MINISCREW ASSISTED SLOW EXPANSION OF MATURE SUTURES

A Thesis

by

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ABSTRACT

The purpose of this study was to evaluate whether complex, mature sutures could be separated using skeletal anchorage and light, continuous forces. Twelve adult female New Zealand White (NZW) rabbits, 8 to 9 months old, were randomly assigned to three groups (0 g, 42 days, n=3; 100 g, 42 days, n=7; or 100 g, 105 days, n=2). Open-coil nickel-titanium springs delivered constant forces of 100 g across the sagittal suture to miniscrew implants (MSI's) placed bilaterally in the frontal bone. Sutural separation was measured bi-weekly. Bone formation (mineral apposition) on both the endocranial and ectocranial surfaces was measured with fluorescent labels and micro-computed tomography (μCT). Qualitative histologic analyses of the suture tissues were performed using H&E staining; osteoclasts were evaluated using tartrate resistant acid phosphatase (TRAP) staining. All 24 MSIs remained stable throughout the experiment. There was no statistically significant sutural separation in the control group. In the experimental groups, sutural separation was significant ($p < .05$) at all time points up to 42 days. The rate of separation was linear during the first 42 days, and decreased between 42 to 105 days. There were moderate correlations ($R=0.59-0.89$; $p=<.05$) between MSI separation and bone marker separation. Mineral apposition rate (MAR), which was not measureable in the control group, showed significant deposition of bone in the experimental group. MAR was greater between 14-28 days than between 28-38 days, and it was greater on the ectocranial than endocranial surface, but neither of these differences were statistically significant. Based on the μCT analysis, 3D sutural volume of the experimental group increased significantly (p=0.02), whereas the increase in surface area was not significant (p=0.26). Based on these results, it is possible to separate the sagittal suture of mature rabbits. Sutural separation is limited, indicating involvement of other sutural articulations.

DEDICATION

This thesis is dedicated to my wonderful family.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Orthopedic maxillary expansion has been reported in the literature for over a century. While the initial reports of the procedure were in the United States, the treatment was not commonly used in this country until decades later. More recent popularization of the procedure has been based on an understanding of the suture biology and the tissue reactions at the sutural level. Maxillary expansion has predominately been used in juveniles and young adolescents, where the process has been compared to distraction osteogenesis, in which the suture is mechanically separated with heavy forces. Traditional expansion uses high residual forces which are delivered to the teeth via the expansion appliance. Many authors condemn expansion in adults based on the idea that the suture will not split and that the expansion forces will push the teeth out of the bone. For adults in need of maxillary expansion, surgically assisted rapid palatal expansion or orthognathic surgery is commonly prescribed. Both procedures are associated with a host of morbidities. The practitioners who are willing to try non-surgical expansion in adults have had limited success with a number of concomitant complications.

Currently, there is no effective, predictable, non-surgical method to separate the mature midpalatal suture in humans. A review of the literature involving mature sutures gives evidence of the feasibility of obtaining sutural expansion provided the force system is appropriate. There is strong evidence in the literature to suggest that facial sutures maintain their patency until much later in life than cranial sutures. However, sutures are thought to become increasingly complex and interdigitated, hence traditional orthopedic maxillary expansion has been discouraged beyond the age of 18. However, by diverging from traditional expansion techniques, such as using tooth-borne anchorage, a miniscrew implant (MSI) supported appliance can provide forces resulting in increased skeletal, rather than dental changes. With the use of MSI's, it is possible that decreasing the force level will allow for the necessary remodeling of sutural interdigitations to take place and promote sutural expansion.

The purpose of the present project is to evaluate whether a complex, mature suture can be remodeled and separated using skeletal anchorage and light continuous forces and to quantify the process using histology and microcomputed tomography (microCT).

A Brief History of Maxillary Expansion

Maxillary expansion has had a controversial history, beginning over 150 years ago. This procedure, which separates the two halves of the maxilla orthopedically, was first reported in 1860 by Emerson C. Angell.[1] Angell's case report documented the effects of a device that was fitted, but not cemented, to the maxillary posterior teeth with two contra-rotating screws to expand the maxilla of a 14-year old female patient. Angell noted a diastema between the central incisors after two weeks of using the device, which he interpreted as separation of the maxilla at the midpalatal suture. At the time this caused a great deal of controversy among his colleagues and there was a long period of time where the dental profession in the United States did not accept his interpretation. [2] Andrew J. Haas credits Korkaus with reintroducing the procedure to the U.S. in 1956.[3] Haas himself is credited with the surge in popularity of maxillary expansion in the US with his publication of case reports documenting 10 of his 45 patients treated in this manner. More than a century and a half after Angell first described the procedure, maxillary expansion in various forms is used by a majority of orthodontists in the United States.[4]

Indications for Maxillary Expansion

Orthodontists prescribe maxillary expansion to alleviate a number of clinical problems that patients present with, including transverse deficiencies, tooth size-arch length discrepancies (TSALD), and crossbites. Haas stated early on that the procedure could be used to treat unilateral and bilateral crossbites in days, rather than the years it may take with conventional braces.[3] He argued the superiority of this method because the denture bases themselves are widened; the teeth are not simply tipped buccally. Wertz, among others, found a gain in nasal cavity width with a subsequent increase in nasal capacity.[5] Adkins et al. quantified a gain in arch perimeter as a result of maxillary expansion and found that for every millimeter of increase in transpalatal width of the premolars, there is a 0.7 mm increase in arch perimeter, which may be used to correct a tooth size arch length deficiency (TSALD).[6] Further studies corroborated this finding and have found sutural expansion to be stable in the long term.[7, 8]

Anatomy and Histologic Structure of Craniofacial Sutures

The maxillary suture and all other animal sutures are classified as synarthroses. In sutures, two bones come together and are joined by fibrous tissue such that minimal movement can occur.[9] According to Persson, the main biologic function of sutures is to unite bones while allowing minor movements, to act as areas of growth, and to absorb mechanical stresses in order to protect osteogenic tissue. [9]

In 1956, Pritchard et al. performed histologic studies of craniofacial sutures in a number of animals, including rats, sheep, rabbits, pigs, cats, and man, at various stages of development.[10] They found that the different animals shared the same basic components of craniofacial sutures. The group identified five intervening layers of cells and fibers between adjoining bones- two outer cambial layers, two fibrous capsules, and the loose cellular middle zone. The cambial layer contains fine collagen bundles, which are ‘osteogenetic’ and will later become Sharpey’s fibers. This is a highly cellular area with an outer zone of pro-osteoblasts and an inner zone of definitive osteoblasts, which make this a site of active osteogenesis. The periosteal fibrous capsule contains collagenous fibers interspersed with elongated fibroblasts at the advancing edge perpendicular to the advancing edge of bone, and parallel to it elsewhere. The loose cellular middle zone is highly vascular with loose collagen fibers. Surrounding these sutures are two uniting layers of fibrous laminae- both on the internal and external surfaces of the bone.

More recent studies have shown this five-layered sutural structure to be correct but only in its early stages.[11] As sutural development progresses, the suture is reduced to only three layers: two interconnecting fibrous layers with a highly cellular middle zone. At the sutural margins there are three zones of ossification: an inner mineralized zone, a middle area of osteoid accumulation, and a peripheral zone of cells undergoing differentiation.[11] This peripheral zone is rich with fibroblasts and osteoprogenitor cells with a capacity to produce osteoid matrix.

Although cranial and facial sutures develop in a slightly different manner, once formed there is no difference structurally, functionally or in how they respond to mechanical intervention.[9]

Sutural Growth

Sutures of the facial skeleton in humans are fully formed by week 17 in utero.[11] As growth of the face proceeds, the sutures are constantly being separated by other body parts or organs (e.g. the brain). This separation causes stretching of the fibers crossing the suture, which leads to differentiation of osteoprogenitor cells. These cells at the advancing bone fronts produce osteoid, which is eventually mineralized, initially becoming woven bone. This new bone maintains the suture at an approximately constant width.[12] As suture growth is determined extrinsically, these are growth sites, not centers, and bone formation occurs by intramembranous ossification.[12] There are osteocytic and fibrocytic cell populations responsible for the generation and remodeling of bone that takes place. As the two halves of the suture approach one another, inductive signals are released from the bone fronts which prevent its obliteration.[12] Depending on anatomic location, these sutures may overlap, as seen in beveled sutures or simply abut one another. The latter is the case with midline sutures. There is no immediate overlapping which classifies them as butt-end sutures. [13]

The Aging Suture

After initial growth at a suture ceases, there are a number of morphologic and biologic changes that take place, which many authors and clinicians cite as the reasons for increased difficulty in obtaining orthopedic maxillary expansion. The orthodontic text by Graber et al., states that maxillary expansion is increasingly difficult with age.[14] Further, when growth of the cranium is complete, most sutures ossify and orthopedic intervention at sutures is no longer possible.[14] Critics have cited increasing sutural complexity with age, fusion of the suture, decreased cellular capacity, and inhibition by the aging sutures that the maxilla articulates with. The data must be closely evaluated to discern why some authors have come to these conclusions and to see if it really is possible to expand the mature maxillary suture.

Morphologic Changes

Numerous studies have shown increasing sutural complexity and bony interdigitations with age. A classic study by Melsen histologically evaluated palatal specimens removed at autopsy from 33 boys and 27 girls aged 0 to 18 years.[15] She classified the development of the midpalatal suture into three stages. During the infantile period, the suture is short, broad and y-shaped. During the juvenile period, the suture becomes more sinuous with interdigitations. Finally, during the adolescent period, she found that the interdigitations became so pronounced that separating the two halves of the maxilla would be impossible without fracturing these processes. Following rapid maxillary expansion in adolescents, she showed that there were fractured bone spicules histologically.[16]

It is further believed that the changing sutural morphology is related to functional demands on the suture. Jaslow, who evaluated the strength of goat cranial sutures and their energy absorption, concluded that patent cranial sutures in adult animals act like shock absorbers.[17] With increasing interdigitation, the ability of the suture to absorb energy, as well as their ultimate strength, is greatly increased.

