Short Term Stability and Muscle Adaptation in Mandibular Lengthening by Distraction Osteogenesis

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Thesis Title Short Term Stability and Muscle Adaptation in Mandibular Lengthening by Distraction Osteogenesis

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ชื่อวิทยานิพนธ์ การศึกษาการคงตัวของกระดูกและการปรับตัวของกล้ามเนื้อในการยืดกระดูกขากรรไกรล่างด้วยวิธีดิสแทรกชั่นออสติโอเจนเนสิส

ผู้เขียน นายธงชัย นันทนรานนท์

สาขาวิชา ศัลยศาสตร์ช่องปากและแม็กซิลโลเฟเชียล

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บทคัดย่อ

การศึกษาการเกิดกระดูกใหม่ในกระดูกขากรรไกรล่างโดยขบวนการดิสแทรกชั่นออสติโอเจนเนสิสทำในกระต่ายขาวพันธุ์นิวซีแลนด์ อายุ 5 – 7 เดือน น้ำหนักระหว่าง 2.5 – 3 กิโลกรัม เครื่องมือแยกกระดูกดัดแปลงจากเครื่องมือขยายกระดูกเพดานของทันตกรรมจัดฟัน เครื่องมือนี้จะยึดกับกระดูกแต่ละข้างด้วยสกรูชนิดไทเทเนียมขนาดเล็ก กระดูกจะถูกแยกออกจากกันอย่างช้า ๆ ครั้งละ 0.5 มิลลิเมตร วันละ 2 ครั้ง เป็นเวลา 10 วัน ต่อเนื่องกัน เพื่อให้ได้ระยะทางโดยประมาณ 10 มิลลิเมตร ผลการศึกษาพบว่าไม่มีอาการแทรกซ้อนหลังการให้ยาสลบหรือการผ่าตัด เครื่องแยกกระดูกสามารถยึดติดแน่นกับกระดูกที่ตัดแยกออกจากกัน โดยมีการหดกลับของเครื่องแยกกระดูกโดยเฉลี่ยเพียง 0.56 มิลลิเมตร (ร้อยละ 2.95) การศึกษาทางเนื้อเยื่อวิทยา พบการเรียงตัวของไฟโบรบลาส และคอลลาเจน ขนานกับแนวการแยกกระดูก การเกิดกระดูกใหม่ในระยะแรก จนถึง 2 สัปดาห์หลังการแยกกระดูก พบการเกิดกระดูกชนิดกระดูกอ่อนร่วมด้วย หลังจากนั้นการเกิดกระดูกใหม่จะเป็นชนิดอินตราเมมเบรนัส การวิเคราะห์ภาพจากภาพรังสีและการทดสอบการแข็งผิวแสดงให้เห็นว่า การเกิดกระดูกใหม่จะเริ่มทันทีหลังจากการแยกกระดูกเสร็จสิ้นลง และเพิ่มขึ้นอย่างรวดเร็วจนใกล้เคียงกับกระดูกปกติในระยะเวลา 4 – 6 สัปดาห์ กล้ามเนื้อไดแกสตริกส่วนหน้าจะตอบสนองโดยฝ่อลงเมื่อเริ่มแยกกระดูกออกจากกัน และกลับมีขนาดใหญ่ขึ้นจนมีขนาดเท่าปกติเมื่อเวลาผ่านไป อย่างไรก็ตามการศึกษาในระยะยาว พบว่ากล้ามเนื้อบางชนิดฝ่อลงอย่างมากเช่นเดียวกับที่พบในการฝ่อของกล้ามเนื้อในกรณีที่ไม่ได้เคลื่อนไหวเป็นระยะเวลานาน กล้ามเนื้อด้านที่มีการแยกกระดูกจะมีขนาดใหญ่กว่าด้านตรงข้าม แต่การศึกษาทางเนื้อเยื่อวิทยาไม่พบการเกิดเซลล์กล้ามเนื้อใหม่ แต่กลับพบเนื้อเยื่อไฟบรัสแทรกอยู่ในกล้ามเนื้อ ดังนั้นการที่กล้ามเนื้อมีขนาดใหญ่ขึ้น อาจจะเกิดจากการแทนที่ด้วยเนื้อเยื่อดังกล่าว ไม่ใช่กล้ามเนื้อมีขนาดใหญ่ขึ้นจริง การที่ไม่พบการเกิดเซลล์กล้ามเนื้อใหม่ แสดงให้เห็นว่าขบวนการดิสแทรกชั่นออสติโอเจนิสิสน่าจะไม่มีบทบาทในการสร้างเซลล์กล้ามเนื้อใหม่แต่อย่างใด ในส่วนการคงตัวของกระดูกพบว่ามีการหดกลับของเนื้อเยื่อกระดูกที่สร้างขึ้นใหม่น้อยมาก

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Abstract

Sixteen healthy, 5-7 months of age, weighting 2.5-3 kilograms, New Zealand white rabbit were used to study distraction osteogenesis process in the mandible. The modified orthodontic palatal expansion screw was fixed with two bicortical self-tapping titanium microscrews on each side. The fragments were gradually separated for 0.5mm twice a day for 10 consecutive day to achieve a 10-mm distance. The result showed that the animals were well tolerated to the surgical operation and the anesthesia. The modified palatal expansion screw was very stable permitting easy distraction with the relapse distance only 0.56 mm (2.95%). Serial histologic analysis demonstrated longitudinal orientation of collagen with osteoid material forming parallel to the distraction vector. In the early stage of ossification, several areas of cartilagenous foci were seen up to 2 weeks after completion of distraction. The image analysis of the radiographic examination and the Vicker's surface hardness testing revealed new bone formation started immediately after completion of distraction and rapidly increase to the normal level in 4-6 weeks later. The anterior belly of digastric muscle adapted by transient atrophy with initiation of distraction and resolved with time. However in long term observation, some specific myofiber underwent severe atrophy as seen in type II muscle atrophy, which resulted from prolong immobilization. From the gross morphological observation in 6 months after complete distraction group, the muscle of the distraction side was remarkable larger when compared with the opposite side. However, in the histological study, no regeneration of myofiber was found. In the other hand moderate area of fibrosis was seen replacing normal muscle tissue. This finding was probably account for the pseudohypertrophy of the affected muscle. No regeneration of myofiber was detected in any stage of the experiment. This highlights the fact that distraction osteogenesis perhaps no role in genesis of the myofiber. The lengthened segment in 6 months after completion of distraction group showed good stability with no significant statistic different compared to the immediate distractor removal.

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Thongchai Nuntanaranont

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Chapter 1

INTRODUCTION

Introduction

Distraction osteogenesis is a method of producing unlimited quantities of living bone directly from a special osteotomy by controlled mechanical distraction. The new bone spontaneously bridges the gap and rapidly remodels to a normal macrostructure the local bone.(1,2,3,4,5)

Distraction osteogenesis, also called similar to Callostasis (generating new bone by stretching the callus , as in a fracture) ,this concept of bone lengthening was first described by Codivilla(6) in 1905, who used it to elongate a femur by repeated pulling forces. Other investigators also applied the technique but it remained undeveloped because of associated complications such as nonunion, nerve damage, local edema, skin necrosis, and pin tract infection.(7,8) However, the technique of bone lengthening by gradual distraction was further developed and refined by G.A Ilizarov in 1951.(3,9) He treated large number of patients in Kurgan, Siberia with fractures and nonunions following World War II. He utilized a primitive external ring fixator to compress the injured bone ends. By chance, a patient reversed the compression rods , thereby distracting the bone fragments. Ilizarov observed new bone formation radiographically and pursued this new method both experimentally and clinically.(9) Initially his patients were treated for fractures and nonunions . Without the benefit of antibiotics, Ilizarov was also able to cure cases of chronic osteomyelitis using only this method. Later he even developed the technique of bone transportation [10,11,12,13] and limb lengthening.[14,15]

Sporadic recognition of his work has appeared in the English medical literature,[16,17,18,19] but in the early 1980s a group of orthopaedic surgeons in northern Italy dedicated themselves to the understanding Ilizarov's treatment principles and applying his method.[20,21]

Ilizarov used ring external fixators and small diameter (1.5 to 1.8 mm) Kirschner wires under tension, he can generated more than 18 cm of new bone from a single operative intervention, often doubling the baseline bone length.[5,22] These highly modular fixators can direct the new bone formation in any plane, as the distraction osteogenesis always follows the vector of the force.[3,4] Age seems not to be a limitation so long as the patient has the potential to heal a fracture . The indications for this surgical technique are similar to those for traditional bone grafting and include limb lengthening , nonunions, pseudarthroses, and any osseous defect from trauma , tumor, or infection.[23] Because this method used local host tissue to regenerate new bone, it offers many potential advantages over bone grafting. Sources of autograft are limited and may leave local morbidity at the donor site. Allografts may transmit unknown antigens, bacteria, or even viruses. As dead foreign bodies, allografts may not be desirable in infected wounds. Bone grafts must be resorbed and replaced by "creeping substitution" leaving then structurally vulnerable to fracture.

Distraction osteogenesis has been shown to regenerate completely vital bone, capable of bearing load, at about 1 cm of bone length per month in children and 1 cm per 2 months in adult.[22,24,25,26]

Ilizarov used the canine tibia to study distraction osteogenesis . By varying the stability of fixation , the energy of the osteotomy (ie, degree of vascular damage) , and the rate and rhythm of distraction, he has postulated that all four factors are critical to osteogenesis. [3,4]

The use of distraction osteogenesis in the craniofacial skeleton was first reported by Snyder *et al.*[27] Who used monofocal distraction to lengthen canine mandible. They described the experimental mandibular lengthening, to repair a 1.5 cm bony defect at the mandibular body.

Successful clinical bone lengthening in the craniofacial surgery was first described by McCarthy *et al.* in 1992.[28] They begun their trial in 1989 on bone lengthening involving the human mandible using a external device. They reported the using of distraction osteogenesis by extraoral appliances to lengthening congenital hypoplastic mandible in children.[28] They applied the experimental model described by Karp *et al.*[29]for the lengthening of unilateral congenitally deformed mandibles in children. They produced a unilateral corticotomy in four children with Nager's syndrome and found bone to form without relapse. The overall effect on nerve and muscle function was not recorded although they postulated that bone lengthening might ultimately affect the functional matrix in development.

Since then several clinical reports followed such as Havlik and Bartlett[30] ; Losken *et al.*[31] and Klein and Howaldt.[32] Now a wide variety of techniques are available to lengthen segments or entire maxillary or mandibular arches.[33,34,35,36]

Hypoplasia of the mandible can result from a congenital defect such as hemifacial microsomia or can be acquired after traumatic deformation of the mandible in early childhood. The growth potential of mandible can be impaired after destruction and subsequent ankylosis of the TMJ caused by condyle fractures or by inflammation extending from the middle ear. In both instances early treatment is necessary to avoid secondary deformities of the midface.(37) Mandibular asymmetry becomes worse in time because of the normal growth of the non-affected, contralateral side. This leads to secondary malformation of the maxilla, nose , and orbit. There is a progression of the facial deformity and psychological problems may occur with time. This is why these patients have to be treated as early as possible. The mandible should be operated on in order to support the growth of the adjacent structures and to avoid or minimize secondary deformities. If there is missing bone , especially a missing joint process , early grafting of a costochondral transplant has proven to be of significant benefit.

In the past, the gold standard for reconstruction of the severe hypoplastic mandible in young children is usually done with a costochondral bone graft. This extensive surgical procedure requires intermaxillary fixation for some weeks and possibly a tracheostomy.(38,39) For that reason younger children with severe malformations preferred not have any operation. One of the drawbacks of a costochondral graft is the unpredictability of its growth, producing an excessive increase in mandibular length in some cases; inadequate rate of growth in others; and in some cases no growth at all.(40,41,42) Other complications with costochondral grafts are infection or resorption,(43) as well as the potential for such donor site morbidity as pneumothorax ,scarring , a mild chest wall contour defect, and postoperative pain.(44)

Sliding osteotomy to advance the hypoplastic mandiblewithout bone graft is a useful alternative. But the use of the sagittal split ramus osteotomy to correct mandibular retrognathism has been reported to involve a postsurgical neurosensory complication rate ranging from 0% to 54%.[(45)] Block *et al.*[(46)] showed that distraction osteogenesis can be applied to the mandible with minimal neurosensory effect. The slow nerve stretching in distraction osteogenesis may result in minimal injury because less axonal tearing and less complete compression of the vasa nervorum. The other drawback of the orthognathic surgery to advance of the hypoplastic mandible is that the patients should be postponed at least until the end of the growth period. By waiting so long, the child will be burdened with a functional and aesthetic malformation that may severely influence its psychological development. When the deficient mandible need a major advancement, the use of graft cannot be avoided. This means increasing then morbidity in both the donor and recipient sites.

In this situation , gradual callus-distraction of the underdeveloped mandible can be helpful in restoring the rudimentary part of the posterior mandible. This procedure of bone lengthening was presented by McCarthy *et al.*[(28)]. Through this small surgical intervention, it opens up a new perspective of treatment, especially in younger children with severe deformities. They require early treatment to avoid secondary malformations of the midface resulting from growth restriction by a small mandible.[(38)] Furthermore , the potential for bone-building of the hypoplastic mandible is best achieved in young children.[(47)]

Several animal models were used for the experimental studies to reveal the controversial aspects in distraction osteogenesis. In the present study, the rabbit model is proposed as the animal model for the experimental study of distraction osteogenesis by lengthening the mandible using a modified orthodontic palatal expansion screw. The anesthetic method, the surgical operation and the use of the modified orthodontic palatal expansion screw to lengthen the mandible of rabbit model by distraction osteogenesis principle will be studied. The rabbit model is more suitable than the other animals because of its ease to keep and less harmful when compare with the other kind of animals such as dog, sheep or monkey. A well-established protocol on our rabbit model could also be used in the further research in cranio-facial distraction osteogenesis. The modified palatal expansion screw is proposed as the device for distraction instead of the normal distractor. The serial histological examination of the new bone formation in the distraction gap will be studied, particularly on the process and type of ossification. The serial radiographic evaluation of the expanded gap will be also performed by both qualitative and quantitative measurement. The strength of the new bone, the response and adaptation of the related muscle to the distraction vector and the stability of the lengthened segmented will be studied.

Review of Literatures

Distraction osteogenesis defines the technique of bone generation and osteosynthesis by the distraction (stretching) of native preexisting bone. This technique was pioneered, although not invented, by the late Gravil A. Ilizarov, the Russian orthopedic surgeon. Ilizarov used novel approaches to treat thousands of patients with various orthopedic disorders. He described in detail of the clinical uses of distraction in orthopedic reconstruction, as well as the experimental basis for such techniques. By varying the stability of fixation , the displacement of the osteotomty, the rate and rhythm of distraction , Ilizarov postulated that all four factors were critical to osteogenesis.(3,4)

Definitions

- **Distraction osteogenesis** is the regeneration of bone between vascularized bone surfaces that are seperated by gradual distraction . The bone is most commonly seperated by osteotomy or corticotomy and stabilized by external fixation. Following a 5 -day latency period, the daily rate of distraction of 1 mm is divided into a rhythm of 0.25m four times a day.
- **Corticotomy** is an osteotomy of the cortex only while preserving the local blood supply to the periosteum and the medullary bone.
- **Latency** is the time period between the osteotomy and activation of the distractor.
- **Rate** is the number of millimeters per day at which the bone fragments are stretched.
- **Rhythm** is the number of distractions per day.
- **Transformation osteogenesis** is the conversion of non-osseous interpositions (eg., fiborcartilagenous in non-union, synovial cavities in

pseuo-darthrosis , or connective tissues in delayed unions) into normal bone by combined compression and distraction forces, sometimes augmented by a nearby corticotomy.

- **Bone transportation** is the regeneration of a bone defect by combining the distraction and transformation osteogenesis.
- **Healing index** is the number of months or days from the operation to full ,unprotected loadbearing for each centimeter of new bone length.

Aronson and Harrison[48] and Aronson *et al.*[49] have reproduced Ilizarov's original model of a 28 -mm lengthening of the adult canine tibia following a 7-day latency to study the histology ,physiology, and radiology of bone formation during distraction osteogenessis .In over 60 experimental lengthenings , he had varied the rate ,rhythm , latency, and fixator stability to test Ilizarov's theories and to gain further insight into the bone biology.

His experiments tested the critical factors of rate and rhythm on distraction . 7 days after external fixation of a tibial corticotomy, the entire distraction gap was created by day 7 to see if new bone would bridge between the cut surfaces. All animals went on to an atrophic nonunion. This experiment also reproduced the original method of limb lengthening reported by Codivilla at the turn of the century, that routinely resulted in an empty gap.[6]

To further examine rate and rhythm, the next group underwent sporadic daily distractions of 1.0 to 1.5 mm similar to the standard and currently accepted technique of limb lengthening developed by Wagner.[50,51] More bone was produced, but again all trials resulted in nonunion.

The Wagner's technique was purposely planned in three stages to distract, to graft the defect by internal plate fixation, and then years later to remove the plate when all of the graft is incorporated and remodeled.[50,51]

When the corticotomized fragments were distracted at the rate and rhythm recommended by Ilizarov (0.25mm every 6 hours), all bones healed and went on to normal lengthened bone. Using whole bone gravimetrics to confirm that new bone was actually added, the average 12% lengthening produced an average of 27% increase in mass and an 26% increase in volume.[1,2,48,49,52]

Rate was further tested by comparing two groups distracted at 1 versus 2 mm per day. All animals in both groups bridged the gap and remodeled to normal-appearing bone; however , noninvasive monitoring techniques, quantitaive technetium scintigraphy and quantitative computed tomography, detected a trend toward decreased osteoid production and mineralization at weeks 4 and 5 in the more rapid (2mm/day) group.[53]

Distraction osteogenesis created by the same protocol of corticotomy, latency , rate and rhythm was then tested for fixator specificity by comparing the tension wire ring external fixator modeled of Ilizarov with the half-pin fixator of Wagner. The radiographic and histologic appearance of the new bone formation was found to be identical, except that the medially placed half-pin device tended toward uncontrolled valgus during the distraction process.[52,54,55]

Histology

Histologic preparation from biopsy and whole bone sectioning were made normally mode in the coronal and transverse planes.

The initial latency period appears to be no different than routine fracture healing. Fibrin-enclosed hematoma and inflammatory cell infiltration fill the gap at the corticotomy site. At the start of distraction , mesenchymal cells begin to organize forming a bridge of collagen and immature vascular sinusoids.

As distraction begins, the fiborvascular bridge seems to organize itself parallel to the direction of distraction. The collagen network becomes more dense and less vascular, almost resembling tendon, while the vascular channels remain at the edges closely related to the cut corticotmy segments. (1,2,48,49,55,56)

During the first week of distraction, this central zone of relatively avascular fibrous tissue bridges the entire 6- to 7- mm gap , which is called the fibrous interzone (FIZ) . Spindle -shaped cells resembling fibroblasts are loosely interspersed between collagen bundles; neither osteoid nor osteoblast is present(Fig. 1). Bone mineral is distinctly absent. [1,2,48,49,55,56]

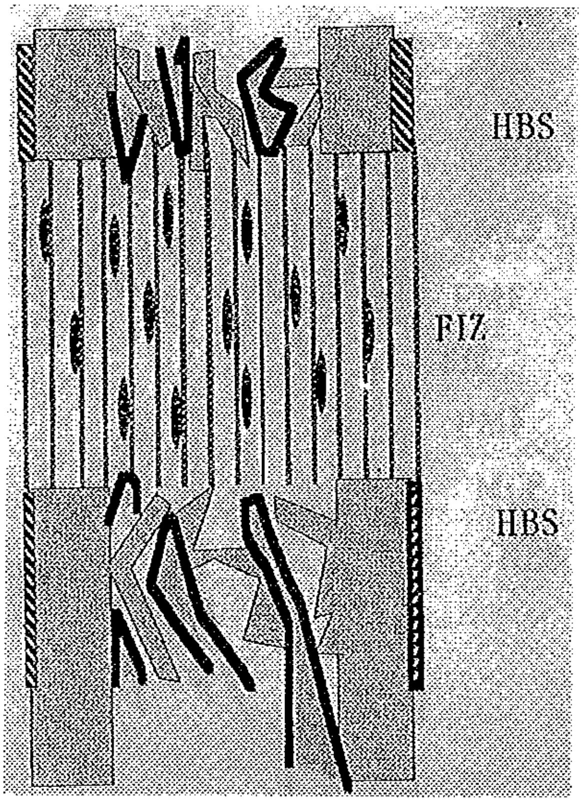


FIGURE 1. Distraction osteogenesis , week 1. Spindle-shaped fibroblasts are interspersed among parallel bundles of collagen that bridge the two cut bone surfaces. This bridge covers the entire host bone surface (HBS), including periosteum, cortex, and cancellous bone in the medullary canal. The majority of blood vessels are located in the intramedullary canal. The majority of blood vessels are located in the intramedullary region but do not cross the fibrous interzone(FIZ). (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:875,1993)

During the second week of distraction , osteoblastic cells appear in clusters adjacent to the vascular sinuses on either side of the FIZ . Collagen bundles become fused with a matrix resembling osteoid. The osteoblastic cells initially rest on the surface of these primary bone spicules and eventually become enveloped within, as the spicule is gradually enlarged by circumferential apposition of collagen and osteoid.

By the end of the second week , the osteoid begins to mineralize. These early bone spicules, called the primary mineralization front (PMF), extend from each corticotomy surface toward the central FIZ like stalactites and stalagmites.[56] This osteogenic process is seen uniformly covering the entire cross section of the cut bone, including periosteum, cortex , and medullary spongiosa(Fig.2).

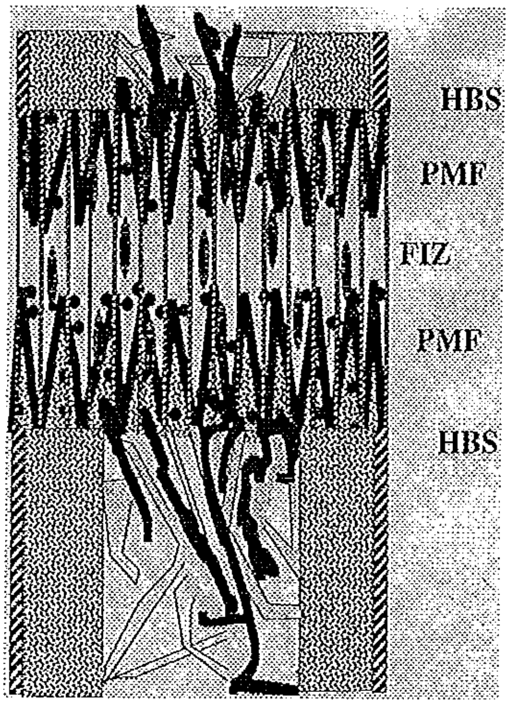


FIGURE 2. Distraction osteogenesis, week 2. As the gap widens, the local blood supply intensifies on either side of the fibrous interzone, where microcolumns of new bone are produced by clusters of osteoblasts. These new bone cones form two primary mineralization fronts (PMF) located on both sides of the fibrous interzone. (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:875,1993)

This process continues in the third week, with the FIZ undulating across the center at an average thickness of 6 mm. As the distraction gap increases, the bridge is formed by the elongation of the new bone spicules. The tips of the spicules begin at a diameter of about 7 to 10 microns, while expanding to diameters of up to 150 microns toward each corticotomy surface. Each micro-column of new bone is surrounded by large thin-walled sinusoids ; this zone is called microcolumn formation (MCF) [57] (Fig. 3).

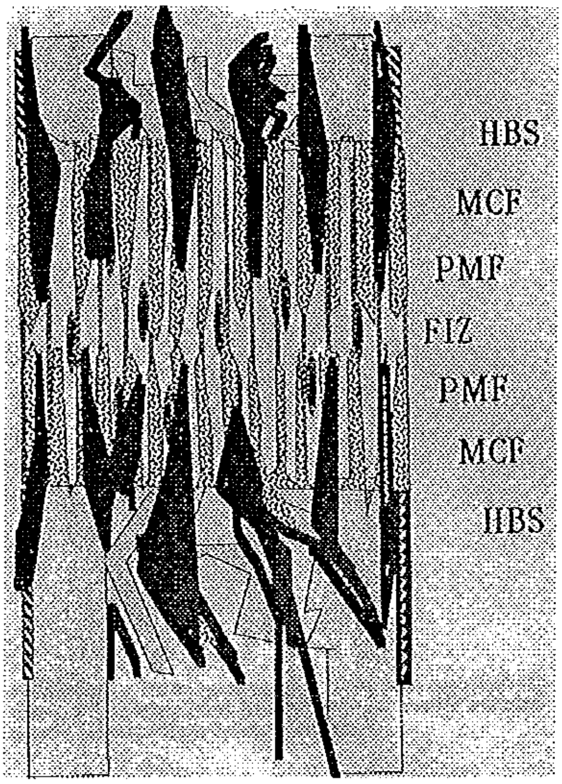


FIGURE 3. Distraction osteogenesis , week 3. As distraction continues, length is gained by increasing the length of individual bone columns in the zones of microcolumn formation (MCF). The central fibrous interzone remains relatively avascular, while the large vascular sinuses continue to supply the areas of new bone formation at the primary mineralization fronts. (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:876,1993)

At the conclusion of distraction , the FIZ ossifies, creating one zone of MCF and completely bridging the gap (Fig. 4).

