was drilled through the alveolar bone and both maxillary incisors at the mid-root level using a drilling bur. A stainless steel ligature wire (diameter 0.3 mm) was inserted through the hole. Bonding was applied until the buccal and palatal wires were completely embedded in the bonding material, then it was light cured.[44]

In 2006, Yoshimatsu et al used a variation of the Ren's model by using Nickel-Titanium closed coil spring. The appliance was inserted between the maxillary incisors and the first molar. It was fixed with a 0.1 millimeters wire around each tooth using a dental adhesive agent. To prevent detachment of the maxillary incisors during the experiment, a shallow groove of 0.5 millimeters from gingival was made. A 10 grams force level of the coil spring was estimated.[43]

Rat molar physiological distal drifting and distal alveolar walls are characterized by alveolar wall resorption. The interradicular bone is cancellous type which composed of osseous trabeculae and the vascular network, some of which are continuous with the PDL, there is no distinct lamina dura.

Objectives

1. To compare the rate of tooth movement between recent and healed extraction sockets with corticotomy-assisted orthodontic tooth movement in rats.
2. To histologically compare between recent and healed extraction sockets with corticotomy-assisted orthodontic tooth movement in rats.

Hypotheses

1. Rates of tooth movement in recent and healed extraction sockets with corticotomy-assisted orthodontic tooth movement are not different.
2. The histologic appearance in recent and healed extraction sockets with corticotomy-assisted orthodontic tooth movement in rats at 0, 7, 21 and 60 days after orthodontic force application are not different.

Significances of the study

1. To basically understand the histological changes of corticotomy-assisted tooth movement in recent or healed extracted socket types, examining correlation to regional accelerate phenomenon.
2. To provide the information for clinical application in extraction case for the rate of tooth movement in different socket conditions associated with surgical intended orthodontic.

CHAPTER 2

RESEARCH METHODOLOGY

Samples

This study was split mouth randomized control trial experimental design which had been approved by Animal Ethic Committee, Prince of Songkla University (No. MOE 0521.11/272). Thirty two adult male Wistar rats, aged 3-4 months, weighing 150 to 250 grams were randomly using simple random technique divided into 2 groups (healed or recent groups)

1. Healed socket group: extraction maxillary first molar at least 2 months until socket is completely healed before starting corticotomy and orthodontic tooth movement

2. Recent socket group: extraction maxillary first molar with corticotomy and orthodontic tooth movement

After random allocation of rats into 2 groups, the thirty two Wistar rats in each group were randomly divided into 4 subgroups depending on the days after starting the corticotomy and orthodontic tooth movement as shown in figure 1.

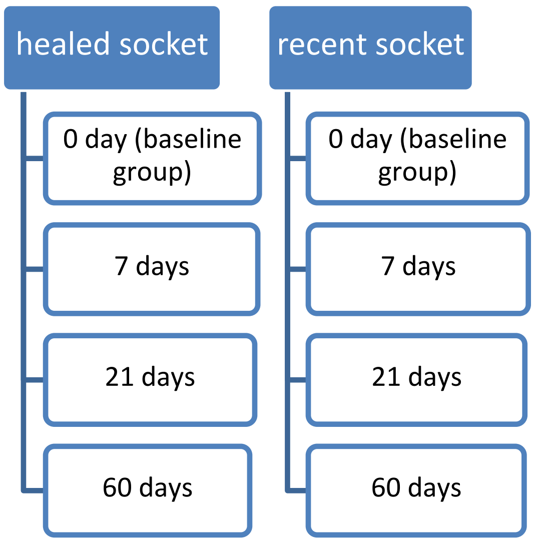


Figure 1 Diagram comparing healed and recent groups

The sample size was calculated from the equation as describe previously[18], it was calculated as one-tailed t-test formula as shown.

$$\text{Sample size (n/group)} = \frac{(Z_{(1-\alpha)} + Z_{(1-\beta)})^2\, 2\sigma^2}{(\overline{X}_2-\overline{X}_1)^2}$$

When σ^2 = square of standard deviation = 1.8

$(\overline{X}_2-\overline{X}_1)^2$ = square of mean different between experimental and control group = 1.76

Significant level of 0.05 and the power of test is 90%.

From this calculation, the sample size of each group would be 8 rats. Because 4 subgroups were classified, totally 32 Wistar rats were used in this study.

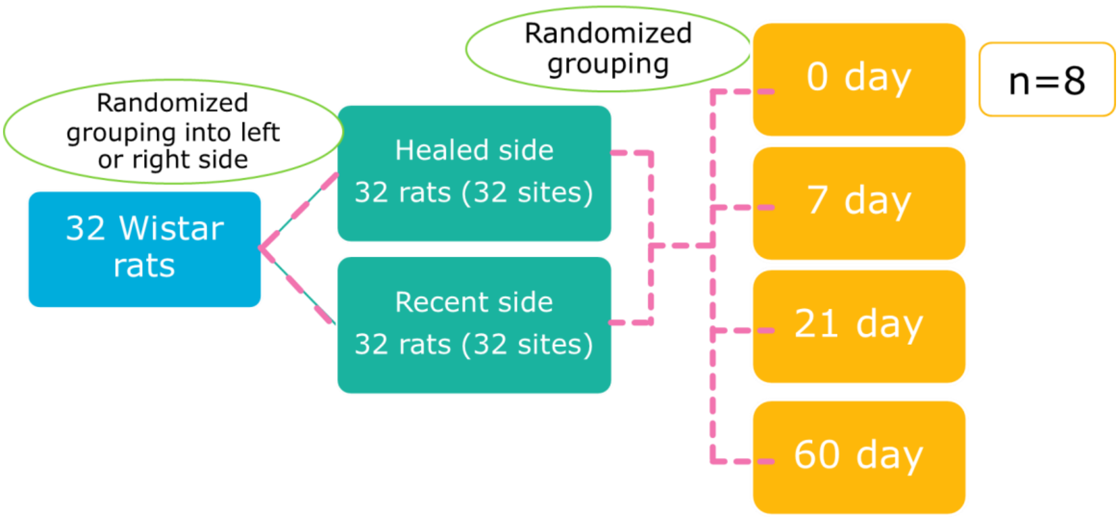


Figure 2 Diagram represents the study design in summary

The protocols consisted of two parts which were clinical and histological procedures. In the clinical part, the surgical and orthodontic procedures were done before proceeding in histological part.

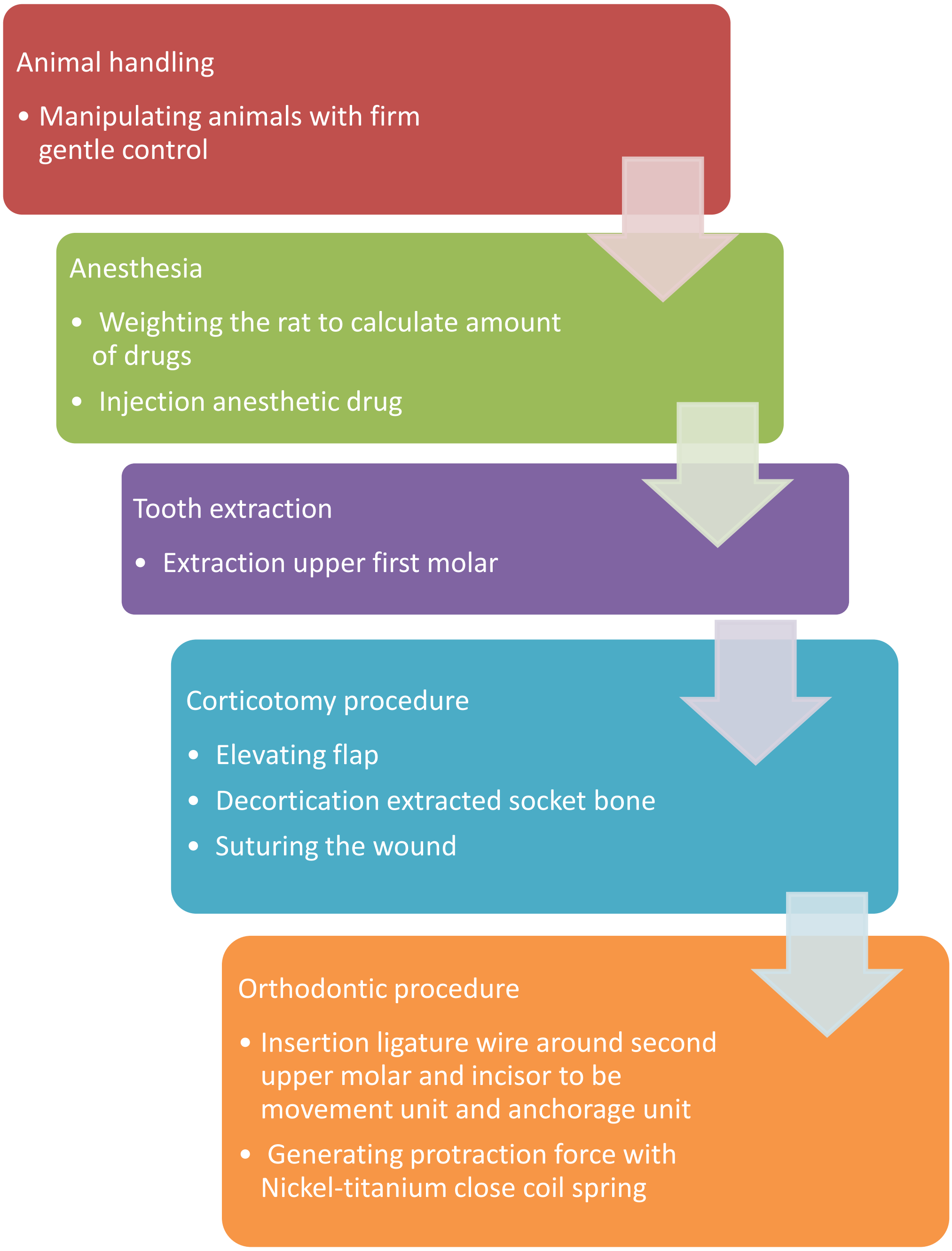


Figure 3 Clinical part of surgical and orthodontic procedure

Part I clinical procedure

First step: Anesthetic procedure

The rats were weighing to measure accurate weight for anesthetic injection. The dosage of anesthetic drug per weight of rat was 1 milliliter of combined drug per 100 grams of rat.

