Prevention of Maxillary Collapse During Sutural Distraction Osteogenesis for Cleft Palate Closure

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ABSTRACT

Sutural distraction osteogenesis (SDO) has been proposed as a novel approach for cleft palate closure in an effort to avoid the shortcomings of traditional surgical repair. In this thesis, we present data that confirms that attempted distraction of the palatomaxillary suture (PMS) achieves cleft closure preferentially by alveolar arch collapse, and not by intended SDO. To that end, we have designed a novel custom-fit intraoral splint that successfully prevents maxillary collapse while facilitating cleft defect approximation via sutural distraction. Preservation of maxillary dimensions was confirmed via intraoral measurements and craniometrics. New bone deposition secondary to SDO was quantified with histomorphometry and microCT, while the effects of distraction on the PMS and palatal bone were assessed with histology and Dual-energy Xray Absorptiometry (DXA). In summary, approximation of palatal defects via SDO in a canine model without maxillary collapse is possible, and may be a promising therapeutic approach for the repair of cleft palates in human infants.

RESUME

L'ostéogenèse d'élongation de la suture a été proposée comme une approche originale de fermeture de la fente palatine afin de pallier aux insuffisances de la réparation chirurgicale traditionnelle. Dans cette thèse, nous présentons les données qui confirment qu'au moyen d'une élongation tentée de la suture maxillo-palatine, on réalise avec succès la fermeture de la fente aux dépens de l'effondrement maxillaire statistiquement important dans un modèle de palais fendu canin. Dans ce but, nous avons conçu une nouvelle attelle intra-buccale, réalisée sur mesure, qui évite avec succès l'effrondrement maxillaire tout en facilitant le rapprochement de la fente par l'élongation de la suture. L'effondrement maxillaire a été mesuré in vivo en intra-buccal et par craniométrie post mortem. L'ostéogenèse d'élongation de la suture a été corroboré par l'histomorpho-métrie et microCT. En résumé, la réparation des fentes palatines au moyen d'une élongation de la suture a des implications cliniques passionnantes pouvant être réalisée à la suite de la prévention de l'effondrement maxillaire, l'attelle intra-buccale décrite en complément.

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General Introduction

1.1 The Cleft Palate – Current Care and Future Prospectives

Cleft Palate (CP) is a congenital birth defect where children are born with a physical communication between the oral and nasal cavities. In addition to the notable aesthetic element (visible when the child cries, frequent nasal regurgitation when feeding), the development of normal feeding mechanics (generating intraoral suction) and speech is significantly disturbed.[1-3] It is this functional disability that proves to be the most severe consequence of a cleft palate.

Currently, all popular methods of cleft palate closure rely on the extensive mobilization and transposition of palatal soft tissue flaps in order to close a combined bony and soft tissue defect and achieve functional reconstruction of the soft palate musculature.[1-8] Problems associated with this approach include: **1.** Extensive scarring along planes of dissection which subsequently restrict facial growth leading to midfacial deformity,[9-17] and **2.** An unaddressed bony palatal defect which contributes to crossbites secondary to unstable dentoalveolar arch form,[18-21] and frequent fistulization.[17,22-25] These maxillofacial and orthodontic complications are often significant, requiring corrective surgery and/or complex orthodontic attention.[23,24] In addition to being time-consuming and costly to repair, they are a source of significant morbidity for these patients.

1.2 Sutural Distraction Osteogenesis for Cleft Palate Closure

To address the aforementioned shortcomings of conventional surgical repair, surgeons have experimented with novel techniques of cleft palate closure. One promising technique proposed is based on the principles of tissue expansion, known as sutural distraction osteogenesis (SDO).[26,27] In short, a distractive force is placed across an immature bony suture (present in the developing craniofacial skeleton), causing it to expand. The mechanical distraction of the suture causes the production of osteoinductive growth factors which, in turn, stimulate proliferation and

differentiation of osteogenic cells (osteoblasts) contained within the immature bony sutures.[28-40] The result is the deposition of new bone within the bony suture.[27] The soft-tissues enveloping the bone also undergo expansion.[27,41-44] Applied to cleft palatal defects, in theory, this approach could approximate the bony defect in addition to the traditionally repaired deficient soft tissues. Definitive surgical closure could then be completed with minimal tissue dissection followed by suturing of the approximated cleft edges. The benefits would potentially include significantly decreased infant surgical morbidity, improved orthodontic outcomes and reduced facial growth abnormalities contributed to by scarring along surgical planes.[45]

The first attempt at closure of cleft palate defects using the technique of SDO was described by Liu, Song and Song in a canine model.[26] The authors reported successful closure of surgically-induced cleft palates via SDO of the longitudinal palatomaxillary suture (PMS). The investigators, however, consistently observed that animals who underwent sutural distraction had an "underdevelopment of the width of the midface", which was not present in the control group.[26] In spite of the latter observation, they concluded that the cleft defects were approximated by new bone generation via bilateral distraction of the PMS, which they documented histologically.[26] ***Contrary to the authors, however, it is our hypothesis that cleft closure was primarily achieved by the medial collapse of the maxilla/alveolar arches as a result of the medially-directed distractive force, and not by intended SDO.*** The latter is a complication that limits the clinical applicability of this approach to cleft palate disease, as it could contribute to further facial distortion and orthodontic deformity. However, we support the belief that SDO could be a viable therapeutic option pending elimination of associated maxillary collapse.

To that end, we have designed and tested a novel custom-fit, intraoral palatal splint, aimed at providing mechanical support to the alveolus during the phase of force application for sutural distraction. ***It was our goal to demonstrate that the use this intraoral splint could achieve closure (or approximation) of palatal defects by desired sutural distraction osteogenesis by preventing deleterious maxillary/alveolar arch collapse in an experimental canine cleft palate model.***

To achieve our goal, distraction of the longitudinal (PMS) was carried out in splinted (anchored) and non-splinted (non-anchored) dogs with surgically-induced cleft palatal defects. Maxillary width was carefully monitored for evidence of collapse during the course of osteodistraction with *in vivo* intraoral measurements and *post-mortem* using craniometry (direct measurement of standardized bony landmarks) on prepared skulls. Bone deposition secondary to SDO at the distracted suture was quantitated with histomorphometry and micro-computed tomography. Structural integrity of the distracted palatal bone and the architecture of the distracted PMS were also assessed by harvesting palatal specimens at the time of animal sacrifice for tissue analysis. Gross tissue histology was employed to ensure the reconstitution of normal suture architecture following the distraction phase, while bone mineral density (BMD) was measured in the palatal bone using Dual-energy Xray Absorptiometry (DXA), a useful technique of assessing osteodistracted bone quality and strength.[46]

Pending the successful approximation of cleft defects via SDO (i.e. defects closed by newly deposited bone in the absence of maxillary collapse), the secondary goal of the tissue analysis was to confirm that both suture morphology and bone composition was not permanently altered. *The latter is important because evidence of the deposition of structurally poor or abnormal bone (contributing to possible palatal/maxillary instability) or deranged suture anatomy (potentially contributing to growth disturbances) could limit the clinical applicability of this novel experimental technique for cleft palate closure.*

1.3 Research Objectives

Our research objectives were as follows:

1) To definitively demonstrate that attempted distraction of the longitudinal palatomaxillary suture for cleft palate closure without intraoral splinting (as described by Liu et al[26]) causes significant alveolar/maxillary collapse.