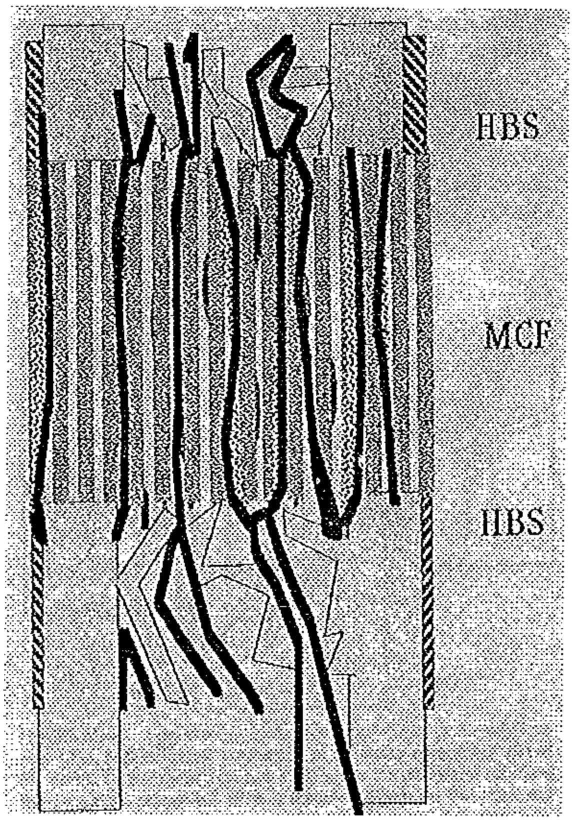


FIGURE 4. Postdistraction consolidation. The microcolumns of bone and vascular sinuses bridge the fibrous interzone , leaving a uniform cross-sectional bridge of living bone tissue. (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:876,1993)

During the 6 weeks after frame removal , the osteogenic area remodeled into cortex and medullary canal(Fig. 5). The bony columns took on the staining characteristics of mature lamellar bone with cement lines and smaller osteocytes resting in lacunae. The fibrovascular tissue that filled the spaces around bone columns was replaced by normal appearing marrow elements.(1,2,49,55,56)

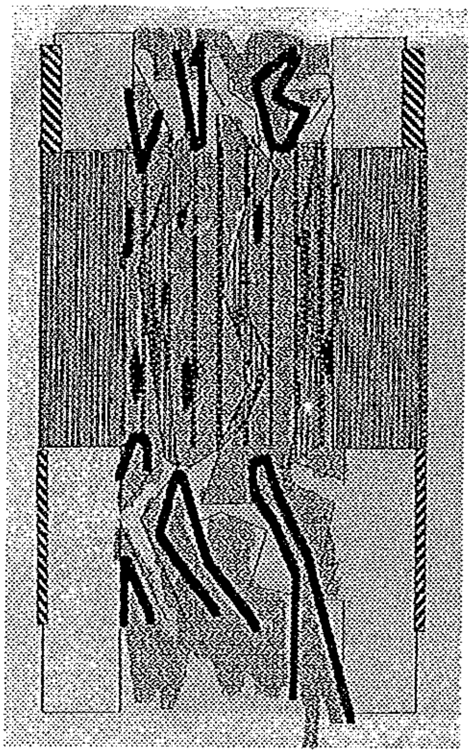


FIGURE 5. The microcolumns of bone are easily remodeled into cortex and medullary canal. Neovascularity has receded and bone marrow elements fill the intramedullary spaces. (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:877,1993)

Some authorities referred distraction osteogenesis as a growth plate, and in the clinical sense of bone regeneration it is. However, distraction osteogenesis is histologically an intramembranous ossification in its purest form. The only part of a growth plate that resembles distraction osteogenesis is at the zone of Ranvier, where periosteum stretched across the physis, undergoes direct appositional bone growth.(58) In the rabbit model, the distraction site is frequently bridged with columns of plump cells surrounded by a matrix that resembles cartilage.[59] This observation may be species-related artifact. In all of the experimental canine tibial lengthenings, as well as those reported by Delloye and colleagues[60] and in two human biopsies , intramembranous ossification has been the predominant finding.

Physiology

The most important physiologic factors in successful distraction osteogenesis are the regional and local blood supply.[57] Each column of new bone is completely surrounded by large vascular sinusoids. The appearance of clusters of osteoblasts at the tip of each column is in close proximity to these sinusoids. India ink injection studies with Splateholz clearing technique demonstrated that these vessels were parallel to the bone columns and the distraction force, but very few vessels actually crossed the FIZ . [1,53]

Technetium scintigraphy of the osteogenic area shows an intensely hot region with a central cool area corresponding to the FIZ . Quantitative technetium scintigraphy has been developed to measure these changes. The initial or flow phase of the scan correlates best with blood flow. [61] During the 4 weeks of distraction, the experimental side peaks to 7 times the normal side and then decrease to 3 times normal for at least the next 3 months.[53] The delayed or bone phase of the scan correlates to osteoid production , which demonstrates a 12- fold increase in uptake during distraction and that falls to a plateau at 5 times normal for the next 3 months.[53] The massive increase in regional blood flow must still circulate evenly on the microscopic level, as described in the histologic section. The orderly zones of bone formation seen in normal distraction osteogenesis involve collagen deposition, osteoid formation, and mineralization.

Delloye *et al.*[60] and Yasui *et al.*[62] have looked at the importance of preservation of blood supply in performing the osteotomy. By locally inhibiting either the medullary blood supply, the cortical blood supply or the periosteal blood supply, these investigators have demonstrated that the periosteum is probably the most important structure for the successful regeneration of bone.

In both experimental and clinical cases where distraction osteogenesis failed, the histologic preparations found that ischaemia is a consistent process. Each bone surface surface that is being distracted must contain viable osteocytes in the bony lacunae with intact blood supply in order to initiate and perpetuate the distraction process. Although it is not mandatory to perform the classical corticotomy, certain technique such as the oscillating saw , which may create thermal necrosis of bone, should be avoided in order to maximize the available blood supply to the bony surfaces.

Pathophysiology

Certain factors that reliably lead to poor osteogenesis are : excessive rate, sporadic rhythm , initial diastases, frame or bone-fixator instability , inadequate consolidation period, poor regional or local blood supply, and a traumatic corticotomy. It is easy to postulate that an initial diastases would inhibit the formation of a primary fibrovascular bridge . Instability results in macromotion, especially shear forces that can disrupt the delicate vascular channels. The importance of rate and rhythm may well involve the biosynthetic pathways on a cellular level by rate-limited steps, such as protein synthesis and cellular mitosis. Ischaemic vascular diseases can reduce regional vascularity, and a traumatic corticotomy can severely disturb the local vascular flow.

Biopsies from sites of failed osteogenesis reveal ischemic , atrophic fibrous tissue when the cut bone surface is devoid of osteocyte and red cells. If the ends are initially separated more than 1 cm or are distracted too quickly, islands of cartilage proliferate in the gap. Instability of the distraction frame prematurely can also lead to breakdown of the microcolumns and subsequent replacement by nonunion.

Noninvasive Monitoring

During the process of distraction osteogenesis it is sometimes helpful to assess the progress of bone formation. Early on, the surgeon can adjust the latency to enhance osteogenic potential . During distraction , rate or rhythm adjustments may be necessary to optimize osteogenesis. During consolidation, it is good to know when the osteogenic area is strong enough to remove the fixator.

In long bone, the surgeons should assess the corticotomy for completeness by intraoperative fluoroscopy , distracting no more 2 mm, angulation no more than 10 degree to 15 degree , and rotating no more 20 to 30 degree . Often , the far cortex is only greensticked, allowing some diastasis , but multidirectional angulation will be blocked and rotation will be eccentric. The corticotomy must be complete to allow uniform distraction.

The corticotomy should be well reduced radiographically as the frame is fully assembled, to decrease local hemorrhage and to ensure that the osteogenic bridge is not compromised(Fig.6). Either excessive bleeding or lack of bleeding at the time of corticotomy may result in a local vascular deficiency . In these cases, the latency should be extended. However, premature consolidation may occur as early as 14 days in the metaphyseal region and as early as 21 days in the diaphyseal region.

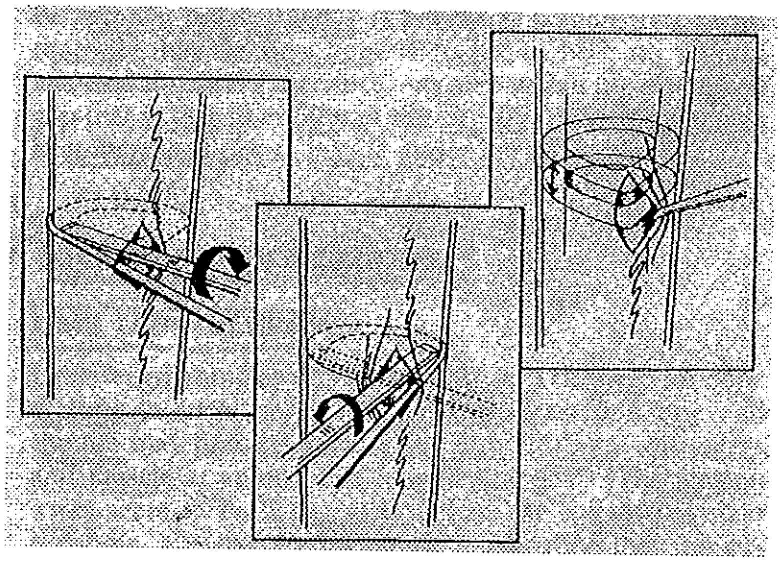


FIGURE 6. low-energy corticotomy technique in the adult canine tibia. Subperiiosteal placement of a narrow osteotome allows the cortex to be cracked on two sides while the periosteal tube and spongiosa are preserve. With a torquing maneuver the third and final side can be separated. A temporary diastasis of 2 mm is acceptable to ensure complete separation of the cortices. The cortical surfaces should be reopposed with the external fixator and the periosteal tube closed. (From Aronson J. The biology of distraction osteogenesis. In: Chapman MW. (eds): Operative Orthopaedics. 2nd ed. Philadelphia,JB Lippicott:877,1993)

Standard radiography provides a good weekly or biweekly check on the progress of the distraction gap (length and alignment). Usually by the third week of distraction new bone mineral appears as fuzzy , radiodense columns extending from both cut surfaces toward the center. Orthogonal views parallel to adjacent rings and between connecting rods should be specified and reproduced for adequate comparisons.

As distraction proceeds, the central FIZ remains an undulating radiolucent zone of 4 to 8 mm wide, while increasing amount of new bone is added from each end. The new bone spicules should span the entire cross-sectional area of the host bone surfaces on both orthogonal views. If the new bone appears to be bulging and the FIZ is narrowing, the distraction rate should be accelerated.[63] If the new bone forms an hourglass appearance and the FIZ is widening, the distraction rate should be decelerated.[63]

The absence of new radiodensity by the third week of distraction may be a cause for concern.[2,56,63] Ultrasonic examination is sensitive to mineral deposition within the cartilage before bone appears on plain radiographs. Unfortunately, ultrasound during distraction osteogenesis was found not reliable. Vascular channels may mimic the new microcolumns of bone. The high-resolution probe may not fit easily between rings , even with silicone spacers. Circumferential examinations are usually obscured by the rings or rods. Occasionally , the distraction gap appears empty on ultrasound, suggesting a cystic cavity. In this rare instance, distraction should cease and the gap should be gradually closed until the corticotomy surfaces engage again for a second latency before re-distraction

Quantitative computed tomography (QCT) is a new technique that can be useful. The histologic zones of distraction osteogenesis create a predictable pattern of mineralization that can actually be measured by QCT.[56] The number of Hounsfield units per pixel can be measured in a region of interest drawn around the perimeter of the osteogenic area at each transverse cut spanning the distraction gap. These values are reproducible with minimal interference by the connection or telescopic rods.[64] Heavy metals such as rings, wire fixation bolts, or the steel head of the clicker units cause significant interference, so avoid any measurement in these areas. When compared to a similar region on the contralateral normal side, the average QCT density is converted to a percentage of normal . The FIZ usually should be about 25% to 35% of normal , the PMF usually rises to about 40% to 55% of normal, and the MCF remains at about 60% to 75% of normal during distraction.[56] If this uniform sequence is presented by QCT, then distraction can proceed despite radiolucency on plain films.

When bone mineralization cannot be demonstrated by plain radiography or QCT, the triphase bone scan can potentially be quite useful. Again based on experimental evidence , both sides of the distraction gap should be very hot in all three phases. In cases of poor vascularity or traumatic corticotomy, a triphase bone scan can also be performed after the predicted latency to confirm that both sides of the corticotomy have adequate vascular flow . If the scan is cold, distraction should be stopped and any local problems carefully assessed. Arteriography is rarely indicated in these circumstances.

During consolidation , plain radiography can be obtained on a monthly basis until the osteogenic area has cortex and medullary canal on orthogonal views. Despite the appearance of these radiographic findings, the overall bone density may be severely reduced . QCT is helpful to demonstrate quantitatively that the new bone is stable. Any area within the distraction gap that is less than 60% of the density of the normal side is at an increase risk to buckle under normal load.

Influencing Factors

Fink *et al.*[65] in the study of osteoneogenesis and its influencing factors during treatment with the Ilizarov method , using a digital radiograph processing system to evaluated in 57 patients who had undergone 58 callus distractions and 13 epiphyseal distractions. During the course of treatment, the density of the distraction areas on radiograph was measured. The densities of the whole distraction length were correlated to the following parameters. Age of the patient, start of distraction after corticotomy , mean distraction speed , mean weight loading during the periods of distraction and consolidation , location of corticotomy (distal femoral metaphysis versus the proximal tibial bone), and analgesic medication. Except for the location of corticotomy and the analgesic, the density was influenced by all other parameters. Age of the patient and weight loading were found to bethe most important parameters. Patients with leg shortening caused by poliomyelitis and a patient with a shortened leg after amniotic membrane strangulation showed a slower rise of the density curve on radiographs than the other patients. The amount of bone regeneration varied at different regions because of the non-homogeneous soft tissue coverage of the bone and the impairment of its local blood supply by the surgical exposure for the corticotomy. The radiographic density of the distraction space decreases during the distraction period until its completion because of the increased central radiolucent connective tissue growth zone than of the surrounding mineralized front-lines during the distraction phase. This gradient is considered a better quantitative parameter on the bone regeneration than the healing index that indicates the healing time per centimeter of distraction. Thus, distraction is also influenced strongly by different individuals and timing of the apparatus removal. A great variance though less objectivity than the gradient of density trend curve during the consolidation phase. The factors are patient age, the length of latency period, and weight load during distraction and consolidation phase. Among the factors that influent on the new bone formation in: Age and weight loading were noted to be the key factors. Though testing each parameter yields only low correlation coefficient of significance, the extent of bone regeneration could be predicted with a certainty of 95% by the summation of 5 parameters

The influencing factors during treatment with the Ilizarov method can be divided into the followings:

1. Surgical Factors

Surgical factors include the surgical technique and the location of bone transection.[9,66,67,68] Ilizarov[9] considered the technique of corticotomy to be of primary importance for the callus distraction. He attributed great significance to the intactness of the nutrient artery. His resulting demand to preserve the medullar vascular system by the method of corticotomy technically is not controllable . The intraoperative verification of the success of the corticotomy is conduced either radiologically by showing a diastases or clinically by showing slight movement between the bony fragments. Both methods suggest at least a partial destruction of the intramedullar vascular system.

Moreover Yasui *et al.*[68] were able to prove through microangiograph, a complete reconstitution of the intramedullary vessels 10 days after osteotomy. Delloye *et al.*[60] showed the lack of distinction between corticotomy and osteotomy concerning the method of bone healing and the amount of newly formed bone. Numerous authors were able to prove the efficacy of osteotomy for callus distraction.[59,69] It is important to use a chisel instead of a saw during osteotomy to avoid local heat damage of the bone ends that might lead to a reduction of the bone regeneration.

Most authors observed an improved osteogenesis after metaphyseal bone transsection when compared with diaphyseal transsection.[66,67,70,71] Aronson and Shen[66] attribute this to the greater osteogenic potency of the metaphyss because of its large amount of spongiosa and its excellent blood supply. Bone formation seems to be better in the distal femoral epiphysis than in the proximal tibial metaphysis, as numerous authors report lower healing indices for the femur than for the tibia after callus distraction.[67,72,73,74] Even though slightly shorter healing indices for the femur than for the tibia, no significant difference was seen between the gradients of the radiograph-density trend curves, which suggests equal ossification rates for both bones. The healing index is influenced strongly by the subjectively determined day of removal of the fixator, which has large individual variations.

2. Activation Factors

The start of distraction , the distraction rate, and the pace of distraction are considered important factors.(4,9,68,69,75) Ilizarov(4,9)and White and Kenwright (69,75) indicated the significance of the postoperative latent period before distraction and observed lower quantities of newly formed bone when the latent period had been short or was missing altogether. Aronson and Shen(66)alone were able to induce a sufficient callus formation in animal even distraction started immediately. Wagner technique also advocated an immediate distraction after bone transsection but did not result in adequate bone regeneration, which necessitated spongoiosa grafting to fill the bone defect (76). Longer latent periods leads to slight rise of the density-cure gradient, and improved bone formation after an interval of >8 days when compared with the group with a latent period of ≤ 8 days.

Various authors reported that a daily distraction rate of 1 mm yielded the best results.(2,9,60) Slower rate often leads to early ossification of the distraction space. Faster rate (>1mm per day) coincides with less new bone formation, most likely attributable to microangiographically tearing of endosteal and periosteal vessels.(4,9,68) The daily distraction generally should be accomplished in as many rhythm as possible.(4,9,68) Ilizarov(4,9) stated that the highest bone regeneration was effected by an autodistractor at 60 steps a day and that distraction of the same daily length in 1 single step was followed by the lowest bone formation. These results led to the general recommendation of a distraction rhythm of 4 times, 0.25 mm each time, during clinical use of the Ilizarov method. (4,9,72,73)

3. Mechanical Factors

The mechanical features of the distractor used (ring fixator or unilateral fixator) (3,9,55,60,68,77)and the amount of weight put on the extremity(3,9)play an important role in the bone formation. A certain axial dynamic, permitted by the ring fixator,(78,79), help callus formation in cases of fracture healing that also include the callus distraction.(80) Multidirectional instability of the fixator leads to a change in osteogenic activity and to the formation of fibrous connecting tissue and great shares of cartilage during callus distraction. (3,9)

The ring fixator permits full loading of the extremity, which causes increased bone regeneration.(3,9) Bone formation was influenced by the load during treatment with a ring fixator. The positive influence of the load was slightly greater during the activation phase than during the consolidation phase. The axial stress seems to improve bone formation during the period of consolidation and also at the time of distraction, which justifies the need for an increased weight load (full load if possible) of the extremity in the ring fixator even during the phase of distraction.

4. Patient Factors

Paley[81] discovered that the healing index in adults can be as much as twice as high as in children. Fischgrund *et al.*[67] showed that bone formation occurs sooner and more rapidly in patients younger than 20 years old than in persons between 20 and 29 years old. The latter again shows a higher ossification activity than the group of patients older than 30 years of age. The patients younger than 20 years old did not differ concerning osteoneogenesis in the age groups of 1 to 9 years old and 10 to 19 years old.[67]

The etiology of the leg shortening seems to be of some importance for new bone formation. The patients with poliomyelitis and a patient with amniotic membrane strangulation and a combined thigh-shank dystrophy possessed a less effective bone regeneration during callus distraction. Here trophic disorders mainly of the musculature nature might have been involved . The loss of muscle tonus and active movement leads to diminishing arterial perfusion and stagnation of venous drainage.[82] The impaired circulation is held responsible for growth retardation and marked inactivity-osteoporosis in poliomyelitis[82] and may even slow down new bone formation. The crucial significance of adequate local blood supply for bone regeneration has been confirmed by different authors.[9,59,68]

The local blood supply might have a key function concerning the varying bone formation processes that have been observed. Brutscher *et* *al.*[83] attributed the reduced bone formation in the ventral and medial tibia (during callus distraction of the shank) to the inadequate soft tissue coverage and the poor local blood supply of the bone in that area. In addition injury of the soft tissue during corticotomy plays a crucial role in the reduction of osteogenesis by harming the local circulation. This is supported by the findings of having less bone formation in the surgical access sites at the thigh (despite excellent soft tissue covering) and the tibia. Soft tissue covering is more abundant in the ventrolateral thigh than in the ventromedial shank, which might explain the more pronounced radiograph density differences in the tibia. This hypothesis can be further supported by the observation of smaller differences between lateral and medial or dorsal and ventral radiographic densities respectively in epiphyseal distraction (without surgical exposure of corticotomy) than in callus distraction (with corticotomy). Concerning these 2 surgical methods, bone-forming processes cannot be compared without reservation. Callus distraction induces an intramembranous ossification whereas epiphyseal distraction induces an intramembranous or endochondrol ossification depending on the rate of distraction.[84,84]

In conclusion, good distraction osteogenesis by corticotomy should be done with careful handling of the soft issue, especially the periosteum, in order to avoid local bone defects. Callus distraction should best be done before18 year of age, and a latent period of at least 8 days should be observed. The mean distraction rate is preferred to be 4x0.25 mm per day, and that, when using a ring fixator, full weight load should be achieved if possible. All parameters, most importantly the onset timing of distraction and rhythm, should be adapted individually . If necessary , the distraction rate and rhythm should be varied during the treatment according to the situation.

Technical Applications

1. Limb lengthening

Limb lengthening by distraction osteogenesis obviate the need for bone grafting in most cases. The gradual distraction rate and rhythm may prove to have fewer complications with nerve and muscle at comparable lengthening goals. The tension wire seem to be better tolerated by the soft tissues than half-pins and this allows for the longer time that the distractor must stay in place.(86) Limb lengthening has traditionally been limited to children, but the Ilizanchov method has expanded the age group to adults with proven clinical success, even though adult take longer time to consolidate than children.

Standard lengthening ranges from 4 to 7 cm , leaving more severe conditions for amputation.(26,50,51,87) Because the results with distraction osteogenesis seem more reliable, longer lengthening is considered as first choice in various surgery. As a result, conditions such as proximal femoral focal deficiency, fibular hemimelia, and radial clubhand may now be considered indications for lengthening.(10,22,24,25). The circular fixator maintains mechanical control over the bone fragments in three planes (6 degree of freedom), so that angular and translational corrections are possible in addition to the standard axial distraction. This method is ideal for angular lengthening, as from partial growth arrest, and may be considered for shortening as little as 2 cm if angulation is part of the deformity. The modularity of the external fixator may minimizes some expected complications during lengthening , such as joint subluxation , contracture, and angulation of the fragments. Despite these advances , the complication rate with limb lengthening remains very high.[81] These procedures should be attempted only by surgeons experienced in limb lengthening. The actual external fixator is completely modular and must be designed , constructed, and applied in custom fashion for each patient. The system is not user friendly initially, but after a steep learning curve, this fixator becomes user-friendly to both the surgeons and patients for dealing with more complex problems.[54]

2. Nonunion

Nonunion is classically treated by bone grafting and compression loading with either plates , nails, or casting . If we consider the histology and physiology of a hypertrophic nonunion that has well-vascularized , live bone ends stiffly connected by a fibrocartilage interface , it strongly resembles that as seen in distraction osteogenesis.

Using the Ilizarov method, primary distraction across a hypertrophic nonunion stimulates renewed osteogenesis, which can then be compressed and rapidly healed. Since nonunions often have associated shortening and angulation, distraction not only re-stimulates osteogenesis but also corrected the underlying deformity. Both distraction and compression in these instances are applied gradually at the standard rate and rhythm of optimal biology.[88]

On the other hand atrophic nonunion requires additional treatment. Since the bone ends are hypovascular and contain dead bone with fatty interposition, primary distraction will not stimulate osteogenesis. Primary compression with or without open debridement to bleeding bone must be augmented by a corticotomy in the same bone to increase the local blood flow and also provide a source of new bone to compensate for the lost length. The modular fixator can be adapted for multifocal treatment such as simultaneous distraction osteogenesis and compression of a nonunion. (11)

3.Bone transport

Bone transport is a unique application of this biology. Large intercalary defects in bone from trauma, infection, or tumor can be regenerated by simultaneous distraction osteogenesis and transport of a living segment of bone across a defect.[11] As in the case of the atrophic nonunion, this multifocal treatment involves distraction and eventual compression of the transported bone segment to the other bone segment. Transport can be carried out proximally , distally , or even transversely.

The transport segment is usually the entire cross section of bone but can be a partial fragment to fill a partial gap , such as in cavitary osteomyelitis . If the biological principles are observed (that is , each cut bone surface has a blood supply and distraction is carried out at the proper rate and rhythm by stable external fixation following an appropriate latency), distraction osteogenesis should produced new bone that rapidly remodels to normal bone.

Distraction Osteogenesis in Craniofacial Skeleton

The use of distraction osteogenesis in the craniofacial skeleton was first reported by Snyder *et al.*[27], who used monofocal distraction to lengthen the canine mandible. They described the experimental mandibular lengthening in dogs to repair a 1.5 cm bony defect in the mandibular body.

This work was repeated by Michieli and Miotti[89] in 1977, in the study of "lengthening of mandibular body by gradual surgical-orthodontic distraction". They illustrate lengthening of the mandibular body in two dogs using intraoral appliances.

Kutsevlink and Sukachev[90]reported lengthening of the mandible in the study of "distraction of the mandible in an experiment"

In 1992, successful bone lengthening in craniofacial surgery was first described clinically by McCarthy *et al.*[28]. They begun first clinical trial in 1989 on bone lengthening involving the human mandible using a external device. They reported the use of distraction osteogenesis by extraoral appliances to lengthen congenital hypoplastic mandibles in children.[28] They applied the experimental model, as described by Karp *et al.*[29] and produced a unilateral corticotomy in four children with Nager's syndrome and found bone to form without relapse. The overall effect, on nerve and muscle function was not recorded although they postulated that bone lengthening might ultimately effect the functional matrix for development.