Figure 4 Weighing and anesthetic injection

After weighing the rat to calculate the dose of anesthetic drug, the intramuscular injection was done at gastrocnemius muscle of 90 mg /kg of Ketamine hydrochloride (Calypsol,Gedeon Richter Ltd., Hungary) and 10 mg/kg of Xylazine hydrochloride (Xylavet, Thai Meiji Pharmaceutical Ltd., Thailand) in ratio 7:3. During the surgery, animals were closely monitored of steady respiration and skin color.

Second step: extraction procedure

To assure the sedation was effective, the reflex of mouth and extremities when inserting the retractor or touching was not detected and the respiratory rates were consistent. After confirming that the rats were sedated, the first surgical step was tooth extraction. The chosen side to healed or recent sockets would be randomly selected. The maxillary first molar was loosen and elevated with spatula no.7 and extracted with mosquito forceps.

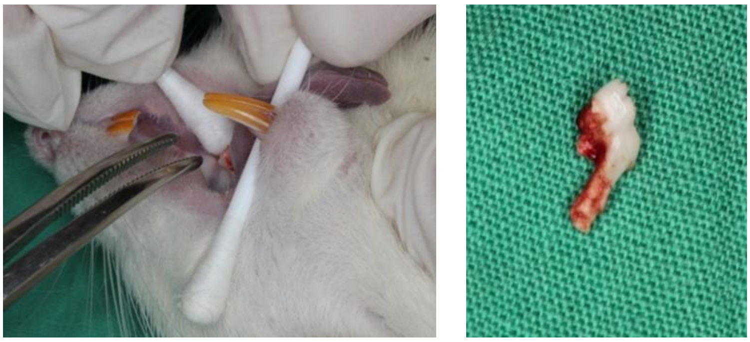


Figure 5 Extraction of maxillary first molar and the extracted tooth

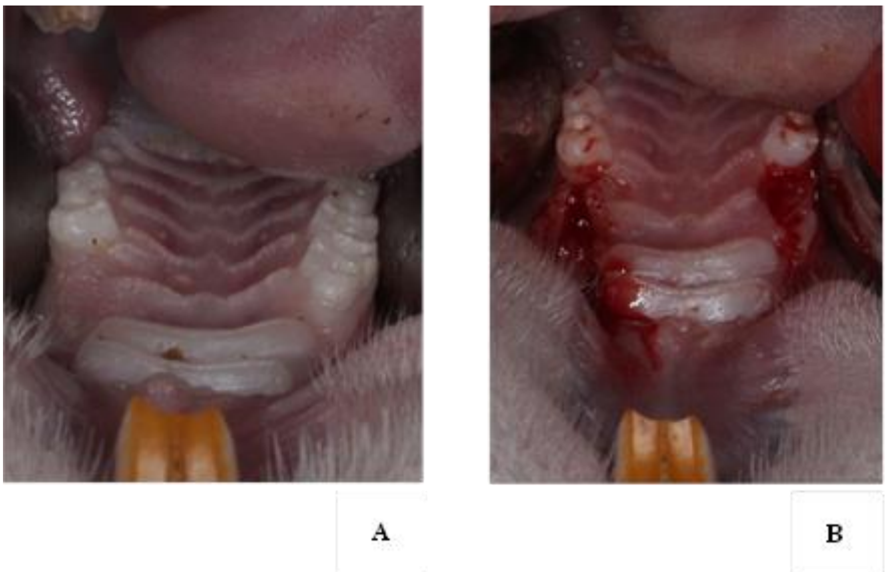
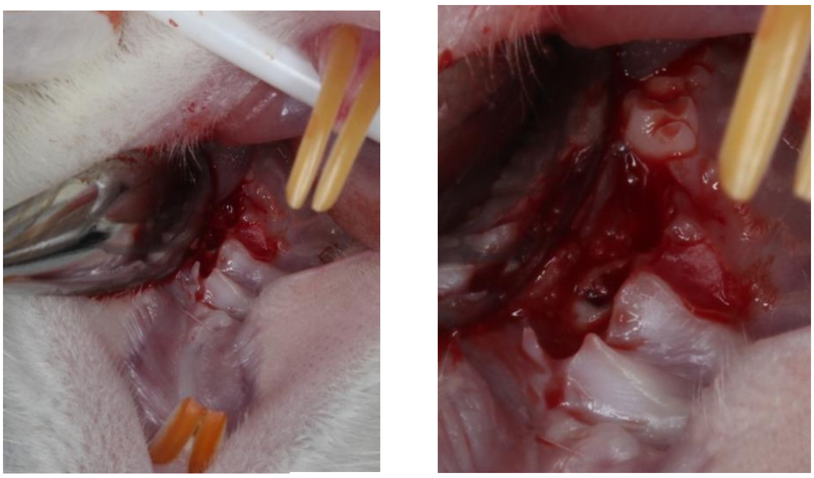


Figure 6 Clinical photographs (A) follow up at least 2 months for complete healing of healed side (B) after extraction recent side and decortication both recent and healed side (left side as healed side, right side as recent side)

Third step: Decortication procedure

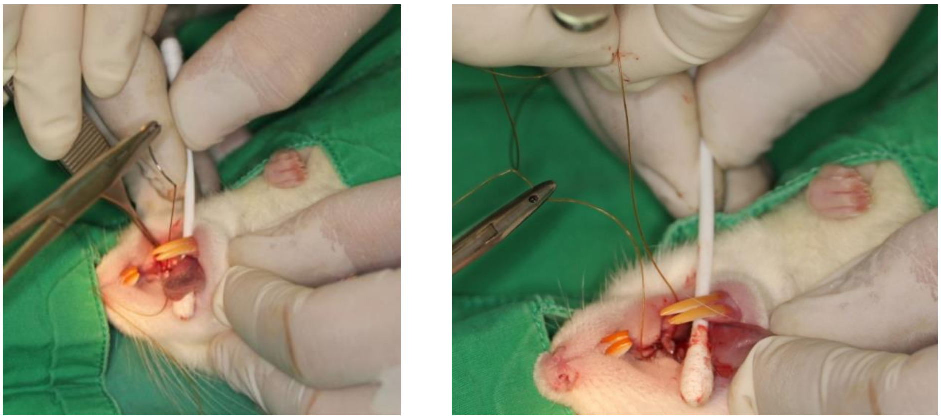
The operations were done by the same operator. Selective alveolar decortications was performed first by making sulcular incision with blade no.11 from mesial aspect of the maxillary second molar, then extended 5 mm (measured by a periodontal probe) in a mesial direction from the tooth line-angle extending to the edentulous area of extracted first molar.

Full-thickness, triangular-shaped periosteal flap was elevated on the maxillary buccal and palatal aspects adjacent to the maxillary second molars. The two-point decortications were operated on midalveolar ridge by using a slow speed handpiece and a small carbide round bur diameter 0.5 mm under sterile water irrigation. Each decortication injury was drilled half ofround bur depth or about 0.25 mm in diameter. The periosteal flaps were sutured with bioresorable 3-0 (3 metric 25 mm) chromic catgut sutures (Chromic Catgut, Shanxian runte medical instruments Ltd, China), and then primary closure was achieved for primary tissue healing.[43]



A B

Figure 7 Decortication protocol (A) incision line extended mesially form upper second molar and full thickness flap elevation (B) mid-alveolar two-point decortication



A B

Figure 8 Decortication protocol (A and B) suturing the wound with bioresorable 3-0 chromic catgut

Fourth step: orthodontic procedure

After the surgical operation, the 0.008-in ligature wires were placed at the second molar around the cemento-enamel junction. The light (Blue) nickel titanium close coil spring (Dentos, Dentaplex, USA) which was 8 millimeters in length and 1.5 millimeters in diameter was used. Then one side eyelet of nickel-titanium closed-coil spring was ligated to the ligature wire, while another side of eyelet was ligated to the incisor area.

In the incisor area, the retention was often inadequate. To prevent slipping of ligature wire, the shallow drilling groove 0.5 millimeters in height from incisor gingiva were made and flow able light-cured composite bonding (Flow Tain, Reliance Orthodontic products inc, USA) was applied to fix the wires strongly to the tooth. The light nickel-titanium closed-coil spring was kept free of the bonding material and the spring was extended 5 millimeters to generate 10 grams force.

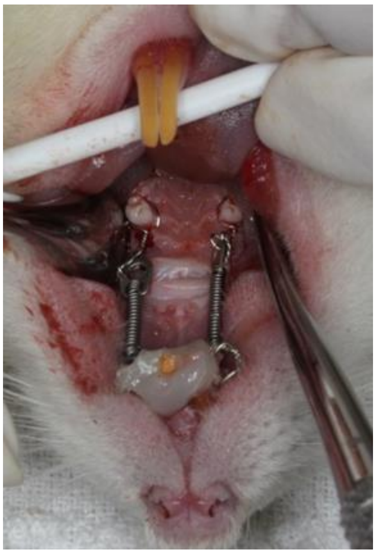


Figure 9 Orthodontic protraction force was applied by insertion NiTi closed coil spring from maxillary second molar extended 5 mm to incisor to generate 10 grams of force

Fifth step: Measurement of tooth movement

Initially, before begin surgical procedure; the impression of initial reference models was taken with light body silicone (Affinis, Coltene/ Whaledent Inc., Switzerland).

At 0,7,14 and 60 days after spring placement as shown in study design, the intraoral photograph records were taken to evaluate the appliance attachment including hard and soft tissue appearances, after that, the orthodontic appliances were removed and the photographs would be taken again.