2) To describe the design and fabrication of a novel custom-fit intraoral palatal splint and demonstrate that its use can prevent medial maxillary collapse while facilitating cleft palate closure via sutural distraction osteogenesis (in a canine model).

3) To attempt to demonstrate that distraction of the palatomaxillary suture with the addition of an intraoral splint (i.e. without maxillary collapse) results in an increase in bone deposition in the palatal bone.

4) To demonstrate that approximation of cleft palate defects using the technique of SDO resulted in the deposition of compositionally normal bone.

5) To demonstrate the reconstitution of pre-distracted palatomaxillary suture anatomy following distraction.

CHAPTER 2

Literature Review

2.1 The Cleft Palate - Anatomical & Functional Derangement

Cleft Palate (CP) is a congenital deformity caused by failed fusion of the medial and lateral palatine processes during embryogenesis, resulting in an open communication between the oral and nasal cavities.[1,2] Cleft palate defects occur posterior to the *incisive foramen* (the site where the lateral maxillary bones meet the midline premaxilla) and are known as *secondary* or *palatal* clefts. Defects of the *primary* or *prepalatal* cleft (anterior to the foramen) are referred to as a "cleft lip". The normal palate consists of an anterior bony or *hard palate* and a posterior *soft palate* which culminates in the *uvula* posteriorly. Any portion of the hard and/or soft palate can be deficient, in addition to being *complete* (bone and soft-tissue missing) or *incomplete* (such as a *submucosal* cleft palate, where only the soft-tissues are absent). Finally, clefts of the primary palate can be *unilateral* or *bilateral*, in the absence or presence of a cleft palate.[1,2]

Functionally, the open communication between the oral and nasal cavities secondary to the cleft palate results in difficulties feeding (frequent regurgitation of feeds through the nose), further complicated by an inability to generate sufficient intraoral suction. Development of normal speech is also disturbed as a result of the inconsistent pressure generated.[2,47] In addition, clefting of the palate causes re-orientation of the *levator palatini* muscles which normally cross in the midline in a transverse orientation into a more longitudinal position (see figure 2.2).[47] These muscles play a paramount role in achieving velopharyngeal closure (where the soft palate is elevated and pulled posteriorly to make contact with the posterior pharyngeal wall). Thus, clefting often results in velopharyngeal incompetence (VPI), causing hypernasality.[1,2,47]

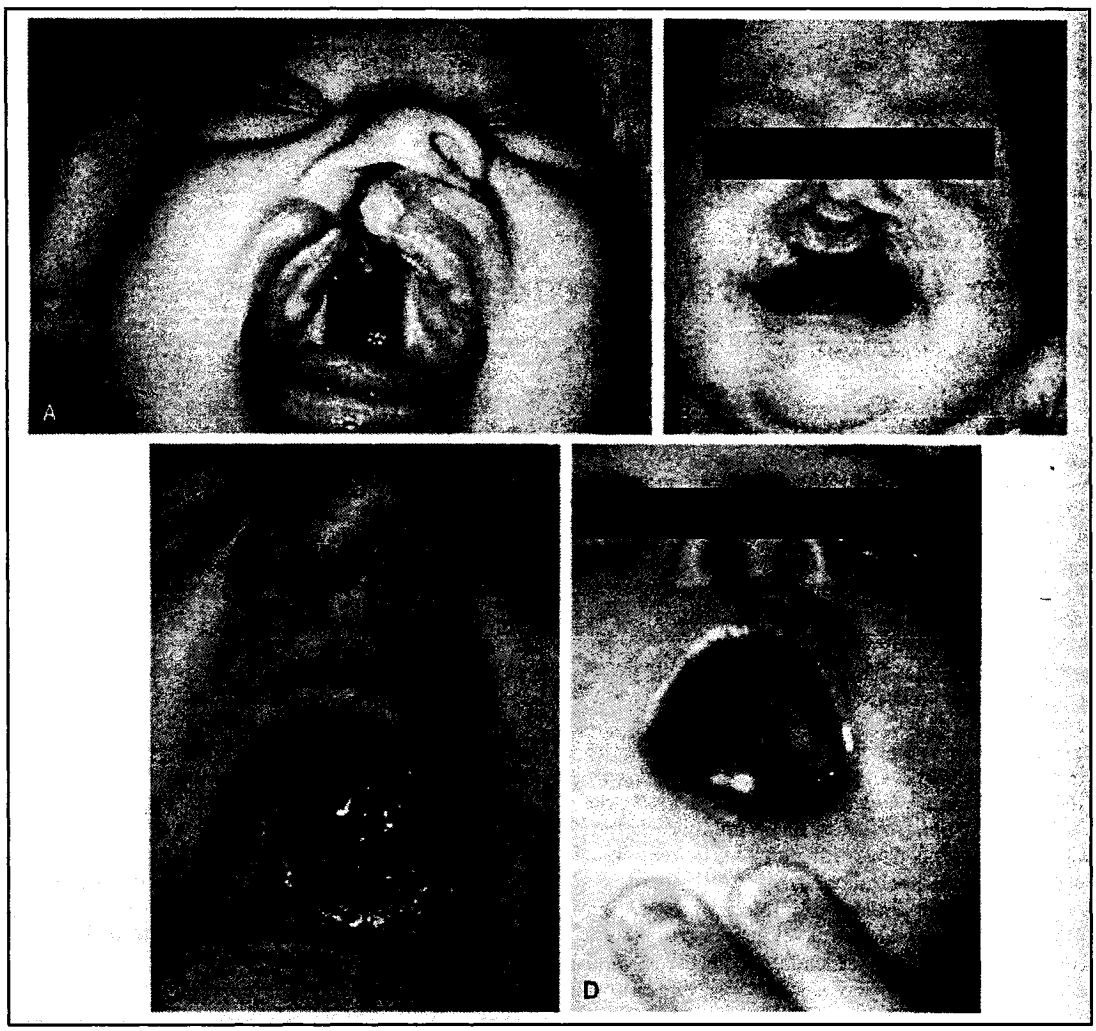

Figure 2.1. Photographs of cleft lip and palate (above); cleft palate post-cleft lip repair (below left); isolated cleft palate (below right) in human infants.