Since then several clinical reports followed such as Havlik and Bartlett;[30] Losken *et al.*[31]and Klein and Howaldt.[32] A variety of techniques have also been reported to lengthen either segment or an entire maxillary or mandibular arches.[33,34,35,36]

Karp *et al.*[29] described the application of an external fixator to distract a unilateral corticotomy site in the canine model. Distraction was produced by 1-mm advancement once a day. They demonstrated longitudinal orientation of collagen , with calcification forming along the tension lines. They showed the existence of osteoblasts along with undifferentiated mesenchymal cells in this "zone of extended bone formation". Likewise , there was a "zone of remodeling " where osteoclasts were prominent and shown to be reshaping the unorganized bone. The "zone of mature bone" demonstrated an area of compact bone adjacent to the well-formed membranous bone of the mandible . This cascade of bone formation paralleled with the findings in long bone. They believed bone development was a result of intramembranous ossification. [91] Others have believed bone formation requires cartilage as a precursor and that the distraction produces bone by endochondral bone formation.[59]

1. Innovation of Intraoral Devices

Although the application of the Ilizarov technique to maxillofacial skeleton shows promise , its use has not been widespread . Extraoral appliances have been effective in clinical cases, but their use has been hampered by many complications.[28,92] These include skin or bone necrosis, pin tract infection, scarring ,facial nerve and inferior alveolar nerve injury and the poor predictability. [28,93]

The complications of extraoral devices such as scarring and formation of granulation tissue can be avoided to a certain degree by proper planning and positioning of the transcutaneous pins, Their presence remains an increased risk of trauma and it is often necessary to construct rather cumbersome external protection. The external nature of the device impair professional or scholastic activities during the period of distraction and consolidation.[94]

Michieli and Miotti[95]have addressed these concerns by the use of a specially fabricated intraoral tooth-borne appliance to provide the necessary distraction. This technique might result in unwanted tooth movement and an unavoidable creation of spaces between teeth in the location of the distraction. The development of intraoral appliances occurred in several centers and authorities as reported by Guerrero,[96] McCarthy *et al.*[97], Chin and Toth[33]and Diner *et al.*[94,98]

McCarthy *et al.*[97] investigated the use of miniaturized bone lengthener that was suitable for intraoral placement along the buccal surface of the mandible of growing canine mandible. They suggested that their intraoral method of mandibular lengthening offered the same advantages of the extraoral lengthening but without the need for a cutaneous incision and resulting scar.

Chin and Toth[33] demonstrated the feasibility and potential advantages of using internal devices for distraction osteogenesis in the management of maxillofacial skeletal deficiencies. The internal distraction devices were used to correct a variety of maxillofacial skeletal deformities in five patients. One patient underwent bilateral Le Fort III advancement by distraction, three patients underwent mandibular ramus lengthening, and one patient underwent segmental alveolar reconstruction by distraction. Development of internal distraction devices is important to address the limitations of currently available biphasic systems . Potential benefits of internal devices include 1) elimination of skin scars caused by translation of transcutaneous fixation pins, 2) improved patient compliance during the fixation or consolidation phase because there is no external component , and 3) improved stability of the attachment of the device to the bone.

Diner *et al.*[98]reported a case in "Intraoral distraction for mandibular lengthening : a technical innovation", used an intraoral distraction device for mandibular lengthening in a 7 year-old girl with hemifacial microsomia. Correction of vertical deficiency of the ramus was associated with expansion of the soft tissue of the jaw, and without visible scar. An intraoral miniaturization of the distraction device was constructed (10g,4.5 mm width,12 mm height and 40 mm length). Through an intraoral vestibular incision, the mandible was exposed in a sub-perriosteal plane, from the condylar neck to the gonion and the distal mandibular body. A complete corticotomy was possible with minimal dissection of the periosteum. The distraction device was placed on the external cortex of the ramus. It was held in position by two bicortical pins (1.6mm diameter) placed percutaneously on both sides of the corticotomy line. The distraction device was connected with a flexible rod (length 30 mm) that turned the distraction jackscrew. This rod was located in the submucosa up to the canine tooth. The rod was intraoral and extramucosal, in contact with the dental crown where it could be gripped for rotation. The rod was covered with silicone to protect the soft tissues of cheek and lips. The material used (stainless steel , TeflonR, Resin,silicone) was sterilized by dry heat. The first prototype of Dinner *et al.*[64]was used in December,1993 in their department to lengthening the hypoplastic mandible ,almost always the hemifacial microsomia patients, and since 1995 (with assistance of Leibinger co.) they have modified the device to its present standardized model(How Medica Leibinger GmbHR, Freiburg,intraoral distractor).[94]

Diner *et al.*[94] reported a case series in nine young patients using intraoral distractors. Seven patients had hemifacial microsomia , one patient had ramus hypoplasia after TMJ ankylosis and one patient had Treacher-Collins syndrome. The amount of mandibular lengthening ranged from 12 to 28 mm depending on the duration of expansion . Retention after expansion , to allow ossification to take place, lasted for 3 week on average . The follow-up period ranged from a minimum of 5 months to a maximum of 44 months. They concluded that insertion of the intraoral distraction apparatus in juvenile cases of mandibular hypoplasia yielded excellent results. No complications occurred, and the method can be highly recommended.

2. Tooth VS Implant Support Distractor Design

Osseointegrated implants were used as distractor to lengthen the canine mandibles was reported by Sawaki *et al.*[99]Five adult dogs were used for the experiment. After the extraction of the left mandibular premolar and molar teeth, two osseointegrated implants were placed. Abutment connection, attachment of the intraoral distraction device , and an osteotomy in the region between the implants were performed 3 months after implantation. The distraction was done at rate of 1mm/day for 10 consecutive days to elongate the mandible to 10 mm. The animals were sacrificed at 2 ,3 ,and 4 weeks after the distraction, radiographic and histologic examinations were done. They found that the longer the time after completion of distraction , the more uniform the new bone that was observed radiographically and histologically in the gap created by the distraction. The titanium implants remained stable during the course of mandibular lengthening. They concluded that an intraoral device using osseointegrated dental implants can serve as a mechanism for distraction osteogenesis in the maxillofacial skeleton.

Block *et al.*[100] reported of using implants for anchorage during maxillary distraction in a canine model. In 1995 they reported use of a tooth-supported distractor to advance an anterior maxilla of a dog. [101] The results of the study indicated that a tooth-borne maxillary distractor will result in significantly greater dental movement than skeletal movement and that skeletal fixation was needed for distractor used to advance the maxilla.

Yamamoto *et al.*[102]reported the feasibility of maxillary advancement by distraction osteogenesis using osseointegrated implants. After extraction of the premolar and molar teeth , four titanium implants were installed in the maxillary alveolar bone. Three months later, the distraction device was connected to the abutments , and osteotomy in the medial portion of maxilla between the implants was performed. Distraction was carried out at the rate of 1 mm per day to obtain a 10-mm elongation. Morphological, radiographic and histological examinations showed that successful maxillary advancement was achieved. New bone was primarily formed by intramembranous ossification and partial endochondral ossification. Titanium implants placed for anchorage of the distraction device remained stable during the course of maxillary advancement. They suggested that this technique can provide significant advancement of the maxilla with improved stability .

3. Reconstruction of Jaw Defect

Distraction osteogenesis seems to be a promising method, not only for correcting mandibular hypoplasia such as that in hemifacial microsomia by lengthening the deficiency mandible, but also for reconstructing segmental bone defects in the mandible.[103]

All of the studies of the mandibular lengthening involved the placement of a single osteotomy through a continuous length of mandible with subsequent distraction across that site to stimulate bone growth. This type of distraction is referred to as monofocal . Monofocal distraction osteogenesis is appropriate only for the lengthening of linear bone. It is not applicable to the reconstruction of substantial segmental skeletal defects.

Reconstructing segmental skeletal defects requires either bifocal or trifocal distraction osteogenesis. The stumps of bone on either side of a segmental defect do not need to be brought into direct contact with bifocal or trifocal distraction, as is required with the monofocal type. Instead, these forms of distraction utilize a transport disk of bone that is cut from one , or both, ends of segmental skeletal defect. The vascular supply to the transport disk is preserved by maintaining the continuity of the periosteum across the osteotomy that was used to create the transport disk of bone. The movement of that vascularized transport disk across the segmental defect leaves a regenerative callus in its wake at a rate of at least 1 mm/ day per transport disk.

The first experimental regrowth of bone using distraction osteogenesis within segmental mandibular defects was reported by Costantino *et al.*[103] In that study, bifocal distraction osteogenesis was found to be successful in filling 2.5-cm mandibular body defects in a canine model with structurally stable bone of diameter comparable with the preexisting mandible. Controls demonstrated that preserving periosteum across a segmental canine mandibular defect that was held in external fixation would not result in functionally significant bone in the time required to fully fill a similar defect with bone by distraction osteogenesis. The dogs were followed for 4 weeks after removal of their distraction appliances, and all demonstrated normal oromandibular function during that time.

Constantino *et al.*[104]used the same canine models to describe the long term 12 month functional ,morphologic and biomechanical results when bifocal distraction osteogenesis was applied. In this long term study , three canines had 2.5 cm unilateral segmental mandibular body detects filled with structurally stable bone using bifocal distraction osteogenesis. These dogs exhibited normal oromandibular function for 1 year following segment regrowth and external fixator removal. Macroscopic and histologic evaluation of the regrown segments revealed a re-formation of the cortical and medullary architecture. Stress testing demonstrated the average ultimate strength of the regrown segment at 53 Mpa, which corresponded to 77% of normal mandibular bone. The data suggest that clinical trials applying this technique to segmental mandibular reconstruction are warranted.

Annino *et al.*[105]demonstrated the feasibility of curved trifocal distraction osteogenesis for reconstuction of mandibular symphyseal defects in the dog. Shvyrkov *et al.*[106]reported the application of distraction osteogenesis in gunshot defects of mandible.

Block *et al.*[107]in "Bifocal distraction osteogenesis for mandibular defect healing" reported the use of bifocal distraction osteogensis to repair the continuity defect of four patients who had previously infected mandibular angle fractures requiring appropriate debridement and fixation and resulting in 15 to 35 mm full-thickness continuity defects in the body region of the mandible.

Sawaki *et al.*[108]reported a clinical case in which an irradiated mandible was reconstructed by trifocal distraction osteogenesis. The authors reported a case in which a mandibular segmental defect, about 60 mm in length, was reconstructed by distraction osteogenesis. The patient was a 45-year-old man who had been treated for an oral floor cancer . After preoperative chemotherapy and irradiation therapy , the mandible had been resected from the second incisor on the right side to the first molar on the left side, and had been reconstructed with a titanium plate and a vascularized rectus-abdominis compound flap. However , an infection developed around the titanium plate and this plate had to be removed. Therefore, trifocal distraction using an original three - dimensional distractor was performed, at the rate of 1 mm per day (0.5 mm in the morning and 0.5 mm in the evening). During the distraction period, the skin flap was pushed out from the bone defect. Although small free bone transplants were needed for complete continuity, the segmental bone defect was almost filled by the regenerated bone with the lengthened gingiva. Radiographic observation showed successful new bone formation in the lengthened area.

Oda *et al.*(109)in the study of "Segmental mandibular reconstruction by distraction osteogenesis under skin flaps" . In five adult dogs , molar were extracted and skin flaps from the neck prepared for delayed transplantation . Two weeks later, a 25-mm segment of the mandible was excised with surrounding periosteum and gingiva . The mandible was stabilized with a reconstruction plate and the intraoral defect repaired with a pedicled skin flap. A proximal transport segment was created and an external distraction device was applied. After one week, distraction of the transport segment began at a rate of 1 mm/day . After distraction was completed, the distractor was left in place for 12 weeks until the dogs were sacrificed. Radiologic and histologic examinations confirmed new bone at the distraction site. The intraoral skin flap was pushed out of the defect as the distraction progressed. Bony union of the transport segment to the distal stump was not achieved due to intervening soft tissue. These results suggested that was feasible to bridge a mandibular defect that was covered with a skin flap bydistraction osteogenesis.

4. Experimental Studies in Distraction Osteogenesis

4.1 Animal model for experimental study of distraction osteogenesis in membranous bone

Study	Animal model	Site
Karahaju-Suanto *et al.*,1990	Sheep	Mandible
Karahaju-Suvanto *et al.*,1992	Sheep	Mandible
Snyder *et al.*,1973	Dog	Mandible
Michieli and Miotti,1976	Dog	Mandible
Costantino *et al.*,1990	Dog	Mandible
Karp *et al.*,1992	Dog	Mandible
Yamamoto *et al.*,1997	Dog	Maxilla
Kumoro *et al.*,1993	Rabbit	Mandible
Califano *et al.*,1994	Rabbit	Mandible
Guerissi *et al.*,1994	Rabbit	Mandible
Stewart *et al.*,1998,1999	Rabbit	Mandible
Persing *et al.*,1990	Rabbit	Craniofacial(skull base)

4.2 Rabbit as the experimental model in distraction osteogenbesis

Bone	Study
Long bone	Alho *et al.*,1982 De Bastiani *et al.*,1986 White and Kenwright,1990
Mandible	Kumoro *et al.*, 1993 Guerissi *et al.*,1994 Califano *et al.*,1994 Stewart *et al.*,1998,1999
Cranifacial	Persing *et al.*, 1990

4.3 Distraction protocol in rabbit model

Study	Bone	Protocol	Length gained
Kumoro *et al.*,1993	mandible	0.36mm/day latency 2wks	8.64 mm
Califano *et al.*,1994	mandible	1mm/day latency 12hours	14 mm
Guerrisi *et al.*,1994	mandible	1mm/day	10-20mm
Stewart *et al.*,1998	mandible	1mm/day* latency 3 days 3mm/day latency 3 days	15 mm 15mm.
Stewart *et al.*,1999	mandible	1mm/day* latency 3 days 3mm/days latency 3 days	15mm 15mm

** better results of new bone formation*

4.4 Apparatus used in rabbit mandibular distraction osteogenesis

Study	Distraction device
Kumoro *et al* .,1993	Unilateral external fixation device (Ortho fix M-100) One self tapping screw on each side
Califano *et al.*,1994	Unilateral external fixation device Two K-wires pin on each side
Guerrisi *et al.*, 1994	Unilateral external fixation device Two K-wires pin on each side
Stewart *et al.*,1998,1999	Bilateral external fixation custom device Two K-wire pin (diameter 1.6 mm)on each side

4.5 Type of ossification in distraction osteogenesis

Bone type	Animal	Site	Type of ossification	Study
Long bone	Dog	Forelimb	Intramembranous	Delloye *et al.*,19990
	Rabbit	Hindlimb	Endochondral*	Kojimoto *et al.*,1988
Membran-ous bone	Dog	Mandible	Intramembranous	Karp *et al.*,1992
	Rabbit	Mandible	Endochrondral* + intramembranous	Kumoro *et al.*,1993
	Rabbit	Mandible	Small foci of endochondral*+intra membranous	Stewart *et al.*,1998

* Endochondral ossification in origin

4.6 Muscle adaptation to distraction Osteogenesis

Study	Bone	Muscle tissue changed
Ilizarov,1989	Long bone, Dog	Hypertrophy and hyperplasia of myocyte
Carlson *et al.*,1989	Mandible, Monkey	Hypertrophy in Type I myofiber (slow twitch)*
Fischer *et al.*,1995	Mandible, Dog	Transient atrophy with initiation of distraction but adapts with compensatory regeneration and hypertrophy completely after 48 days of fixation

* Immediate advancement by traditional 'one stage' orthognathic surgery (saggital split)

In the present study, rabbit is proposed as the model for distraction osteogenesis to lengthen the mandible. Rabbit as an experimental animal is cheap, easier to keep, and ethically better accepted for experiment than dogs or sheep. Moreover, because the bone served as the histologic specimen is very small so it would have been very helpful to see a histologic picture of the whole distracion area on a single histologic section.

Aims of the Study

1. To determine the mineralization process and the mechanical strength of the regenerated bone in distraction osteogenesis process using rabbit mandible model.
2. To determine the effect of distraction osteogenesis process on related muscle following distraction.
3. To assess the stability of the regenerated bone following distraction osteogenesis in rabbit mandible.

Chapter2

MATERIAL AND METHOD

Material

Sixteen healthy 5-7 months of age, New Zealand White rabbits weighting 2.5-3 Kilograms served as the experimental subjects.

Equipment

The distraction device used in the present study was Modified from an orthodontic palatal expansion screw with a capacity of opening up a 10 mm.(Fig. 7). The wire ends of the device were bend and solder to the alloy extension rods which could be fixed to the bony segment by microtitanium screw on each side (Fig. 8).

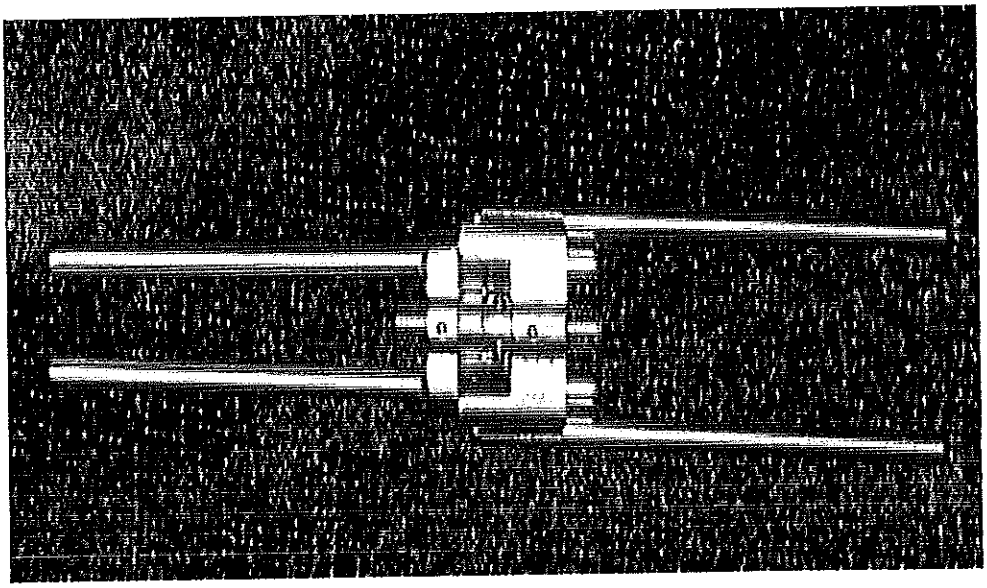


FIGURE 7. Orthodontic palatal expansion screw

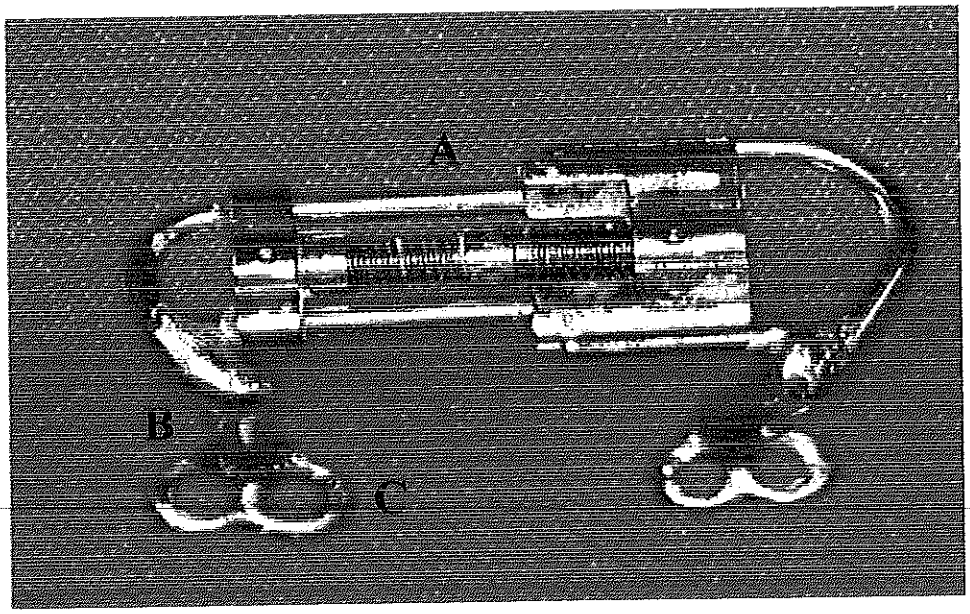


FIGURE 8. Modified distraction device. A= active part; B= extension rod and C= fixation hole for micro-titanium screw.

Method

The surgical procedure was performed under aseptic condition. All animals were anesthesized with an intramuscular injection of Ketamine Hydrochloride (25mg/kg) and Diazepam (5mg/kg) The medications were repeated if needed. The animal was observed to breath spontaneously . The hair at the right submandibular region was shaved . The surgical field was painted with iodine solution , and the animal was draped to allow aseptic access to the right submandibular area. Penicillin G Sodium 0.5 million unit was administered intramuscular preoperatively and each day postoperatively for a total of 3 consecutive days.

The skin in the operative field was injected with 1 ml of 2% Lidocaine Hydroclodride with 1:100,000 epinephrine solution. A linear incision was made along the inferior border of the mandible, approximately of 2 cm in length. The subcutaneous tissue was approached by sharp dissection . The plastysma muscle was reflected in the supraperiosteal plane . Caution was made to avoid injury to the large vessels, especially in the posterior region near the angle of the mandible. Any bleeding was stopped by pressure or ligation. A minimum of periosteal tissue was incised longitudinal along the inferior border of the mandible and then carefully elevated . The subperiosteal dissection was carried on until exposed the body of the mandible from the anterior region to the antegonial notch(Fig.9). The mental nerves were carefully preserved. The vertical osteotomy cut was located vertically from the area posterior to the mental nerve and just anterior to the premolar teeth downward to the lower border of mandible(Fig.10). After the cortex was marked by a small round bur to be the guideline a buccal corticotomy was made by using a small fissure bur. The corticotomy were bounded by bicortical notching on the upper and lower margin of the mandible in order to weaken the more stable areas of the mandible, thus facilitating the subsequent "green stick fracture" . Irrigation by normal saline was used to decrease the heat produced from the rotary bur when the bone was cut. Every effort was made to preserve the inferior alveolar nerve. The bone was fractured by working carefully with a chisel . The mobility of the fragments was checked by rotational movement with the chisel until the complete separation was obtained(Fig.11).

FIGURE 9. Subperiosteal dissection for exposure of the lower border of mandible

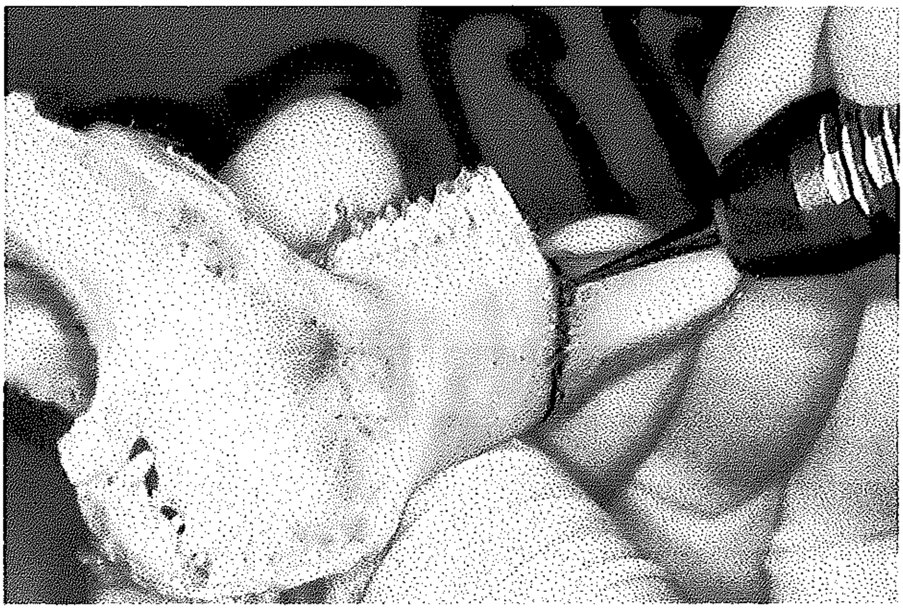


FIGURE 10. Demonstration of the osteotomy line in a dry rabbit mandible

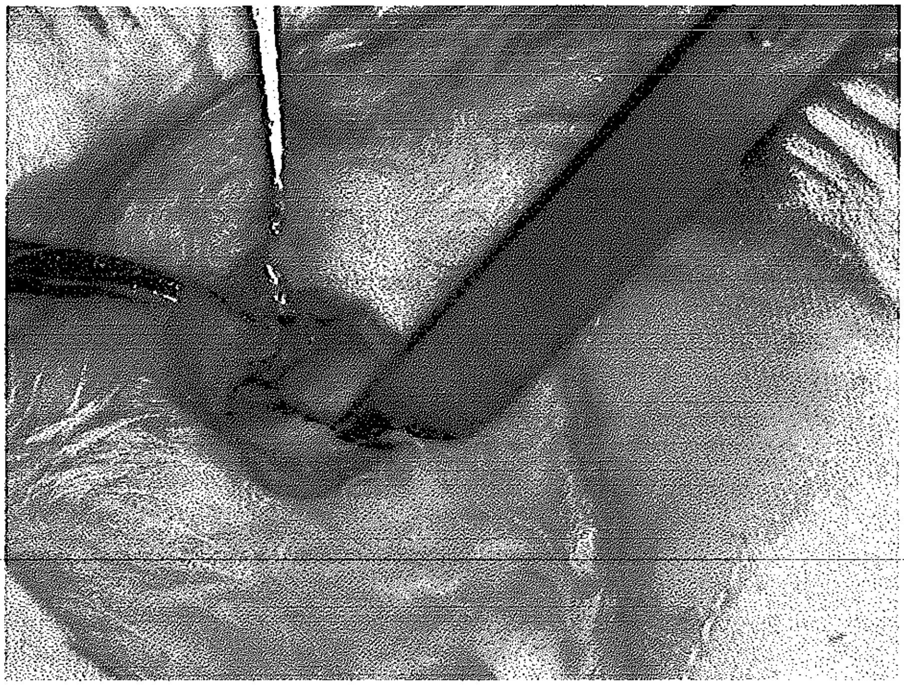


FIGURE 11. Complete separation of the oteotomy with chisel

The distraction device was fixed to the proximal and distal segments of the fracture site with two bicortical self-tapping titanium microscrew on each side(Fig.12). The lingual periosteal envelop was not disturbed during the surgical procedure. After placement of the distraction device was complete, the surgical wound was irrigated with normal saline and all of the bleeding points were checked. The periosteum, plastysma muscle, subcutaneous tissue were closed in layers using resorbable 3-0 sutures. Finally the skin layer was sutured with 3-0 nylon suture with the activation part of the distractor left outside the wound so that it can be used to expand the distraction device(Fig.13). The upper incisor teeth were ground down by approximately on third of their projecting length.