A B

Figure 10 Clinical photographs in the post-treatment phase (A) before orthodontic appliance removal (B) after appliance removal

Before sacrificed the animals, the post treatment impressions was taken by using an injection type dental light body silicone impression material. The rats were then sacrificed with high dose anesthetic drug and cervical dislocation. Within 10 minutes after euthanasia, the maxillas were removed and the tooth movement measurements were evaluated. The amount of tooth movement was measured from the distance between distal surface of second molar to mesial surface of third molar with both direct and indirect techniques. The direct technique was measured with 0.01 millimeter accuracy digital veneer caliper (Mitutoyo, Sumipol Co.Ltd., Thailand), while the indirect technique was measured with reference model to compare the accuracy with direct technique. The comparison was done with initial reference models that done at T0.[45]

Figure 11 Direct measurement of tooth movement

Figure 12 Indirect measurement of tooth movement with light body silicone

Part II histological procedure

Subsequent to measuring amount of orthodontic tooth movement, maxillas were fixed in 10% neutral buffered formalin within 48 hours after maxilla removal. Then, the maxilla was decalcified with prepared 10% ethylenediaminetetraacetic acid Disodium salt Dihydrate (Fluka, Sigma-Aldrich inc., Singapore) in pH 7 at room temperature for 21 days and dissected into recent and healed side or half maxilla. After that, the samples were prepared to be 5 millimeter thickness and embedded in paraffin block. Paraffin-embedded samples were sectioned by placing the occlusal surface of second and third maxillary molar toward the paraffin block. Each section was 3 micrometers thickness.

Figure 13 The maxilla after formalin fixation and EDTA decalcification

Figure 14 The maxilla was dissected into recent and heal side

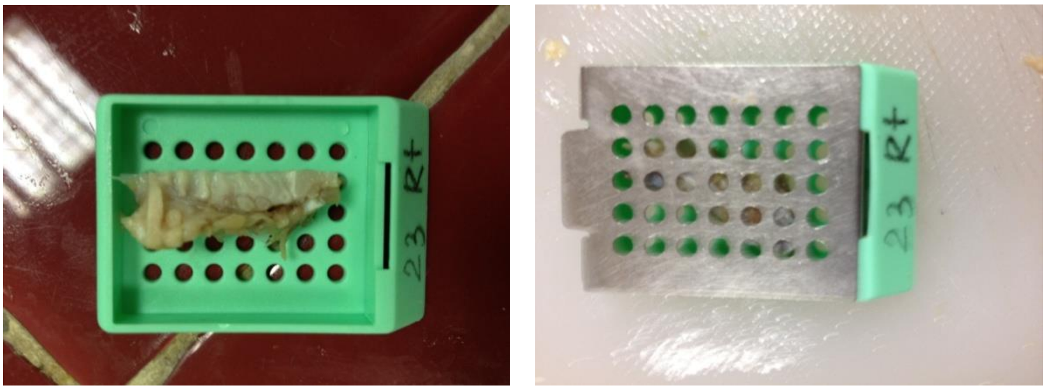


Figure 15 Five millimeters thickness of sample which inserted into block storage

The histological sections were consisted of 10 sections passing from extracted maxillary first molar through maxillary third molar area which horizontally parallel to occlusal plane. Each section was 3 micrometers thickness and departed from the next one 144 µm because the average mesial rat molar root length is 1415 µm±430 µm.[46] Although the root anatomy of the second molar differed from the first molar, the pilot sectioning was done to figure out the proper length of section which covered total root length of second molar. The pilot sectioning was performed as same procedure as describe above. Nine pilot sections included all the roots of second molar and the tenth section was beyond the root area. Therefore, this histological protocol was based on the previous study and the pilot sectioning.

The sections for general histological examination were stained with hematoxylin and eosin and examined for overall morphology as well as the catabolic activity. The overall appearances were described to comparatively explain the pressure and tension of maxillary second molar roots of recent and healed groups in every period of study.[18]

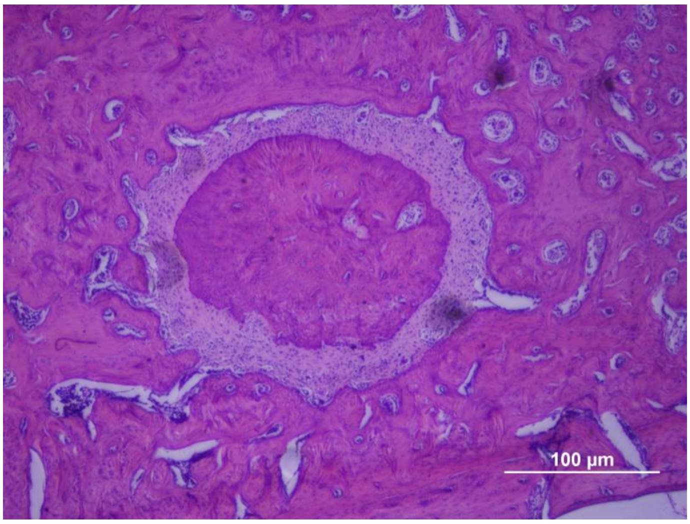


Figure 16 Example of histological section at 40x magnification (Bar = 100 micrometers)

To confirm the area of periodontal space, the program of CAD-KAS Measure-Pictures 1.0 version was used for counting the pixel of periodontal space around mesiopalatal root of second maxillary molar of the 8^{th} and 9^{th} sections or 912 – 1,260 μm from occlusal surface were used.

This technique was applied to measure the bone and PDL surface from the study of Sebaoun[18] which study the effect of selective alveolar decortication mesial to the first molar area by using captured picture at 25X magnification of hematoxylin and eosin staining. Then a standard grid of 15 mm^2 was used in first molar area defined by and center within boundaries of five roots of first molar. Within the standard grid, the trabecular bone surface and total PDL surface was recorded in square millimeter. The width of periodontal space was also recorded for each root per quadrant (mesial, palatal, lingual and buccal).

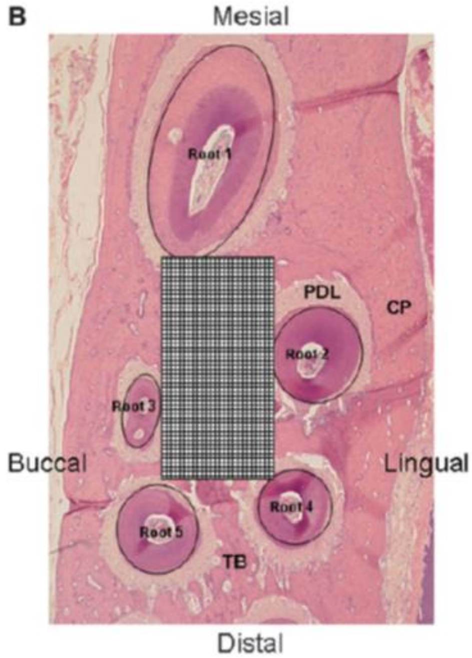


Figure 17 Histomorphological analysis for bone and PDL surface (25X magnification): CP = cortical plate, TB = trabecular bone from study of Sebaoun et al[18]

The calculated value of periodontal space of mesiopalatal root of second molar was done by freeware of CAD-KAS Measure-Pictures 1.0 version. The calculated value was come from the formula as shown below

Pixel area value of periodontal space = (Pixel area value of root and PDL space)-(Pixel area value of root)

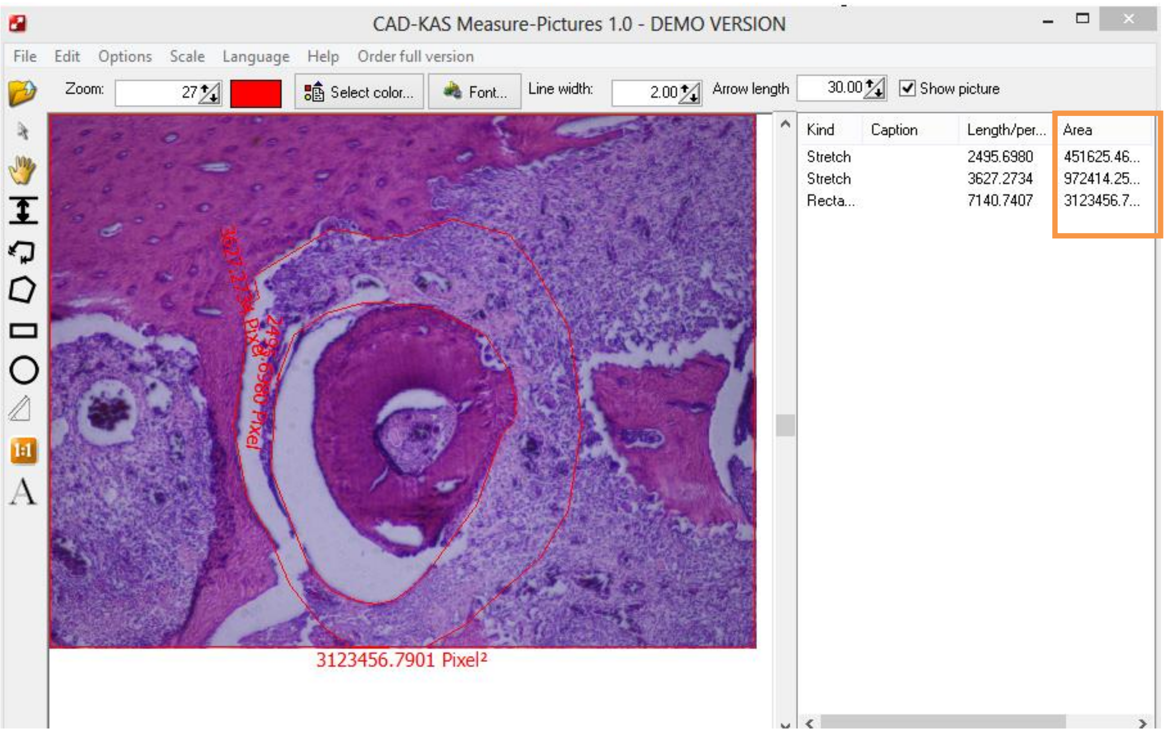


Figure 18 Example of pixel area measurement of mesiopalatal root of second molar

Then, the pixel area values of periodontal space were transformed into percentage of PDL / total PDL + root area to be compared between healed and recent group in each period of time and to compare within the group with varies timing.

Statistical analysis

The measurements of all samples were repeated twice and intraobserver reliability was done by using intraclass correlation cofficient. The normality was tested with Shapiro-Wilk test to examine distribution of data. Because the data results were not normal distribution, the nonparametric test was used. To compare the different between healed and recent extraction socket group, Wilcoxon signed rank test was used. Kruskal-Wallis was used to compare different duration after orthodontic force application of recent and healed group. The significant differences were determined at $\alpha < 0.05$.