Herring et al. showed that the type of force placed on a suture correlates with its distinct morphologic structure. [18] They studied the zygomatic arch, due to its relatively simple force system. There are two distinct forces experienced by the suture within its distinct vertical and horizontal components. The vertical segment of the suture was found to be under compression and, histologically, it had bony interdigitations with fibers running obliquely. These interdigitations, they maintained, prevented slippage in any direction and increased the surface area for fibrous attachment. The horizontal segment, which is under tensional forces, had no such interdigitations, even though the force levels were the same. They concluded that it is not the force level, but the force direction, which leads to a particular sutural adaptation. It has been proposed that the longer these sutures remain active, the greater the development of these interdigitations.[13, 19]

While sutures become increasingly complex through adolescence, they do not continue to increase in complexity beyond this age. A recent study by Korbmacher et al. evaluated autopsy palatal specimens from 28 individuals aged 14-71.[20] These authors used microCT to evaluate the interdigitation of the palatal suture and determine if any relationships exist between age and interdigitation. The specimens were divided into three groups: <25 years, 25-30 years, and >30 years. They found no significant differences in interdigitation among these three groups. They did, however, note a large inter-individual variation in the youngest and oldest groups. This study suggests the suture increases in complexity up to a point, after which functional demands direct the complexity of the suture.

Sutural Ligament Changes

It has also been suggested that it becomes increasingly difficulty to obtain intermaxillary separation with age due to changes in the ligament at cellular and tissue levels. Ten Cate et al. described the cellular components of growing sutures based on his histological observation of growing rat calvaria.[21] The osteoblasts lining the suture margins of growing animals are large cells highly filled with cellular components. This is in contrast to non-growing animals where the osteoblasts are inactive and show a dramatic reduction in cytoplasmic organelles, rendering them less capable of secreting matrices.[21] Although the secretions were less in the non-growing animals, they were still present to some extent.

Kokich, who evaluated the frontozygomatic sutures of humans 20 to 95 years of age, found decreasing numbers of fiber bundles, osteoblasts and fibroblasts with increasing age.[19] The areas of deposition and resorption were also reduced, but the areas were present to some extent at all ages.

Brin and colleagues[22] evaluated rapid palatal expansion in young and old cats focusing on cyclic nucleotides. Nucleotides fluctuate in bone cells undergoing tensile strain. They participate in the initial activation of bone producing cells and are involved in the continuous cellular response that leads to bone remodeling. These authors found that in the young animals, the staining intensity for both cAMP and cGMP were elevated, whereas in the older animals, the staining was faint and the bony deposition at the sutural margins was less than in the younger animals. The authors concluded that the bone cells of older animals are not as responsive to tensile forces as those in younger animals, but they were still responsive. Unfortunately, the expansion in this study only lasted for 15 days, making it impossible to evaluate longer-term effects on the adult cells. Although the regenerative cells appear to be decreased in number and quality, the suture appears to maintain its regenerative capacity.

Fusion

Fusion is the eventual endpoint of all sutures of the craniofacial complex.[13] However, there is much controversy concerning the ages at which maxillary sutures close in humans. Most cranial vault sutures of humans tend to become obliterated during the second or third decade of life. [23, 24] However, facial sutures tend to remain patent until much later in life. [25] It has been suggested that the patency of these sutures is maintained as a result of masticatory forces. Behrents et al. studied the effects of masticatory forces on the sagittal suture of growing monkeys.[26] They found that even with a small bite force, the temporalis muscle delivers enough tensile strain to separate the suture.

In the aging palatal suture, obliteration takes place by the formation of bony bridges across the suture. Persson et al., concluded that these bony bridges, or spicules, occur after growth at the suture has ceased and that the bridges are preceded by strong collagenous fiber bundles that run uninterruptedly across the suture.[27] They further noted that osteoclastic resorption was present adjacent to the areas of obliteration and that undermining resorption of these spicules contributed to the reopening of an obliterated suture.

Persson and Thilander sought to quantify sutural closure with age.[28] Using palatal autopsy specimens from humans 15 to 35 years of age, they developed an obliteration index, calculated from histologic sections as the obliterated suture length relative to the entire suture length. While there was large inter-individual variability, palatal sutures showed a moderate increase in obliteration throughout the age range studied. Further, they speculated that greater than 5% obliteration would be required before orthopedic separation of the maxilla becomes impossible, which they found at 25 years of age.

A number of studies do not support the notion of increasing sutural obliteration with age. Sicher stated that fusion of the intermaxillary suture does not begin until the mid thirties.[29] An early study by Wright held a similar view, contending that the suture remains unossified and relatively easy to split up to 35 years of age.[30] Kokich and colleagues found that, in monkeys, midpalatal suture closure was not present in any of their specimens, from 4 to 22 years of age.[31]

The study by Korbmacher et al.,[20] dicussed previously, also evaluated sutural obliteration. They used the obliteration index proposed by Persson and Thilander[28] and found no age effect for obliteration of the palatal suture. The mean obliteration was found to be low in all groups with a minimum of 0% and a maximum of 7.3%. Once again, large inter-individual variability was noted, with the oldest specimen, a 71 year-old female, having an obliteration index of 0%.

A study by Knaup et al. produced similar findings.[32] Using histology, they evaluated 22 palate autopsy specimens from individuals 18 to 63 years of age, divided into groups <26 years and >26 years of age. Like Korbmacher et al.,[20] they found that the obliteration was low in all subjects. However, they did find a statistically significant difference. The median obliteration values were 0% and 3.11% for the younger and older groups, respectively. However, they concluded that this increased ossification was not a valid reason for increased resistance to maxillary expansion in younger or older subjects.

The field of forensics has also attempted and failed correlate maxillary suture fusion with age. One study using fusion of the midpalatal suture in autopsy specimens as a means of estimating age found that beyond age 33, predicted ages based on sutural obliteration show ‘no demonstrable trend in accuracy when compared to predicted ages.’[33]

In summary, the literature does not produce strong evidence for age-dependent fusion of the midpalatal suture and, further, that fusion does not appear to preclude its separation in older individuals.

Circummaxillary Sutures

Changes of the sutural articulations of bones surrounding the maxilla have been proposed as a limiting factor for maxillary expansion among older individuals.[25, 34] Like the maxillary suture, these sutures show the same changes in morphology, cellularity, and fusion with age.[19]

A study of maxillary expansion in dry skulls of adults showed that there was little difficulty in separating the midpalatal suture.[34] It was the circummaxillary articulations that prevented widening of the maxillary halves. This study is of limited value, however, because the dry skull is not capable of remodeling.

A study by Starnback et al., evaluated the effect of rapid maxillary expansion on circummaxillary sutures in monkeys, with ages comparable to humans 7 to 9 years old.[35] They found that the frontonasal, zygomaticomaxillary and zygomaticotemporal sutures showed increased cellular activity with greater numbers of osteoclasts and osteoblasts and a wider sutural area. This supports the notion that maxillary expansion has at least some effect on its surrounding sutures.

It has been established that both the length and number of interdigitations of the craniofacial sutures increase with age,[19] but it appears to be possible to remodel these bony processes even into adulthood. Brandt et al. performed an experiment using adult

Macaca fascicularis monkeys, in which a posteriorly directed force was delivered to the maxilla via headgear.[36] The authors concluded that even in these adult, non-growing, animals the maxilla was displaced posteriorly, and its craniofacial articulations were altered in all three planes of space. Histologically, the facial sutures showed remodeling of their interdigitations when compared with control animals. They showed remodeling at the zygomaticomaxillary, zygomaticotemporal, and zygomaticofrontal sutures, with the zygomaticomaxillary interdigitations being almost completely remodeled away.

Other authors have stated that, due the morphologic and biologic changes, we can expect a slower rate of expansion in adults.[36, 37] This finding applies to both palatal and circummaxillary sutures. In their study of headgear use on adult monkeys, Brandt and colleagues found decreased effects when compared to the same forces used in younger individuals.[36] Storey came to the same conclusion when separating the maxilla of adult rats.[37] These findings make sense due to the fact that sutures that are more interdigitated have forces acting over a much larger surface area, and are thus have less force per unit area. The decreased cellular components available for remodeling also play a role, thus taking longer to achieve separation.

Looking closely at the evidence, it appears that adult sutures do have the capacity to remodel, albeit at a slower rate than in younger individuals.

Current Treatment in Adults

Currently, there are more and more adults seeking orthodontic care.[14] Musich et al. estimated that approximately 20% of these patients need maxillary expansion for ideal treatment.[38, 39] However, because of the real and perceived sutural changes that occur with age, many clinicians opt to perform surgically assisted rapid palatal expansion (SARPE) when transverse increases are needed.[40, 41] A survey of 441 orthodontic offices that use maxillary expansion showed that age was the greatest limiting factor for prescribing traditional maxillary expansion versus a SARPE, with 79% of offices using age as the primary criterion. [40] Further, the average age for prescribing SARPE was 19 years, with a range from 7 to 32 years. Another survey of 105 orthodontic practitioners in Germany found that the average age after which they believed traditional RPE to be ineffective was 17 years, but they prescribed SARPE from 10 to 35 years of age. These are rather large ranges and no clear consensus appears to have been established by practitioners.