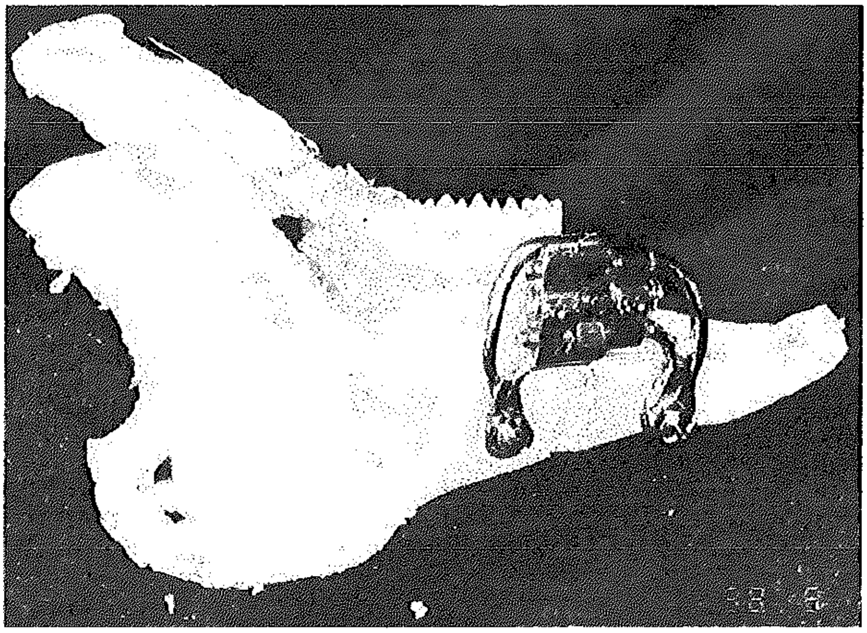


FIGURE 12. Complete fixation of the distractor to the osteotomized mandible

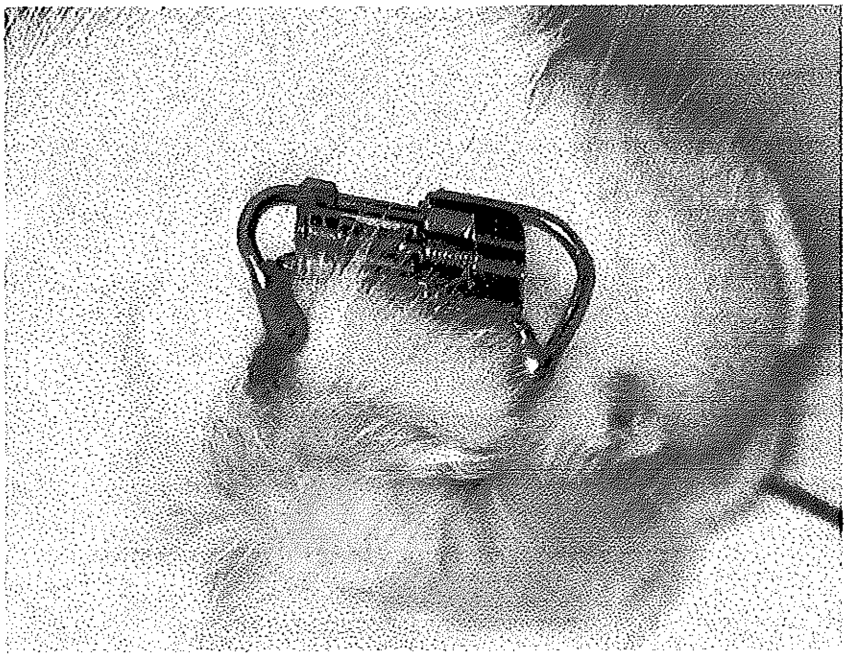


FIGURE 13. Active part of the distractor was left outside the surgical wound

The rabbits were maintained on a diet of milled pellet food from postoperative day 2 until their distraction appliance was removed under intramuscular Ketamine Hydrochloride and Diazepam 6 week later . In this group of experiment , the device was removed and then one screw adjacent to the distracted bone on each side was reinserted into the bone. The distance between the center of the head of screws was measured by the digital caliper (Digital Caliper, Mitutoyo, Tokyo, Japan) and recorded to compare with the distance after the 6 month later in the same animal to determine the stability of the expanded segment. Once the distraction appliance was removed , the rabbits were placed on its regular food which they ate for the remaining part of the study . The surgical site was cleaned once daily with normal saline and betadine solution for the first postoperative week. The antibiotic ointment was applied around the pins to prevent possible pin track infection. The extracutaneous part of the device was protected from the animal scratching by individual face shield made from normal saline plastic container(Fig.14).

FIGURE 14. Plastic face shield

After the osteotomy, the distraction was delayed for 3 days , "the latency period " as per Ilizarov's protocol. Distraction was then initiated with separation of 0.5 mm performed twice a day (1 mm distraction each day) for 10 consecutive days. The activation was performed with gentle restraint of the rabbit but without causing any discomfort.

Before and after placement of the distractor, the lateral cephalogram using the occlusal film and intraoral mandibular cross-sectional occlusal film using pediatric periapical film were taken (10 mA , 50 KVP , 0.34 sec and 10 mA, 50 KVP, 0.42 sec respectively) by dental radiographic machine(Gendex, Gendex Co., Illinois, USA). The parallel cone film holder were used in all of the radiographic examination so that the same position and distance could be reproduced . The radiographs were repeated taken at day 5 ,10 ,and at 2,4,6 weeks after completion of distraction, and 6 months after the distraction appliance was removed. All of the radiographic films were taken by the same dental radiographic machine and then processed by a automatic film processor (Dent X 9000, DentX/Logetronics GmbH, Kornberg, Germany)

The animals were sacrificed in 6 groups for the gross morphologic study, histologic analysis and biomechanical testing as the following sequence: group I (n=2) after 5 days of distraction, group II (n=2) after 10 days of distraction, group III (n=2) 2 weeks after completed distraction, group IV (n=2) 4 weeks after completed distraction , group V (n=2) 6 weeks after completed distraction, group VI (n=4) 6 months after completion of distraction(Fig.15).

Scheme of the experiment (16 rabbits)

Day	Sacrifice time(rabbit No)
0	**insert distractor**
3	**begin distraction**
	day 5 (n=2)
	day 10 (n=2)
13	**complete distraction**
	2 wk (n=2)
	4 wk (n=2)
	6 wk (n=2)
37	**removal of distractor**
	6 months
193	**sacrifice** (n=4)

Note: *Two rabbits were set for control group*

FIGURE 15. Scheme of the experiment

The animals were sacrificed painlessly by intravenous administration of sodium pentobarbitone 1ml/kg via ear vein and then their mandible were removed. The middle part of the anterior belly of diagastric muscle (1 centimeter long) was sharply cut in the cross section plane from the operated side. This muscle specimens were served for the histologic study using cryostat technique to examine the cross section of the muscle fibers. The muscle specimens were cut by the cryostat technique to a 5 micron thickness and stained with hematoxylin and eosin for the study of the cross-sectional muscle fiber area. The histogical cross-sectional muscle fiber of the specimen from the light microscopic image were captured and transferred to the personal computer by the digital camera (JVC TK-C1380, JVC Co., Tokyo, Japan). Three random regions of the same frame area from each slide were served to calculate the average cross-sectional surface area of the muscle fibers using Leica Qwin image processing and analysis system (Leica Imaging System Ltd, Cambridge, England).

Finally , the mandible was separated at the symphysis using the bone cutting machine(Exakt-Cutting Grinding System, Exakt Apparatebau, Norderstedt, England) to form two halves of hemimandible.

Lateral films of each hemimandible were taken (10 mA, 50 KVP, 0.26 sec, 12 inch FFD). These hemimandible lateral films and the previous taken intraoral mandibular cross-sectional films were used for the densitometer measurement. The films were attached to the lighted viewing box, then the image was captured and transferred to the personal computer by the digital camera. The Leica Qwin image processing and analysis system were used to perform the gray measurement. The gray measurement produce information about the gray level (ie. The brightness) of all the pixel in the selected image area. The measuring frame was placed around the lengthened bone area both in the lateral and occlusal films. The program will measure and calculate the mean gray level (Mean Gray = Sum of Gray/Number of pixels measured).The radiograph of the bone just anterior to the premolar tooth in the opposite non-operated side was also captured by the digital camera and processed in the same manner. So that they could be compared with the lengthened side.

The Lengthened bone were cut from the mandible and then divided into the upper and lower parts(Fig.16). The upper part was reserved for biomechanical testing using the Microhardness Tester (Buehler Micromed II, Digital Microhardness Tester, Beuhler Co., England). This specimen was stored frozen in 0.145 M NaCl solution and was thawed to room temperature in the day of testing.The normal bone just anterior to the premolar tooth at the non-operated side were processed in the same manner. The lower part was served as gross morphological examination. The specimens were fixed and decalcified in 10% Formic Acid and embedded in paraffin blocks. Sections of 5 microns thickness of tissue were stained with Haematoxylin and Eosin and examined under light microscope.

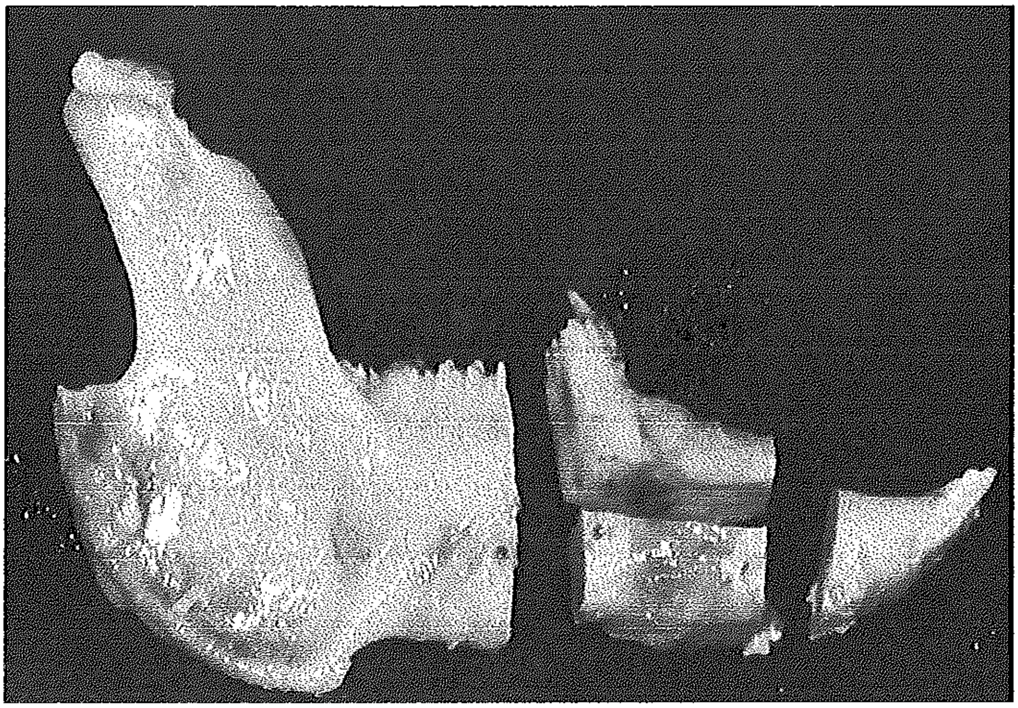


FIGURE 16. Bony specimens for the study

Two rabbits were used to serve as control. The bone from the anterior region to the premolar teeth and the anterior belly of digastric muscle were harvested from both sides of the mandible in the same manner for comparison with each experimental groups.

The quantitative data of the experiment were statistically analyzed by mean of Mann-Whitney test, after combining group I, II and III (n=6) and group IV, V and VI (n=8), in order to achieve a sufficient *n* for statistically analysis. These two combinations was analyzed in order to assess the different between the early stage (group I, II, III) and the late stage group (group IV, V and VI) of the distraction process. The Mann-Whitney U test was used to test the different between these two groups. The statistic significant different was determined when p was lower than 0.05.

Chapter 3

RESULT

Clinical Evaluation

The animals tolerated well with the surgical operation and the anesthesia. There was no accidental death of rabbit encountered. The animals recovered well from anesthesia, a period of anorexia for a couple of days was frequently observed. After this period they were able to eat independently without any disturbance from the distraction device.

The modified palatal expansion screw fixed to the osteotomized bone by 4 micro-titanium screws was very stable permitting easy distraction and minimized any infection problem. The surgical wound healed without evidence of infection. The face shield prevented dislodging of the distractor from the animal scratching. However, some rabbits developed localized pin track infection, which was treated by wound dressing and application of chloramphenicol ointment. The activation of the device twice a day was easy and well tolerated by the animal without any restraint. After 10 consecutive days of distraction, the rabbits developed a severe cross bite of the anterior teeth and overgrowth of the incisors caused by the continual eruption of the teeth (Fig.17).

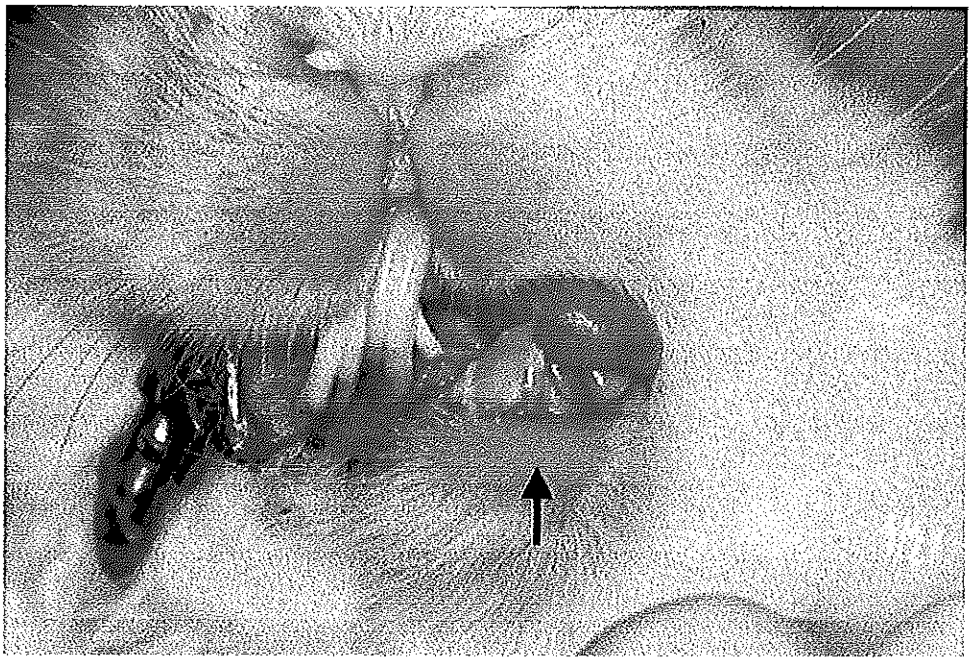


FIGURE 17. Clinical appearance of the rabbits after completion of activation, the clinical obvious cross bite of the incisor teeth and the deviation of the chin to left side (arrow) were developed as the result from the lengthening of the mandible on the right side. The overgrowth of the incisors teeth also was obviously noted.

After completion of activation, the animals were able to take its regular food for the remaining part of the study. No significant weight loss was observed in the experimental animals.

The 10 consecutive days of distraction were achieved in all animals without any failure of the distractor. Mechanical testing on the strength and the relapse of the device after used in the experiment were studied. The results were shown in the Appendix.

Gross Morphological Evaluation

Distraction produced bone elongation up to 10 mm. Clinical observation of the mandible after removal of the attached soft tissue showed a lengthened gap filled with new callus-like tissue. The distracted side of the mandible was significantly longer than the non-operated contralateral side (Fig.18).

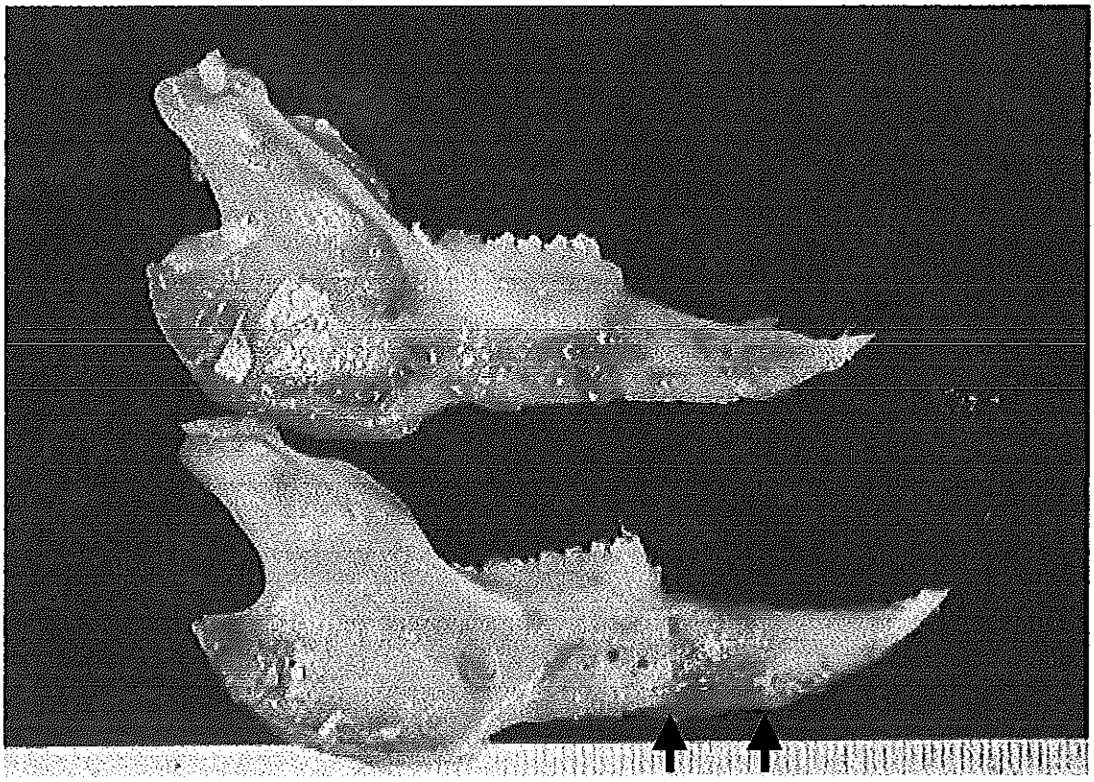


FIGURE 18. Comparison of the length of the hemimandible in same animal after completion of distraction. The distraction hemimandible (lower) was marked longer than the non operated side (upper). The arrows showing the regenerated tissue in the lengthened gap.

At completion of distraction

The regenerated tissue in the distraction gap had the grayish-red color, and this made it easily distinguished from the normal adjacent bone stump. The tissue presented with the consistency similar to fibrous connective tissue. The shape and size of the newly formed tissue corresponded to the adjacent osteotomized bone end (Fig.19).

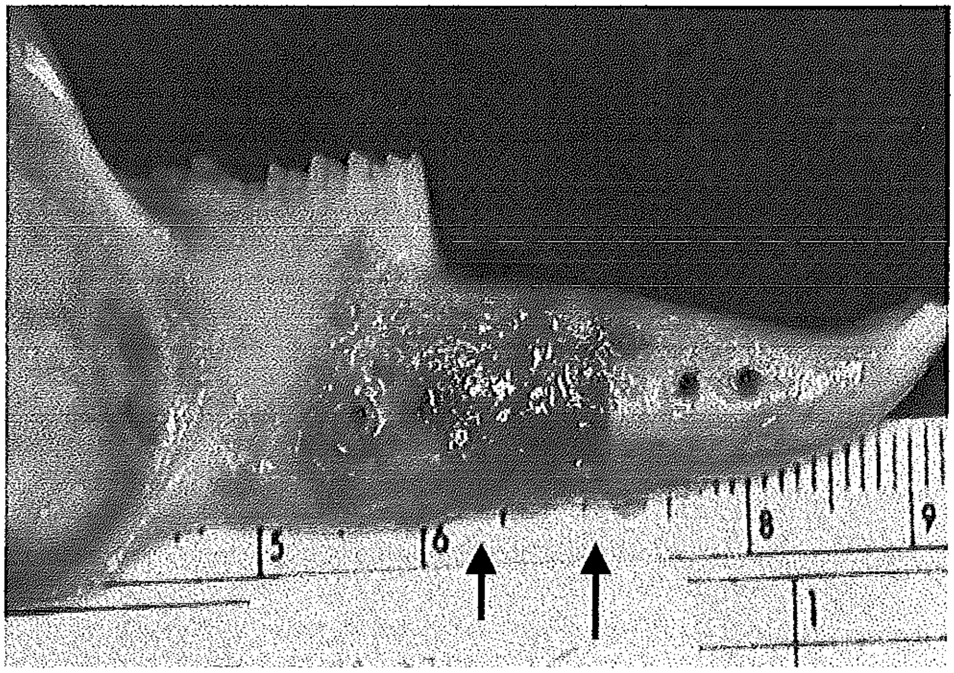


FIGURE 19. Clinical feature of the hemimandible at completion of distraction. The newly formed tissue between the arrows was demonstrated. Fibrous connective tissue appearance and consistency were noted.

At 2 weeks after completion of distraction

By 2 weeks after completion of distraction, the soft grayish-red tissue in the expanded gap was decrease in length due to replacement by callus-like tissue from both osteotomized bone stumps. The appearance of the outer surface of the lengthened bone could be distinguished from the adjacent normal bone. The consistency of the regenerated tissue became firmed than at completion of activation. However, the hardness was less when compared with the normal side of mandible (Fig.20).

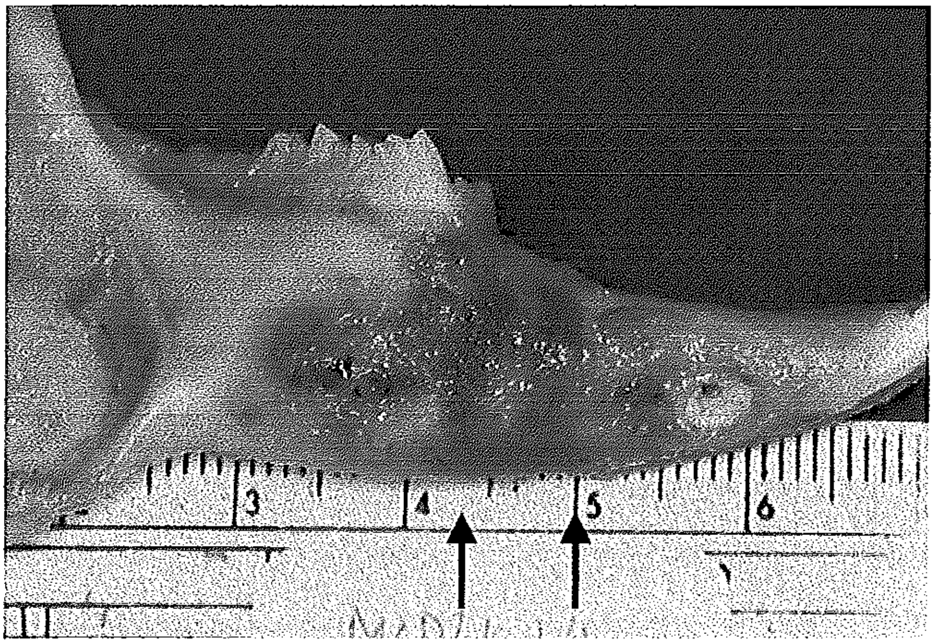


FIGURE 20. Clinical appearance of the hemimandible at 2 weeks after completion of distraction. The soft grayish-red soft regenerated tissue was decrease in size resulted from formation of callus-like tissue on both bony stumps (arrows).

At 4 weeks after completion of distraction

By 4 weeks after completion of distraction, the clinical appearance of the tissue in the lengthened portion became more difficult to be distinguished from the adjacent normal bone. The tissue looked like callus bone formation than fibrous connective tissue as in the earlier stage. Although the consistency of the newly formed tissue in the gap became harder than the 2 weeks period, it was still softer than the normal adjacent bone (Fig.21).