CHAPTER 3

RESULTS

Measurement error analysis

All measurements were repeated 1month apart and calculated to determine the intraobserver reliability. The intraclass correlation coefficient of one-way random effect model was used to determine the measurement error in both parts of this study. In the first part, total sample was re-measured the rate of tooth movement and the average measurement intraclass correlation was 0.955 which indicated great reliability. In the second part, the 20% of the sample was used to test reliability of the percentage of periodontal space's calculation. The intraclass correlation in this part was 0.916 which also presented great reliability.

Part I: Rate of tooth movement

To detect the health of animals, the animal's weight was evaluated every 2 weeks to consider if the animal could maintain usual living. After 2 months follow up, all extracted wounds were healed with no infection or inflammation. When considering the animal care, no weight loss was detected when compared with initial (0 weeks). Every animal were incremental increased in weight throughout the experimental period.

Table 1 Weight of the animals after tooth extraction of the healed side which monitored every 2 weeks

Sample Number	Weight (grams)				
	0 wk	2 wks	4 wks	6 wks	8 wks
1	300	313	345	350	358
2	300	335	327	340	369
3	310	350	373	390	407
4	339	365	393	417	429
5	290	300	335	346	366
6	290	337	363	369	373
7	315	332	353	380	389
8	294	332	366	386	399
9	217	304	327	336	349
10	213	288	325	337	349
11	228	295	330	360	367
12	220	322	371	399	408
13	200	285	350	379	405
14	215	284	307	331	343
15	210	285	340	357	380
16	326	328	382	402	413
17	320	326	383	422	418
18	320	319	371	399	414
19	315	329	362	390	420
20	300	304	352	364	354
21	300	301	337	358	380
22	308	328	357	370	384

Table 1 (Continued)

Sample number	Weight (grams)				
	0 wk	2 wks	4 wks	6 wks	8 wks
23	273	287	336	360	381
24	229	316	358	367	400
25	229	348	372	375	391
26	218	335	382	400	431
27	234	348	414	435	461
28	233	317	384	397	416
29	212	334	367	390	407
30	216	303	356	378	425
31	213	317	394	417	447
32	225	313	362	380	422

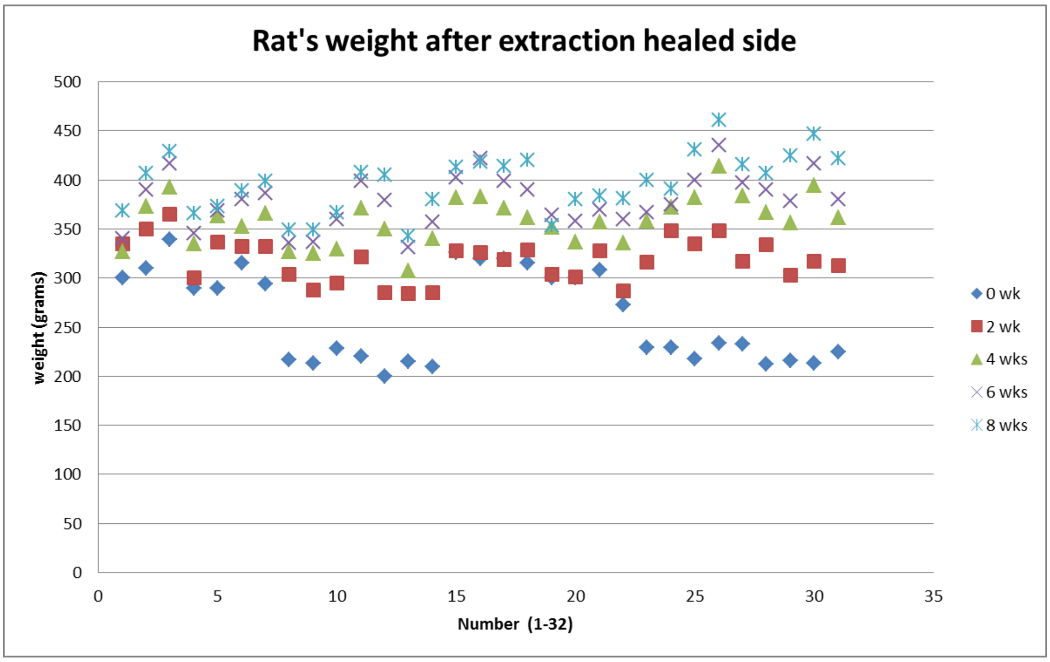


Figure 19 Graph demonstrated rat's weight after extraction of the healed side (every 2 weeks)

Evaluation of the tooth movement

In the pretreatment phase, the mean distance between distal surface of second molar to mesial surface of third molar between recent and healed extracted socket groups were not statistically significant different. There was no space between second and third molar on both groups in every collection period as shown in table 2.

Table 2 Comparison between distances of tooth movement in pretreatment phase

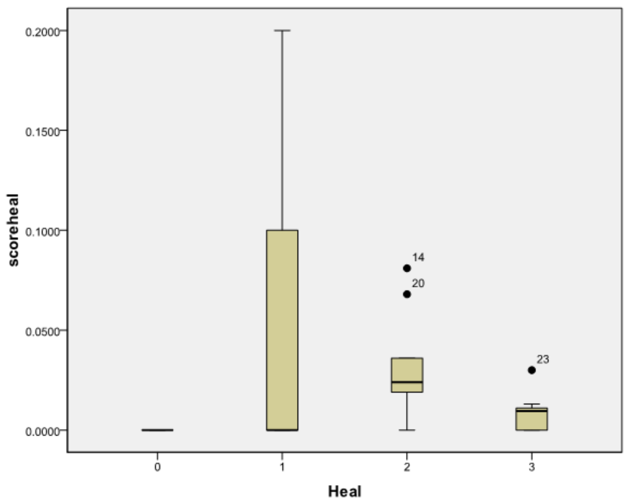
Collection period (group)	Recent socket group (mm)	Healed socket group (mm)	P-value Wilcoxon Signed Rank test
0 day	0	0	1.00
7 days	0	0	1.00
21 days	0	0	1.00
60 days	0	0	1.00

After surgical and orthodontic procedure, the distances of tooth movement in each group were measured and calculated for the rate of tooth movement. In the post-treatment phase, tooth movement velocities after the corticotomies at 0, 7, 21 and 60 days were not significantly faster on the recent side than on the healed side as shown in the table 3.

Table 3 Comparison between the rates of tooth movement (mm /day) in post-treatment phase

Collection period group	Recent socket group (mm/day)	Healed socket group (mm / day)	P-value Wilcoxon Signed Rank test
0 day	0	0	1.00
7 days	0.035 ± 0.064	0.049 ± 0.71	0.345
21 days	0.029 ± 0.019	0.033 ± 0.024	0.500
60 days	0.018 ± 0.011	0.0089 ± 0.009	0.114

However when the rate of tooth movement was compare within healed extracted group, there were significant different in different period of tooth movement as shown in figure 20.



Test Statistics[a,b]

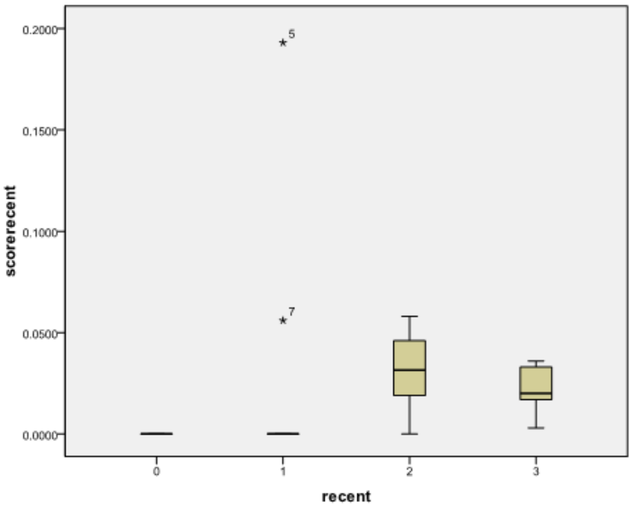
	scoreheal
Chi-Square	8.158
df	3
Asymp. Sig.	.043

a. Kruskal Wallis Test

b. Grouping Variable: Heal

Figure 20 Boxplot presenting the rate of tooth movement among the healed group (0 = day 0, 1 = 7th day, 2 = 21st day, 3 = 60th day)

On the contrary, when the rate of tooth movement was compare within recent extracted group, there were not significant different in different period of tooth movement.



Test Statistics[a,b]

	scorerecent
Chi-Square	7.758
df	3
Asymp. Sig.	.051

a. Kruskal Wallis Test

b. Grouping Variable: recent

Figure 21 Boxplot presenting the rate of tooth movement among the recent group (0 = day 0, 1 = 7th day, 2 = 21st day, 3 = 60th day)

In conclusion, the rate of tooth movement between recent and healed extracted site was not significant different in 0, 7th, 21st and 60th day. Also; the second molar protraction rate within recent extracted group in different period of collection was not significant different. However; when comparison the rate of tooth movement in the different collection period within healed group, there was significant different as shown in figure 21.

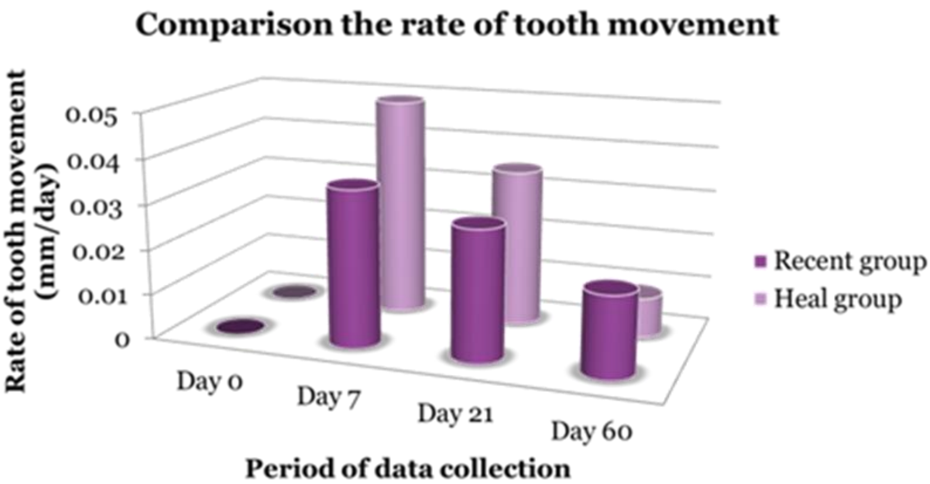


Figure 22 Graph demonstrate the rate of tooth movement in different period of data collection at post-treatment phase

Part II: Histological results

Part 2.1 Histological appearances

Because of the small size of the jaws, marrow space are usually limited to the bone at the level of the apical third, in which our study found out that the marrow space was detected at the 8th to 10th section which estimated1.15 millimeters from the occlusal surface.