Even the recommendations from the literature are ambiguous. As stated earlier, Persson et al. reported that 25 years was the age limit for orthopedic maxillary expansion. In the oral and maxillofacial surgery literature, Epker and Wolford advocated SARPE for individuals over the age of 16.[42] A recommendation by Timms and Vero put the age for SARPE at 25. The orthodontic text by Proffit states that above the age of 15, the chances of opening the maxillary suture declines due to increasing interdigitations, and that the upper age of gaining significant expansion is 15 to 18 years.[43]

Although SARPE is a valid treatment modality for maxillary expansion in non-growing individuals,[44] it does not come without significant morbidities. A review by Suri and Taneja found significant hemorrhage, gingival recession, root resorption, injury to the branches of the maxillary nerve, infection, pain, devitalization of teeth and altered pulpal blood flow, periodontal breakdown, sinus infection, alar base flaring, extrusion of teeth attached to the appliance, relapse, and unilateral expansion associated with SARPE.[44] If hemorrhages are significant, they may be life threatening, requiring blood transfusions, and additional hospital stays. They also noted some unusual complications with SARPE, including compartment syndrome resulting in permanent blindness, bilateral lingual anesthesia, and a nasopalatine canal cyst.[44] Furthermore, SARPE is associated with an additional surgical cost to the patient, and many patients are unable or unwilling to undergo this surgical procedure. With all of these possible costs and complications in mind, it behooves clinicians to look for a more conservative means for orthopedically expanding the adult maxillary suture.

Biology of Maxillary Expansion

Early histologic studies of maxillary expansion sought to prove that Angell was right; that the maxillary suture was indeed separated. An early study by Cleall et al. evaluated the maxillary expansion of 10 juvenile rhesus monkeys, and two control monkeys, at 2 weeks post expansion and 3 months post expansion.[45] These authors concluded that there was ‘no doubt’ that the maxillary suture had been split, that the bony defect created in this area was filled with bone, and that it eventually returned to its normal form.[45]

At the cellular level, Ten Cate et al. studied the histology of rat sutures at 20 time points post expansion, ranging from 1 hour to 42 days.[21] These authors chronicled the sequence of events that occurs at the cellular level, beginning with small tears within the suture, while the periosteum, or uniting layers, remained undamaged. The torn collagen fibrils were associated with an exudate that was introduced into the defects. Death of some fibroblasts and osteoblasts occurred near the tears. By 24 hours, an influx of macrophages and pioneer fibroblasts had occurred, with fibroblasts creating collagen and macrophages taking up the exudate. Within 3-4 days the pre-existing and undamaged osteoblasts began forming new bone at the suture margin. For the next 1-2 weeks, lamellae were formed along the margin. Once the expansion force was removed, remodeling of the bone and suture began until a normal sutural morphology and dimension was achieved.

Expansion in humans has been studied as well. Melsen obtained biopsy samples from the maxillary suture of children undergoing expansion at different time points.[16] Interestingly, she found that expansion in the pre-pubertal children, the histology showed signs of a ‘true stimulation’ of normal growth. That is, the expansion did not result in a traumatic response, but increases in osteoclasts and osteoblasts and an even, smooth, deposition of bone along the sutural margin. However, in older children (12-13 years), due to the shape of the sutures, a traumatic response was seen with multiple microfractures. This was followed by a resultant repair and healing phase.[16]

Slow Expansion vs. Rapid Expansion

The two different types of biologic response that Melsen observed, stimulation of normal growth and traumatic response followed by repair,[16] are found in different age groups as well as with different types of expansion.

Most early expansion devices, as well as the majority used today, are of the rapid expansion type, whereby a jack-screw is activated a specific amount per day, usually between 0.2 to 0.5 mm per day, until the desired expansion is achieved. [1, 3, 4, 8, 16, 46] These activations produce a rapid increase in force with each activation until the suture separates. In preadolescents, whose sutures are not yet complex, separation stimulates normal growth[16] and the force level dissipates quickly in relation to the separation of the suture. In adolescents, the complexity of the suture described by Melsen et al., causes a rapid increase in force values placed on the teeth and maxillary complex. This occurs until the interdigitating processes fracture and the two halves of the maxilla separate, causing a rapid decrease in the force. The force response to these activations varies with age and can be exceedingly high in older individuals, up to 34.8 lbs.[47]

An alternative to rapid expansion with a jack-screw is slow maxillary expansion (SME), which is generally produced using nickel titanium or TMA wires and springs which produce a constant force level.[48] These forces in humans have been measured around 2lbs, with lower rates of expansion (0.4-1.1mm/week).[37, 49] This type of expansion has been shown to maintain sutural integrity with less tissue damage and produce similar clinical results over longer periods with less relapse potential.[37, 49, 50]

Traditional Maxillary Expansion in Adults

There have been numerous case reports documenting successful and unsuccessful non-surgical maxillary expansion in adults. To understand the difference between reports, it is necessary to closely evaluate their methods of expansion and results.

Capelloza et al. performed rapid maxillary expansion with tooth-borne appliances in 38 consecutive patients (males over 17 years and females over 15 years).[51] The rate of expansion was high, with four quarter-turns immediately, and two quarter-turns morning and night for the next five days. Using a clinical diastema as evidence of sutural expansion, the authors reported success in 81.5% of the patients. However, these authors noted only a moderate amount of sutural separation, with the majority of the expansion due to alveolar bending.[51] Only 31% of the patients experienced no complications, with the remainder experiencing some combination of pain, edema, and palatal lesions. They concluded that expansion at the apical base level was small and that it was accompanied by large amounts of buccal tipping. They suggested that patients with a compromised periodontal situation should not consider this procedure. For only moderate gains in suture width, patients underwent, in some instances, severe pain. Further, the force levels placed on the suture with this rate of expansion were extremely high.

Handelman reported the effect of expansion in five adults.[52] Rather than calling it rapid maxillary expansion, he called the procedure rapid maxillary alveolar expansion (RMAE). Successful maxillary dental width was obtained, which he attributed it to bending of the alveolus rather than opening of the suture.[52] His activation schedule was less than that of Capelloza et al.,[51] which may account for the decrease in complications that were reported. He further advocated that this procedure should not be performed on individuals with a compromised periodontium.

In a later study, Handelman et al.[53] reported on 47 adults treated with RME. This time, consecutively treated patients and a control group were used. Nine of the 47 patients experienced complications, such as pain, on which basis the author suggested lowering the activation schedule to one quarter-turn every other day to minimize patient discomfort. However, no mention of sutural expansion, or diastema formation was noted in their study, again indicating that their expansion was dentoalveolar and not sutural.

Numerous other studies have shown variable successes with RME in adults. Wertz[34], in his study of 82 adolescents of various ages, showed decreased skeletal changes in the older patients and was unsuccessful in expanding the maxillary suture of one 16 year-old female. Alpern and Yursoko[54] reported that they were able to expand up to the age of 20 in females and 25 in males, beyond this age, their patients required surgical intervention to separate the suture. Haas[3] performed rapid expansion in 80 individuals and was unable to obtain sutural expansion in two of his older male patients, one 17 and the other 19 years-old.

Based on these studies and case reports, there is a large degree of variability among studies reporting expansion among young adults. Many older adults experienced significant complications when large amounts of force were used. Further, the predominant form of expansion appears to be at the level of the alveolus, which is concerning for adults who are more prone to periodontal issues than their younger counterparts.

Proper Expansion Appliance for Adults

The idea that the maxillary suture separation in adults is difficult has been based on studies using traditional tooth-borne rapid maxillary expansion (RME), which are fraught with potential complications.

It has been well established that rapid maxillary expansion causes an accumulation of large forces on the maxillary dentition, especially in adults, where the time required for the load to dissipate is increased.[47] Zimring and Isaacson found that the load produced in their oldest patient undergoing RME, a 15 year-old female, was increasing so rapidly that they reduced their activation schedule from 2 turns to one turn per day, and then again to every other day when the load would not decrease. Even with such a reduced activation schedule, the residual load was 34.8 lbs of force, which necessitated premature removal of the appliance. In many of the case reports previously described, the authors also suggested reducing the activation schedule in order to reduce patient discomfort because the residual loads do not decay rapidly.[3, 34, 51]

RME has been suggested because it is thought that a heavy force is needed to overcome the resistance of the maxillary suture and to, at least temporarily, minimize dental translation.[37] Unfortunately this force appears to be incompatible with expansion of the adult suture and tooth-born appliances. As has been shown, high forces cause mild to severe discomfort. Recent advances in mini screw implants (MSI's) have allowed for the development of bone-born expanders, with similar results both short and long-term when compared to tooth born maxillary expansion.[55] This type of appliance coupled with a nickel titanium expansion device[56-58] should allow the type of continuous dissipating forces so as not to overwhelm the reduced osteocyte and fibrocyte populations while remodeling the bony processes.

An Animal Model for Studying Mature Sutural Expansion

The goal of the present study was to mimic maxillary expansion in adult humans experimentally. There is clearly a need for expansion in the adult population and there are difficulties and complications associated with traditional approaches. A number of studies have recently used the sagittal suture of growing rabbits to study bone-borne slow sutural expansion. [59-61] These studies used light continuous forces and were able to quantify sutural separation and bone formation in younger animals.

The present study uses the rabbit animal model. Storey noted that the rabbit model was ideal for evaluating adult sutures because rabbits also exhibit increasing interdigitation with age. [37] Persson et al. confirmed the similarity of rabbit and human sutures based on their histologic comparisons of the adult rabbit sagittal suture with the adult human palatal suture.[27] The human material consisted of palatal autopsy specimens of 24 adults, aged 15-35. Their rabbit specimens included the sagittal sutures of rabbits 24-36 months of age.[27] They concluded that closure of these sutures in the humans and rabbits proceed in essentially the same manner: dense collagen bundles crossing the suture precede the development of bony spicules or areas of obliteration. Further, in both sutures they noted osteoclastic resorption adjacent to the bony spicules which, they maintained, contributed to opening of the suture by undermining resporption at both sides of the calcified bridges.[27]

The age at which the sagittal suture of rabbits ceases growth has also been studied. Mooney et al. placed bone markers on either side of the sagittal suture of New Zealand White (NZW) rabbits at 10 days of age and followed them for 18 weeks.[62] They found increases in width of the markers across the sagittal suture through 12 weeks of age. The average increase from 12 to 18 weeks was 0.1mm. Alberius and Selvik, who performed a similar study on NZW rabbits, found that the sagittal (inter-parietal) suture had completed its growth before the age of 8 months.[63] Unfortunately, the time-points at which their measurements were taken were 112 days and 231 days, so an exact age of completion of sutural growth cannot be obtained. Based on the dramatic decline of growth rate between days 84 and 112, it can be assumed that completion of growth was closer to the four-month mark.