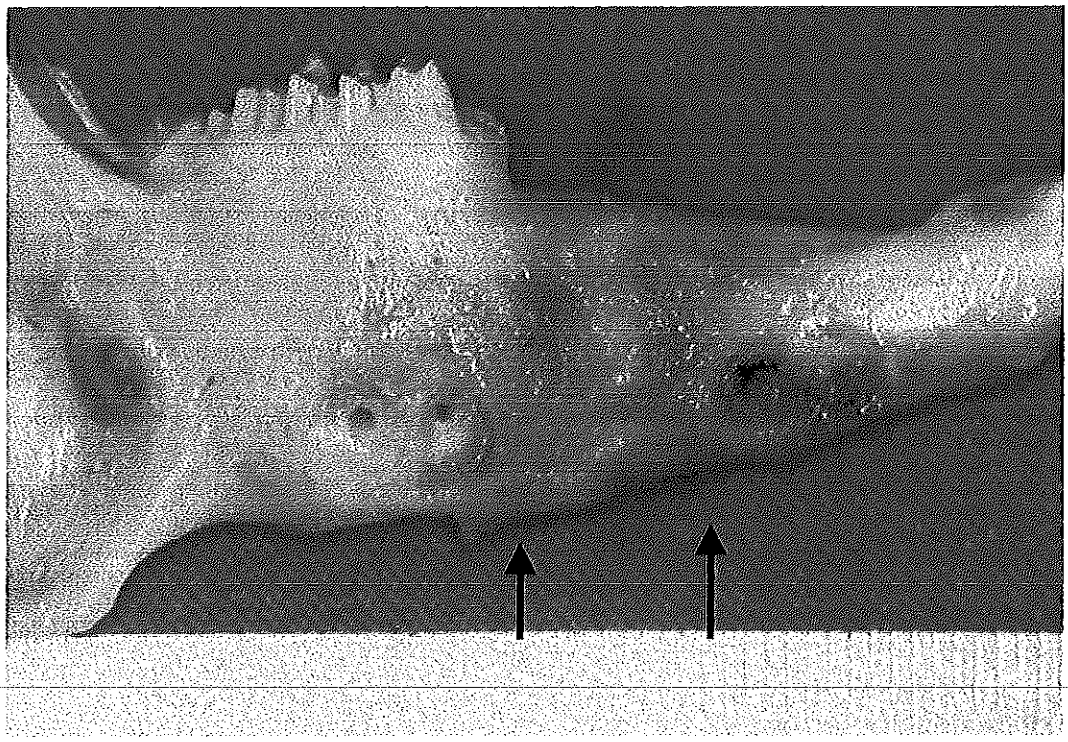


FIGURE 21. Clinical feature of the hemimandible at 4 weeks after completion of distraction. The tissue in the gap (arrows) increased in hardness and the clinical appearance became look like the preexisting normal bone stump both sides.

At 6 weeks after completion of distraction

By 6 weeks after completion of distraction, the expanded area was not readily discriminated from the preexisting bone. The regenerated tissue had both the structure and consistency nearly similar to the normal mandible (Fig.22).

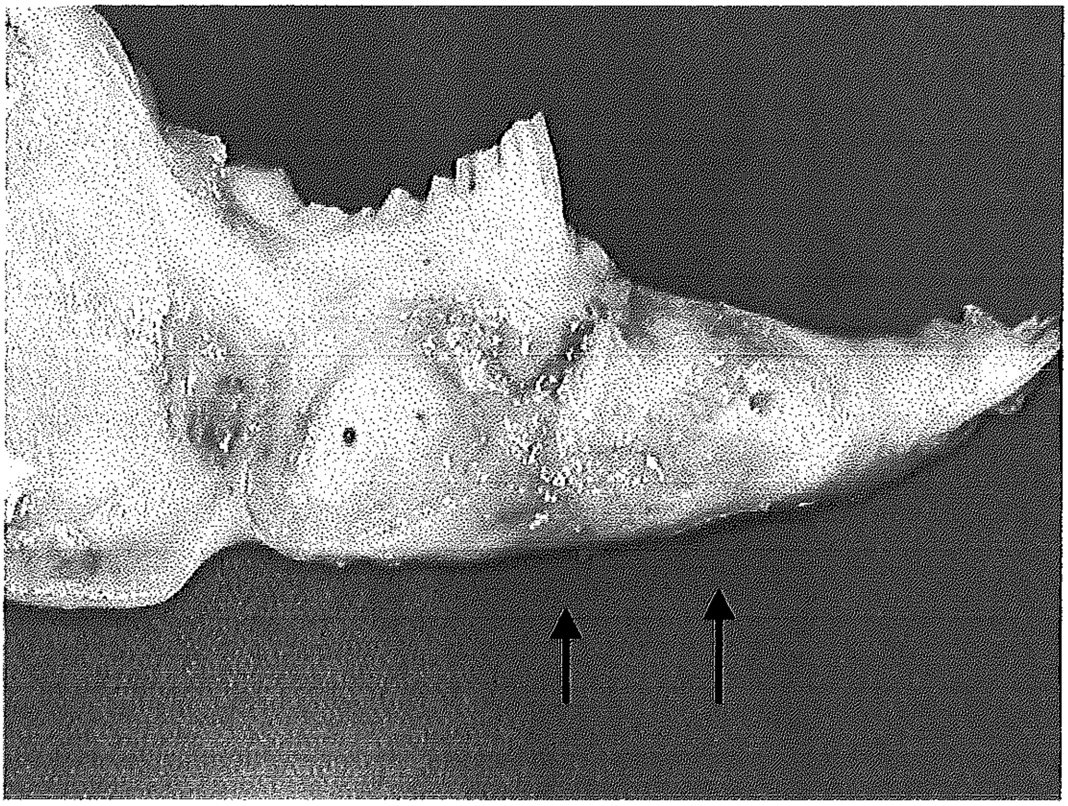


FIGURE 22. Clinical feature of the hemimandible at 6 weeks after completion of distraction. The gap tissue (arrows) had both the physical structure and the hardness nearly similar to the normal mandible.

At 6 months after completion of distraction

By 6 months after completion of distraction, the regenerated tissue had both the gross morphological structure and hardness as same as the normal mandibular bone. At this stage, the lengthened tissue in the mandible was completely indistinguishable from the adjacent osteotomized bone stump on both sides (Fig.23).

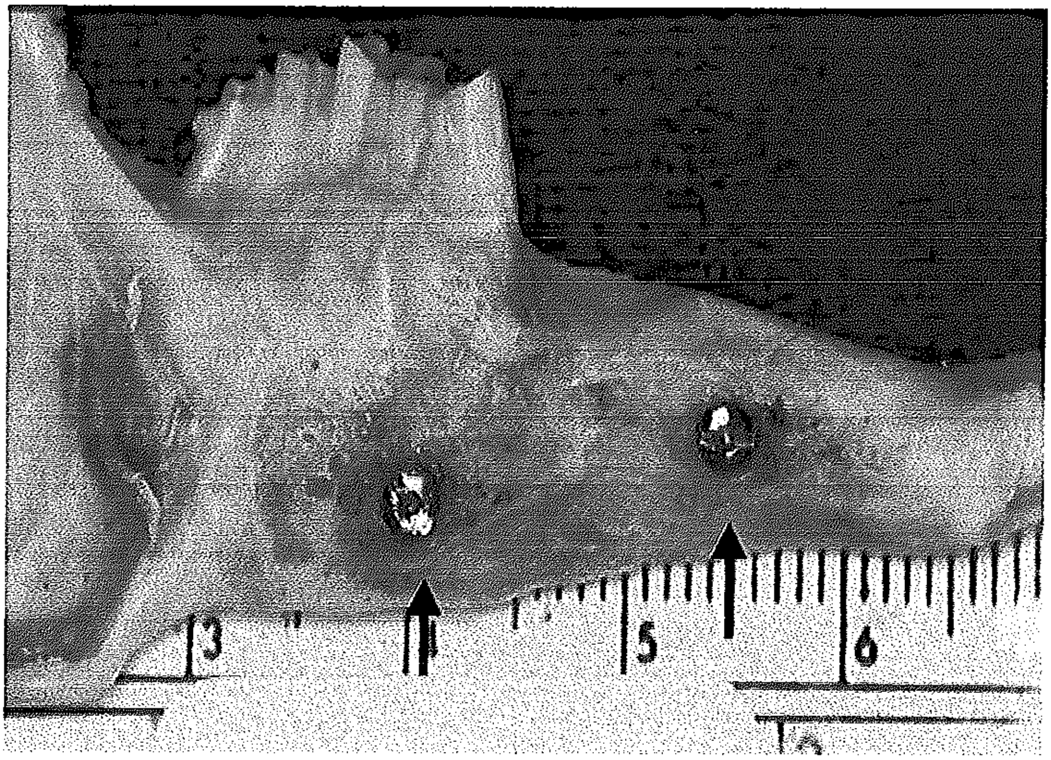


FIGURE 23. Showing clinical appearance of the hemimandible 6 months after completion of distraction. The newly formed tissue between two titanium screw marking (arrows) posed the gross morphological appearance and hardness similar to the adjacent normal mandible. Note the discrimination between the regenerated tissue in the gap and the normal osteotomized bone on both sides was completely impossible.

The summary of the sequence of gross morphological features was showed in Table 1.

Table 1. Summary of the gross morphological feature according to timing of the experiment.

Parameter	**Time**				
	Complete distraction	**2 weeks**	**4 weeks**	**6 weeks**	**6 months**
Gross morphology	Soft grayish-red	Callus-like tissue on both side of gap	Increase hard tissue appearance and consistency	Structure and consistency almost nearly the same as the normal mandible	Completely undistinguishable from adjacent osteotomized stump

Radiographic evaluation

The radiographic examinations were performed during the distraction and after the distraction experiment using the intraoral cross-sectional occlusal film when the animals were still alive and the lateral hemimandible view after the animals were sacrificed.

At 5 days of distraction

By 5 days of distraction, a 5 mm radiolucent gap was established. No radiopaque area was showed in the distraction gap. The micro-titanium screws secured the distraction device penetrated bicortically (Fig.24).

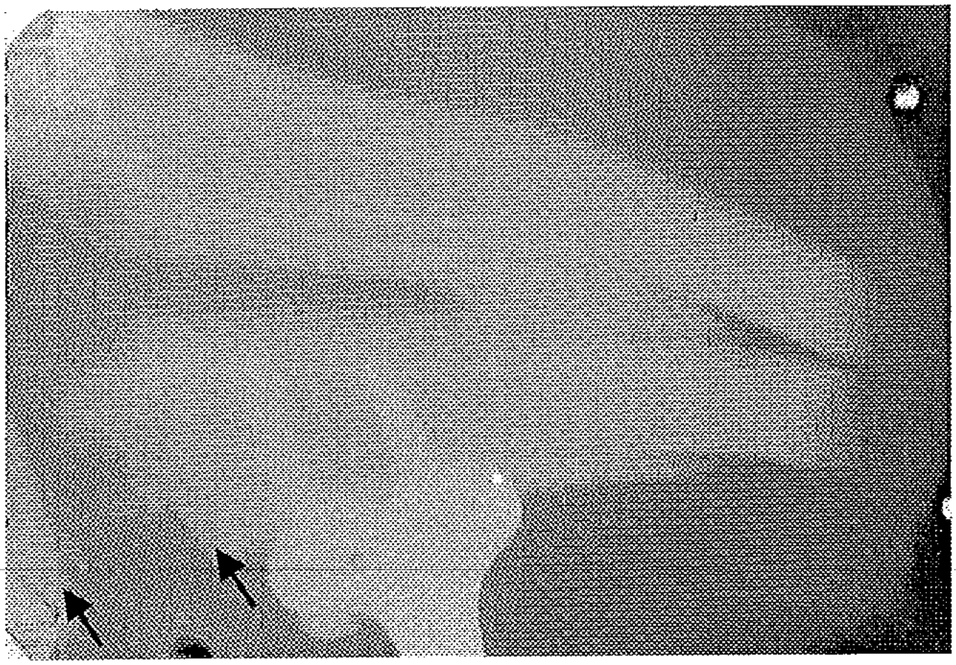


FIGURE 24. Intraoral cross-sectional occlusal film at 5 days of distraction demonstrated radiolucent gap between the osteotomized bone stump (arrows). No any radiodensity are was noted throughout the expanded gap in this stage.

At completion of distraction

At completion of distraction, both lateral and occlusal film demonstrated three zones which were quite readily separated from each other and from the adjacent bony stump. This zonal structure comprised of an anterior and a posterior radiopaque region with a central radiolucent band. These radiopaque and radiolucent zones were approximately equal in size (Fig.25,26).

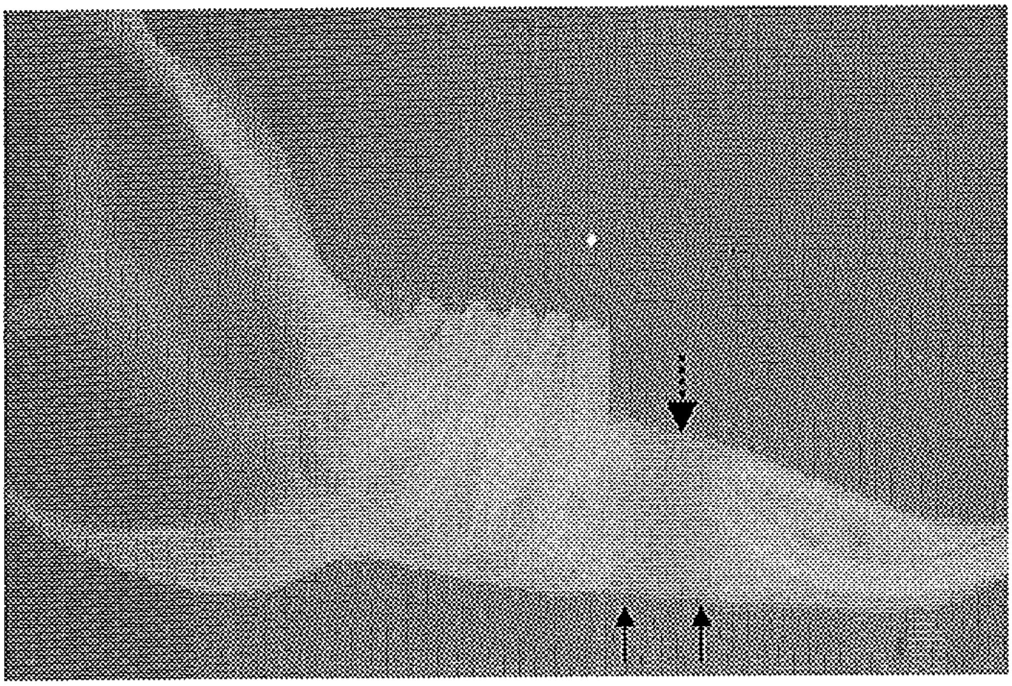


FIGURE 25. Lateral hemimandibular film at completion of distraction, the three zonal structure in the expanded gap which comprised of 2 peripheral sclerotic zones near the osteotomized bone stump (black arrows) and central radiolucent zone (dash arrow) were demonstrated.

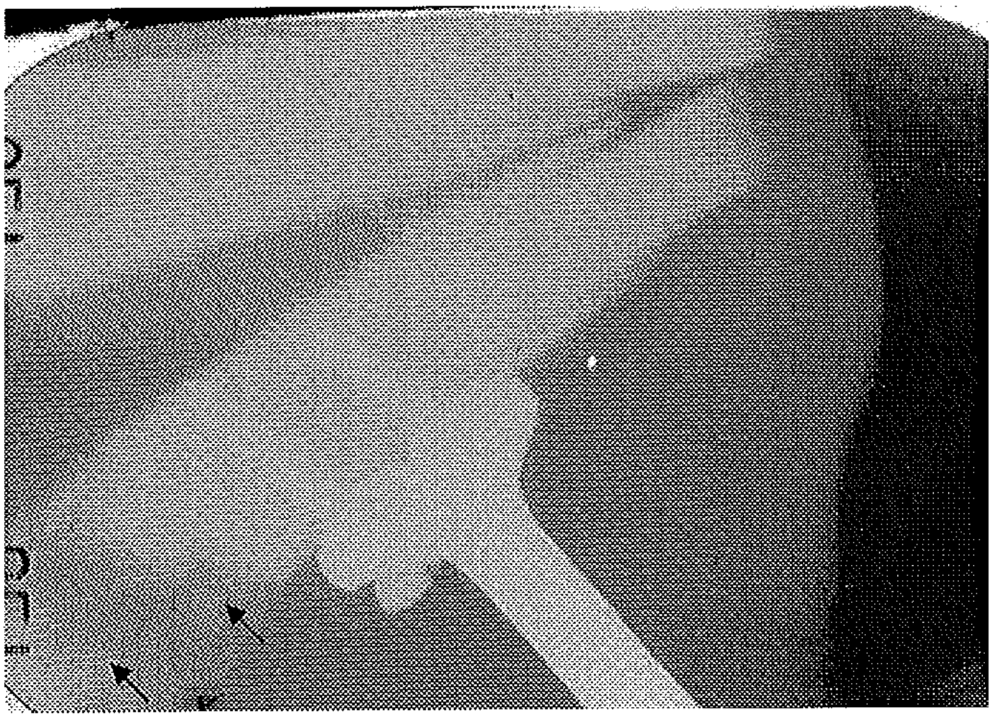


FIGURE 26. Intraoral occlusal film at completion of distraction, showing the same characteristic structure as in lateral hemimandibular film. Note the delicate radio-opaque streak extending from the osteotomized bone stump on both sides (arrows) toward the radiolucent central region but not cross this zone.

At 2 weeks after completion of distraction

By 2 weeks after completion of distraction, the radiopaque area was scattered throughout the distraction gap. As a result, the radiolucent central zone disappeared in this stage. The scattered radiodensity was distributed evenly in both cortical and medullary regions (FiG.27). The radio-opaque streaks extending from the osteotomized bone stumps tended to run parallel with the distraction vector and this was clearly noted in the cross-sectional occlusal film (Fig.28).

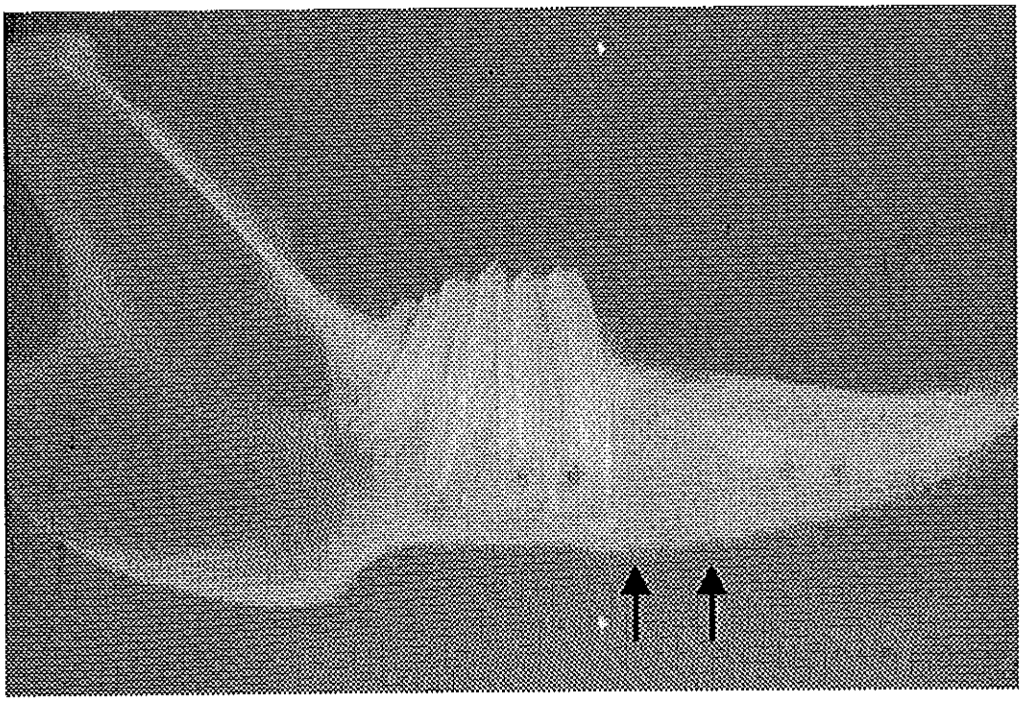


FIGURE 27. Hemimandibular lateral film at 2 weeks of distraction, showing radiopaque area scattered throughout the expanded gap (arrows). Neither radiographic structure of both cortical layer nor medullary cavity of normal mandible was seen in this stage.

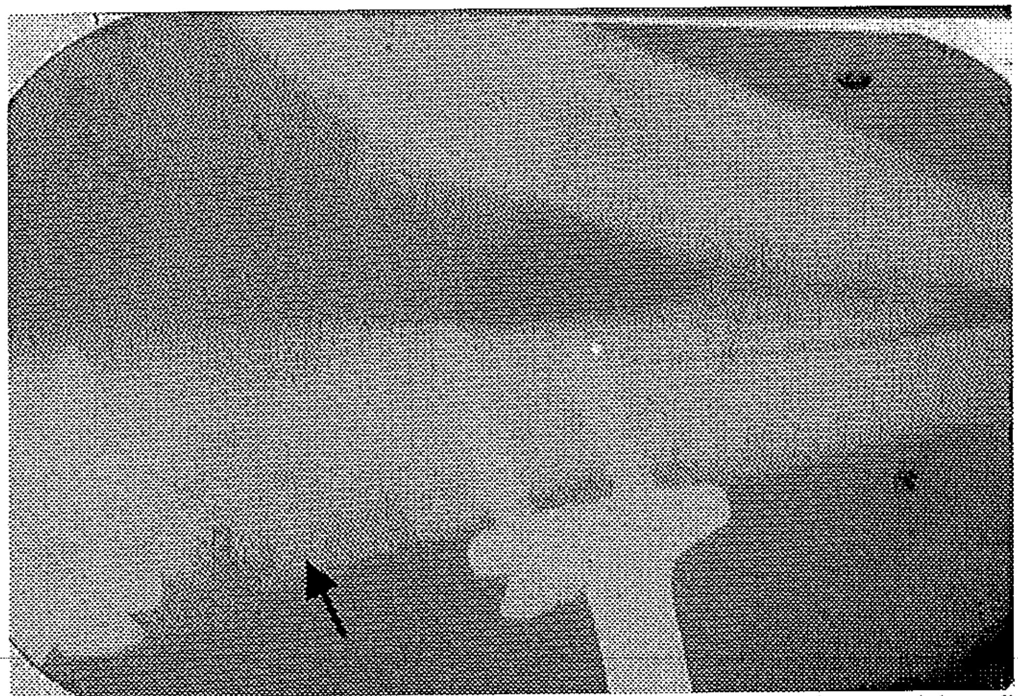


FIGURE 28. Intraoral occlusal film at 2 weeks after completion of distraction, noted the radio-opaque streaks which tended to aligned themselves in a parallel manner with each other and with the distraction vector. (arrow)

At 4 weeks after completion of distraction

At 4 weeks after completion of distraction, the thin radiopaque cortical layer was seen on both upper and lower border in the distracted segment (Fig.29). The radiodensity area in the distracted site was increased but still slightly less than the normal mandible. However, the typical aligment of radio-opaque streaks being parallel to the vector of distraction was still observed (Fig.30).

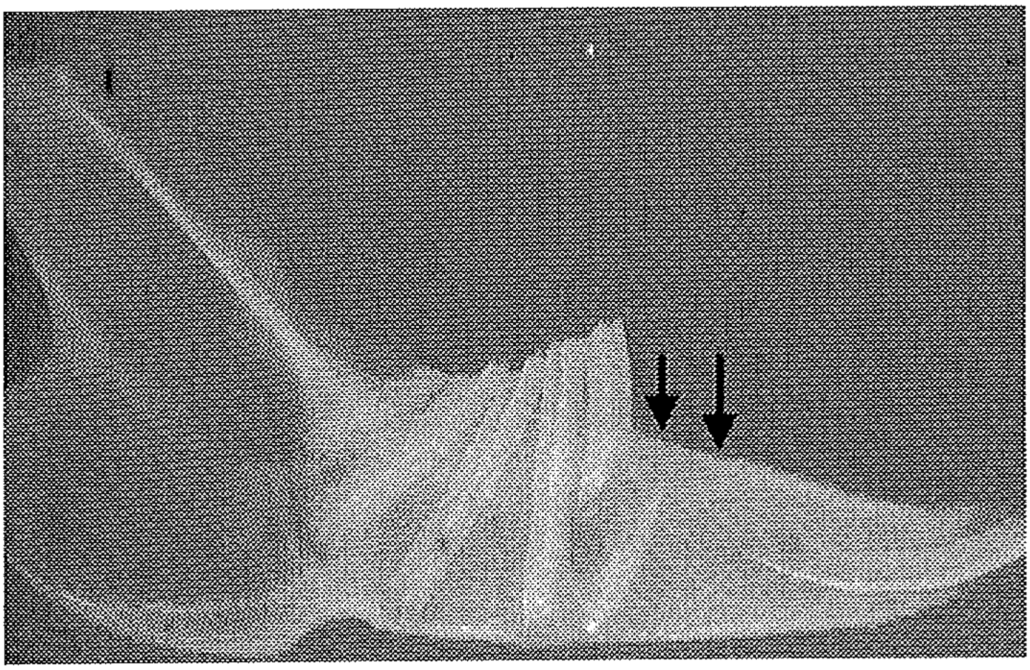


FIGURE 29. Lateral hemimandibular film at 4 weeks after completion of distraction , noted the thin delicate radiopaque layer at the upper and lower border of the lengthened gap.(arrows) The expanded area showed slightly less radiodensity than did the preexisting mandible.