Proper orientation of the levator palatini and *tensor palatini* muscles are also important for optimal function of the *eustachian tubes* in the ears.[1,48] Thus, children with cleft palates frequently have accumulation of fluid in the middle ear (serous otitis media), nasopharyngeal content reflux as well as difficulties equalizing middle ear pressure. Middle ear infections, or otitis media, are common, as is the need for

myringotomy tubes. Permanent hearing loss can result in children whose ear problems are overlooked.[47,48]

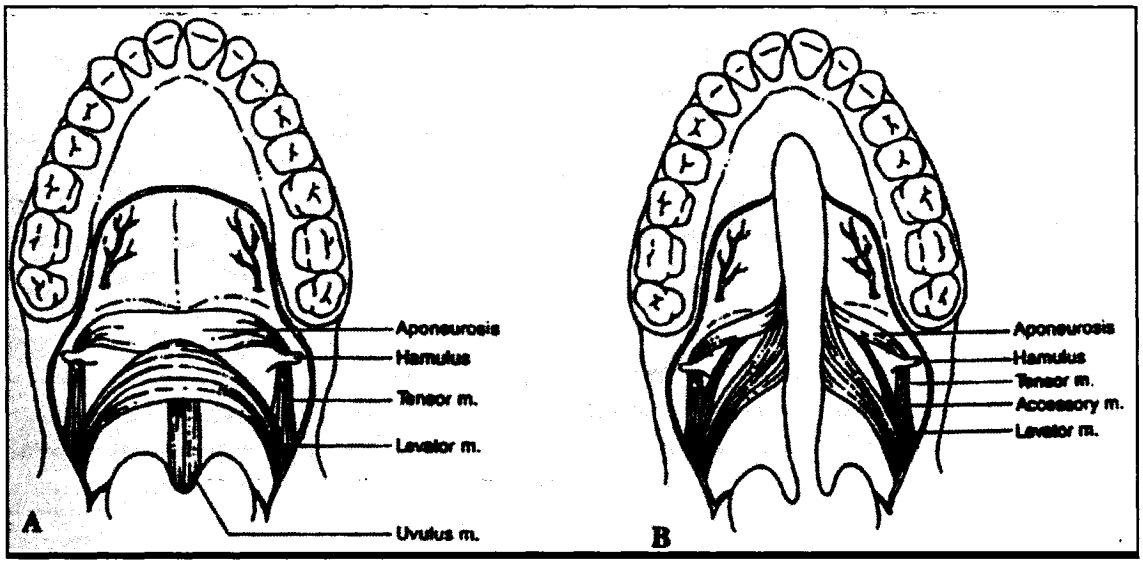

Figure 2.2. Musculature of the soft palate. A) Normal musculature, B) Abnormal musculature of the cleft palate. Note the more longitudinal orientation of the levator muscles which insert on the posterior edge of the palatal bone and cleft edges. Figure from Plastic Surgery, McCarthy.[47]

2.2 Incidence and Etiology of Cleft Palates

Cleft palates occur with an incidence of approximately 1.4 per 1000 live births in Caucasians, and are seen more often in females.[2] The incidence is lower in Blacks but higher in Asians, with rates reaching 3.2 per 1000 live births.[2] Although the exact etiology is unknown, cleft palate is thought to be a multifactorial defect. Genetic etiology is suggested by a high incidence in select families. For example, the likelihood of having a child with a cleft increases to 5% if a first- or second degree relative is affected, and to 15-25% in those parents with two first- or second degree relatives.[49] A recent study also noted the increased risk of clefting in consanguineous marriages.[50] Cleft palates also form part of the constellation of deformities in various syndromes such as Stickler's syndrome, velocardiofacial syndrome, fetal alcohol

syndrome, DiGeorge syndrome and trisomies. From the standpoint of environmental influences, no single teratogen has recognized as a causative agent although a number have been shown to cause cleft palates experimentally. Examples include alcohol, insulin, corticosteroids, carbon monoxide, tretinoin, anticonvulsants, phenobarbital, salicylates, oxygen deficiency, and possibly smoking.[47,51] Mothers who give birth to children with cleft lips and/or palates were also more likely to have had dietary deficiency during the pregnancy according to a recent epidemiologic study.[50] Specifically, an increased risk of cleft palate and/or lip has been found to be associated with poor maternal vitamin B_6 status but not consistently related to folic acid intake.[52]

2.3 Diagnosis and Standard of Care

Diagnosis is usually by the obstetrician or pediatrician at birth, although more recently *in utero* diagnosis via ultrasound has been described.[47] Consultation with a surgeon who will ultimately perform the repair is sought shortly after birth. Management planning is, however, a combined effort coordinated by the various members of the "cleft palate team".[53,54] This multidisciplinary group, usually consists of a plastic surgeon, a pediatrician, a speech pathologist, an audiologist and pediatric otalaryngologist, orthodontist and dentist. A social worker and cleft palate nurse frequently provide initial care instructions and additional coordination of specialists and family support that is necessary. Therapy involves a combination of presurgical orthopedics (oral appliances used to non-surgically approximate tissues maximally), staged surgical correction, followed by close monitoring of speech, feeding and psychosocial adaptation by the cleft palate team.[1-3,55] The cleft palate team is also involved in the long term follow-up with respect to the patient's growth and development and the timing of appropriate secondary operative procedures (see section 2.6.6)

2.4 Surgical Repair of the Cleft Palate

Cleft palate repair has undergone significant evolution over the last 100 years.[3,51] There now exist a number of techniques from which the cleft surgeon can draw his/her preferred method.[3] In general, however, these techniques are all based on mobilization of existing soft tissues to approximate the nasal, oral and muscle layers over the combined soft tissue and bony defect. More specifically, the levator muscles are dissected free from the oral and nasal mucosa and re-approximated in their normal anatomic position in the midline. The nasal mucosa is then sutured to itself, while mucoperiosteal flaps based on the *greater palatine* artery are mobilized to approximate the oral mucosa.[1]

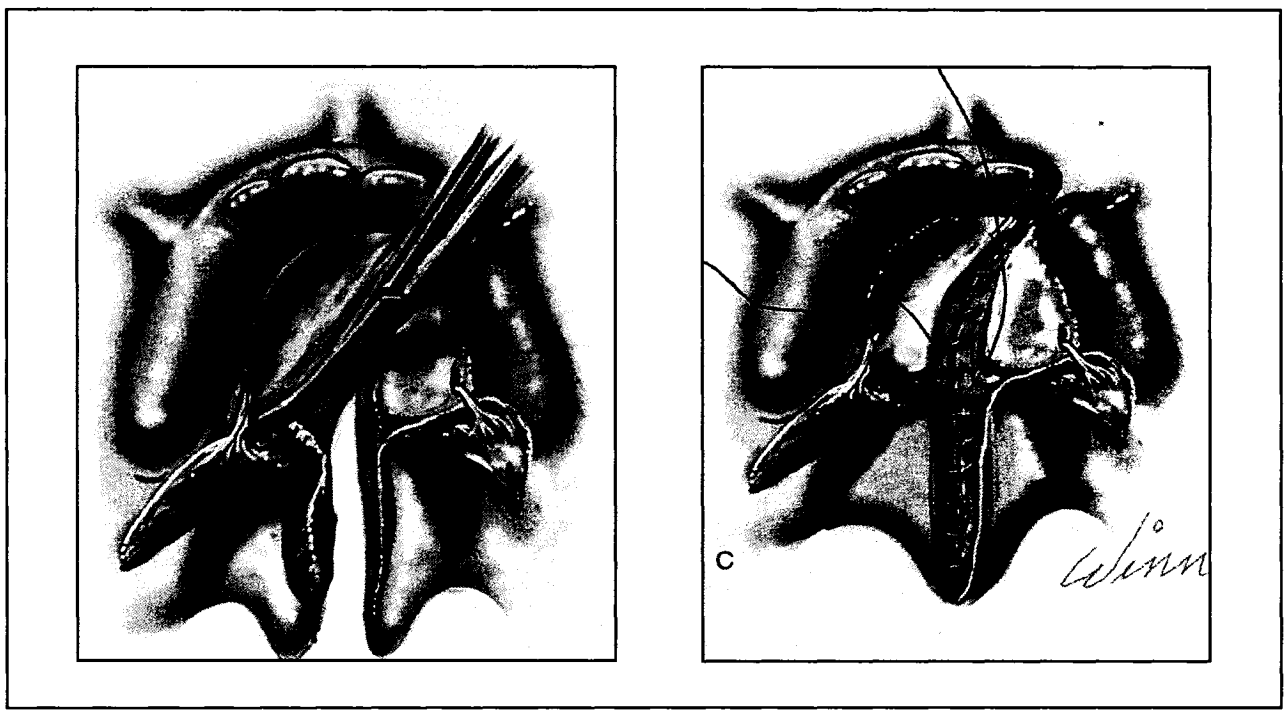

Figure 2.3. Basic surgical approach for cleft palate repair. Elevation of mucoperiosteal flaps (left). Approximation of mucosal and palatal soft tissues (right). Figure from Cleft Craft, Millard[3].