Specific Aims

Currently there is no predictable method of expanding the adult maxillary suture non-surgically and without significant morbidities. A different method of expansion of mature sutures is necessary due to the distinct sutural characteristics of adults. The following research questions will be evaluated to better understand sutural expansion in this age group:

1) Is it possible to expand a complex, non-growing suture slowly using continuous, light forces and an MSI-born appliance?
2) What is the rate of bone formation compared to bone separation?
3) How much remodeling of the bone takes place and where?

4) What does a mature suture look like in 3D before and after expansion?

An animal model previously used in suture expansion studies was adapted to study mature sutures. The midsagittal suture was chosen for this study because of its noted similarity to adult human midpalatal sutures, both in terms of its structure and its histology.[27, 64]

CHAPTER II

BACKGROUND

Orthodontists have expanded midpalatal sutures of the maxilla for over a century. While initially introduced in the United States during the 1860s, palatal expansion was not commonly performed until decades later.[2] Its popularization has been based on the profession's understanding of the suture biology and the tissue reactions at the sutural level.[2, 3, 46] Maxillary expansion has predominately been used in juveniles and young adolescents, whose sutures are less complex and easily separated. For older adolescents, whose sutures are more complex, expansion requires higher forces, delivered to the teeth via the expansion appliance.

Expansion in adults has been limited by the belief that the midpalatal suture will not split,[14] and that the expansion forces may negatively affect the periodontium.[51] There have been many attempts to separate the sutures of adults,[51-54] but only sporadic cases have been successful. The majority of the expansion in adults has been shown to be due to bending of the alveolus, which is associated with significant morbidities.[52, 53] For adults in need of maxillary expansion, surgically assisted rapid palatal expansion or orthognathic surgery are commonly prescribed. Both procedures are costly and also associated with a host of morbidities.[44]

Currently, there is no effective, predictable, non-surgical method to separate the mature midpalatal suture in humans. Sutural expansion in older adolescents and young adults may be possible because the facial sutures maintain their patency until much later in life than commonly thought. Growth of the midpalatal suture is complete by about 14 years of age in girls and 16 years in boys,[65] and the suture increases in interdigitation up to adolescence.[16] However, sutural complexity is highly variable and does not appear to change in adults, although sutural bone density increases.[20] Most importantly, facial sutures do not fuse until much later in life,[19, 31] and retain the cells necessary for bony remodeling.[19, 22] The sutures of the bones that articulate with the maxilla, and are affected by expansion, remain patent until later in life.[19, 36]

The purpose of the present study was to evaluate whether a complex, mature suture can be remodeled and separated using skeletal anchorage and light continuous forces. Theoretically, skeletal anchorage makes it possible to maximize the skeletal, and minimize dental, changes. Lighter forces may be necessary for modeling to take place under the biological constraints of mature sutures.

CHAPTER III

MATERIALS AND METHODS

Animals

The sample consisted of 12 adult female New Zealand White rabbits (8-9 months old). The housing, care, and experimental protocol were in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee, Texas A&M University Baylor College of Dentistry. The animals were initially quarantined for three days, maintained under standard laboratory conditions and were provided a stock diet and water ad libitum.

Experimental Design

The rabbits were randomly divided into three groups: a 42-day loaded group (n=7), a 42-day control group (n=3), and a 105-day loaded group (n=2). The 42-day period was chosen based on a similar study in immature rabbits.[61] The 105-day group made it possible to preliminarily evaluate longer-term expansion effects. The sutures of both the 42 and 105-day groups were expanded using the same 100 gram force level.

Mini Screw Implant Insertion, Bone Marker Placement, and Force Delivery

All animals were anesthetized IM with ketamine 35mg/kg and xylazine 1mg/kg. The surgical sites were shaved and disinfected. All procedures were performed under sterile conditions. Local anesthesia was obtained with 2% lidocaine with epinephrine

1:100,00. Two regions of skin were removed from the skull using a 3 mm punch biopsy instrument, approximately 5 mm lateral to the midsagittal suture, at a point midway between the anterior and posterior orbital rims (Figure 1). The punch sites were displaced 3-4 mm anteriorly and two 1.0 mm x 0.3 mm sterile stainless steel bone markers were tapped into the skull using a custom-made stainless steel appliance. Two custom-made MSIs (3mm x 1.7mm) were placed through the initial punch sites with a manual driver. Postoperative analgesia was controlled with nalbuphine 2mg/kg/sc BID PRN.

A 20 mm stainless steel wire (0.20) was inserted passively through the holes in the heads of the MSIs and loaded with a 15 mm Sentalloy® nickel titanium open-coil spring, or clipped passive (0g) for the control group (GAC, Bohemia, NY). To keep the wire from sliding, the ends were bent with a stop loop and bonded with composite (Figure 1A). The 100 g force levels were maintained due to the properties of the nickel titanium coil springs, which remained compressed between 8 and 12 mm.[48]

Measurements

Measurements were taken at the time of the initial surgery and every 14 days for the first 42 days, and at 91 and 105 days for the 105-day group. Measurements were taken under the same anesthesia protocol used for the initial surgery and included the animals' weights, ventrodorsal radiographs and inter-MSI measurements with digital calipers. Caliper measurements were taken by two operators (R.P., P.T.) blinded to the experimental and control groups, and averaged. Radiographs were exposed on phosphor plates at 68 kVp and 10 mA for 12 milliseconds from a fixed distance (30 cm) with the animal in the supine position (Figure 1B). Images were transferred to Dolphin Imaging Software 11.5 (Dolphin Imaging, Chatsworth, CA). Calibration and inter-bone marker distances were measured by one blinded examiner (R.P). After four weeks, 24 radiographs were remeasured to establish intraexaminer method error (0.049 mm) using Dahlberg's formula ($\sqrt{\sum d^2/2n}$).

Fluorescent Bone Labeling

To identify bone-forming regions and quantify mineral apposition rates (MAR), the fluorescent labels calcein (10mg/kg; Sigma, St. Louis, MO) and alizarin complexone (20mg/kg/im; Sigma, St. Louis, MO) were administered to all animals. Calcein was given at 14 days in the 42-day group and day 77 in the 105-day group. It was again administered 4-5 days prior to sacrifice (day 37-38 in the 42-day group; day 100-101 in the 105-day group). Alizarin complexone was administered 14 days prior to sacrifice, at day 28 in the 42-day group, and at day 91 in the 105-day group.

Tissue Preparation for μCT and Histologic Analysis

After 42 or 105 days, the animals were anesthetized with ketamine and xylazine, and euthanized through exsanguination by perfusion of saline. Fixation was performed with 4% paraformaldehyde. A 1 cm x 0.5 cm x 2 cm section of the sagittal suture and adjacent bone was dissected between the MSI's and scanned with a desktop μCT 35 system (Scanco Medical; AG, Switzerland) at 55 kVp and 10 μm. The suture was oriented perpendicular to the image plane.

The 3D volume was segmented and analyzed using Analyze 11.0 (AnalyzeDirect, Overland Park, KS) by one blinded examiner (R.P). Three regions of the suture were analyzed in all specimens (anterior, middle and posterior). Each region contained 100 sequential coronal slices (100 * 10 μm =1 mm total), with the middle region located where the wire crossed the suture and the other regions located approximately 3 mm anterior and posterior to the MSIs (Figure 2). The suture was segmented slice-by-slice using the auto-draw feature by setting a seed point within the suture and a threshold of 0-72. Limits were manually drawn at the superior and inferior edges of the suture and where the suture communicated with the medullary space. Any extraneous data was removed in the 3D rendering (Figure 3) and 3D volume and surface area were obtained from this region. Four weeks later, three specimens were re-segmented and analyzed to establish intraexaminer method error for the 3D volume (8×10^{-4} μm).

Three slices from each region (first, middle and last) were exported as TIFF files to Bioquant OSTEO 13.2 (Bioquant Image Analysis Corporation, Nashville, TN) software. The suture was then traced and its length and height were measured. To evaluate the level of interdigitation, the complexity index (CI) was calculated as:

$$\text{CI} = \text{Overall suture length/Suture Height.}$$

The measurements from the three slices from each region were averaged. The width at the endocranial and ectocranial surfaces (1 mm) of the suture were measured in the same manner.

Three control 42-day and four experimental 42-day sutures were randomly selected, as well as two experimental 105-day sutures, for histological analysis. Analysis was performed by one blinded examiner (R.P.). The specimens were sectioned coronally into two parts. The anterior parts were fixed with 70% ethanol for two weeks without decalcification. After dehydration with an ascending series of ethanol (70-100%), they were embedded in methyl methacrylate and sectioned coronally (approximately 60μm) using a diamond saw (3 sequential sections per animal). The images of the sections were digitized using confocal microscopy (Leica TCS-SP5, Buffalo Grove, IL) and collected from a single optical imaging plane. Image analysis was performed with Bioquant OSTEO 13.2 (Bioquant Image Analysis Corporation, Nashville, TN) software. The bone labels were traced at both the ectocranial and endocranial surfaces (1 mm) bilaterally. The distances between the labels were taken at 90° to each other, every 5 μm, and averaged. This bone between the innermost label and the middle label represents the mineral apposition rate (MAR) between 14-28 days, and the bone between the middle and outermost layers is the MAR between 28-42 days (Figure 4).