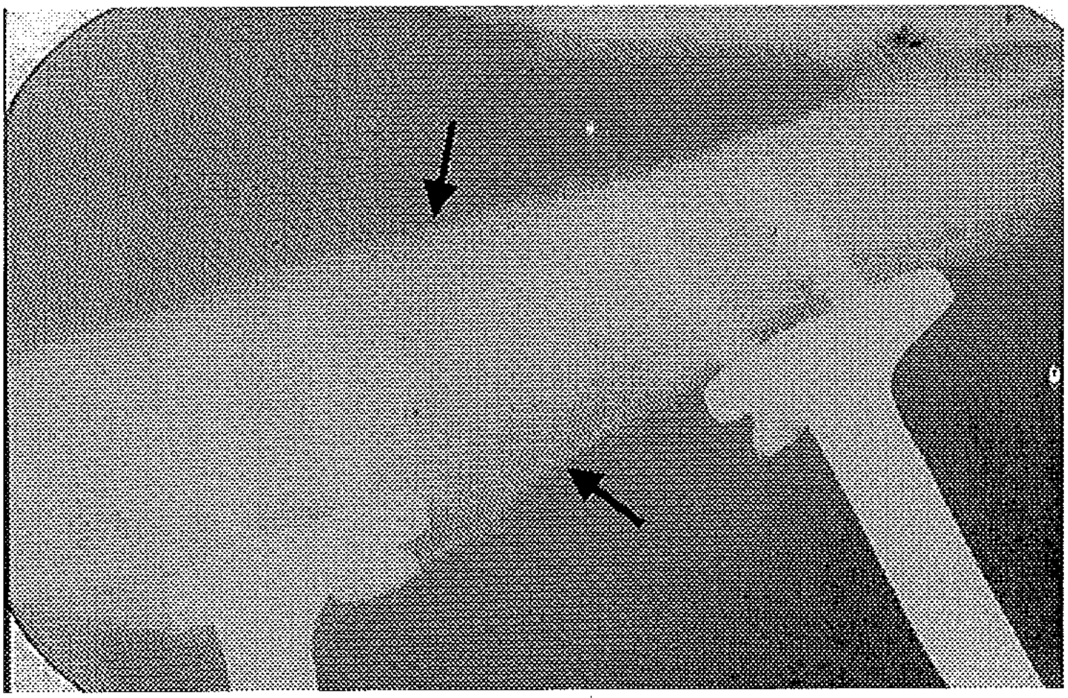


FIGURE 30. Intraoral occlusal film at 4 weeks after completion of distraction, noted the increase of radiodensity when compared with the 2 weeks group. The paralleled radiopaque streaks with the distraction vector still could be observed. The arrows showed the established thin cortical layer on both sides of the outer layer of the expanded gap.

At 6 weeks after completion of distraction

By 6 weeks after completion of distraction, the radiodensity of the distraction gap kept increasing until it achieved the same level of density when compared with the normal mandible. The thickening and the continuity with both adjacent osteotomized bony stumps of the outer cortical layer also were observed (FiG.31,32).This led the distracted bony segment indistinguishable from the adjacent normal mandible in this stage.

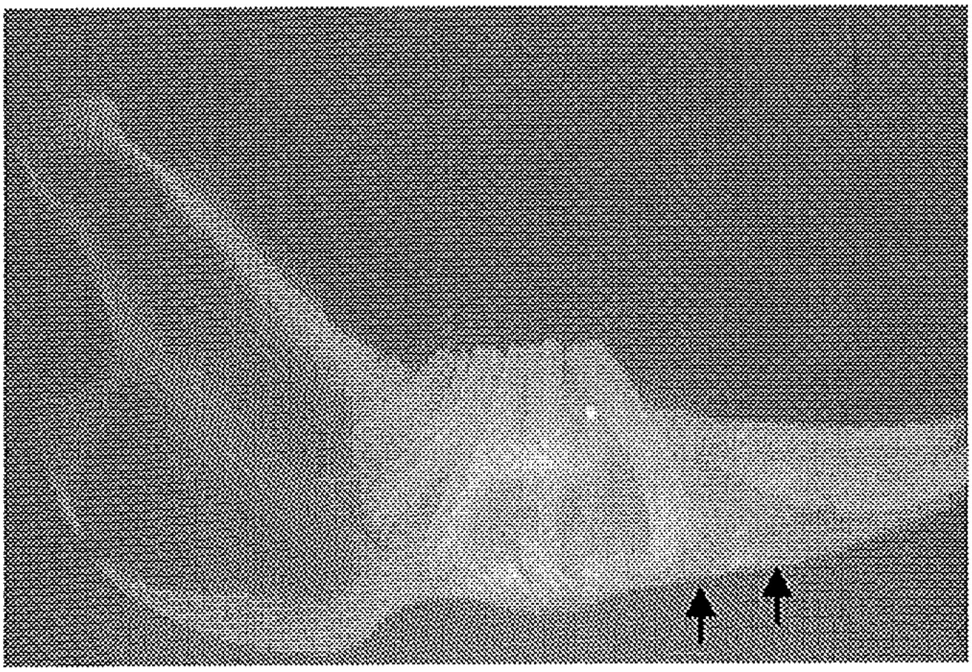


FIGURE 31. Lateral hemimandibular film at 6 weeks after completion of distraction, the radiodensity in the lengthened gap (arrows) increased to the level as the normal adjacent bone. The arrows showed the thickening and continuity to the adjacent bone stumps of the outer cortical layer in expanded gap.

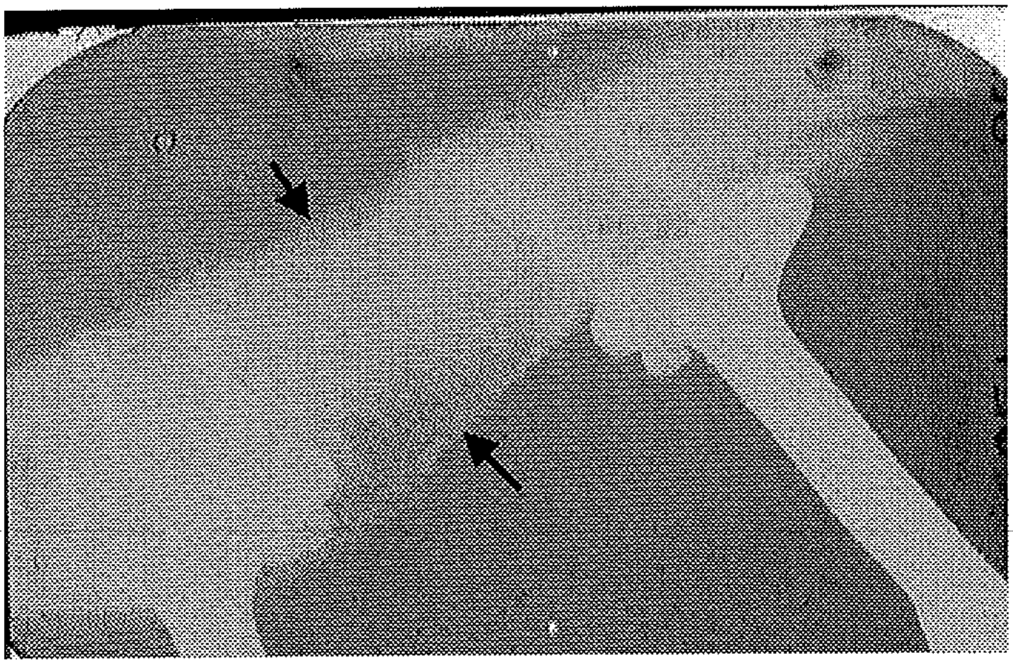


FIGURE 32. Intraoral occlusal film at 6 weeks after completion of distraction, showing the increase radiodensity in the distraction gap. The arrows showed the thickening and continuity of the outer cortical layer with the adjacent bone stumps.

At 6 months after completion of distraction

By 6 months after completion of distraction, the cortical bone was increased in radiodensity and thickening to form a fully mature cortex. It had the same size and continuity with the adjacent normal bone. The marrow cavity in the central region of the expanded gap decreased in radiodensity, resulted from the remodeling process to establish the medullary complex as found in the normal mandible. In this stage, the expanded area possessed all of the radiographic features of the cortico-medullary structure similar to the normal bone (Fig.33,34).

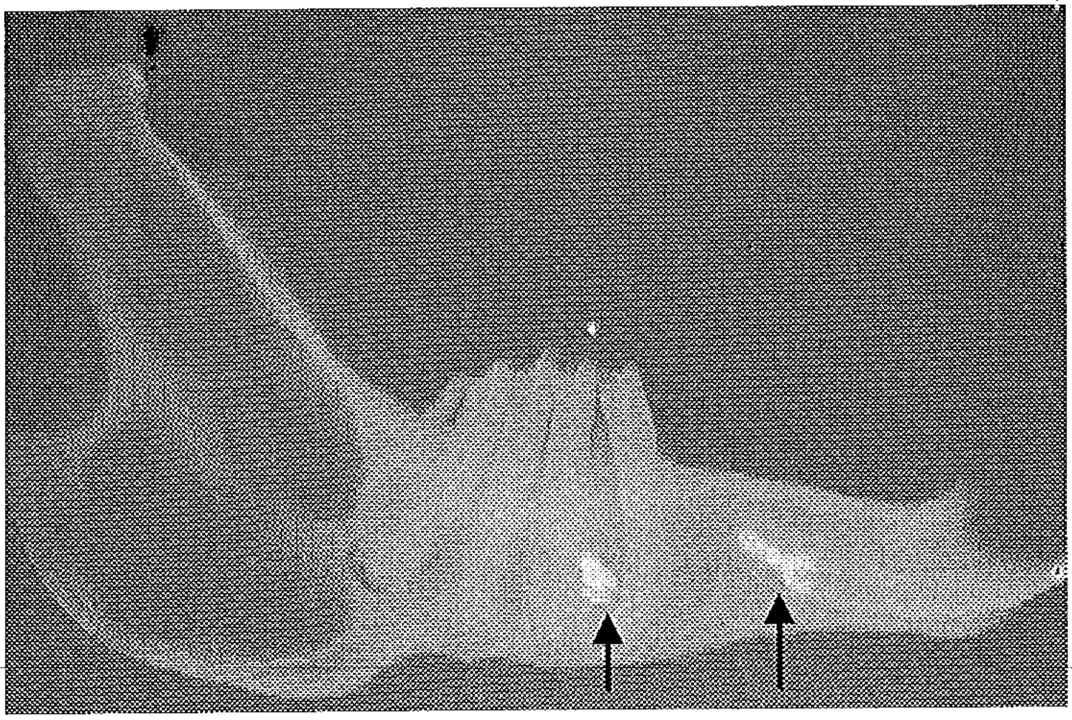


FIGURE 33. Lateral hemimandibular film at 6 months after completion of distraction, noted the definite similarity of the radiographic structure both in outer cortical layer and inner medullary cavity of the expanded gap demonstrated between the two marked screws. (arrows)

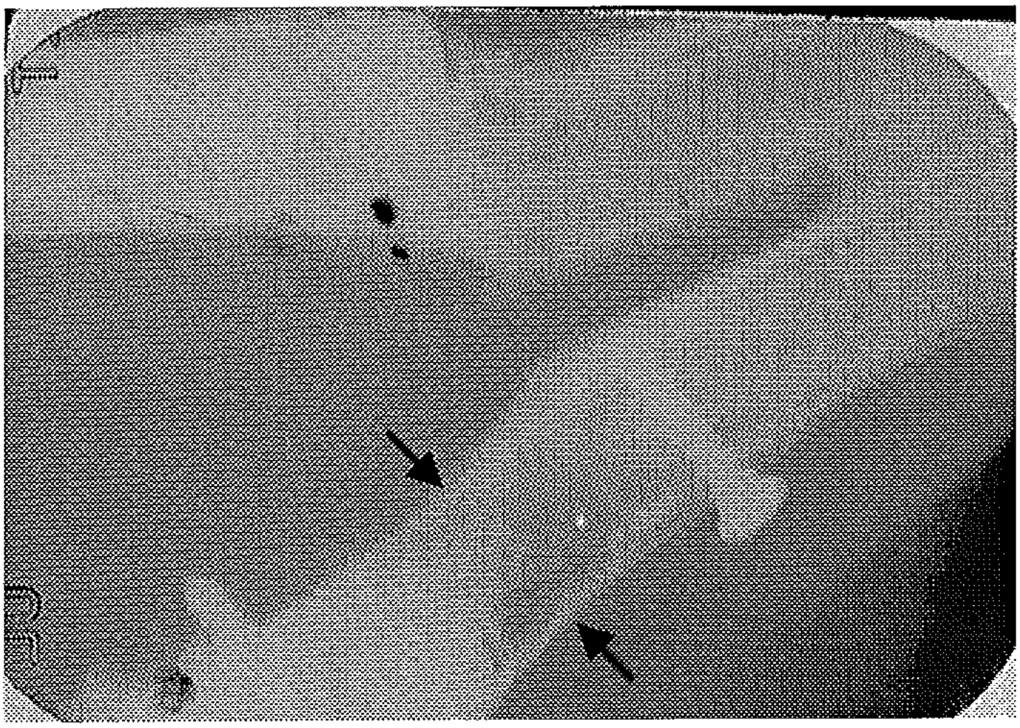


FIGURE 34. Intraoral occlusal fim at 6 month after completion of distraction, showing the fully mature cortex on both sides. (arrows) The radiopacity in the inner region of the lengthened gap was decreased which caused by the normal remodeling process to form the medullary cavity was seen. The parallel radio-opaque streaks feature was disappear. The cortico-medullary complex of the normal mandible was completely established.

The summary of the sequence radiographic features was showed in the Table 2.

Table 2. Summary of the radiographic feature sequence according to timing of the experiment.

Parameter	**Time**				
	Complete distraction	**2 weeks**	**4 weeks**	**6 weeks**	**6months**
Radiograph	Three-zonal structure	Radiopaque area scattered through out the expanded gap	Increase radiodensity in the distraction gap. Thin continuity radiopaque cortical line	Increase radiodensity in the expanded gap to the same level as the normal adjacent bone. Thickening of the cortex	Normal mature radiographic appearance of cortico-medullary structure.

Bone Density of the Distracted Mandible

The radiodensity of the regenerated bone was measured from the serial occlusal films when the animals were still alive and lateral hemimandibular films after the animals were sacrificed. The bone density just anterior to the premolar tooth in the non-operated side was measured in the same manner. The densitometry values presented in mean gray level of the lateral hemimandibular and occlusal films were showed in the table 3 and 4 respectively. These results represented the distraction gap bone density in groups according to the timing of the experiment. The bone density of the bone just anterior to the premolar tooth in the opposite non-operated side was also showed for comparison. Statistic analysis of the distraction gap mean gray level both lateral and occlusal films revealed the early stage group (< 4 weeks group) to be significantly less density than the late stage group (≥ 4 weeks group) ($p < 0.05$). No difference was found between the late stage group and control.

Table 3: Mean (±SD) gray level values of the expanded gap of the lateral hemimandibular films.

Group	Time	
	<4 weeks (n= 4)	≥4 weeks (n=8)
Distraction	100.63±37.87	153.01±17.65
Control	161.35±6.92	164.24±5.73

Table 4. Mean(±SD)gray level values of the expanded gap of the intraoral occlusal films.

Group	**Time**	
	<4 weeks (n=13)	**≥4 weeks (n=8)**
Distraction	110.15±45.24	162.12±8.93
Control	167.51±9.47	169.52±2.48

Biomechanical Evaluation

After the mandibular specimen was thawed to the room temperature, the upper part of the bony specimen was mounted to a acrylic mold with the buccal cortex facing up. The bone surface was smoothed by an abrasive paper as needed to obtain the regular surface for testing. Then the surface hardness of the distracted tissue was tested by the microhardness tester (Buehler Micromed II, Digital Microhardness Tester, Beuhler Co., Ebgland), using the Vicker diamond indentor. The Vicker's hardness of the specimen was calculated by the following formula.

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

$$= \frac{2F \sin (0/2) \times 1000}{d^2}$$

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

HV = Vicker Hardness
F = Test load (gf)
d = Arithmetic mean of the two diagonals d1 and d2 (microns)
0 = Angle between the opposite faces at the vertex of the pyranmidal indentor (136 degree).

Three random areas in the lengthened gap 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. The same procedure was performed in the bone just anterior to the premolar tooth of the opposite non-operated side in the same animal. The Vicker hardness of both the lengthened segment and the normal bone in the opposite side were calculated and recorded. The results were showed in figure 35. The statistical analysis of the expanded gap tissue revealed significantly less surface hardness of the early stage (<4 weeks) when compare to the late stage group (≥ 4 weeks) ($p<0.05$). In addition, the late stage group also showed significantly less surface hardness when compare to control ($p <0.05$).

Table 5. Mean (± SD) Vicker's surface hardness values of the expanded gap tissue.

Group	**Time**	
	< 4 weeks (n=4)	**≥ 4 weeks (n=8)**
Distract	7.50±4.78	14.82±3.66
Control	21.10±1.78	20.78±1.29

Histologic Evaluation

1. Bone Specimens

Bone specimens for the histologic study were harvested included both regenerated tissue in the lengthened gap and the native bone stump near the osteotomy site on both sides. The specimens were sectioned in the coronal and transverse plane.

Latency period

During the latency period, necrotic tissues, fibrin and inflammatory cell infiltration filled the gap of the osteotomy site. The predominant histologic feature of this stage was the presence of the amorphous matrix in the osteotomy site (Fig.35).

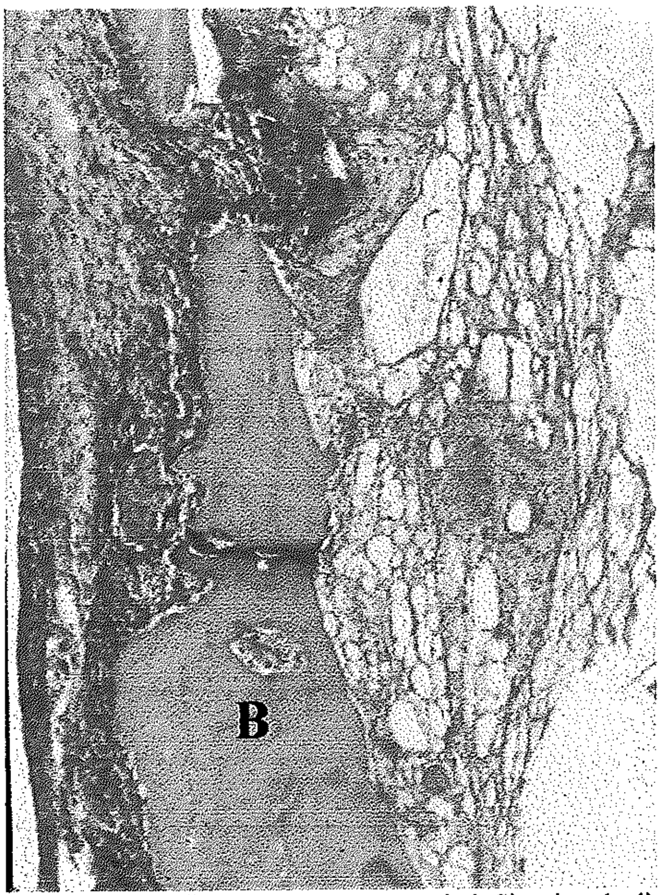


FIGURE 35. Histology at immediate post-osteotomy period. Showing the liquifaction necrosis in the osteotomy site. The fibrin and infiltration of the inflammatory cells were noted. The original bone stump (B) was seen on the left side. (H&E stain)

At 5 days of distraction

By 5 days of distraction, numerous of spindle shaped mesenchymal cells, which resembled to fibrosblast cell, were seen throughout the expanded gap. This spindle cells with dense collagen production arranged themselves parallel to the distraction vector as the distraction begun. In some area, the mesenchymal cells formed buds that led to the development of a primitive microvasculation. In this stage, the predominant histologic appearance was a rich fibrillar matrix of collagen. Neither osteoid nor osteoblast was observed in the expanded gap. In some areas, the collagen bundles became denser and less vascular almost resembling the tendon. Finally, the spindle-shaped fibroblasts and the collagen bundles, which oriented parallel to the distraction force, established bridging of both bone surfaces (Fig.36,37).

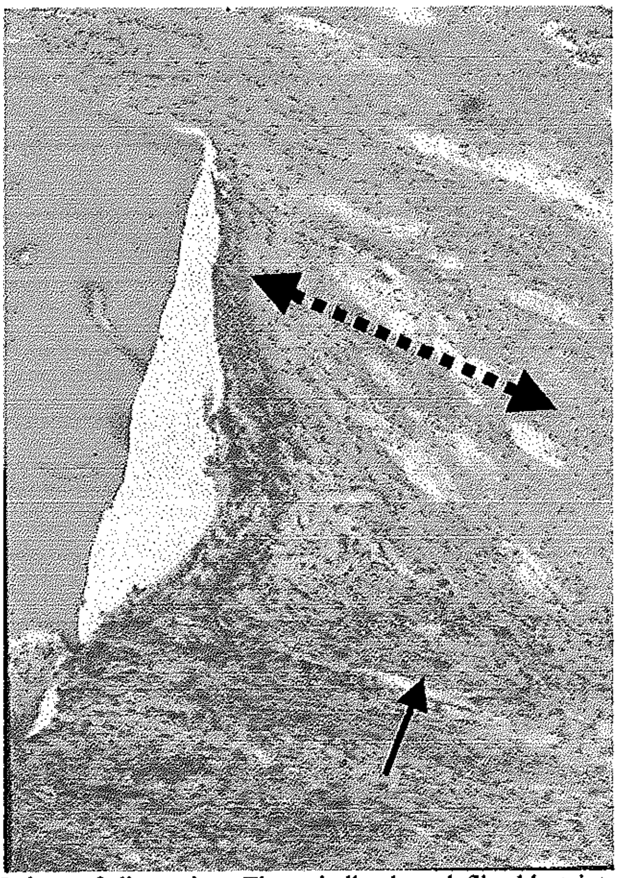


FIGURE 36. Histology at 5 days of distraction. The spindle-shaped fibroblast interspersed among bundle of collagen was demonstrated. The collagen bundles which tended to align themselves to the parallel manner with the direction of distraction (dot arrow) was obviously noted (arrow). The cut bone surface(B) was also seen on the left. (H&E stain)

FIGURE 37. Histology at 5 days of distraction viewed under higher magnification. Showing the spindle-shaped cell resembling fibrosblastic cell with dense collagen production which organized themselves parallel to the vector of distraction (arrow). (H&E stain)

At completion of distraction

On the completion of distraction, the longitudinal new bone trabeculae were seen on both sides near the osteotomized bone surfaces, as well as the area adjacent to the periosteum. The fibrous tissue and collagen were seen mainly in the central region of the gap. The osteoblastic cells were found resting on the primary bone spicules, which resulted from the incorporation of the collagen bundles and the osteoid. These longitudinal new bone trabeculae were seem try to bridge the expanded gap by extending from the cut bone surface and periosteum. In all animals in this group, some foci of cells with the surrounded matrix that resembled the cartilage cells were found in the lengthened gap .The arrangement of the cartilage cells in foci were irregular and did not arrange themselves as orderly as in the epiphyseal plate region (Fig. 38,39)

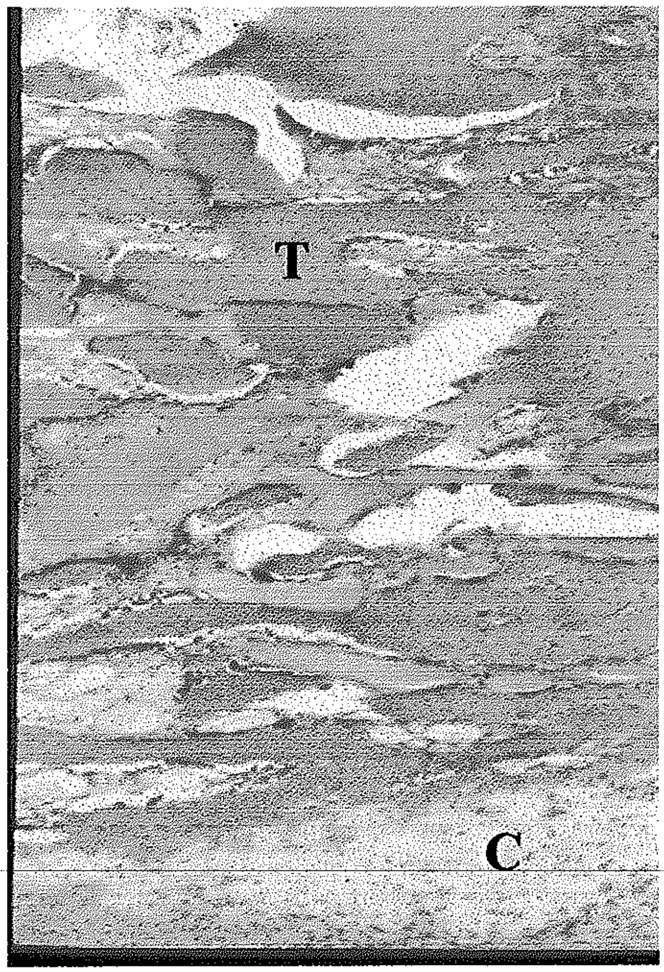


FIGURE 38. Histology at completion of distraction. Showing the longitudinal new bone trabeculae (T) in the lengthened gap. The island of the cartilage (C) was seen in the lower part. (H&E stain)

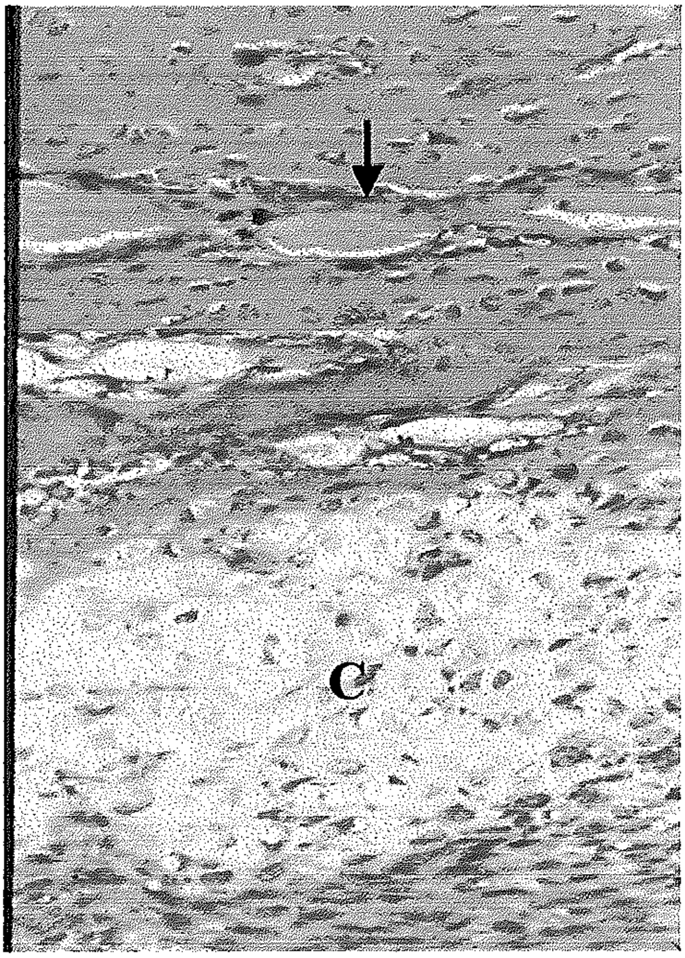


FIGURE 39. Histology at completion of distraction viewed under the higher magnification demonstrated the osteoblastic cells lied on the surface of the new bone spicules (arrow). The island of plump cells surrounded by the matrix that resembled cartilage (C)and arranged in an irregular pattern was seen. (H&E stain)

At 2 weeks after completion of distraction

By 2 weeks after completion of distraction, the primary bone trabeculae became more mature by gradual increasing mineralization of the osteoid. The ostoblastic cells which lied on the surface of these new bone spicules eventually became enveloped within, as the spicules were gradually enlarged by circumferential apposition of the collagen and osteoid. The new bone trabeculae demonstrated continuity in the center of the lengthened gap. As a result, the fibrovascular tissue in the central region disappeared. Some areas of the new bone trabeculae underwent resorption and remodeling were observed. In all animals in this stage, small scattered cartilagenous islands were still seen (Fig.40,41).