The different repairs can be roughly classified according to the types of flaps used for the oral layer closure and are briefly summarized here. The *von Langenbeck* repair is bipedicled with relaxing incisions laterally to allow for medial translation of the flaps.[5] V-Y advancement of bilateral flaps is the basis of the Veau-Wardill-Kilner technique,[4,7,8,56,57] while Furlow's repair utilizes double-opposing Z-plasties.[57] The latter procedure is unique in that it allows for some anterior-posterior lengthening of the palate. ***Note, however, that none of the preceding commonly used techniques address the bony defect.***

2.5 Timing of Cleft Palate Surgery

In addition to multiple techniques of cleft palate repair, the sequence of staged repair of the anomalies often associated with, or caused by clefting of the palate is also variable. The current standard of care and its typical sequence is outlined below:

- 3 months: Lip-Nose Repair (if cleft lip present)
- **6-18 months: Palate Repair**
- >3 years: Pharyngeal Surgery (to correct VPI if present)
- Pre-kindergarten: Rhinoplasty (to correct residual nasal deformity)
- 8-12 years: Alveolar Bone Graft (if cleft lip was present)
- >13 years: Orthodontics +/- Definitive Rhinoplasty
- >16 years: Jaw Surgery in 10-50%

The variable timing of the cleft palate repair (in bold type) is dictated by a number of balancing factors. Traditionally, repair is performed at approximately 12-18 months of age,[58] based on beliefs that later repairs would jeopardize speech development and earlier repairs would involve unnecessary anaesthetic risk and possibly increase the risk of facial growth restriction.[1] There has more recently been a

trend towards earlier repair, supported by studies that have demonstrated improved speech outcomes with surgery performed before phoneme development,[56,58,59] as well as data confirming that the procedure can be safely performed in the neonatal period.[60,61] Nonetheless, other centres advocate delayed palate repair, waiting as long as 27 months with the aim of minimizing maxillary growth attenuation.[58] Although no true concensus exists, presently 6-18 months is the average age at the time of cleft palate repair.

2.6 Pitfalls of Current Surgical Cleft Palate Repair

"The goal of cleft palate surgery is to close the palate with a technique and timing that produce optimal speech and minimize facial growth disturbances."[1]

Shortcomings of current surgical cleft care can be divided into two main categories: ***1)*** Complications common to all significant surgery in the orofacial area, and include bleeding, wound dehiscence, respiratory obstruction and infection. The preceding complications are not specific to cleft palate surgery and therefore need not be discussed in the context of this particular experimental project and thesis. ***2)*** Problems specific to cleft palate surgery, detailed individually below.

2.6.1 Morbidity and Mortality

Secondary to the significant tissue dissection needed to achieve approximation of the soft-tissue cleft edges, pain control and special feeding requirements often necessitate hospital stays of one day or more.[62-64] Post-operative hospital monitoring is also recommended to monitor for airway compromise.[1] Although rare and a recognized complication of any head and neck surgery, fatality secondary to airway compromise or hemorrhage is a specific problem related to aggressive tissue dissection in the oral cavity, and specifically in the soft tissues of the palate and posterior pharynx.[1,3,56]

2.6.2 Facial Growth Restriction

While early cleft palate repair is essential for the development of normal speech, as well as proper aesthetic and psychosocial adaptation, one recognized long-term complication of such early intervention is stunted facial growth.[1,22,47,58] Midface hypoplasia, the most commonly noted deformity, is the result of maxillary growth restriction which, in turn, has been directly linked to palatal surgery, documented clinically in numerous long-term follow-up studies.[2,19,23,58,63,65-67] Ortiz-Monasterio[17,25,68] and others[69] provided further evidence of the iatrogenic nature of the maxillary hypoplasia (i.e. a direct correlation between palatal surgery and growth restriction), demonstrating significantly less midfacial underdevelopment in patients with clefts left unrepaired into adulthood. Indeed, delayed hard palate closure (until 11-12 years of age) such as performed by Schweckendiek,[70,71] has been shown to produce more satisfactory facial growth, unfortunately at the expense of poor speech outcomes.[72]

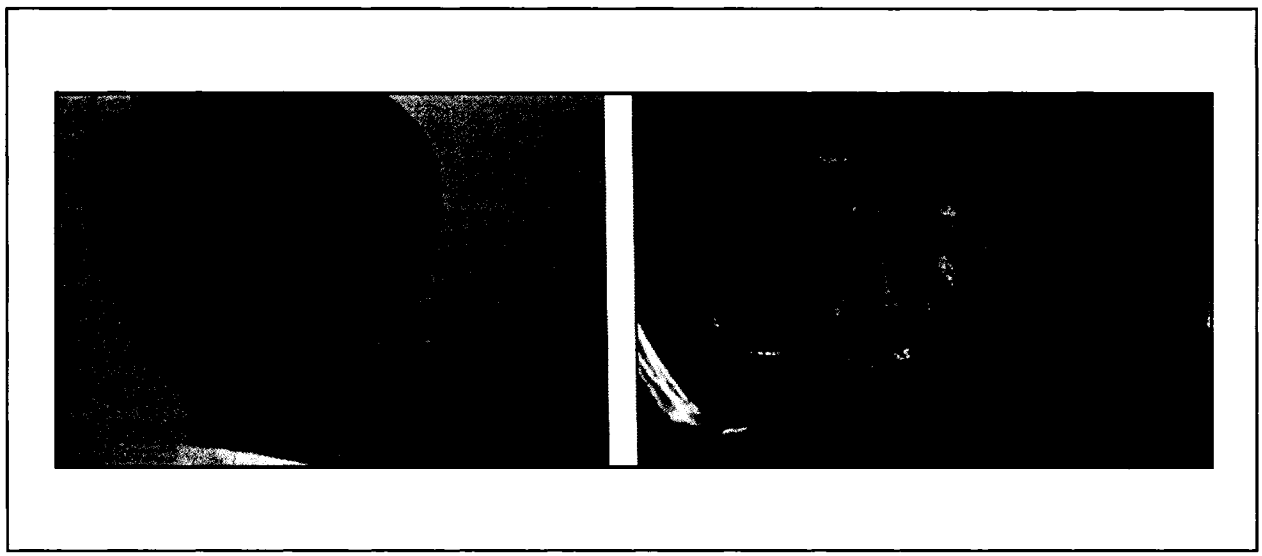

Figure 2.4. Midface hypoplasia (left) and orthodontic complications (right) following cleft palate and lip repair. Photos from Ortiz-Monasterio[17].