The posterior parts of the same sutures were decalcified and embedded with paraffin and ribbon sectioned (6 μm) coronally into 15 sections. Sections 1, 6, and 11 were H&E stained. Sections 2, 7, and 11 were TRAP stained to visualize osteoclasts. All H&E and TRAP sections were digitized using a SPOT 5.1 Advanced Camera (SPOT Imaging Solutions, Sterling Heights, MI.)

Statistical Analysis

Statistical analysis was performed using SPSS® 22.0 (Chicago, IL). Non-parametric statistics were used due to the small sample sizes. Mann-Whitney U tests were used to compare groups. Wilcoxon signed-rank tests were used to compare MAR within animals. A Friedman test was used to compare changes in weight over time. Spearman correlations were used to evaluate the relationship between MSI and bone marker separation. Significance was set at $p < 0.05$.

CHAPTER IV

RESULTS

The ages and weights of the control and experimental animals at the beginning of the experiment did not differ significantly (Figure 5). Neither the control nor the experimental rabbits exhibited statistically significant changes in weight during the experiment. All MSIs (24 of 24) remained stable throughout the experiment.

Biometric Analysis and Measurements

The biometric measurements showed significant increases in sutural separation in the experimental animals at each time point up to 42 days, following a linear pattern for both the MSI's and bone markers (Figure 6). The bone markers showed significantly ($p< 0.002$) less separation than the MSIs at each time point. At 42 days, bone marker separation was 0.63 mm, and MSI separation was 1.2 mm. Rates of separation decelerated after 42 days. The correlations between bone marker separation and MSI separation were statistically significant at each of the three time points evaluated (Table 1).

Histologic and Histomorphometric Comparison

The H&E sections of the control group showed complex sutural interdigitations. There was no disruption of the sutural fibers, and cement lines were found along the entire lengths of the margins, with evidence of only small amounts of new bone (Figure

7A). In the experimental groups, stretching and disruption of sutural collagen fibers was evident, with numerous Sharpey's fiber insertions and immature woven bone visible at the sutural margins. Cement lines were present with evidence of undermineralized lamellar bone at the suture margins (Figure 7B).

TRAP stained sections of both the control and experimental animals showed osteoclasts within the suture, especially near the ectocranial surfaces (Figure 8). The numbers of osteoclasts present were minimal, with no obvious group.

In control bone, distinct and consistent calcein and alizarin label lines were seen on the endocranial surface, but not at the edges of the sutures (Figure 9A). The limited, sporadic deposition of some labels within the control sutures was inadequate for quantification. The fluorescent bone labeled sections in the experimental group displayed distinct, narrow, label lines along the entire sutural margin (Figure 9B), indicating calcification after days 14 (calcein), 18 (alizarin red), and 37-38 (calcein). MARs were greater on the ectocranial than endocranial surface, but the differences were not statistically significant (6.52 ± 0.57 μm/day from days 14 to 28 and 3.66 ± 0.12 μm/day from days 28 to 37-38, compared to 4.3 ± 0.52 μm/day from days 14 to 28 and 3.14 ± 0.36 μm/day from days 28 to 37-38, respectively; p= 0.144). The MAR was greater during the first than second time period, but the difference was not statistically significant (p= 0.068).

Control sutural complexity was greater in the middle and anterior regions than in the posterior region (1.31 compared to 1.59 and 1.44, respectively; p = 0.068) (Table 3). The complexity in the experimental group was significantly greater than in the control group in the posterior region (p=0.019), but not in the anterior or middle regions (p=0.476; 0.476).

MicroCT analysis of the sutures revealed no significant group differences in the sutural surface area (Figure 10). Overall sutural volume was significantly greater in the experimental animals than the control animals (p= 0.02), with statistically significant (p= 0.02) differences in the posterior region only (Figure 11).

CHAPTER V

DISCUSSION

The age of the animals used in this study was appropriate for evaluating the effects of forces on mature sutures. The control sutures were complex with bony interdigitations, and showed no separation over time. There were only sporadic fluorescent bone labels along the sutural margin of the control animals, indicating normal remodeling of bone rather than sutural separation. The cement lines seen in the H&E sections indicated previous appositional bone growth indicating that bone was laid down prior to the start of the experiment, and certainly prior the first round of calcein (at 14 days). The cortical remodeling period of mature rabbits has been estimated to be around 70 days,[66] which would not have been sufficient to secondarily remodel the cement lines in these 8-9 month-old rabbits over the experimental period. The lack of growth observed supports previous studies showing that the sagittal suture of rabbits stops growing between 6 and 8 months.[63]

Most importantly, it is possible to separate the sagittal suture of mature rabbits using light, continuous, forces and skeletal anchorage. The sutures of both the 42 and 105-day experimental groups separated (Figure 6). While both the MSI's and bone markers were used to evaluate separation, the bone markers were more accurate due to the potential for tipping and bodily movement of the MSI's through the bone.[61] The increases observed were not due to sutural stretching, as previously claimed for maxillary expansion in adults.[52, 53] Theoretically, the width of the control suture limits the amount of sutural stretching (i.e this is when bony contact might be expected to occur) that is possible without breaking the bony interdigitations. Stretching alone cannot explain the changes observed in the present study because sutural separation at 42 days was approximately 9 times the width of the control suture at the endocranial surface, and 14 times the control width at the ectocranial surface. In other words, the separation that occurred far exceeded the limits of stretching, indicating that bony remodeling of the sutural interdigitations occurred.

The rate of sutural separation was linear up to 42 days and was consistent with the rate of bony remodeling. The slow expansion performed in the present study supports previous slow expansion studies showing steady separation over roughly the same time period.[49, 64] Although the pattern was similar, the actual rate was less than reported for younger animals. Using the same force levels and expansion apparatus, Liu et al. achieved 5 times as much expansion in 6-week old rabbits (3.2mm vs. 0.63mm in the present study).[61] Their rabbits were growing and the sutures had not become sufficiently complex to limit separation. Due to the bony remodeling necessary for sutural separation in the present study, a diminished rate of separation might have been expected.

A decreased rate of sutural separation among older animals appears to also be due to age differences in cellular response levels, both in the PDL and sutures. Based on cyclic nucleotide levels, bone cells in the maxillary sutures of older cats have been shown to be less responsive to tensile forces than bone cells in younger animals.[14] Bone remodeling cells (osteoclasts) are also slower to respond to tensile forces in the periodontal ligaments of older animals,[67] which results in a slower initial rate of tooth movement.[68]

The rate of sutural separation was maintained up to day 42, after which it decelerated. The decreased separation rate after day 42 may have been due to the limitations posed by the articulations of other bones, including the parietal, nasal, squamous and maxillary bones. These sutures might also be expected to be complex and have to model in order for expansion to occur. Similarly, modeling of multiple circummaxillary sutures occurs during traditional rapid maxillary expansion.[35, 69] Increasing the number of sutures greatly increases the surface area that the forces are acting on, suggesting that force levels may need to be increased incrementally. Future studies are needed to evaluate bony remodeling at the adjacent sutures. Since the palatal suture has similar constraints from the surrounding bony structures, it appears that the sagittal suture is a good model for palatal expansion.

The mineral apposition rate was greater ectocranially than endocranially. MAR on the ectocranial surface was 50% greater than on the endocranial surface (6.52 μm/day compared to 4.33 μm/day). The lack of a difference was probably due to the lack of power. During maxillary expansion in humans, there is a triangular shaped separation of the maxillary suture, with greater separation occurring at the oral surface.[3, 34] While the amounts of endo- and ectocranial separation were not measured in the present study, wider separation at the ectocranial surface might be expected due to the bony articulations. Increased rates of sutural separation have been shown to be associated with increased rates of mineral apposition,[61] which was seen in the ectocranial aspect of the suture.

Expansion resulted in greater sutural volume. At day 42, volume of the suture increased, especially in the posterior region. This was unexpected because previous rabbit expansion studies have shown greater sutural separation anteriorly, due presumably to decreased resistance from bony articulations.[61] Expansion in humans is also greater anteriorly, for the same reason.[34] Importantly, the control sutures in the present study were considerably less complex in the posterior than the middle and anterior regions of the sutures. Since the anterior and middle regions of the suture were more complex, they should separate more slowly, allowing bone formation to keep up with separation. This would allow the sutural volume to remain the same over time. Conversely, in the posterior region, less modeling may have been necessary to separate the suture, which would have allowed for more separation. The rate of separation in the posterior region may have been relatively greater than bone formation. 3D sutural surface area also increased relatively more in the posterior than the other two regions, but the difference was limited and not statisticially significant, due again perhaps to the small sample size.

Based on these findings, it appears possible to remodel and separate the human maxillary suture in young adults using light, continuous forces and skeletal anchorage. There have been numerous reports of bone-borne maxillary expanders using various force levels in humans with varying results,[56, 58, 70] but an understanding of what is happening within the suture in this age group is not well understood. Increasing the force level beyond the 100 grams in this study would certainly be indicated due to the larger sutural surface area in humans. While amount of sutural separation obtained in this study was statistically significant, the amount at 42 days (0.63 mm) would not be clinically significant in the human population. It has been shown, however, that the rate of bone remodeling can be increased with the use of the regional acceleratory phenomenon (RAP)[71], which could possibly be used in conjunction with this technique to augment the rate of expansion. Further studies are needed to evaluate the proper force level in humans and ways to increase the amount of separation in mature individuals.

CHAPTER VI

CONCLUSIONS

Within the limits of this study, the results show:

1. Eight-to-nine month old female NZW rabbits provide good models for evaluating the effects of forces on mature, non-expanding, sutures.
2. It is possible to separate the sagittal suture of a mature rabbit using light continues forces and skeletal anchorage, with new bone formation and increases in sutural volume.
3. The amount of sutural separation decreases over time, suggesting that other bony articulations limit the amount of separation that occurs at this force level.