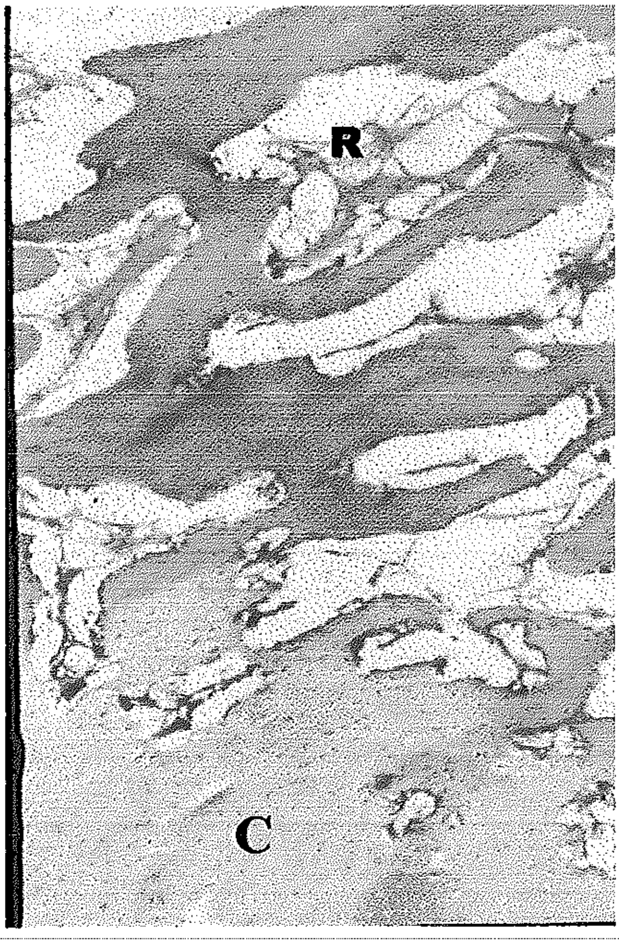


FIGURE 40. Histology at 2 weeks after completion of distraction, showing increasing mineralization of the bony trabeculae. Note the area of resorption and remodeling(R). An island of cartilage (C) was also seen in the lower part. (H&E stain)

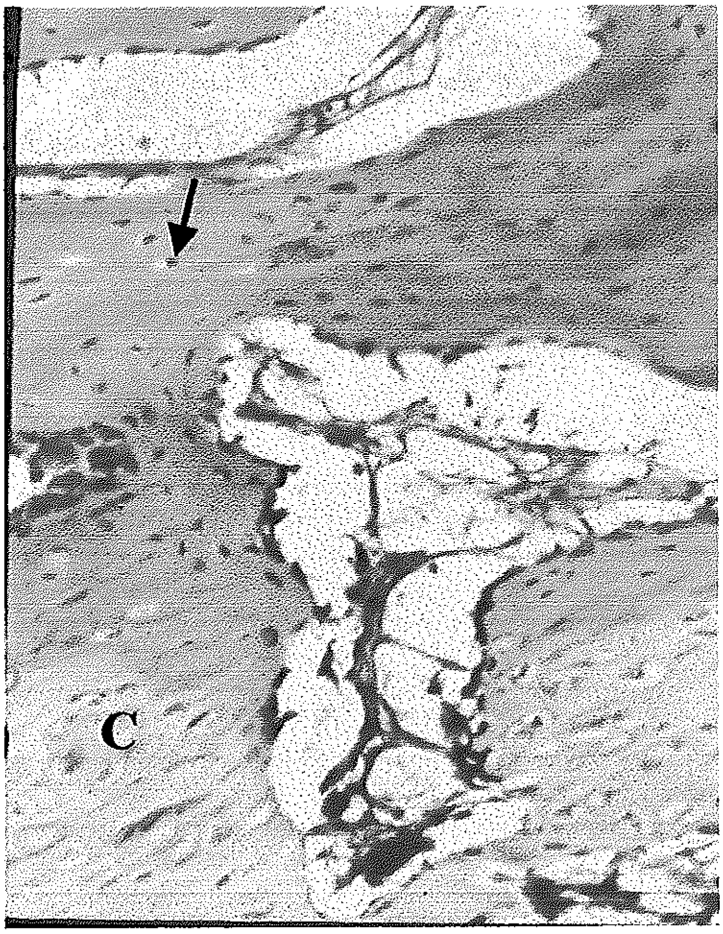


FIGURE 41. Histology at 2 weeks after completion of distraction viewed under higher magnification. Showing the osteoblast (arrow) incorporated into the new bone tissue from the circumferential apposition growth. The island of cartilageous cells (C) was also demonstrated in the lower part. (H&E stain)

At 4 weeks after completion of distraction

By 4 weeks after completion of distraction, the new bone tissues became more mature. The woven pattern of the new bone was noted particularly in the circumferential region of the mandible adjacent to the periosteum. The cartilage foci disappeared by this stage in all the animals (Fig. 42,43).

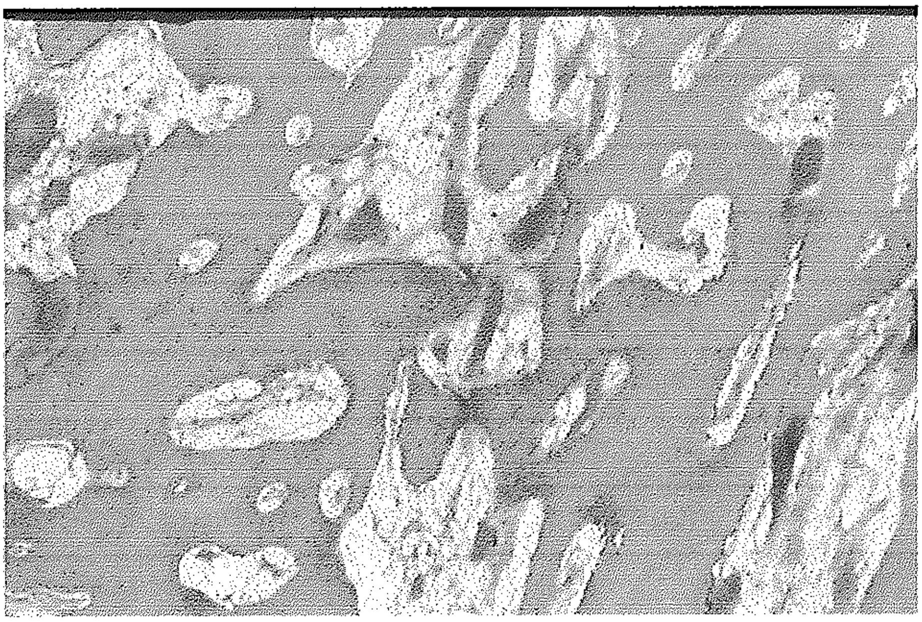


FIGURE 42. Histology at 4 weeks after completion of distraction, the woven pattern of new bone tissue was clearly evident. No cartilage island could be found. (H&E stain)

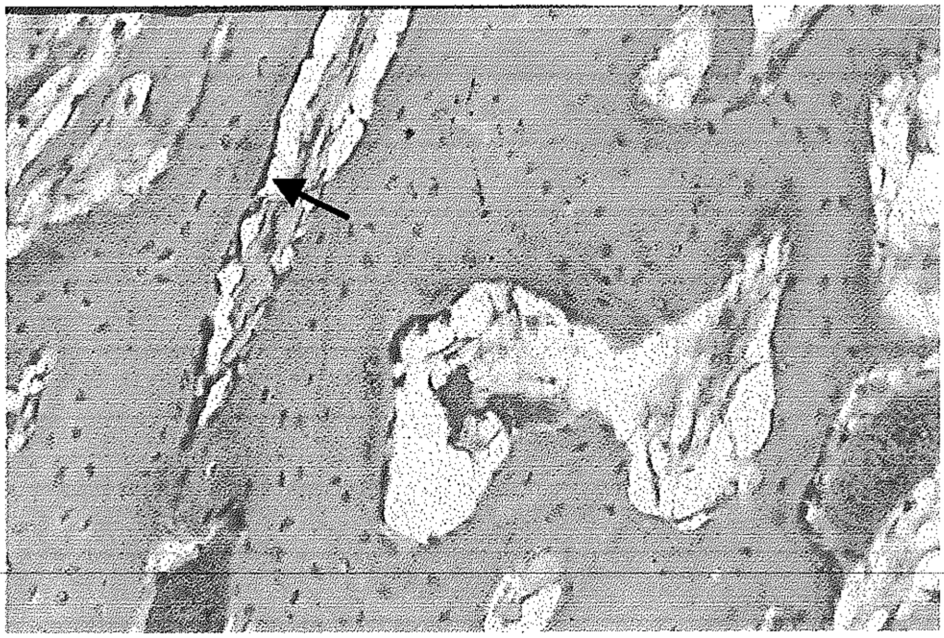


FIGURE 43. Histology at 4 weeks after completion of distraction viewed under the higher magnification. Showing the woven pattern of the new bone formation in the lengthened gap. Note the active osteoblastic cells (arrow) on the new bone surface. (H&E stain)

At 6 weeks after completion of distraction

By 6 weeks after completion of distraction, the cortical area of the lengthened gap became more mature and increase in its thickness when compared with the 4 weeks group. The woven bone pattern was almost changed to cortical bone pattern especially in the peripheral cortical region. Bone trabeculae in the marrow space was absorbed to form medullary cavities (Fig.44,45).

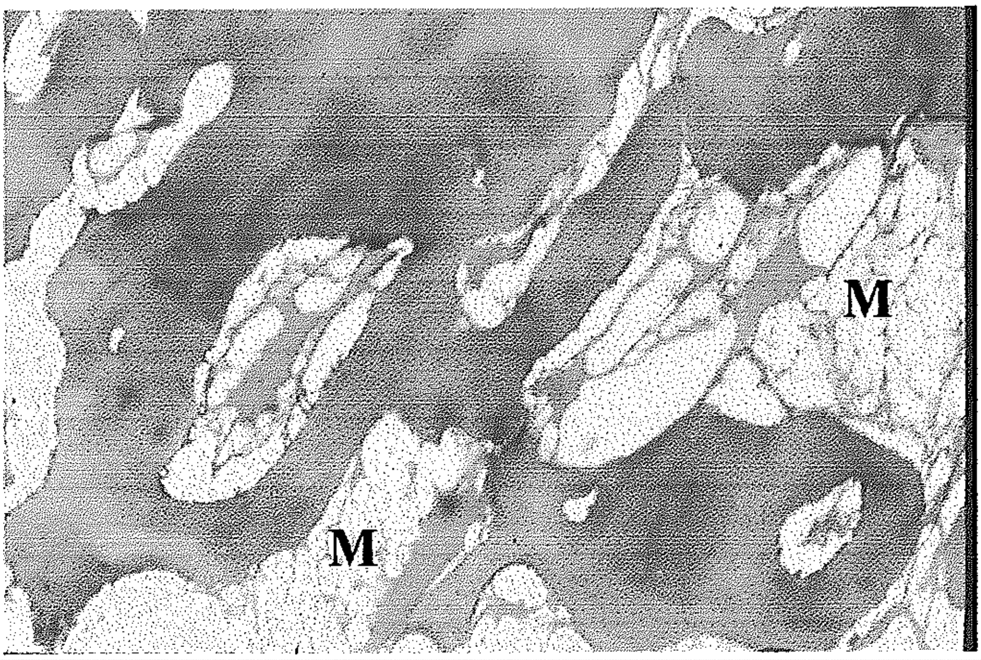


FIGURE 44. Histology at 6 weeks after completion of distraction, noted the mature cortical bone. The resoption of the bone trabeculae to form the marrow cavity and medullary tissue (M) was also demonstrated. (H&E stain)

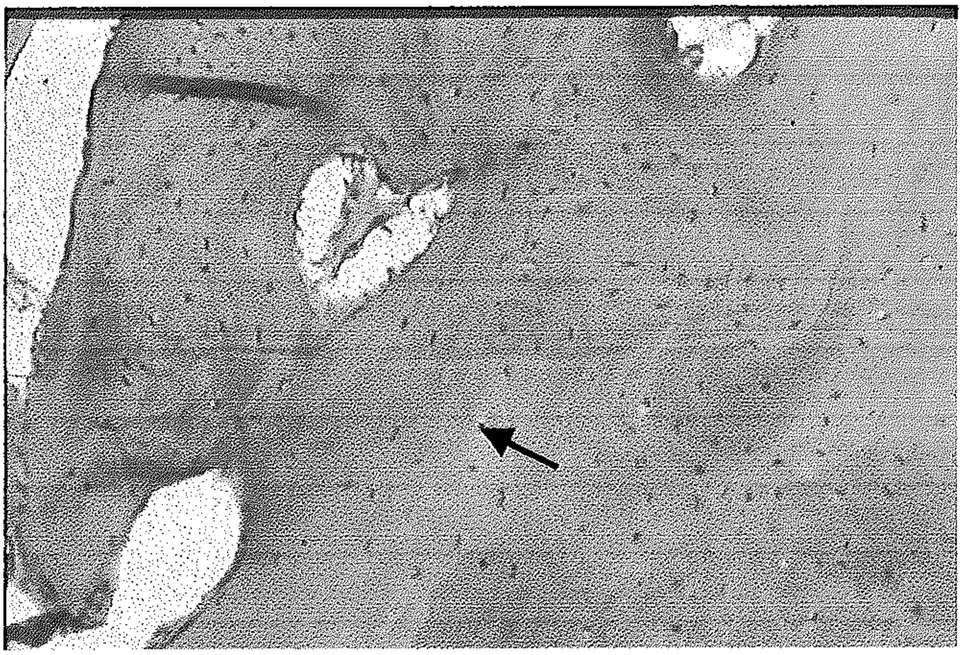


FIGURE 45. Histology of 6 weeks after completion of distraction viewed under the higher magnification, showing the mature cortical bone with lamellar pattern, similar to the normal cortical bone. Note the small, shrinkage osteocyte resting in the lacuna (arrow). (H&E stain)

At 6 months after completion of distraction

By 6 months after completion of distraction, the lengthened bone tissue took on the same staining characteristic of the mature lamellar bone. The lamellae were arranged concentrically around the harversian canals to form osteons. The fibrovascular tissues that previous filled the spaces in the inner part was completely replaced by normal appearing marrow. At this stage, the structure of mature cortical bone and medullary complex were established (Fig.46,47).

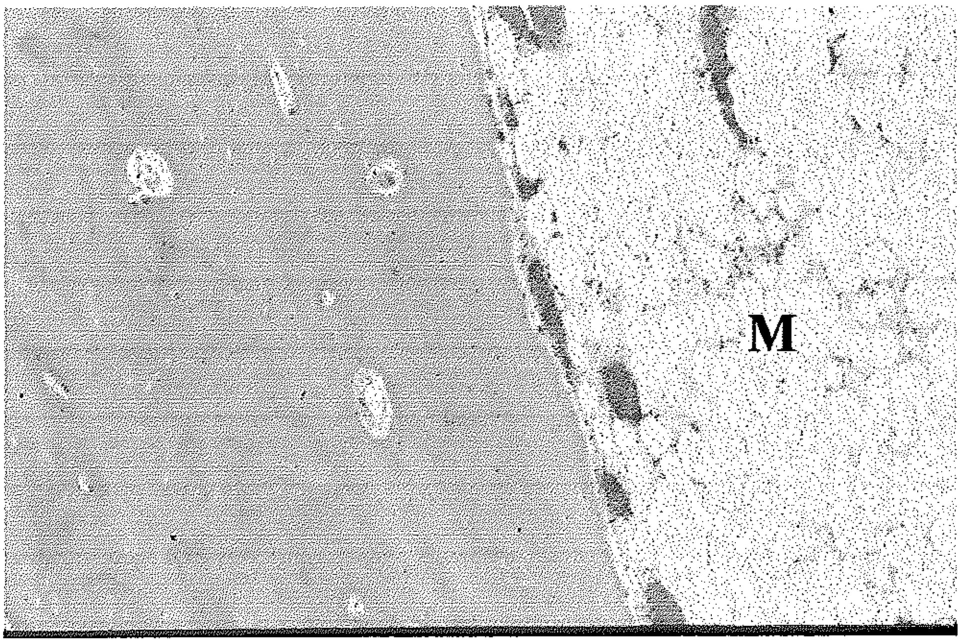


FIGURE 46. Histology at 6 months after completion of distraction. Noted the fully mature cortical bone with the normal lamellar pattern. The harversian pattern of the osteon units also could be noted. The normal marrow tissue (M) was seen on the right side. (H&E stain)

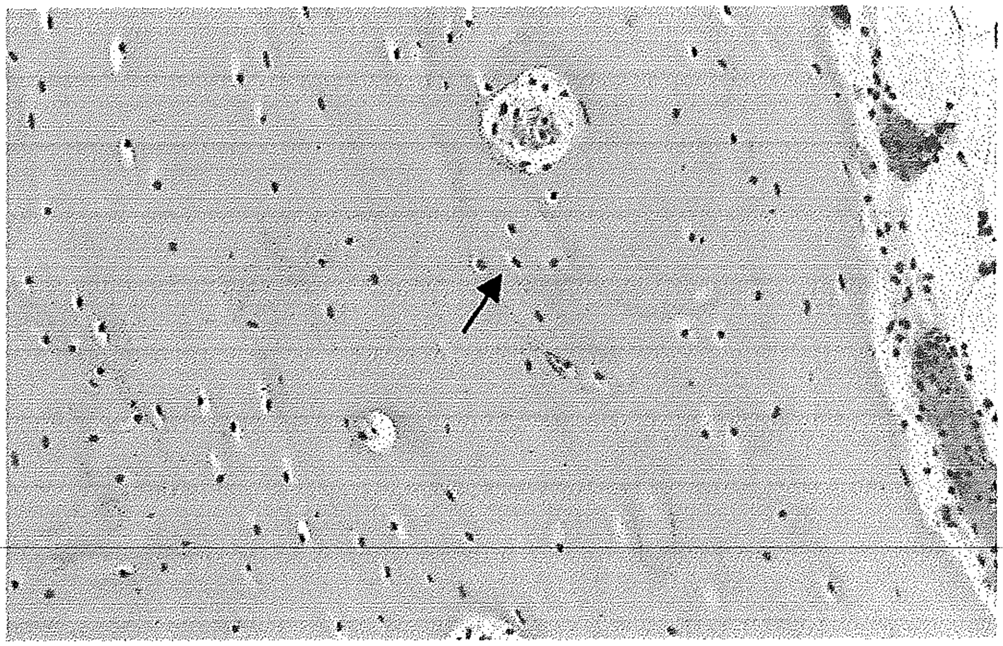


FIGURE 47. Histology at 6 months after completion of distraction viewed under the higher magnification. Showing the lamellae that arranged concentrically around the harversian canals to form the osteons. Note the cement lines and small shrinkage osteocyte in the lacunae (arrow). (H&E stain)

A summary of the histologic changes was shown in Table 6. The histologic study of the distraction gap revealed a gradual changed pattern from an initial amorphous matrix to a fibrous matrix and, finally, an osseous like tissue that eventually developed to the normal mature bone of mandible.

Table 6. Summary of the histologic sequence in the experiment

parameter	Time				
	Complete distraction	**2 weeks**	**4 weeks**	**6 weeks**	**6 months**
Histology	Longitudinal new bone trabeculae	Increase in new bone formation and fusion of new bone trabeculae in the center.	More maturation of bone with woven bone appearance in the peripheral region.	Cortical region increased in thickness and cortical bone pattern. Resorption of bone trabeculae in marrow cavity area	Definite structure of mature cortical bone and medullary complex
Cartilage finding*	2/2	2/2	0/2	0/2	0/4

Note: * The first figure referred to the number of specimens that showed cartilage, while the second represented the number of specimen analysis.

2. Muscle specimens

2.1 Histologic study

At 5 day of distraction

By 5 days of distraction, the significant shrinkage of myofibers in cross section was noted. The atrophic fibers were characterized by angulation, decrease in fiber size and an increase in the number of nuclei per fiber. The space in-between the myofibers was increased and replaced by the fibrous connective tissue. The inflammatory cells were found occasionally infiltrating in the fibrous stroma (Fig. 48).

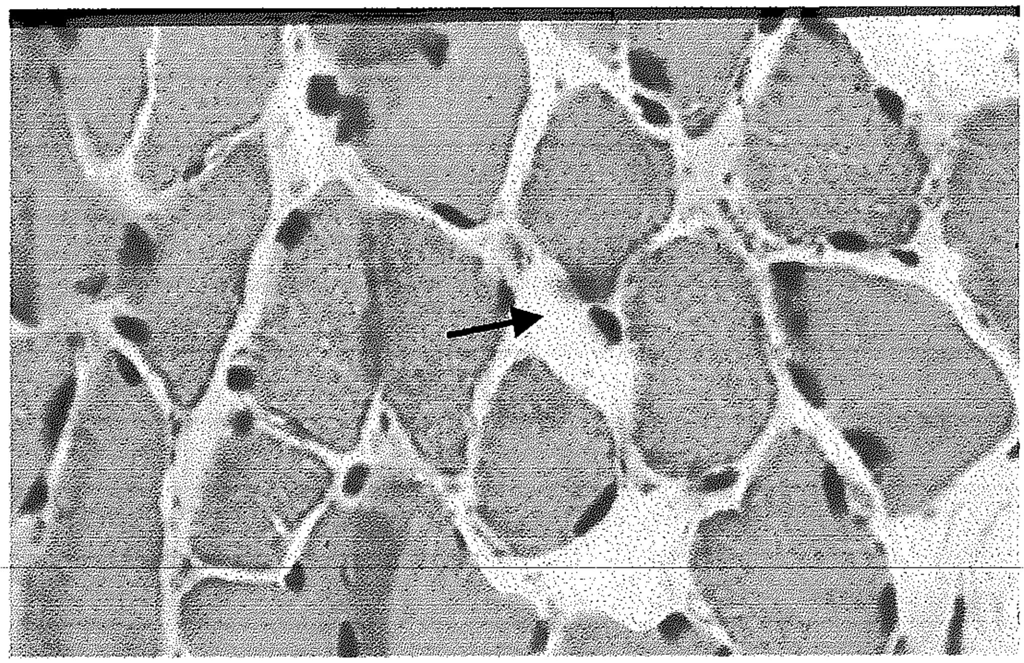


FIGURE 48. Photomicrograph of disgastric muscle cross section 5 days of distraction, stained with H&E. Showing the severe atrophic, angulated myofibers with increase in the number of nuclei per fiber. Increase of the fibrous connective tissue in the endomysial space was noted. (arrow)

At completion of distraction

By completion of distraction, the muscle fibers demonstrated an increase in cross-sectional dimension of the myofibers. The fiber had the more round appearance. The band of the perimysial and occasionally endomysial fibrosis could still be noted, but already marked decrease in amount (Fig.49).