Although clinical studies have clearly demonstrated the presence of growth restriction in patients with repaired clefts, the search for its exact etiology has been based largely on animal cleft model studies.[9-14,73-79] Bardach et al found *a relationship between the amount of soft-tissue undermining involved in elevation of the oral mucoperiosteal flaps and the extent of maxillary retrusion.*[9,10,12-14,78] Anterior-posterior dimension facial growth was most affected in animals that had denuded palate (stripped periosteum) left to heal by granulation (such as in the common Langenbeck-style repair), *concluding that scarring and contracture along planes of dissection was also contributory to growth retardation.*[14,16]

2.6.3 Orthodontic Complications

In addition to midface-hypoplasia, maxillary growth restriction also frequently produces secondary deformities of the jaws and problems with occlusion and dentition (orthodontic malformation).[1,22,47,58,63,65,66] Anterior occlusal crossbites are common, manifested as forward flaring of the upper incisor teeth and lingual tipping of the lower incisors (in an attempt to maintain dental contact) secondary to midface retrusion.[19] A significant proportion of these patients require aggressive orthodontic management with various intraoral appliances, and an additional 25% of repaired cleft patients necessitate orthognathic surgery to reestablish adequate occlusion.[80]

2.6.4 Unaddressed Bony Defect

As alluded to in section 2.4 (Surgical Repair of the Cleft Palate), conventional cleft palate surgery revolves around approximation of mucosal flaps over a combined soft tissue and bony congenital defect.[1,3] In addition to being a non-physiologic/non-anatomic end-point (i.e. bone is not approximated into a normal anatomical position, thus affecting palatal growth and stability), such repair encompasses complications secondary to the lack of a bony scaffold underneath the soft-tissue closure, of which the most significant are *unstable dentoalveolar arch form*[18-21] and *palatal fistulas.*

The former contributes to some degree of alveolar collapse present in almost all repaired cleft palates, further exacerbating existing orthodontic deformity in these patients.[2,18-20,56,63,66] Resultant crossbites require orthodontic intervention, as noted in the previous section (2.6.3 Orthodontic Complications).[2,56,63,65,66,81]

Palatal fistulas are the most common defect in the hard palate after repair and occur in up to 34% of patients.[82] They may occur anywhere along the site of the original cleft, and are thought to be caused by dehiscence secondary to excess tissue tension at the repair site and/or inadequate underlying tissue support.[18] The latter is supported by the finding that fistulization is more common in patients who did not have bone grafting (of the hard palatal defect),[18,83-85] and furthermore by a decreased rate of fistula repair breakdown with the incorporation of a bone graft.[18,86-89] Clinically, oronasal fistulas produce hypernasality and articulation distortion (if large), nasal regurgitation of ingested food and production of socially undesirable sounds.[82] Repair of palatal fistulas is challenging, as even small fistulas require large flaps and a tension-free repair.[58]

2.6.5 Velopharyngeal Incompetence (VPI)

This describes the inability of the soft palate to make contact with the posterior pharyngeal wall to achieve velopharyngeal closure during speech.[47] The result is hypernasality, misarticulation, nasal emissions (escape of air through the nose) and grimacing.[82] Approximately 20% of children who undergo cleft palate repair will require a second procedure for VPI.[82] The most widely used corrective procedure for VPI is the pharyngeal flap, in which a flap of mucosa is elevated from the posterior pharyngeal wall, turned forward, and inset into the soft palate.[58] Although significant improvement with this procedure is common, it involves morbidity related to an additional surgery and anaesthesia, and is also thought to contribute further to a restriction of forward maxillary growth.[47,58]

2.6.6 Surgical Revision of Secondary Deformities of Cleft Palate Repair

Following primary cleft repair and orthodontic treatment, satisfactory facial appearance in not uncommon, followed later by deterioration in facial contour as maxillary growth restriction becomes more pronounced.[2,19,23,58,63,65,66] Clinically, maxillary hypoplasia must frequently be addressed with invasive surgical revision in young adulthood. The procedures of choice are Le Fort I (or less frequently, II) advancement osteotomies.[2,56,63,65,66,81] The latter achieve maxillary advancement, restoring occlusion as well as improving aesthetic lip posture and maxillary form.[81] **Although significant improvements have been made in corrective maxillofacial and orthognathic procedures, the necessity for revisional surgery secondary to iatrogenic facial deformity that complicates traditional surgical cleft palate repair remains a source of significant cost to the health-care system, and more importantly a source of significant morbidity to the affected patient.**[62,63,65,66]

2.7 The Era of Tissue Engineering - Distraction Osteogenesis

The preceding literature review summarized the numerous shortcomings of conventional surgical cleft palate repair. In essence, the majority of the complications can be accounted for by two phenomena: 1) inhibition of normal facial growth, and 2) a non-physiologic/non-anatomic end-point secondary to an unaddressed bony palatal defect – both related to the mechanics and design of current cleft palate surgery. It seems only appropriate then, that surgeons have looked to other methods of repair to circumvent the aforementioned pitfalls and achieve closure of both soft and bony palatal tissue defects. This impetus has penetrated all types of surgery, spawning wide-spread interest in innovative methods of surgical repair.

Tissue engineering has been at the forefront of this evolution, encompassing a wide variety of techniques that represent a shift in the conventional surgical paradigm from "repair" and "replace" to "generate" and "manipulate". *Distraction osteogenesis (DO),* one such technique, exemplifies the fusion of tissue engineering and surgery

into what has been coined the era of "inductive surgery" by Joseph Murray, MD.[44] While this technique dates back to the early 1900's, with Codvilla's discovery of extremity lengthening with the use of traction,[90] the field has exploded experimentally and clinically over the past few decades. Currently, DO has become a commonly used procedure and an indispensable tool in the reconstructive surgeon's armamentarium, and provides the scientific basis and inspiration for this research thesis.

2.7.1 The Biology of Distraction Osteogenesis

Gavril Ilizarov, a Russian orthopedic surgeon, is credited with identifying the physiologic factors involved in tension-stress generated osteogenesis, in addition to introducing this technique into wide clinical acceptance.[91,92] In his pioneering experiments, Ilizarov clearly demonstrated that traction placed across an osteotomy in the lower limbs resulted in osteogenesis, and that successful DO depended on the stability of fixation, the rate of daily distraction and the preservation of the local soft tissue envelope.[91,93-95]

Technically, DO can be divided into distinct phases, outlined below:

1. ***Osteotomy*** – the bone to be lengthened is osteotomized (cut) perpendicular to the axis of desired elongation, stimulating hemorrhage, swelling and initiating the inflammatory cascade.[96]

2. ***Latency*** – The period after osteotomy before which distraction begins, during which migration of inflammatory cells and hematoma formation ensues and the "soft callus" forms.[97,98] Optimal osteogenesis is obtained after a latency of 5 to 7 days,[44] suggested experimentally to be related to the time necessary for sufficient ingrowth of blood vessels and adequate maturation to withstand distraction forces.[93,98-105]

3. ***Distraction*** – traction-induced tension is created across the osteotomy with a "distraction" device, usually consisting of an external frame coupled to a

jack-screw which distracts the bone segments as the screw is rotated. Depending on the bone being distracted, 1-2 mm of excursion can be achieved per day. Fractionated distraction protocols (multiple small advancements of the screw vs. one large movement) achieve significantly less soft-tissue injury and enhanced vasculogenesis.[92,93] Distraction stimulates the production of osteoinductive growth factors including the TGF-β family, basic fibroblast growth factor, insulin-like growth factors and bone morphogenetic proteins, and extracellular matrix molecules such as collagen I and osteocalcin. These factors have been shown to increase in concentration in the distracted callus, and are known to regulate osteoblast proliferation and differentiation, as well as stimulate osseous regeneration in bony defects.[28-40] During this 'distraction' phase, immature bone is deposited.