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APPENDIX A

FIGURES

Figure 1. Diagram and radiograph of expansion apparatus. (**A**) Superior view of CT scan volumetric rendering of pilot rabbit skull with schematic of expansion apparatus. (**B**) Ventrodorsal radiograph showing expansion apparatus and bone markers.

○ Bone Marker ● MSI ⟷ 100g Nickel-Titanium Open Coil Spring

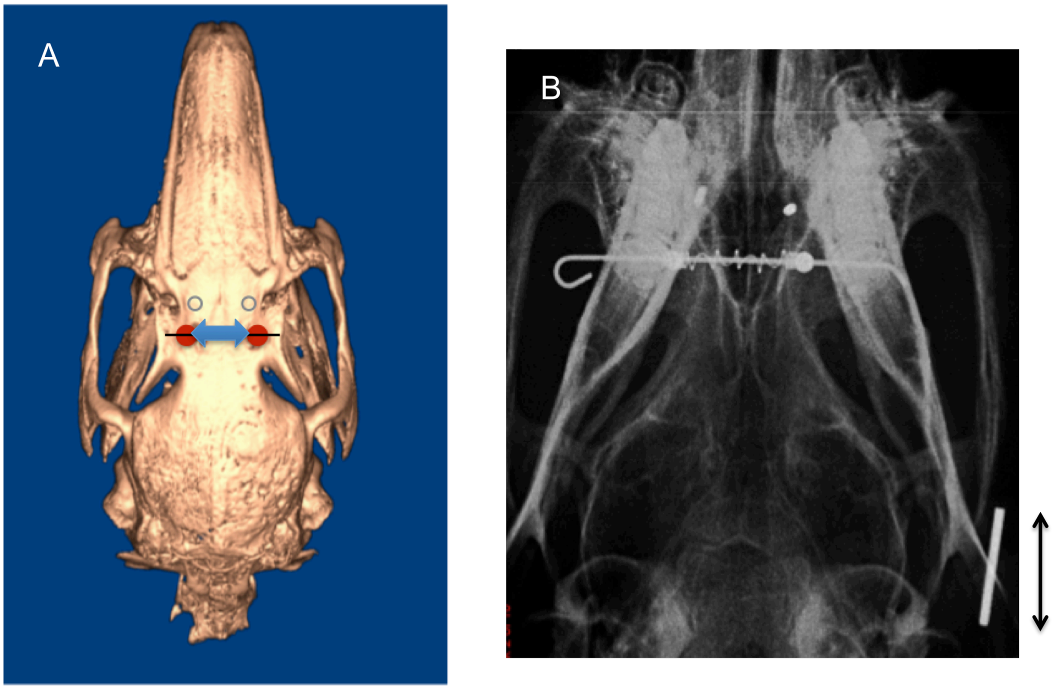


Figure 2. Volumetric rendering of representative suture sample with anterior, middle, and posterior areas of interest highlighted. Superior view of sample (**A**). Sagittal view of sample (**B**).

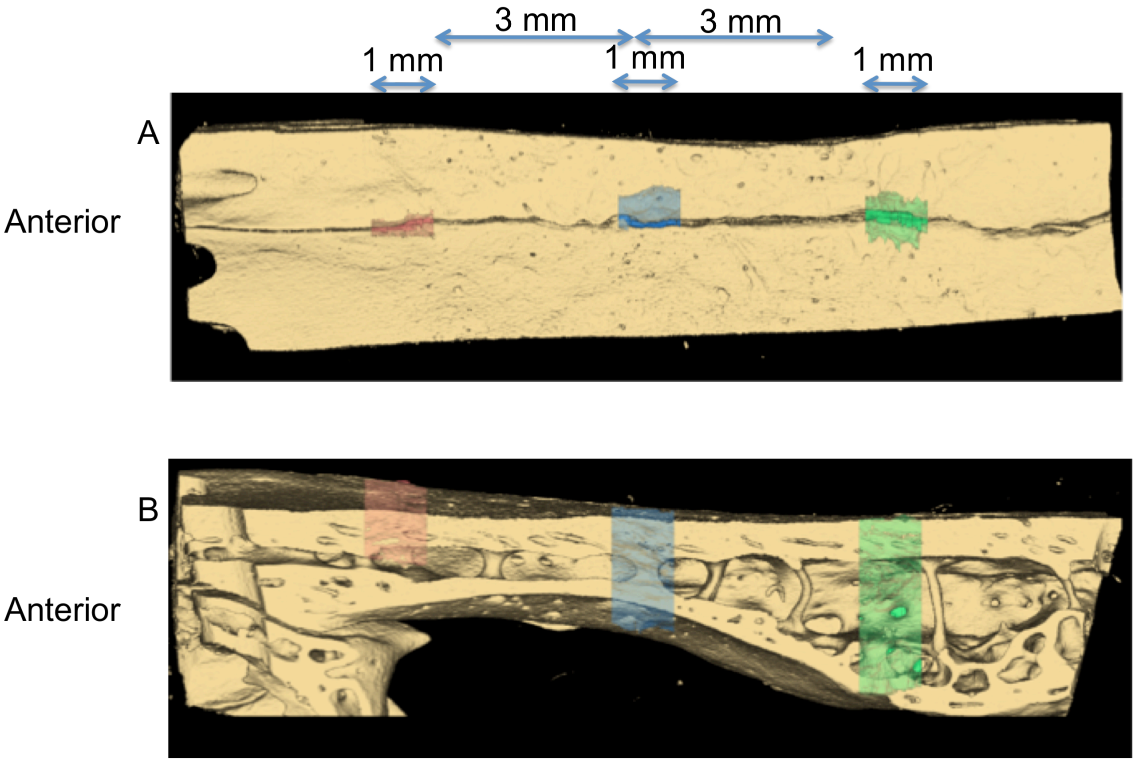


Figure 3. MicroCT segmentation for 3D analysis. 3D rendering of control (**A**) and experimental (**B**) suture. Frontal slice of control (**C**) and experimental (**D**) suture.

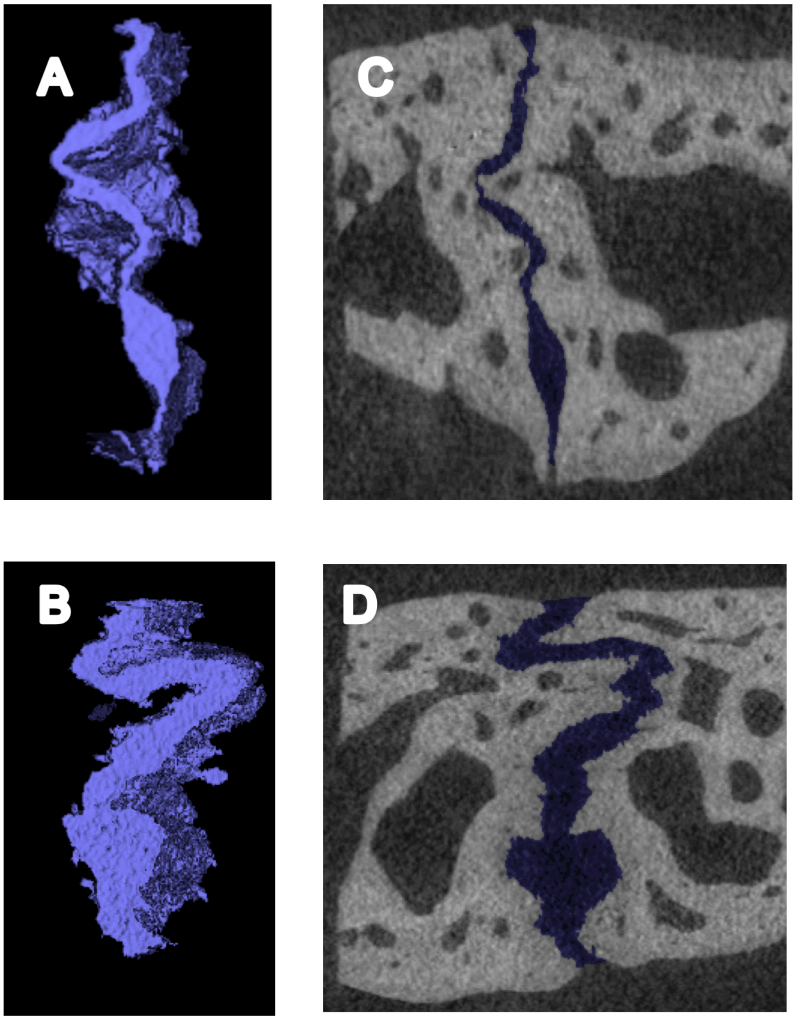


Figure 4. 2D Suture Analyses. (**A**). Histomorphometric analysis of bone labels from days 14 to 28. (Green line: Calcein, days 14, 38; Red line: Alizarin, day 28). (**B**) Representative H&E slide of interdigitated control animal. (**C**) Method of measuring complexity index. Line with arrows is suture height. Curved line is suture length. Sutural Complexity = Suture length/ Suture Height

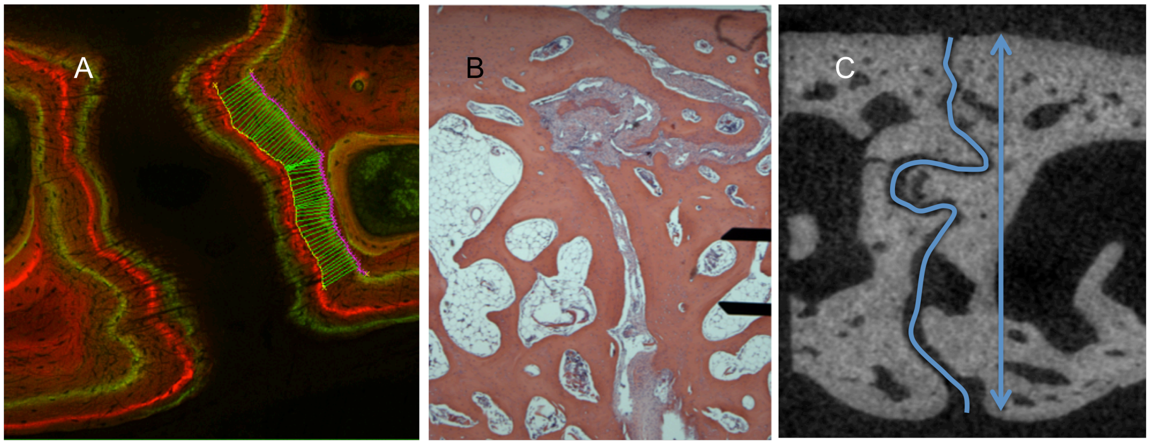


Figure 5: Comparison of ages (**A**) and weights (**B**) of both groups at T0.