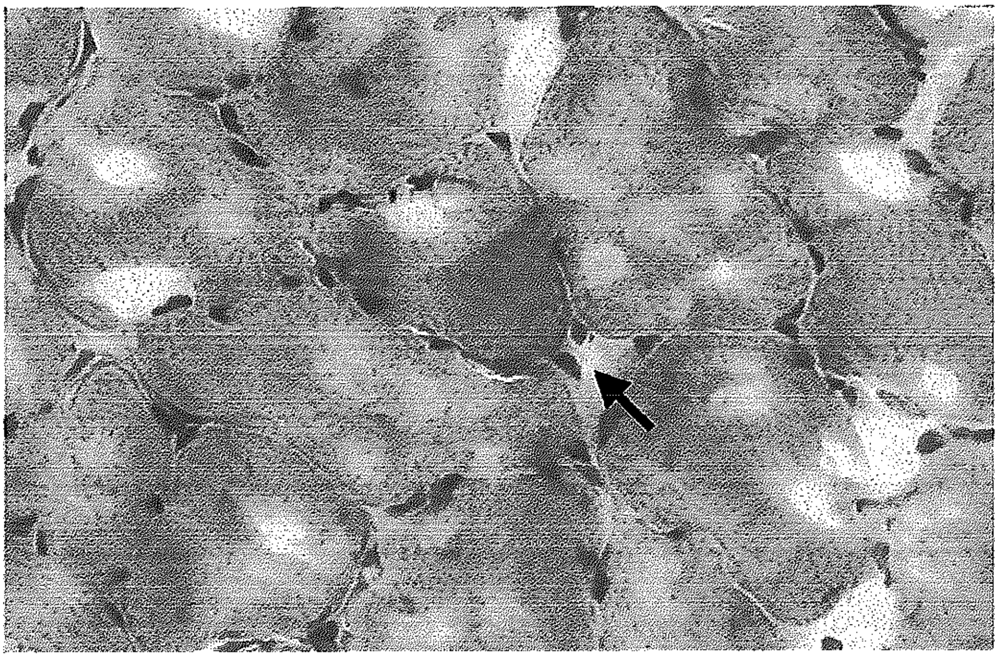
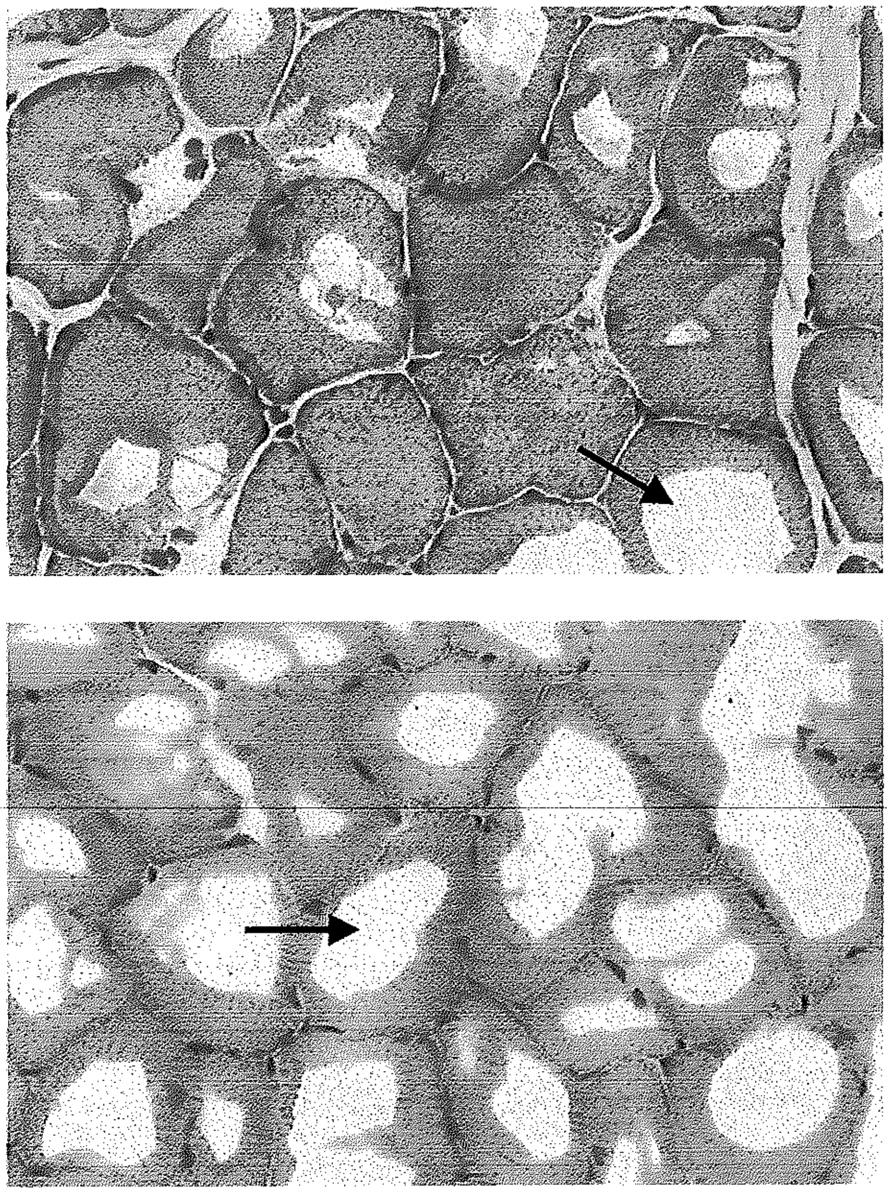


FIGURE 49. Photomicrograph of digastric muscle cross section view at completion of distraction, stained with H&E. Showing the increase in myofibers size. The shape of each fiber became round but some still showed the angulated appearance was noted. The endomysial fibosis (arrow) was marked decrease.

At 2 to 6 weeks after completion of distraction

The myofibers gradually increased in size. The nuclei were found lying on the peripheral location of the myofibers border. The fibers had the round or oval in appearance. Ice crystal artifact was responsible for the punctate clear areas seen within the myofibers (Fig.50).



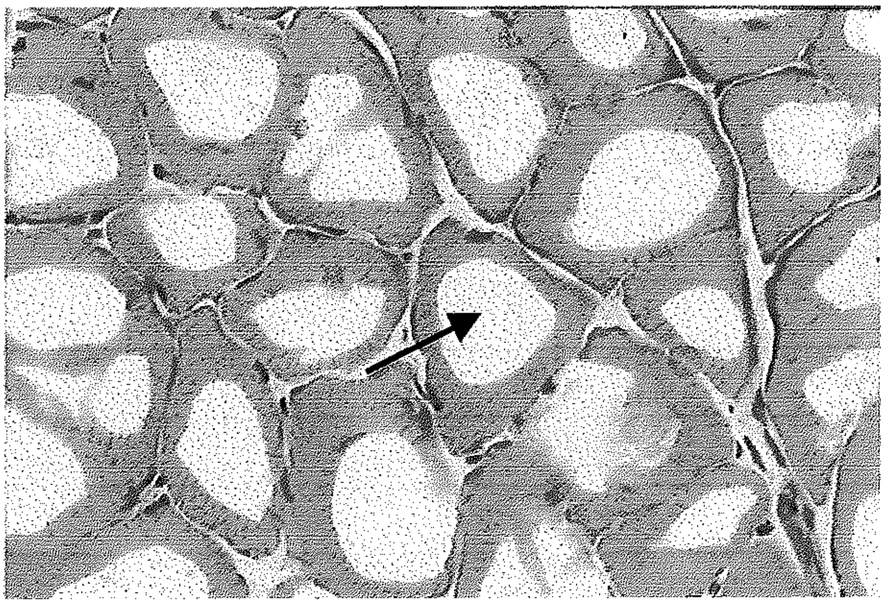


FIGURE 50. Photomicrographs of digastric muscle cross section, stained with H&E. Above, at 2 weeks after completion of distraction. Center, at 4 weeks after completion of distraction. Below, 6 weeks after completion of distraction. Showing the gradually increased in size of cross section area of myofibers. The oval or round shape of the myofibers with the peripheral nuclei were seen. The clear space within the muscle cells resulted from ice crystal formation (arrow).

At 6 months after completion of distraction

By 6 months after completion of distraction, the muscle fibers exhibited a marked abnormal variation in size due to atrophy of some and hypertrophy of others. The atrophic myofibrils severely decreased in size resulted in marked differences, when compared with the adjacent hypertrophic one. These atrophic fibers were found with the hypertrophic fibers throughout the muscle fascicles. No regeneration of muscle fiber was detected. Some areas of moderated fibrosis were seen in the muscle tissue. This gave rise to apparent swelling of the affected muscle and accounted for the pseudohypertrophy (Fig.51).

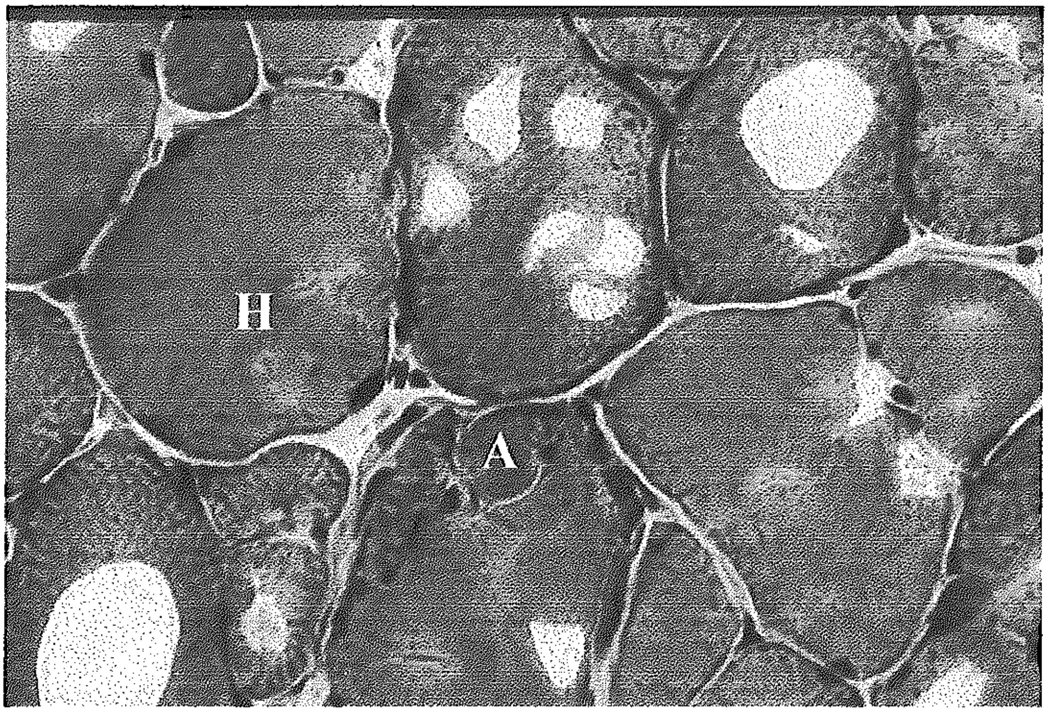


FIGURE 51. Photomicrograph of digastric muscle cross section at 6 month after completion of distraction, stained with H&E(original magnification :x 100) Note the significant different in size between the hypertrophic (H) and atrophic(A) myofiber. The atrophic fiber was scatter in distribution rather than arranged in group.

2.2 Histomorphometric study

Three random areas from each slide were use to measure the mean cross-sectional surface area of the myofibers by the image processing and analysis system. A summary of the myofiber cross section surface changes was showed in Table.7. The statistical analysis revealed the average surface area of the myofibers in the early stage group (< 2weeks group) to be significantly less than the late stage group (≥ 2 weeks group) ($p<0.05$). No difference was found between the late stage group and control

Table 7. Mean (±SD) cross-sectional surface area of the myofibril.

Group	Surface Area (Micron Square)
< 2 weeks (n= 4)	1259.74±528.67
≥ 2weeks (n=10)	2336.20±181.16

Stability Evaluation

The distance between the screws located in the lengthened segment of the six months group (group VI) immediate after distraction device removal and 6 months later was measured and recorded to determine the stability of the lengthened segment. The result outcomes were showed in Table 8. Statistical analysis showed no significant difference between these two groups.

Table 8: Mean(± SD) distance between the center of the screws adjacent to the expanded gap measured immediately after the distractors were removed comparing to 6 months later.

Group	**Distance (mm)**
Immediate distractor removal (n= 12)	14.88±1.01
6 months (n= 12)	14.18±0.57

Chapter 4

DISCUSSION

Technique of distraction osteogenesis involves creation of new bone by gradual movement of two or more bony fragments following their surgical division. This technique was developed in the field of orthopaedic surgery and principally utilized for limb lengthening.[6] In 1950s, Ilizarov [3,4]was credited with having clinically demonstrated in long bone that large amount of new bone could be produced through progressive and continuous distraction. In 1972, Snyder *et al.*[27] applied this principle to the membranous bone of the dog mandible. They used this technique to lengthen the dog mandible. Since then, several experimental studies on membranous bone lengthening by distraction osteogenesis have been reported.[29,89,91,103,110,111] The studies in animal model contributed a great deal of knowledge on the osteogenesis process in membranous bone, basic which raised the possibility of utilizing distraction osteogenesis to treat hypoplastic conditions of the human mandible.[28] However, in practical term, a number of difficulties were encountered, which were related to the type of the animals used for the experimentation.[27,29,95,103,110,111] Some animals such as dog, sheep and monkey are not readily available, difficult to keep and ethically not feasible.

In the present study, rabbit is proposed as the model of the study. Although performing the operation on such a small bone is technically demanding, this problem was overcome by using a device, which was fabricated from the orthodontic palatal expansion screw. The device could adapt easily to fit on the contour of the rabbit mandible in the lower border area, which was the most thickness region of the mandible. Micro-titanium screws were used for bicortical fixation, which provided possible greater stability than the external pin of Orthofix-M or Kirschner wire. [112,113] The small diameter of the microscrew is suitable for the relative small rabbit mandible. Neither unfavorable fracture nor crack of the osteotomized segments, resulting to the loosening of the screws, was encountered. In addition, trauma to tooth root caused by the large pin as in the previous study can be avoided. This prevented artifacts such as suppurative pulpitis, pseudocartilagenous metaplasia at the pin track site or periapical cemental dysplasia. [113]

In contrast to the multiple anesthetic medications used, either via an intravenous route or inhalation with endotracheal intubation, the simple intramuscular injection of Ketamine Hydrochloride and Benzodiazepine supplemented with local anesthetic drugs were used. [115,116,117] A surgical anesthetic state was easily achieved, which seldom required repeated dosage of medications. Neither perioperative or postoperative death of the animal from the anesthetic complication was encountered when compared with the other methods.[113] Despite the service of a skill anesthetist, endotracheal intubation and airway maintenance was technically difficult. The animals were well tolerated to the surgical operation and most of them recovered well from the anesthesia by using this two-combination drug. Therefore, the use of Ketamine Hydrochloride and Benzodiazepine intramuscularly with supplement of the local anesthetic agent is most suitable as the general anesthetic agents for distraction osteogenesis procedure in rabbit.

The rabbits selected for the present study are in a growing state. The growth status is particularly important because the use of the distraction osteogenesis to correct malformation is most suitable to be performed in the young patients.[92] In case of the severe malformation, it would be of great benefit if the correction could be done at an early age to minimize both the physical deformity and the psychosocial impact of the children. The experimental model in the growing period will be a good representation in studying the various factors and techniques of distraction osteogenesis.

In an analysis of animal choice in experimentation, it has been suggested that the choice of the animal in any experiment should be related to the level of the hypothesis testing and that the degree of the phylogenetic affinity between the animal model and humans depends upon the level of experimental manipulation and hypothesis testing. [114] In this study, the rabbit has been used to assess process of bone formation and muscle response after mandibular distraction at different stage. This level of descriptive process only required the use of "generic" animals, such as rabbit, rather than phylogenetically closer animal. [114]

The distraction procedure in the present study is based on the Ilizarov's principle with distraction rate of 1 mm/day (0.5 mm in the morning and 0.5 mm in the evening) as suggested in the previous studies on rabbit mandible.[113,115-117] The latency period was 3 days after the corticotomy with a consolidation period of 6 weeks before removing the instrument. Slower distraction rate leads to early ossification of the distraction space while the faster rate (>1mm per day) coincides with less new bone formation, which most likely attribute to microangiographically visible tearing of endosteal and periosteal vessels.[4,9,68] With regard to the distraction protocol in this study, most of the new bone formation in the distraction gap was normal without any complication of healing.

In principle, non-union will result by creating a bony defect above a critical size, determined by the anatomical location, age, species and integrity of periosteum.[118] Several groups of study have demonstrated incomplete healing of 5mm defects in the rabbit mandible.[119,120] Therefore, a 10 mm mandibular defect of the New Zealand white rabbit would be above the critical size defect for spontaneous bony union.

In the present study, the corticotomy was done to separate the mandible as suggested by Ilizarov,[121] who emphasized the importance of this technique in preserving the intermedullary blood supply. The second reason was to reserve the inferior alveolar neuromascular bundle. Every effort was made to preserve the periosteal envelope during the surgical procedure. Since the osteogenic potential for bone regeneration existed within the periosteum.[59]

With regard to the mode of bone regeneration during mandible lengthening by distraction osteogenesis, some controversial aspects still remain. In long bone lengthening, Kojimoto *et al.*[59] reported that callus distraction in the rabbit hindlimb produced a characteristic zonal structure resembling a growth plate and that the new bone was formed by endochondral ossification. On the other hand, Delloye *et al.*[60] stated that most of the newly deposited bone was formed intramembranous ossification in the dog forelimb. In membranous bone, Karp *et al.*[91] in the lengthening of dog mandible demonstrated that new bone was formed predominantly by intramembranous ossification. However, Stewart[113] reported small foci of endochondral ossification in some specimens of mandibular lengthening in rabbit. Kumoro[112] in histologic analysis of distraction osteogensis of the rabbit mandible demonstrated 9 out of 10 rabbits with cartilagenous formation at an early stage. The cause may be due to instability of the bone fragments (only one screw on each segment was applied because the rabbit mandible was a smaller structure than that to which the Orthofix M-100 is normally applied). In the present study, some small foci of the cartilage were found in all the animal groups in the early stage of distraction up to 4 week after completion of distraction. But according to the good strength and fixation of the well-designed distraction apparatus in the present study, this is probable not the cause of cartilage formation in the process of new bone formation in rabbit mandible. The etiology of the endochondral ossification during the early stages of mandibular distraction in rabbits may be influenced by the inappropriate distraction rate, which resulted in tearing of the microcirculation followed by impairment of the oxygen tension. [112,122]

In the present study, the cartilage foci were found in the new bone formation process as early as the completion of distraction, suggested that the new bone produced by distraction osteognesis were mainly formed by both intramembranous ossification and endochondral ossification. This new bone subsequently underwent remodeling and resulted in mature cortical bone by 6 weeks after completion of distraction. At 6 months after completion of distraction, the definite structure of mature cortical bone and medullary complex were established. So it could be stated that new bone formation in distraction osteogenesis process start with the involvement of endochondral ossification in the early stage, then these cartilage foci disappears up to 4 weeks stage. After this stage, the intramembraous ossification was the predominant finding of the new bone formation process.

The radiographic appearances of the distraction gap at various stages of the present study were similar to those of the previous studies. [112,113] In contrast to the radiographic feature of distraction gap in sheep rabbit distraction gap radiographic appearance was quicker at all stages. , [110] In sheep ,radiopaque streaks extending across the distraction gap did not noted until several weeks after distraction. The same finding was also reported by stewart *et al.*[113] in rabbit mandible lengthening. This difference is likely explained by a species variability.The rapid new bone formation during the early stages of distraction in the present study was also confirmed by the image analysis of the radiographic finding as well as the biomechanical evaluation by Vicker's hardness testing, which showed that new bone formation started immediately after completion of distraction and rapidly increased to the normal level at 4 weeks later.

With regard to the changes of the muscle which relating to the process of distraction, studies in the long bone demonstrated that the associated skeletal muscles adapted to distraction by hypertrophy and hyperplasia of the myocytes.[3] With immediate advancement of the mandible by saggital split osteotomy, studies showed that the muscles undergo hypertrophy in type I-slow twitch fiber.[123] In the study of histopathologic changes in muscle affected by distraction osteogenesis of the rabbit mandible, Fischer *et al.*[124] demonstrated that the cross section of the myocytes in the digastric muscle on the distracted side underwent atrophy during the distraction period. This was followed by subsequent hypertrophy and atrophy noted at 20 days after distraction. No evidence of increasing atrophy was present 48 days following distraction. They concluded that the digastric muscle underwent transient atrophy with initiation of distraction but adapted with compensatory hypertrophy. In our study, myocytes also demonstrated severe atrophic, angulated myofiber occurred during the distraction phase. After completion of distraction, the muscles showed rapid increased in size of the myofibers, until reaching their normal size at 6 weeks after distraction. These results suggested that the associated skeletal muscle that aligned in the same direction of the distraction vector adapted by means of hypertrophy.(124) This was clearly demonstrated by the quantitative analysis in this study.

However, in the 6 months after completion of distraction group, the myofibers exhibited a marked abnormal variation in size due to severe atrophy of some and hypertrophy of the others. These atrophic myofibers were scattered among the hypertrophic myofibers without arranged in cluster or group pattern. These finding is probable related to the selective atrophy of specific myofiber type such as in the type II atrophy often seen in muscle following prolonged immobilization.(125)

The gross morphologic study of the muscle demonstrated slightly larger digastric muscle on the distracted side than the opposite non-operated side at 6 month after completion of distraction. But regard to the histologic section, some area of moderate fibrosis was found in the muscle tissue. Therefore the increase in the muscle mass of the distracted side may be caused by replacement of the muscle fibers due to the fibrous connective tissue. This gave rise to the apparent swelling of the affected muscle and accounted for the "pseudohypertrophy" of the muscle.(126)

According to the histologic evaluation of the muscle regeneration no the new myofibers was not detected. This highlights the fact that distraction osteogenesis, which has the obvious ability in generating new bone, with the potential effect of transforming the mesenchymal cells to osteoblasts,(91) is perhaps no role in the genesis of the myofibers.

In the present study, the image analysis and system was used for the quantitative measurement of the radiographs and cross section muscle fiber area in the various stages of distraction. Image analysis system is the science of making geometric measurement on image from any source. In contrast to qualitative evaluation performed in the previous studies of mandibular lengthening in rabbit model,(112,113,116) the image analysis provided a more reliable statistically significant data, compared to those of the traditional subjective methods.

Most of the previous distraction osteogenesis study stated that the gained length was always less than the amount of the distraction.(113,116) This might resulted from loosening of the pins, pinhole infection and instability of the distraction frame. Our finding showed only a slightly relapse of the expanded segment (0.56 mm, 2.95%) before the distractor was removed. This reflects good physical strength of the distractor, adequate fixation method and the well designed orthodontic expansion screw used in this study. Moreover, evaluation of the segment stability, at 6 month after completion of distraction also demonstrated minimal relapse of the lengthened segment when compared with the measured length obtained immediately after device removal. It implies that distraction osteogenesis in the mandible is a reliable method and, it can be used in maxillofacial region with good stability.

Area worthy of further investigation includes the use of specific muscle enzyme stain to determine the type of the myofibers which affected by the distraction process and the use of osteogenic stimuli, such as growth factors to enhance the process of bone formation. Large sample size and long term studies are also the critical point, which one should consider.

Chapter 5

CONCLUSION

In the present study, rabbit is confirmed as an suitable animal model for the study of distraction osteogenesis in the mandible using the modified palatal expansion screw as the distraction device. New bone formation is formed mainly by intramembranous and partially endochondral ossification in the early stage, although the rigid device and fixation system was used. The ossification process started immediately after completion and had the normal bony histologic appearance in 6 weeks. The radiographic examination and the surface hardness testing also showed new bone formation rapidly begun as early as completion of distraction and reach the normal level in 4 to 6 weeks later.

The anterior belly of digastric muscle on the distracted side adapted by transient atrophy with initiation of distraction and resolved with time after distraction. However, long term observation in 6 months after the distraction was complete, some specific myofibers underwent severe atrophy and areas of moderated fibrosis tissue were found replacing in the muscle tissue. This suggested that the clinical enlarged muscle on the distraction side perhaps merely "Pseudohypertrophy" of the affected muscle. No myofiber regeneration was detected in the muscle specimen of the distracted side in any stage of the experiment. It can be concluded that distraction osteogenesis, which had the obvious ability in induction of osteoblast from the undifferentiated mesenchymal cell, has no role in the genesis of the muscle tissue.

In the long term observation, the lengthened segment showed good stability with minimal relapse. It stated that distraction osteogenesis in the mandible is a reliable method and can be used in the maxillofacial region with good outcome.

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Appendix

The efficacy of the modified palatal expansion screw used for mandibular lengthening by distraction osteogenesis in rabbit model

Orthodontic palatal expansion screw (Hyrax) was modified to use as the distraction device for lengthening mandible in four New Zealand white rabbits by distraction osteogenesis principle. Before the experiment performed in the animals, all devices were tested for hardness and strength by the Universal testing machine (Autograph AG-100 KNG, Kyoto, Japan). The screw of the distractor was activated for 40 turns and tested by moving the extension rod of the universal testing machine for 0.1mm with the speed of 0.5mm per minute. The average compressive force was founded 7.85 newtons as showed in Table 9.

Table 9: Mean (± SD), n=6, of the compressive force of the distractors tested by the Universal Testing Machine.

Distractor	**Compressive Force (Newton)**
A	9.93±0.20
B	7.83±0.12
C	5.56±0.23
D	12.00±0.16
Average	7.85±0.18

Then the distractor was placed in rabbit and activated 4 turns a day for 10 consecutive days to obtain complete distraction. The distance of the extension rods were measured 4 week (A,B) and 6 week (C,D) post-operatively before the distractor removed. The distance was measured by two investigators using the digital veneer caliper (Mitotuyo,Tokyo, Japan). The result in Table 10 demonstrated average distance relapse of only 0.56 mm (2.95%). No significant different in the measurement between two investigators was found by t-test ($p<0.05$)

Table 10. Mean(± SD),n=6, of the extension rod distance in each distractor according to the timing of the experiment.

Distractor	Extension rod distance (mm)		
	Complete activation /before surgery	**Complete activation /before removal**	**Relapse distance**
A	20.70±0.01	20.51±0.10	0.19±0.10
B	20.85±0.03	20.35±0.02	0.50±0.01
C	20.94±0.02	20.68±0.02	0.26±0.01
D	17.96±0.06	16.69±0.02	1.27±0.27
Average	20.12±0.03	19.56±0.08	0.56±0.09

It can be stated that the device in the present study is strong enough and probably use for lengthening the rabbit mandible with the minimal relapse. To obtain the accurate length, additional activated length should be considered.

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