4. ***Consolidation*** – describes the phase of bone maturation and remodeling that occurs after distraction is complete. During consolidation, rigid fixation of the distracted bone is essential to prevent relapse, and to allow proper calcification and solidification of the hard callus into mature bone.[43,44,91,92]

Histologically, one begins to see the appearance of primitive mesenchymal cells and collagen I matrix synthesis in the early stages of distraction.[105-107] Shortly thereafter, fibroblasts initiate a fibrovascular bridge oriented in the direction of distraction. Osteoid synthesis and early mineralization are initiated roughly 10 to 14 days after distraction was initiated. Early bone spicules form starting at 3 weeks,[108,109] followed by continued calcification that leads to closure of the distraction gap. Subsequent remodeling over the period of months restores elements of normal bone including lamellae and marrow.[108,109] **Of note, numerous clinical and experimental studies have confirmed that distraction of both endochondral bone (long bones) and membranous bone (found in the craniofacial skeleton, see Section 2.7.2) result in osteogenesis via *intramembranous ossification* (whereas long bones normally grow and repair via endochondral ossification).[93,105,106,108]**

2.7.2 The Evolution of Distraction Osteogenesis – Application to the Craniofacial Skeleton

Although DO was originally described by Ilizarov for lower limb lengthening, surgeons were soon to realize the potential versatility of this technique for existing reconstructive problems. Deficiency of the craniofacial skeleton was one such domain. Snyder pioneered this entry, publishing successful elongation of mandibles in a canine model in 1972.[110] Following various technical modifications,[107,108,111,112] McCarthy provided clinical proof that DO could be effectively and safely used to treat hypoplastic mandibles,[42,43] opening the door to the era of clinically feasible craniofacial distraction.

Since that time, DO has been applied successfully to treat bony deficiencies in midface (maxillary hypoplasia, craniofacial synostosis)[113-115], temporomandibular joint (ankylosis)[116,117], dentoalveolar segments,[116-120] zygoma (Treacher Collins syndrome)[121,122] and the cranial vault.[123-125]

2.7.3 Sutural Distraction Osteogenesis – Distraction Without Osteotomy

Following a more thorough understanding of the biology and mechanics of distraction osteogenesis, surgeons and researchers alike began investigating whether osteotomies were indeed necessary for distraction to be feasible. Could osteogenic areas of bone, such as those present in epiphyseal plates and immature cranial sutures be distracted without an osteotomy?

The first answers to these questions were provided in the early 90's by various authors that confirmed that physeal plates could indeed be distracted to increase long bone length in animal models.[126-128] More recent investigations demonstrated that the frontonasal[129,130] and cranial sutures[27] in the skull could also be distracted successfully without an osteotomy. In addition, the degree of distraction and new bone formation was shown to be comparable to that across an osteotomy.[27] ***Thus, sutural distraction osteogenesis may offer an advantage over traditional DO, by***

eliminating the need for a conventional osteotomy and the associated morbidity (tissue edema, hemorrhage, pain, etc.).

2.7.4 Sutural Distraction Osteogenesis for Cleft Palate Closure – Current State of Knowledge

The cleft palate, along with many other structures in the congenitally malformed craniofacial skeleton, is not only anatomically abnormal but, in addition, represents a deficiency in the tissues. The latter consists of both soft tissue and bone, but as emphasized earlier in this literature review, current surgical repair of cleft palates focuses on the repositioning of soft-tissues only, leaving the bony defect unaddressed. The potential of DO for the treatment of cleft palates is thus readily apparent. In theory, this approach could permit a composite approximation of a composite defect (the cleft). In addition, the technical nature of DO would facilitate gradual lengthening of bone and soft tissue expansion, thus minimizing the need for extensive tissue mobilization and dissection at the eventual time of definitive closure (which after DO is completed, would theoretically involve simple suturing of approximated tissues). The benefits would potentially include significantly decreased infant surgical morbidity, improved orthodontic outcomes due to increased dentoalveolar stability (re-approximated bony scaffold) and reduced growth abnormalities contributed to by scarring along surgical planes[45]. This was supported in a recent published discussion by J. G. McCarthy, M.D., the pioneer of craniofacial osteodistraction, noting that the application of DO to the treatment of cleft palates could potentially revolutionize the field and minimize the current complications.[45]

Experimentally, the first application of DO to the palate was by Carls et al.[131,132] in 1997, demonstrating the elongation of the hard palate (anteroposteriorly) in adult dogs. While this application was not for cleft palates per se, it demonstrated that the palate and oral soft-tissues could successfully tolerate distraction osteogenesis.

Noting the potential benefit of distraction without osteotomy (sutural distraction)[27,129,130] and the ideal anatomical substrate for such a procedure (the palatomaxillary suture, PMS), Liu et al.[26] recorded the first attempt at closure of cleft palates in an animal model, using the technique of sutural distraction osteogenesis. In dogs with surgically-induced cleft palates, a Nickel-Titanium memory alloy spring was used to apply a traction force across the clefts, in an attempt to medially distract the longitudinal portion of the PMS bilaterally. In doing so, they attempted to generate new bone on either side of the cleft and expand the overlying soft tissues in order to approximate the composite cleft defect medially (see Figure 2.7 below).