The haematoxylin and eosin stained sections showed that at day 0, the normal alveolar bone architecture with intact lamina dura and PDL space were detected in both recent and healed group (Figure 19). On 7th day, the interradicular bone was decreased while the non-mineralized tissue and PDL space was increased. Viable periodontal cells and multinucleated osteoclast-like cells could be detected in some area on the margin of bundle bone adjacent to the compressed PDL. This demonstration could indicate the direct bone resorption. (Figure 20)

On 21st day, the interradicular bone was mostly replaced by non-mineralized tissue that was continuous with the periodontal ligament space. Fewer countable cells were observed when compared with that of 7th day. (Figure 21) Finally, on 60th day, the non-mineralized tissues were more replaced with newly formed bone around interradicular area, the periodontal space was decreased when compare with those of day 7 and day 21. However, the overall second molar areas were still incomplete bone healing because of remaining immature alveolar bone structure. (Figure 22)

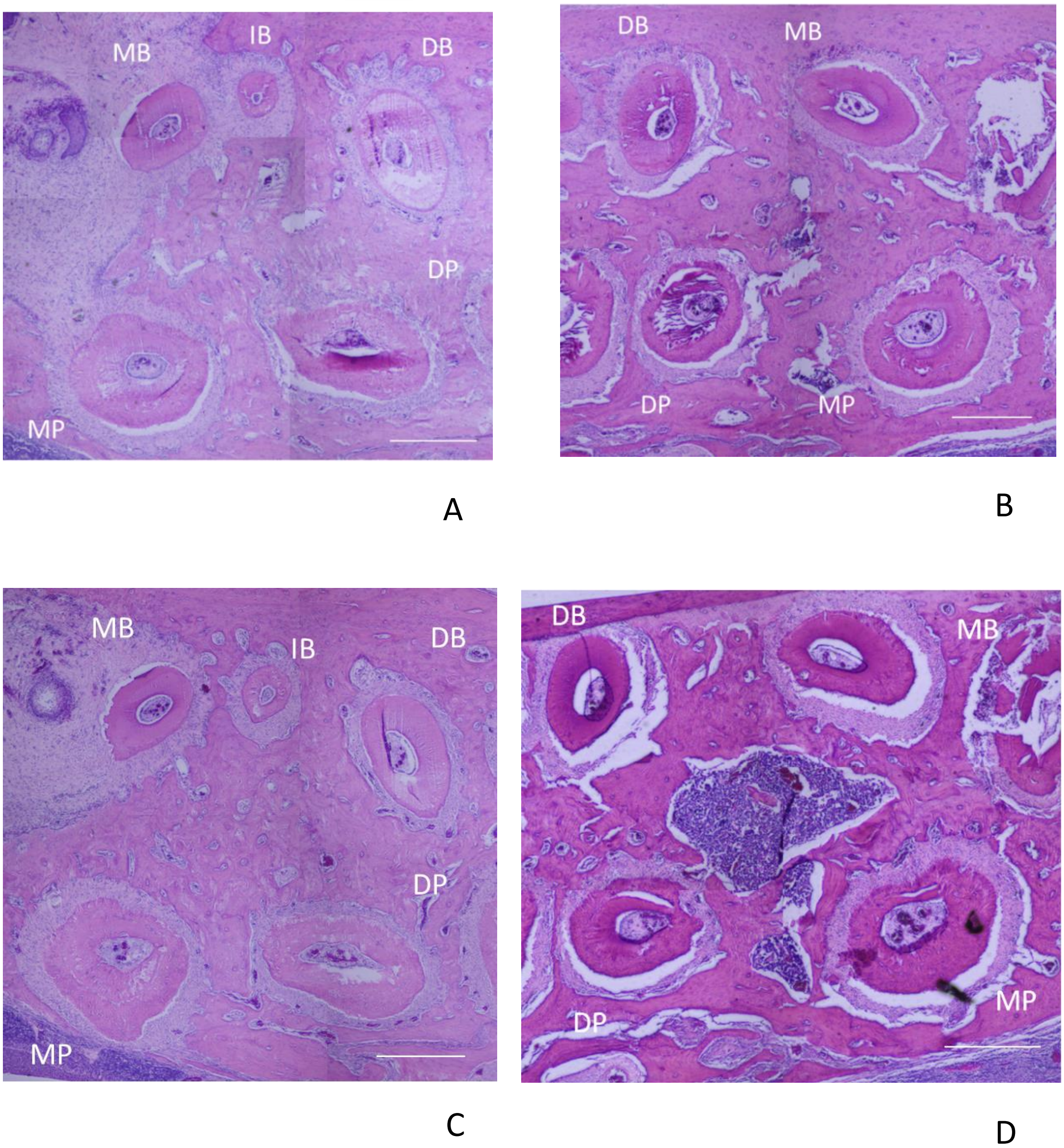


Figure 23 Representative images of the histological appearance at the level of apical third of the maxillary second molar at first day. The second molar had mainly two buccal roots and two palatal roots but in some area, present three buccal roots. (MB = mesiobuccal root, IB = intermediate buccal root, DB= distobuccal root, MP = mesiopalatal root and DP = distopalatal root), Bar = 100 micrometers (A) Healed side at 8th section (B) Recent side at 8th section (C) Healed side at 9th section (D) Recent side at 9th section

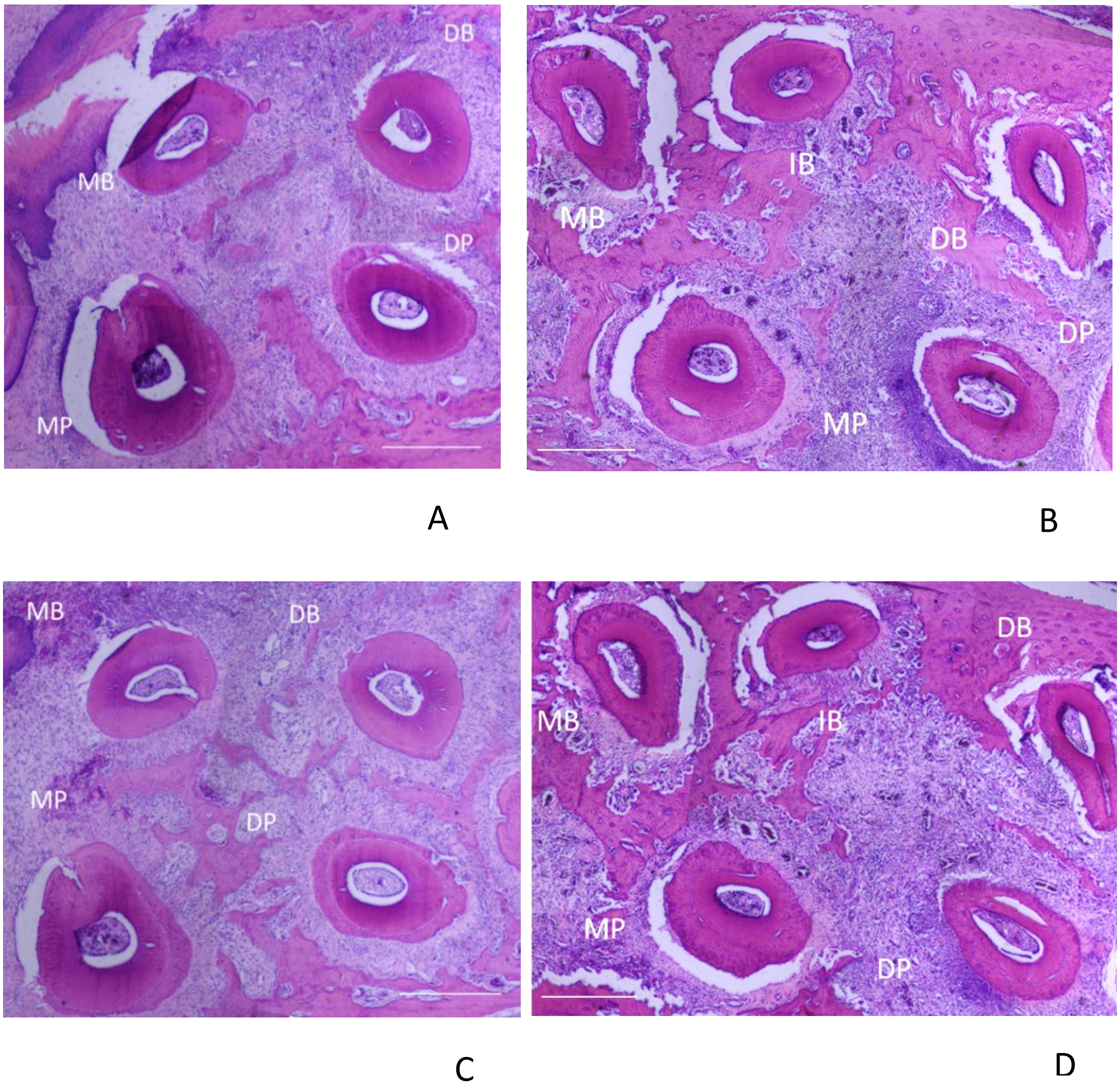


Figure 24 Representative images of the histological appearance at the level of apical third of the maxillary second molar at 7th day. The second molar had mainly two buccal roots and two palatal roots but in some area, present three buccal roots. (MB = mesiobuccal root, IB = intermediate buccal root, DB= distobuccal root, MP = mesiopalatal root and DP = distopalatal root), Bar = 100 micrometers (A) Healed side at 8th section (B) Recent side at 8th section (C) Healed side at 9th section (D) Recent side at 9th section