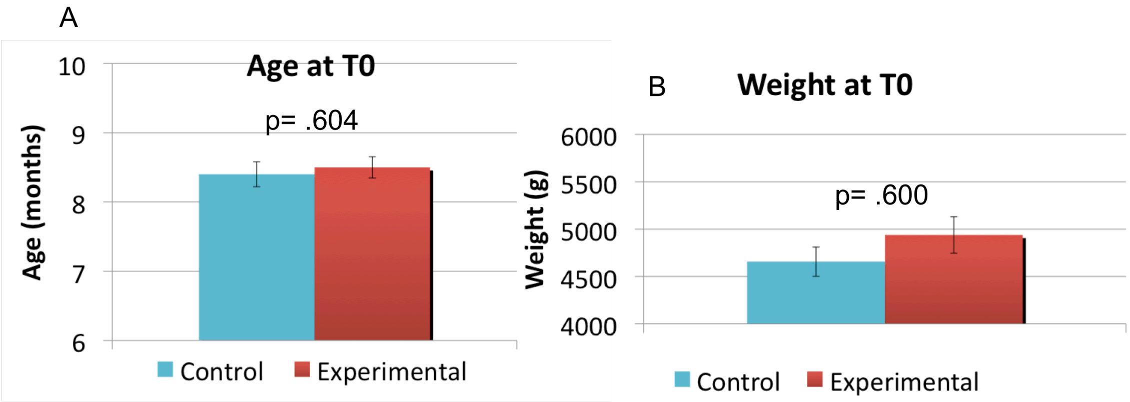


Figure 6: Bone marker and miniscrew separation over time. Inter-bone marker measurements from ventrodorsal radiographs (**A**). Inter-MSI caliper measurements (**B**).

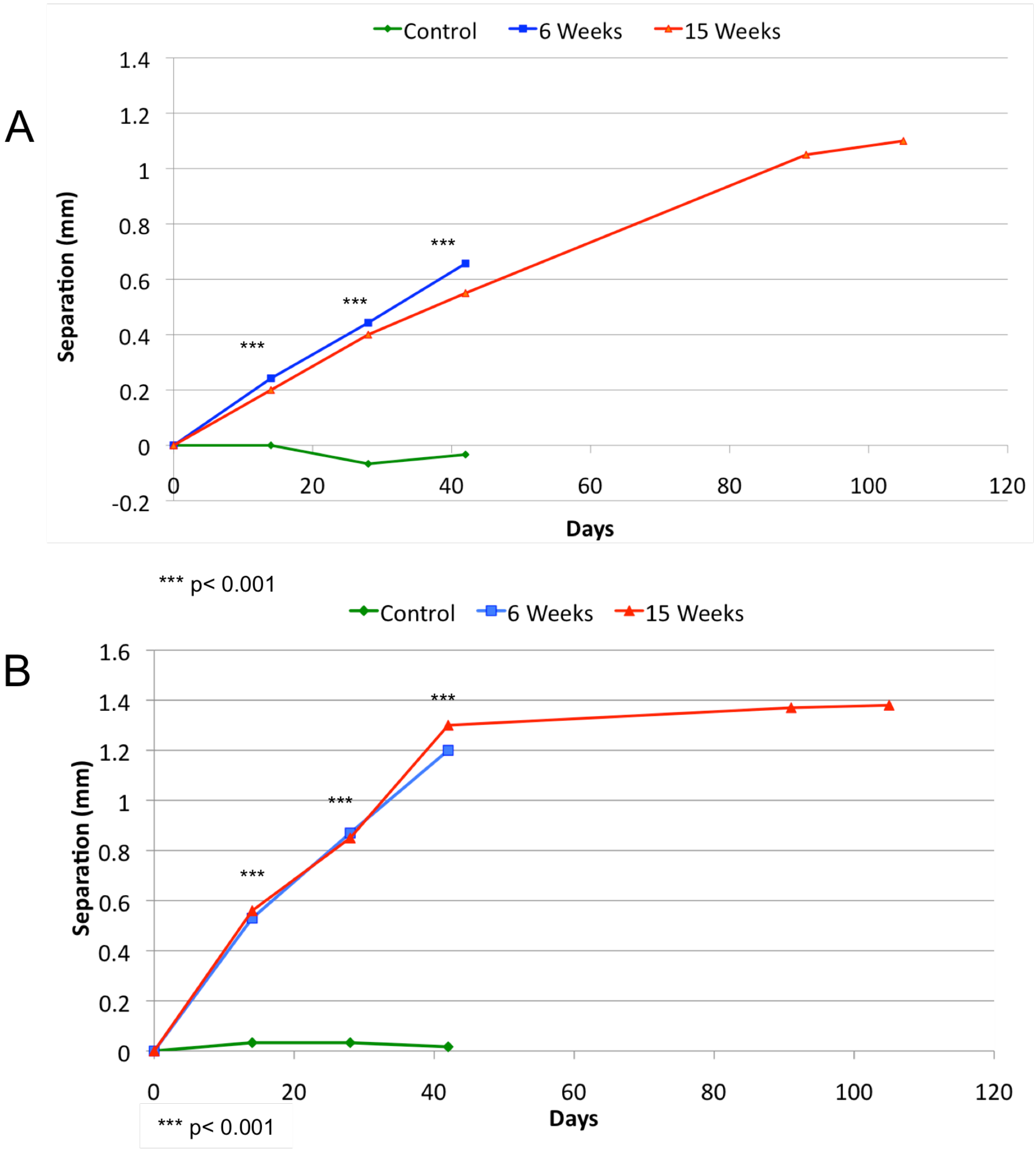


Figure 7: H&E stained sections comparing control (**A**) and experimental (**B**) specimens. Note the mature lamellar bone (M) along the sutural margins in the control section with associated cement lines (C). Undermineralized lamellar bone (U) with elongated Sharpey's fibers (S) was present in the experimental section. The dashed lines enclose the area of undermineralized lamellar bone.

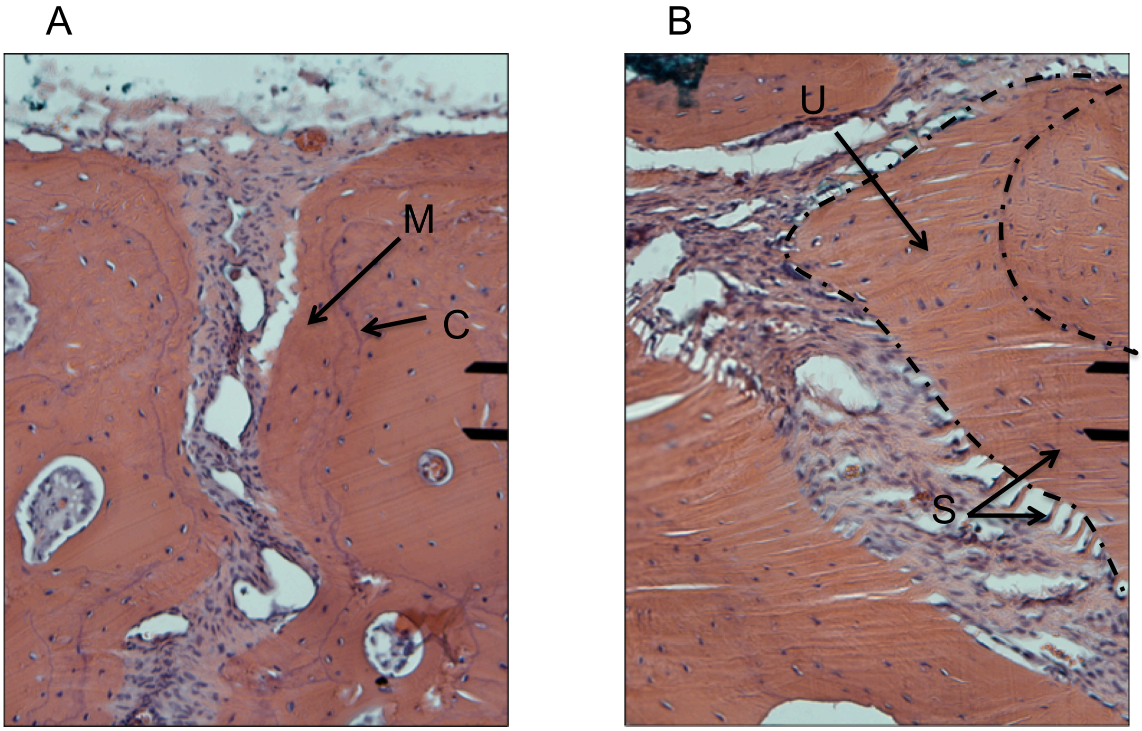


Figure 8: Tartrate resistant acid phosphatase (TRAP) stain of representative control (**A**) and experimental (**B**) sections. Note the presence of osteoclasts (O) within both sutures. Images in boxes are blown up. Dashed lines represent sutural margins.