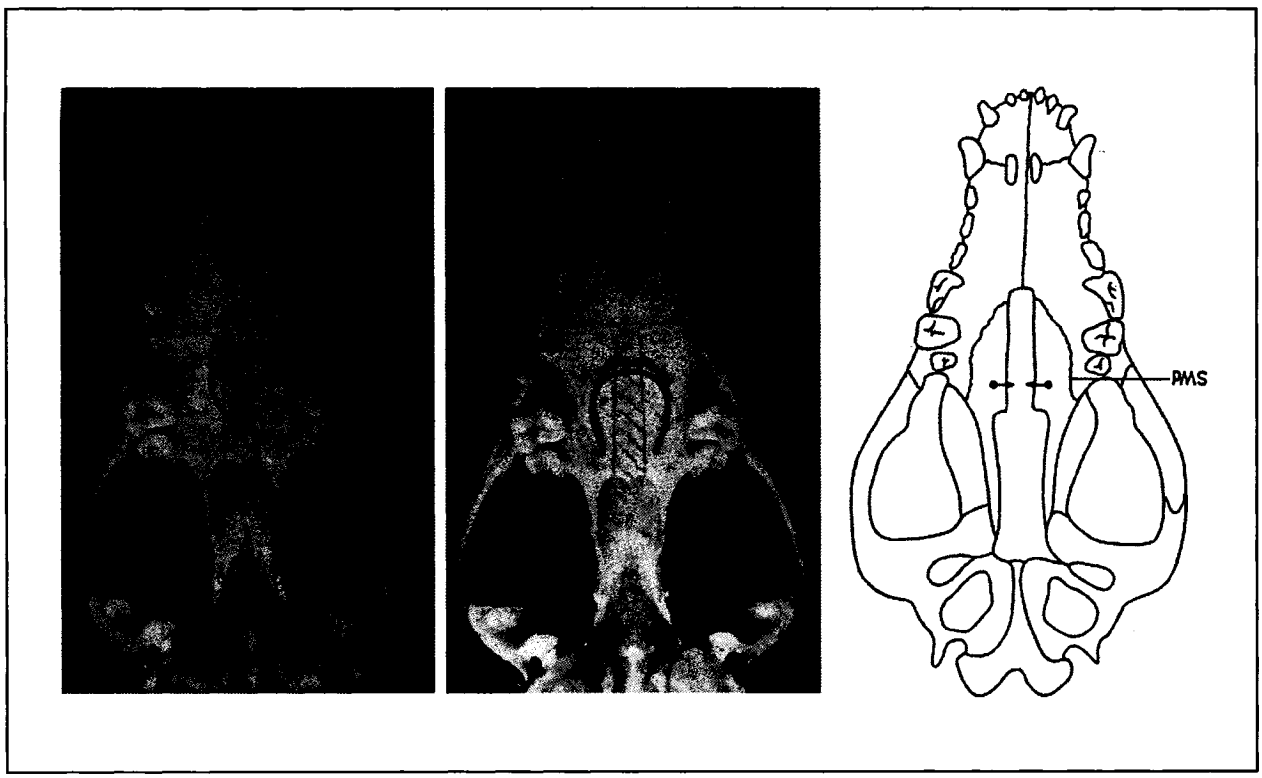

Figure 2.5. Anatomical representation of distraction (expansion) of the PMS (left). Schematic representation of the same procedure (right). Note the positioning of the spring distractor medial to the PMS in an effort to approximate the cleft edges by expanding the PMS bilaterally. Figure from Liu et al.[26]

Grossly, the authors documented impressive closure of the palatal clefts (documented photographically), with complete obliteration of the defects in the majority of the animals (see figure 2.8). Histological analysis of sutural anatomy was suggestive of new bone formation in distracted specimens, prompting the investigators to conclude that the cleft defects had been successfully approximated by sutural distraction.

Figure 2.6. In vivo example of the implanted spring distractor apparatus in a beagle with a surgically created cleft palate at day 0 of distraction (left), midway through distraction (middle), and at completion of distraction (right). Note the complete closure of the cleft defect. Photos from Liu et al.[26]

The authors, however, consistently observed that animals that underwent sutural distraction had an "underdevelopment of the width of the midface", which was not present in the control group.[26] Whether this "underdevelopment" represents

medial collapse of the maxillary bone and dental arches (see figure 2.9 below), a potentially significant complication of the distraction process, is yet unanswered and forms the basis of this research thesis. ***Is closure of cleft palates using this technique achieved primarily by sutural distraction osteogenesis (desired) or by significant alveolar arch collapse (undesired)? And secondly, if the latter is true – can this collapse be prevented?***

Since the initial landmark publication, the same authors have repeated the experiment with an improved distraction device,[133] in addition to reporting the results of a preliminary clinical study in human infants with cleft palate defects.[134] Although the results were again promising, both reports lacked the specific and dedicated monitoring of maxillary dimensions nor any form of alveolar anchorage, thus highlighting the importance of the present study.

2.8 Alveolar Anchorage – Intraoral Maxillary Splint

The orthodontic/dentofacial-orthopaedic concept of anchorage is as old as the practice of orthodontics. Simply stated, all applied treatment forces create a reaction force away from the structures of interest, and anchorage is the resistance that the clinician applies to avoid unwanted movements in these structures.[19,23] As applied to this scenario, we propose to utilize a passive maxillary acrylic splint to preserve the transverse width of the upper dental arch and prevent palatal rotation of the maxillary teeth while tension forces are applied across the cleft palate.[1,23] This custom-fit appliance would fit securely in the roof of the mouth, providing stability against medial maxillary collapse, while a recess in its center would permit the distractor device to medially approximate the cleft edges by expansion of the palatomaxillary suture and enveloping soft tissues (see figure 2.9 below).

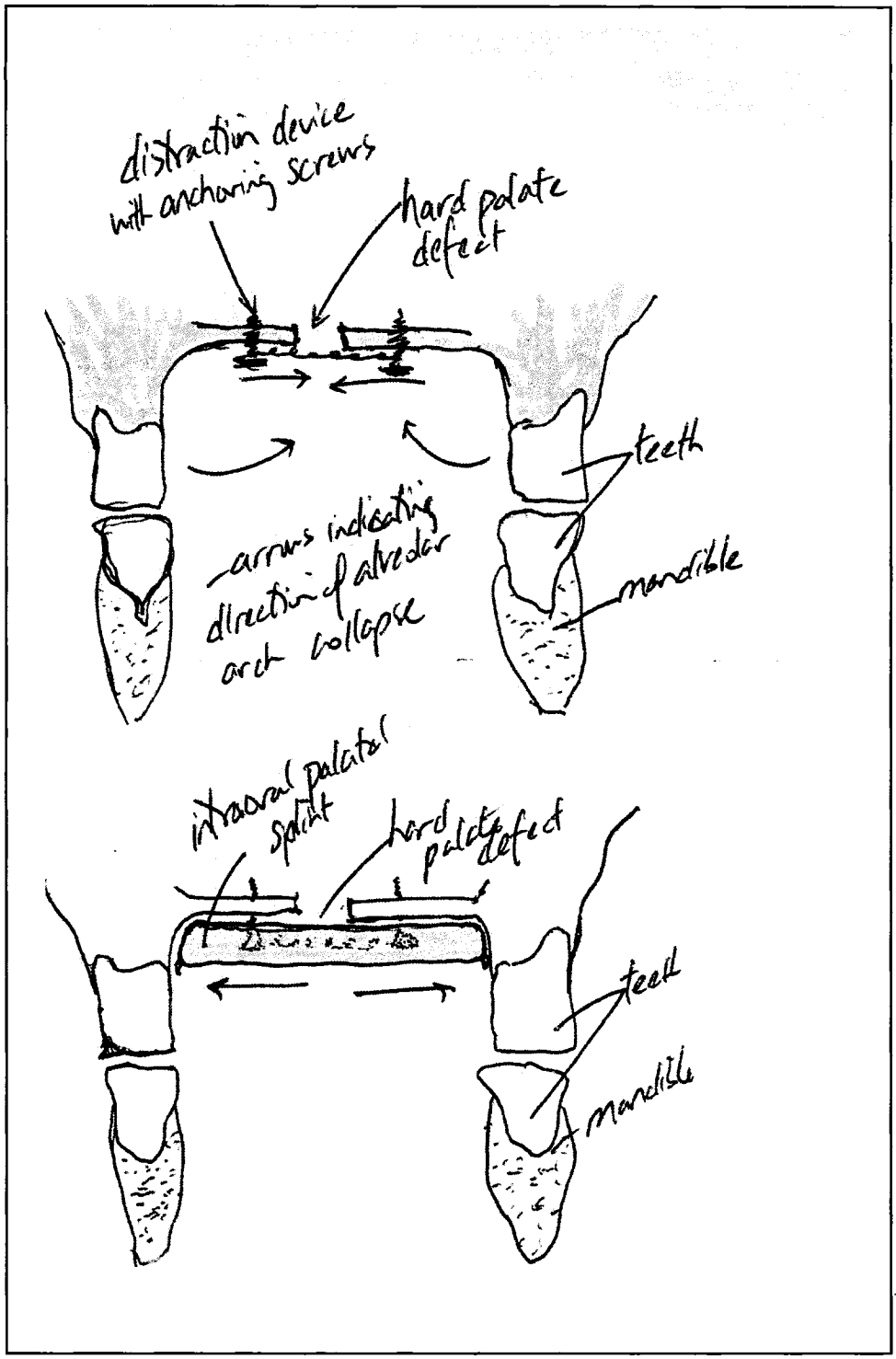

Figure 2.7. (Top) Schematic drawing of alveolar arch collapse (coronal view), demonstrating the medially-directed force applied by the distractor device and the resultant vector of arch collapse. (Bottom) Schematic drawing of the intraoral passive palatal splint designed to provided lateral structural support to the alveolar arches to prevent medial collapse during distraction (note that the splint has a recess within which the distractor and the anchoring screws are situated).