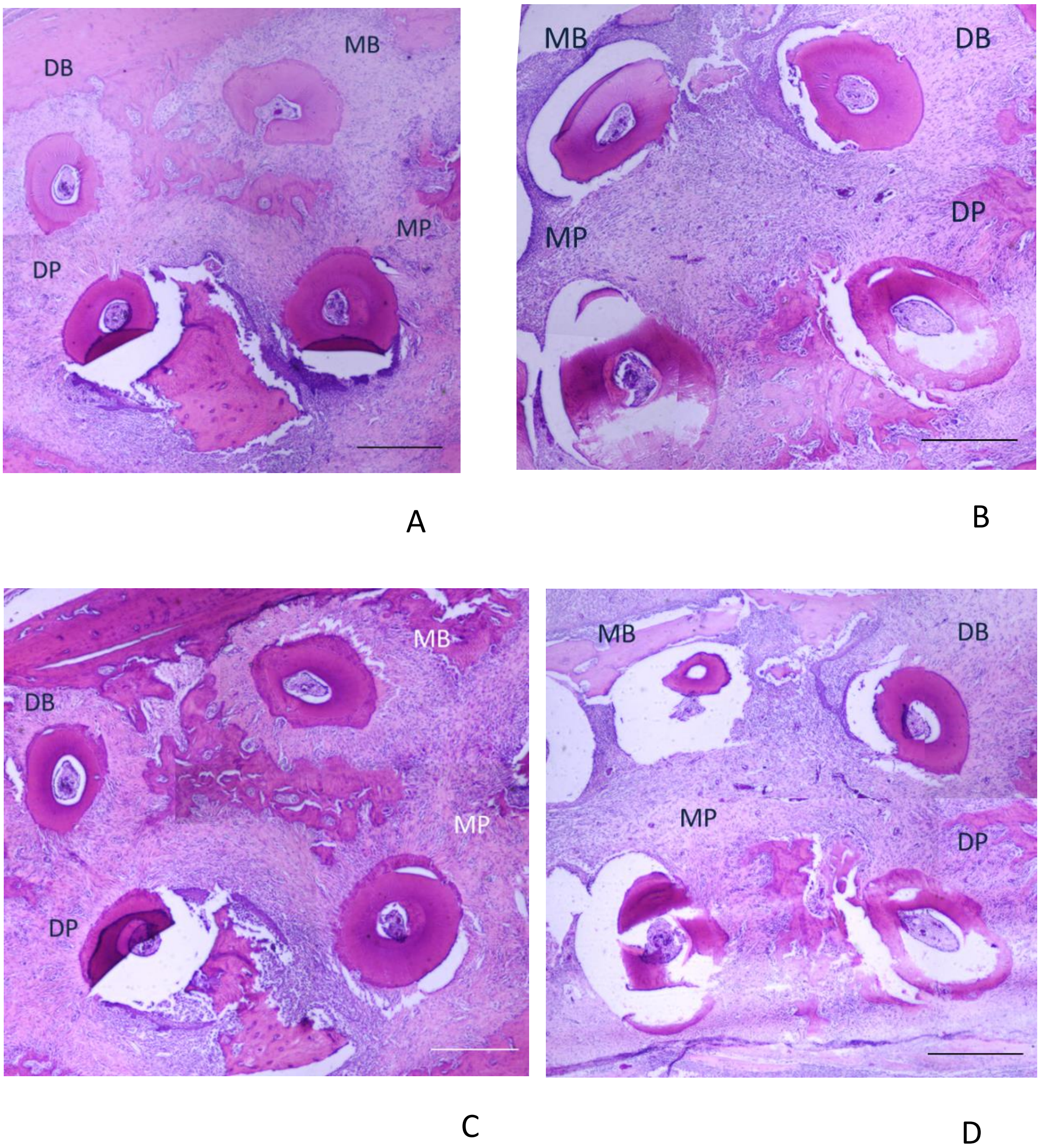


Figure 25 Representative images of the histological appearance at the level of apical third of the maxillary second molar at 21st day. The second molar had mainly two buccal roots and two palatal roots but in some area, present three buccal roots. (MB = mesiobuccal root, IB = intermediate buccal root, DB= distobuccal root, MP = mesiopalatal root and DP = distopalatal root), Bar = 100 micrometers (A) Healed side at 8th section (B) Recent side at 8th section (C) Healed side at 9th section (D) Recent side at 9th section

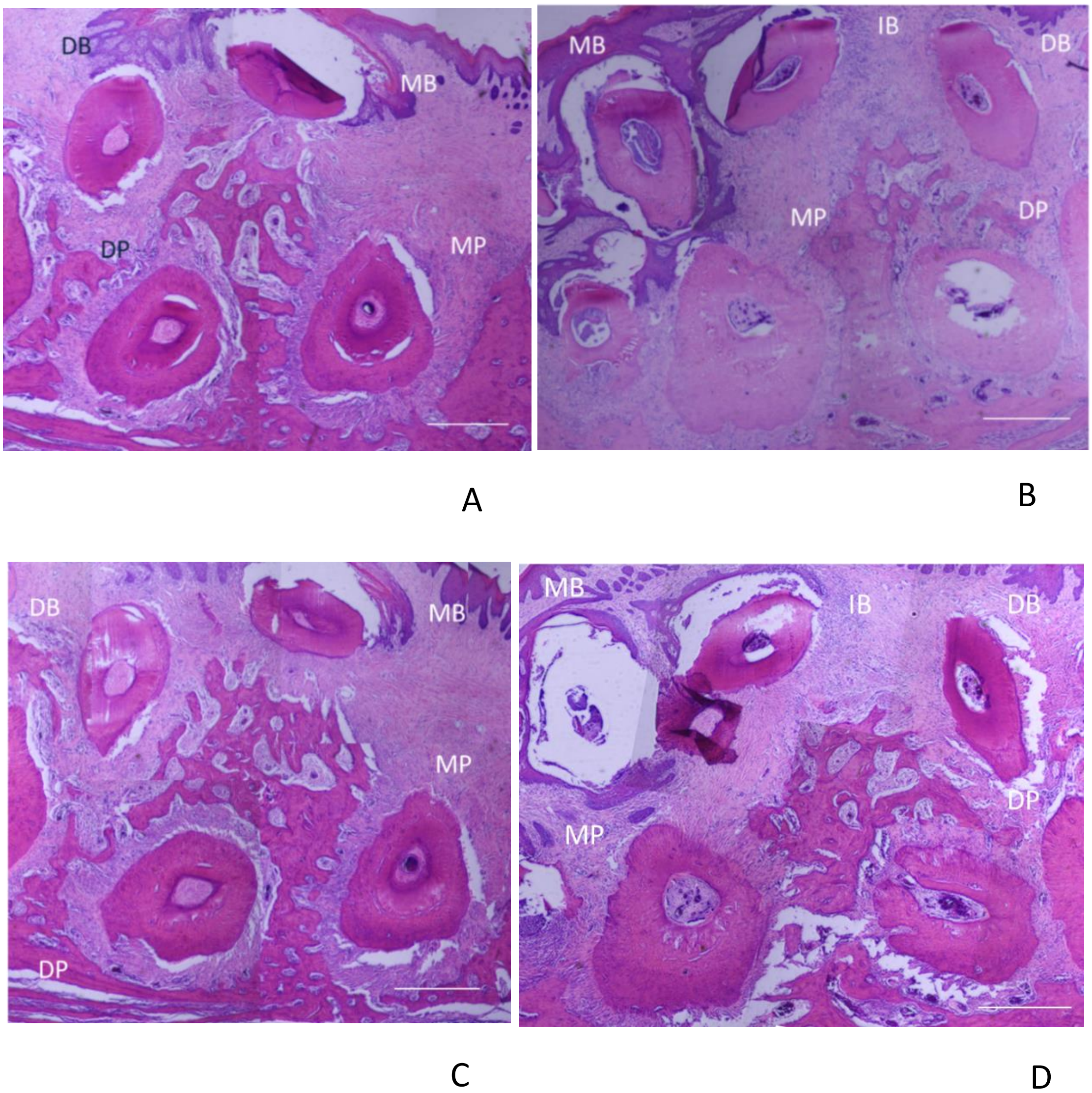
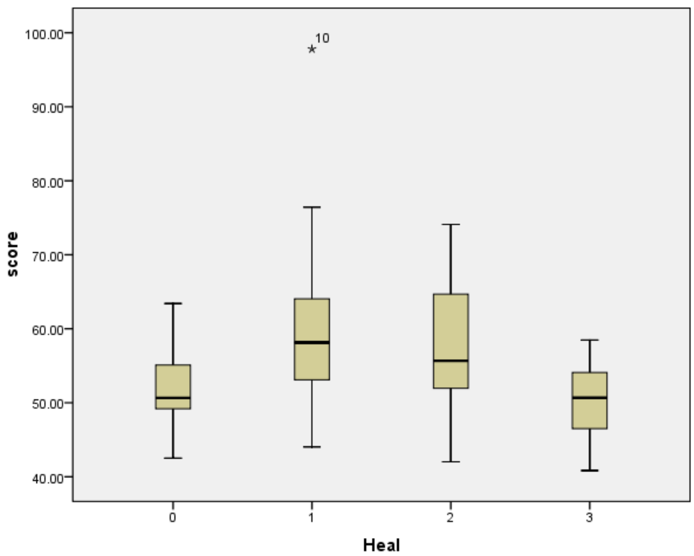


Figure 26 Representative images of the histological appearance at the level of apical third of the maxillary second molar at 60th day. The second molar had mainly two buccal roots and two palatal roots but in some area, present three buccal roots. (MB = mesiobuccal root, IB = intermediate buccal root, DB= distobuccal root, MP = mesiopalatal root and DP = distopalatal root), Bar = 100 micrometers (A) Healed side at 8th section (B) Recent side at 8th section (C) Healed side at 9th section (D) Recent side at 9th section

Part 2.2 percentage of periodontal space measurement

When comparing among different duration of tooth movement of the healed and recent groups, there were statistically significant differences among the periodontal space of different tooth movement timing.