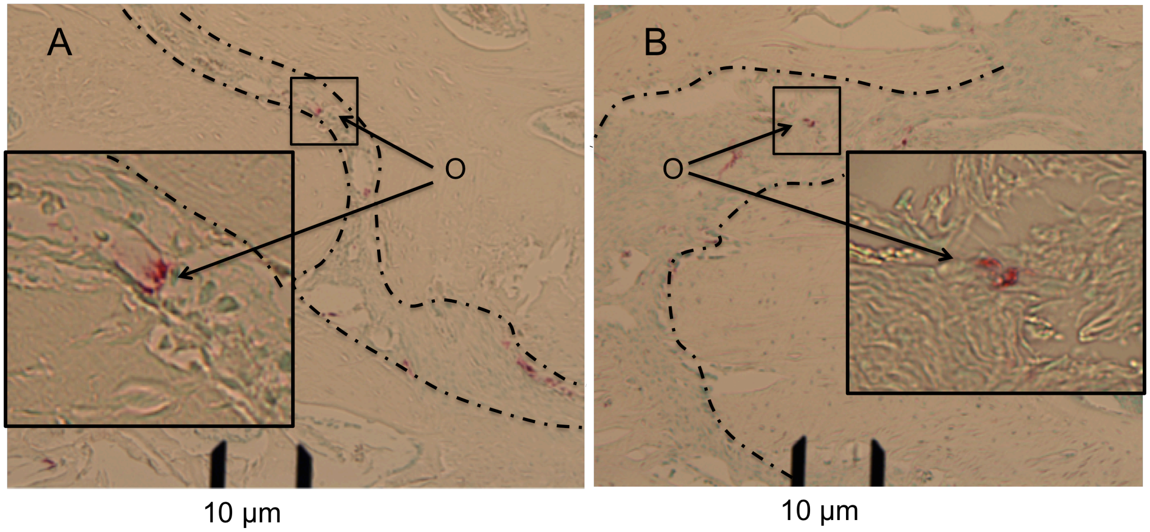


Figure 9: Representative control (**A**) and experimental (**B**) samples of fluorescent labeling.

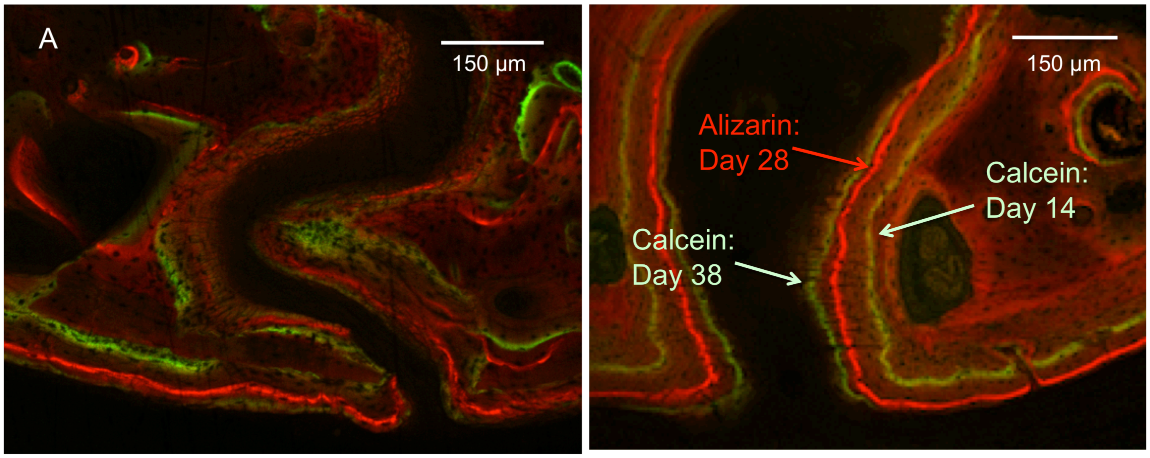


Figure 10: Sutural surface area (mm^2) by region, derived from microCT.

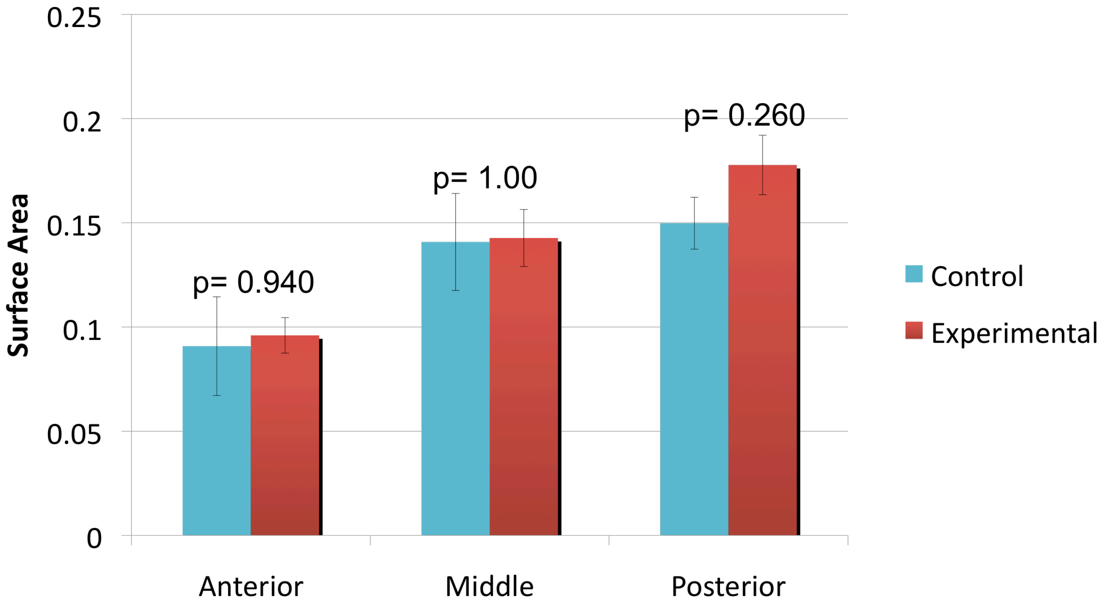
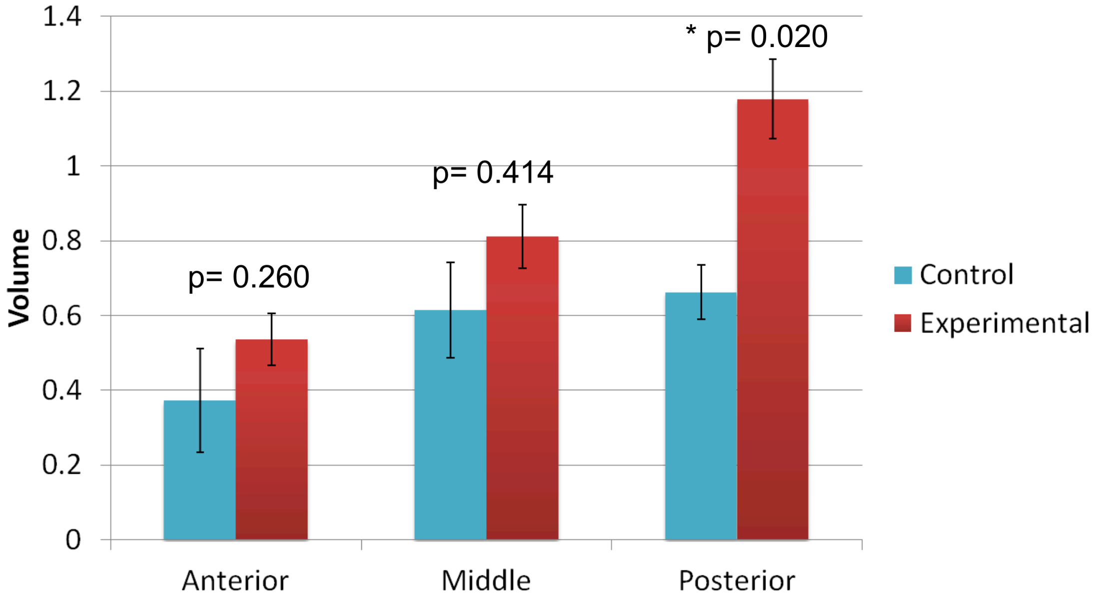


Figure 11: Sutural volume (mm^3) by region, derived from microCT.



APPENDIX B

TABLES

Table 1**:** Spearman correlation between MSI and bone marker separation.

Bone markers

		14 days	28 days	42 days
MSIs	**14 days**	.662*	.871**	.807**
	28 days	.744**	.889**	.849**
	42 days	.590*	.685*	.675*

*p< 0.05, **p< 0.01

Table 2**:** Sutural gap at day 42 and mineral apposition rate (MAR) between days 14, 28 and 38 for experimental and control animals.

	Control Ectocranial	Experimental Ectocranial	Control Endocranial	Experimental Endocranial	Endocranial vs. Ectocranial P values
Sutural gap (µm)	43.3 ± 0.52	142.4 ± 19.4	70.6 ± 2.46	196.4 ± 24.3	
MAR days 14-28 (µm/day)	-	6.52 ± 0.57	-	4.33 ± 0.52	0.144
MAR days 28-38 (µm/day)	-	3.66 ± 0.12	-	3.14 ± 0.36	0.144
MAR differences P values	-	0.068	-	0.068	

- =results not measurable. * = p < 0.05

Table 3**:** Complexity index. Suture height/suture length in the frontal plane.

	Anterior Complexity	Middle Complexity	Posterior Complexity	Intra Group Comparison: P values	Middle vs. Anterior	Posterior vs. Anterior	Posterior vs. Middle
Control	1.44 ± 0.024	1.53 ± 0.030	1.36 ± 0.055		0.144	0.144	0.068
Experimental 42-day	1.61 ± 0.085	1.52 ± 0.149	1.68 ± 0.064		0.917	0.345	0.600
P values	0.476	0.476	0.019*				

- =results not measurable. * = $p < 0.05$