Test Statistics[a,b]

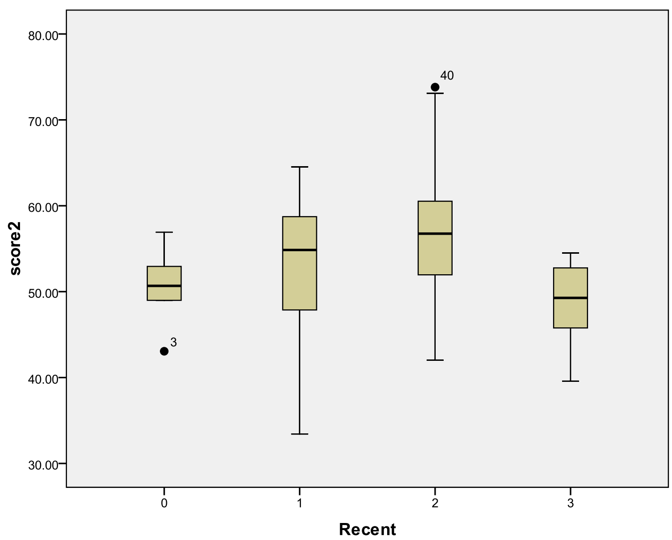
	score
Chi-Square	9.495
df	3
Asymp. Sig.	.023

a. Kruskal Wallis Test

b. Grouping Variable: Heal

Figure 27 Boxplot presenting the percentage of PDL space among the healed group

(0 = day 0, 1 = 7th day, 2 = 21st day, 3 = 60th day)



Test Statistics[a,b]

	score2
Chi-Square	8.168
df	3
Asymp. Sig.	.043

a. Kruskal Wallis Test

b. Grouping Variable: Recent

Figure 28 Boxplot presenting the percentage of PDL space among the recent group

(0 = day 0, 1 = 7th day, 2 = 21st day, 3 = 60th day)

Even though the width of periodontal space was significantly different among the timing of tooth movement, the socket type did not affect the different results. When comparing between healed and recent group in the same period, there were no significant differences in every period of time.

Table 4 Comparison between the periodontal spaces (percentage) in post-treatment phase

Collection period group	**Healed socket group (%)**	**Recent socket group (%)**	**P-value Wilcoxon Signed Rank test**
0 day	51.92 ± 6.95	50.54 ± 4.58	0.936
7 days	61.45 ± 12.65	53.58 ± 8.43	0.114
21 days	58.42 ± 9.50	57.19 ± 8.55	0.752
60 days	50.23 ± 5.82	48.71 ± 4.93	0.462

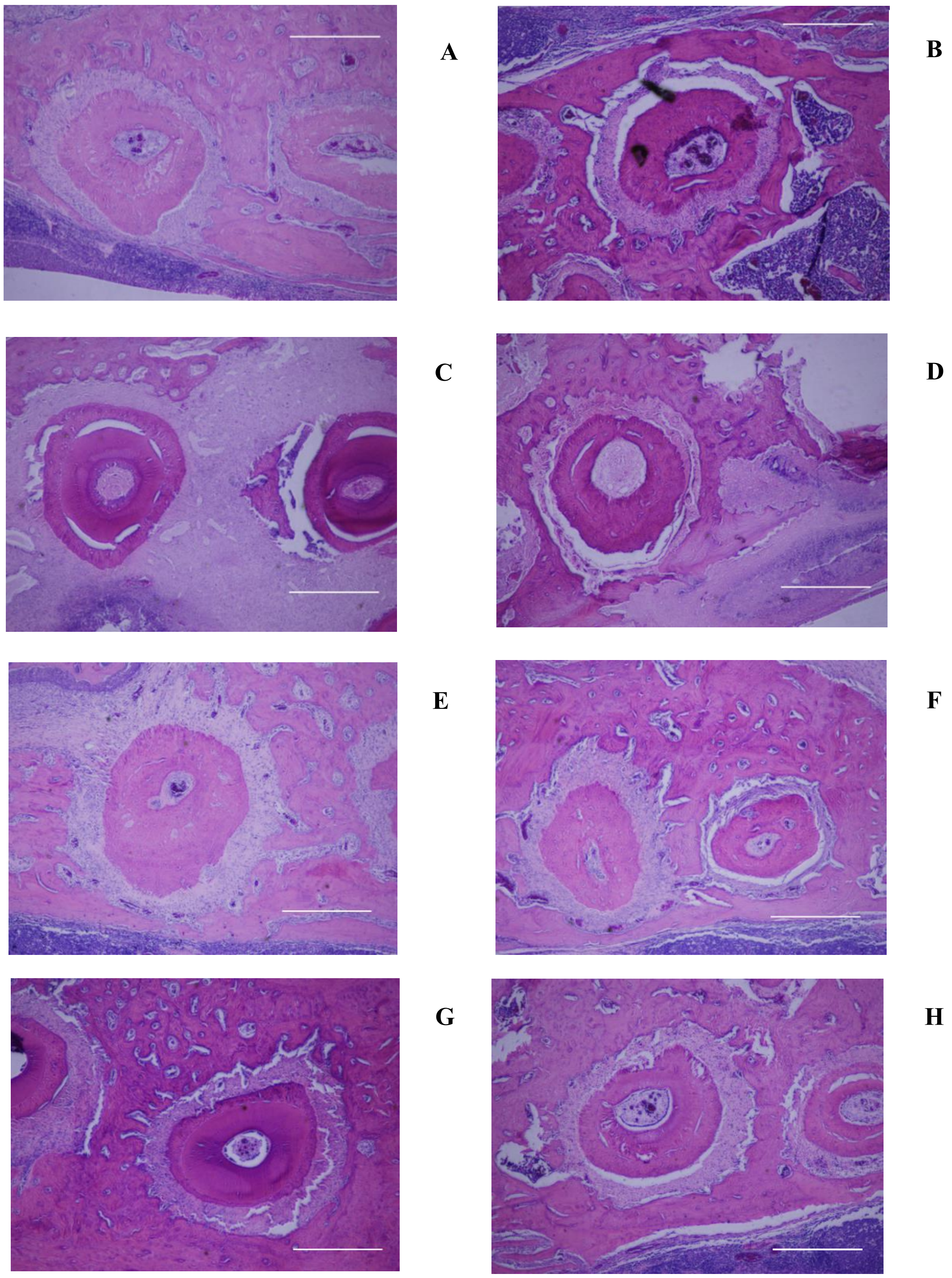


Figure 29 Representative images of the histological appearance of mesiopalatal root of healed and recent group at different time, Bar = 100 micrometers (A) Healed side at day 0 (B) Recent side at day 0 (C) Healed side at 7th day (D) Recent side at 7th day (E) Healed side at 21st day (F) Recent side at 21st day (G) Healed side at 60th day (H) Recent side at 60th day

CHAPTER 4

DISCUSSION

Selective alveolar decortication induces a localized increase in turnover of alveolar spongiosa which rising up the rate of tooth movement. However, there have not been any reports of decortication operated in recent compared with healed socket group. Most reports concentrated on corticotomy at edentulous area or recent extracted socket. Therefore, this study concentrated on comparison between the corticotomy assisted-tooth movement rates through the different socket types.

In this study on the pretreatment phase, the distances between upper second and third molars were not significantly different which would indicate that the preliminary data between two groups were similar. Moreover, the important thing to be considered was the upper second molars movement into the upper first molars extracted sites in the healed socket group before alveolar decortication which would impact on the measurement of tooth movement distances. Fortunately, the timing of tooth extraction was not affected the distance of tooth movement, thus the comparison could be done accurately.

In the post-treatment phase, the rate of tooth movement in each time period between recent and healed group were not different since first to sixtieth day duration of tooth movement. This finding suggested that the decortication could potentially induce a significant increase in tissue turnover in every period of alveolar bone healing. Even though these result similar to the other findings, the comparison with other studies was complicated. There were many factors which affected comparison results such as different animal type, methods of surgical procedure and orthodontic force application, including measurement techniques as well. For example, in the study of Beagle animal model[50],the six maxillary incisors en masse retraction with and without perisegmental corticotomy against single palatal miniplate was done. In decorticated group, the incisors were retracted faster and greater distance when compare with sham group. The rates of retraction were 0.07 and 0.0385 mm/day in decorticated and sham group, respectively. In addition, the study of incremental velocities measurement had also been done in beagles.[51]The extraction of mandibular second premolar and alveolar surgery to reduce the osteal resistance on the mesial side of the extracted socket were performed on the experimental side to compare with nonsurgical alveoloplasty. The first molar retraction with third molar anchorage in experimental side were faster than control side in every period of measurement which were at the first, second, third and fourth week. These results were consistent with the study of Mostafa et al[20] in which the retraction of first molar was operated toward extracted second molar socket with mini-implant anchorage. The corticotomy-assisted technique could double the rate of orthodontic tooth movement. For summation, although the rate of tooth movement could not reference the previous studies directly, it could confirm that the corticotomy was be able to increase rate of tooth movement continuously throughout two months study period.

Although the tooth movement velocities had been reported in animal model aforementioned, the sample size was limited least than 10 samples and the period of tooth movement was limited within one months. Therefore, this study was more precise in method protocol which included the sample size calculation, period of study based on extracted bone remodeling period and the controlled trial split mouth design. Moreover, the comparison of surgical assisted tooth movement toward different socket type had not been demonstrated in previous studies.

The data collection used light body injection silicone impression to be indirect measurement technique and to test reliability of measurement. Therefore, the critical consideration would be dimensional stability of the impression. From Coltene's material instructions, the impression remains dimensionally stable for a practically unlimited period of time (for at least 1 week). Otherwise, from literature review[52], long tern dimensional stability of polyvinyl siloxane was reported. It may be reused to produce stone dies which are as accurate as the originals, as many as seven days later. In this study the first indirect measurement was done 1 hour after impress the sample. Then the impression was re-measured the distance of tooth movement to prove internal reliability after first measurement 1 week. Therefore, the dimensional change of the impression would not be concerned.

When consider the decortication technique, the two point mid-alveolar decortication in the extracted area was used in this study. The advantages of this technique were clear visible surgical areas and controllable size and depth of decorticated point. Even though most authors recommended doing corticotomy both buccal and palatal cortical plates of moved tooth, from literature review[53], there was no suggested evidence that any specific pattern, depth or extent of corticotomy was superior to others. In the other words, it does not matter what the decortication techniques are if it could initiate the RAP response. Obviously, this technique could generate transient osteopenia. Therefore, this decortication technique could be used. Moreover, there were other studies which reported the adjacent area of alveolar surgery to nearby tooth. For instance, Tv et al[54] studied first premolar distalization into alveolar surgery of extracted mandibular second premolar compared with non-surgical control side. The surgical procedure was done at interseptal bone of extracted socket, nearby moved tooth. Active and extensive bone resorption was observed in the compressive area on the experimental side as reported in this study.

Because rat molar's root was thin and interseptal bone was thicker than in human, the effect of tooth extraction in rat would be lesser. To reproduce the extracted socket of human, interseptal bone removal after rat molar extraction could be good suggestion. However, the extracted socket bone was proved to be demineralized throughout the socket wall and interseptal bone. Moreover, the area of tooth movement was minimal distances, the effect of extraction would be enough to replicate the different between recent and healed extracted socket without interseptal bone removal.

Even though this study could not definite the type of tooth movement because the plane of sectioning was extended horizontally in bucco-palatal direction, the highest possibility would be mesial crown tipping. Because the force application was passed above the center of resistance of the tooth, the counterclockwise moment was generate to tip the crown mesially. In addition when consider the periodontal width of mesiopalatal root of 6th section to 9th section on the mesial side, the periodontal space was wider in more occlusally section than in the apically section.

To compare the histological appearance in each time period, the consideration was based on the study of Astrand et al[28]to investigate the healing process of the molar extraction sockets in rats in which there are three phases of healing. The first to fifth days after tooth extraction was blood clotting and granulation tissue formation phase. Then in the next 5-20 days, a bone formation phase was occurred. Finally a bone remodeling phase extended to complete bone healing was 20-60 days after the extraction. Therefore, in this study the seventh, twenty-first and sixtieth day were chosen to represent each healing stage. The seventh day represents the peak of bone resorption, while the twenty first day represents the maximum bone formation. At last the sixty day refers to complete bone remodeling.

Many investigators[18-21]reported the alveolar response to corticotomy with injury of maxillary cortical plate, in which the decortication significantly presented less calcified spongiosa bone surface, greater periodontal ligament surface, higher osteoclast number and greater lamina dura width when compared with the sham group. For summation, the previous results showed that the cortical activation increased the activity of numerous cellular components including formative and resorptive cells.

In addition, when time depended histological changes were considered, both descriptive and quantitative results of this study similar to the results on decorticated site from previous studies. For instant, Kim et al [55] operated experimental split mouth study to compare histological change between control and corticision assisted tooth movement. They found different histological results between control and corticision group in every period of study which consisted of 7, 14 and 21 days after orthodontic tooth movement. On day 7, the control side showed extensive hyalinization of PDL and indirect resorption of the bundle bone adjacent to the compressed PDL on the control side while on the coritcision side demonstrated less hyalinized tissue, more viable cell and multinucleated osteoclast-like cells appeared on the margin of the bundle bone adjacent the compressed PDL or direct bone resorption. On day 14, indirect resorption was widespread and area of local unresorbed bundle bone were observed with remaining hyalinized PDL on control side which related to the lag phase of tooth movement whereas on the coritcision side displayed large resorptive cavities with increased recruitment of osteoclast-like cells. At last, on day 21, the control side showed cessation of resorptive activity in the existing bundle bone which causing markedly wide and traumatic PDL.

The study of Wang et al[19], comparing coritcotomy alone and corticotomy combined with orthodontic tooth movement, found the replacement of interradicular bone with multicellular and nonmineralized tissue with continuous lamina dura in tooth movement combined with corticotomy group but fewer osteoclast were observed in this tissue while on the corticotomy alone side, the multinucleated were presented along the bony margin on day 21. However, on day 60, both group presented extensive replacement of multicellular tissues in the interradicular bone with primary bone. Therefore, the results in this study was close to that of the corticision side in the previous studies which found gradually changes from normal PDL and bundle bone architecture on day 0 then the resorptive area and gathering of multiple cells was occurred on day 7. This inflammatory process was progressed through day 21 and finally the primary bones newly formed in interradicular area on day 60.

Besides the descriptive explanations, the amount of PDL space as quantitative data showed statistical significant different among different period of time. Similar to Sebaoun et al[16] study, the peak of bone resorption had been occurred in the third week which could be detected by increase periodontal space. However the tendency of periodontal space changes was not corresponded. Even though Sebaoun's study indicated that the periodontal space continuously decrease until eleventh week after corticotomy-assisted tooth movement, this study found slightly deduce of percentage of periodontal space from first week until two months after orthodontic force application. The reason why the result was not similar tend might come from different data measurement. While only mesiopalatal area of second maxillary molar was used to evaluate periodontal space in this study, the standard grid of 15 mm^2 was used in first molar area defined by and center within boundaries of five roots of first molar in Sebaoun's study. Therefore the reason why this study only calculated the periodontal space of the mesiopalatal root of second molar came from anterior direction of tooth movement toward incisor area, the calculated periodontal space around mesiopalatal root could represent the effect of tooth movement and decortication more precisely. In addition, the error of measurement and calculation would be less than average the multiple areas as previous methods[18]

Moreover, the anatomy of second maxillary molar is different from the first molar because the roots of second molar mainly consisted of four roots of mesiobuccal, mesiopalatal, distobuccal and distopalatal roots and in some cases could have the intermediate buccal root. While the first molar is basically comprised of five roots which are mesial root, the largest one, and the other roots as second molar. Therefore, most study of tooth movement without extraction in rat mainly concentrated on mesial root of first molar.[1, 47-49]

However, the more crucial point was comparison between recent and healed socket group, they displayed homogenous data in every period of corticotomy-assisted tooth movement phase.

To apply the animal study to be clinical data, the major concern related to animal experiments is whether the finding could represent human situation. The rat alveolar bone is generally denser than that in human. It shows no osteon, its bone plates lack marrow spaces and less amount of alveolar bone surface.[26] However the rats' tissue response to orthodontic tooth movement and healing process appears to be faster than human three times but the principle mechanism is the same.[27]

Despite those differences, rat models are generally considered to be a good model to study orthodontic tooth movement for several advantages. First, they are relatively inexpensive which facilitate to use of large samples and can be housed for a long period of time. Second, the histological preparation of rat material is easier than other animal studies such as dog material. Finally, transgenic strains are almost exclusively developed in small rodents. As mice are too small to place an effective orthodontic appliance, it is clear that rats are the first choice in this field.[44]

For clinical application, the malocclusion such as anterior teeth protrusion or crowding, the extraction is needed to gain space. The appropriate treatment time of extraction is one factor to consider in treatment planning. For example, in crowding case which required extracted space, the timing of extraction first premolar would be extraction before or after the leveling phase. Therefore, the socket wounds are different between recent and heal extraction sites. In healed socket, the tendency of alveolar ridge atrophy would occurred which obstruct the tooth movement passing through this defect. On the other hands, waiting for extraction after leveling phase would cause anterior teeth proclination which would be unsatisfied for esthetic and the undesired direction of tooth movement.

From this study, the timing of extraction might not be considered as indicated factor which affect the rate of tooth movement when combined with alveolar bone decortication. Consequently, the extraction and decortication time would be mainly depended on the treatment sequence which most effective to the patients.

CHAPTER 5

CONCLUSION

1. Corticotomy-assisted tooth movement could stimulate tooth movement into heal extraction sites in the same rate as recent extraction sites.
2. The histological basic of tooth movement of this study presented regional acceleratory phenomenon in every period of tooth movement but there were no differences between opposition sites.
3. The amount of nonmineralized periodontal space was not depended on socket type difference but it is based on period of tooth movement as process of bone healing.

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APPENDICES

PRINCE OF SONGKLA UNIVERSITY
15 Karnjanawanij Road, Hat Yai, Songkhla 90110, Thailand
Tel (66-74) 286958 Fax (66-74) 286961
Website : www.psu.ac.th

MOE 0521.11/๒๗๒ Ref.11/2012

March ๒๘ , 2012

This is to certify that the research project entitled "Comparative clinical and histological changes between recent and healed extraction sites in corticotomy-assisted orthodontic tooth movement in rats" which was under conducted by Asst. Dr. Bancha Samruajbenjakul, Faculty of Dentistry, Prince of Songkla University, has been approved by The Animal Ethic Committee, Prince of Songkla University.

Kitja Sawangjaroen.

Kitja Sawangjaroen, Ph.D.
Chairman,
The Animal Ethic Committee, Prince of Songkla University

ที่ ศธ 0521.11/ 271

สำนักวิจัยและพัฒนา
เลขที่ 15 ถนนกาญจนวนิช
มหาวิทยาลัยสงขลานครินทร์
อ.หาดใหญ่ จ.สงขลา 90110

Ref. 11/55

หนังสือรับรอง

โครงการวิจัย **เปรียบเทียบการเปลี่ยนแปลงทางคลินิกและทางจุลพยาธิวิทยาระหว่างแผลที่เพิ่งถอนฟันและแผลถอนฟันที่หายดีแล้วในการผ่าตัดกระดูกทึบร่วมกับการเคลื่อนฟันทางทันตกรรมจัดฟันในหนูทดลอง**

หัวหน้าโครงการ **ผศ.ทพ.ดร.บัญชา สำรวจเบญจกุล**

ได้ผ่านการพิจารณาและเห็นชอบจาก คณะกรรมการจรรยาบรรณการใช้สัตว์ทดลอง มหาวิทยาลัยสงขลานครินทร์

ให้ไว้ ณ วันที่ 28 มีนาคม 2555

(ผู้ช่วยศาสตราจารย์ ดร.กิจจา สว่างเจริญ)
ประธานคณะกรรมการจรรยาบรรณการใช้สัตว์ทดลอง
มหาวิทยาลัยสงขลานครินทร์

VITAE

Name Miss. Kaviya Kanokpongsak

Student ID 5510820004

Educational Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Mahidol University	2009

Work-Position and Address

Dental Department, Prathai hospital, Nakorn Rachasima, Thailand

List of Publication and Proceeding

Kanokpongsak K., Samruajbenjakun B. The rate of corticotomy-assisted orthodontic tooth movement toward recent and healed extraction sites in rats. Proceedings of The 15th Graduate Research Conference; 2014 March 28; Khon Kaen,Thailand. Khon Kaen University; 2014.