CHAPTER 3

Methods and Materials

3.1 Pilot Study

As per the suggestions of the *McGill University and Montreal General Hospital Research Facility Animal Ethics Committees*, a pilot study consisting of three animals was employed to ensure their safety and viability post-operatively. Following the successful completion of the pilot study period, permission was granted to initiate the experimental portion of this research project.

3.2 Surgical Induction of the Cleft Palate

Twenty-four, 10 week-old male beagle puppies (Marshall Farms©, NY) were utilized for this study. Following a four hour fast, the animals were pre-medicated with a 0.05cc/kg S.C. mixture of the following agents: Butorphanol 40mg, Acepromazine 25mg, Atropine 5mg, and 5 cc of sterile saline. Anaesthesia was induced after a 15 minute delay with Isoflurane given my mask and, following intubation for positive pressure ventilation, anaesthesia was maintained with Isoflurane 1-3% and 2L/minute of oxygen. Cefazolin 30 mg/kg IV (skin/oral prophylaxis) was given at the time of induction. The animals were prepared and draped using sterile technique and secured in a supine position. The endotracheal tube was then fastened laterally in the oral cavity and a retention suture placed in the midline of the protruded tongue to clear the operative field. An 8mm-wide strip of mucosa in the palate midline, extending from the junction of the hard and soft palate posteriorly to the line perpendicular to the 1st molars anteriorly, was excised with a scalpel and elevated with a periosteal elevator. An identical width defect was created in the bone with a taper-fissure burr, followed by removal of any interfering nasal septum with a Rongeur. 10mm long, 1.2 mm diameter titanium self-tapping screws (Synthes Maxillofacial©, Switzerland) were then inserted medially to the PMS on either side of the defect (see figure 2.7), opposite the 2nd molars (for later anchoring of the distractor device). The cleft defect was then extended 1 cm posteriorly into the soft palate using cautery and the edges closed with 4.0 catgut for hemostasis. See figure 3.1. Following confirmation of adequate hemostasis in the surgical site,

anaesthesia was terminated and the animals brought to the post-surgical care facility where they were started on a soft-diet the following day.

Post-operatively, Buprenorphine 0.01-0.02mg/kg SC was administered every 6-8 hours until analgesia was no longer required (animals were self-grooming and eating well, showing no signs of pain). The animals were monitored daily to document healing and/or surgical site infection.

Figure 3.1. Surgically-created cleft palate (intraoperative photo). Hard palate is inferior, soft palate superior. Anchoring screws were inserted opposite the 2nd molars, medially to the PMS bilaterally. Refer to figure 2.7 for a schematic representation of this procedure.

3.3 Construction of the Custom-fit Acrylic Intraoral Maxillary Splint

For those animals in **group 3** (distractor + intraoral splint), acrylic splints were prefabricated the night before distraction was set to begin, so as to ensure proper fit during the distraction period. Under mild sedation (0.05cc/kg S.C. mixture of the following agents: Butorphanol 40mg, Acepromazine 25mg), a dental impression of each dog's maxillary arch was obtained using *Jeltrate*© fast-setting alginate (Figure 3.2) using a light-cured custom-made maxillary acrylic tray (*Triad*©). The positive dental mold was fabricated by pouring the impression with type III dental stone (*Ash Temple*©) (Figure 3.3), followed by application of the cold-cure polymethyl methacrylate (*Dentsply*©) for construction of the intraoral splint. Once cured, the splint was trimmed with an acrylic burr and polished with pumice and rouge. A small recess in the posterior aspect of the oral appliance was created to accommodate the distractor device.

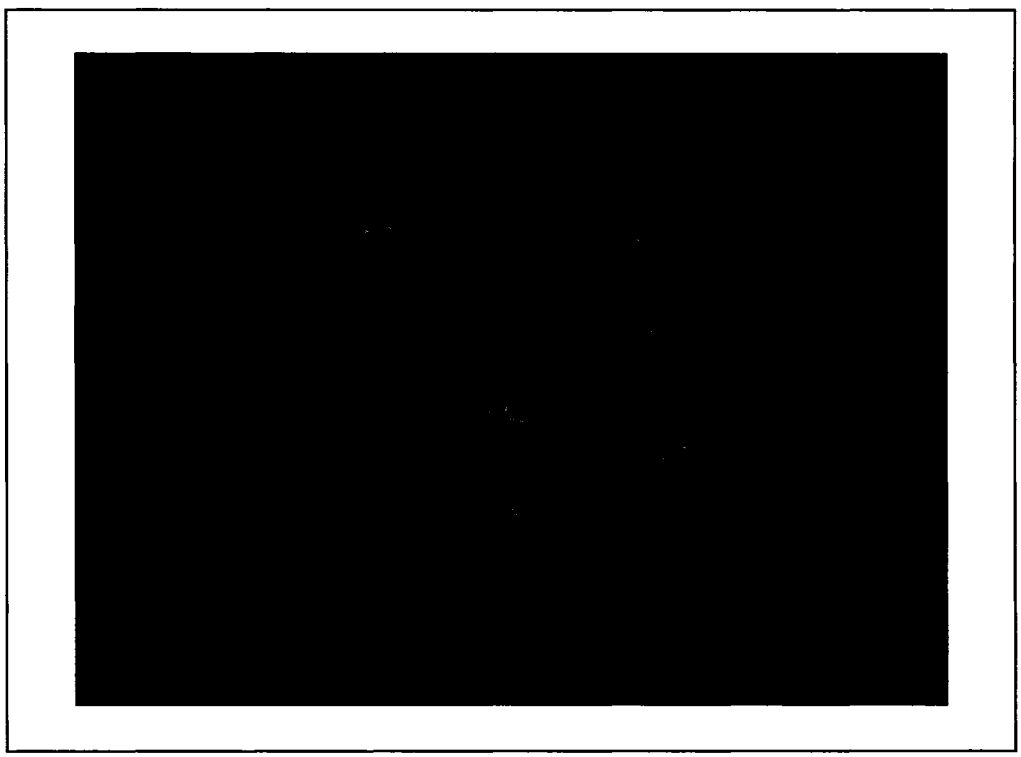

Figure 3.2. Dental impression (negative) fabricated from fast-setting alginate. Note the palatal defect in the midline.

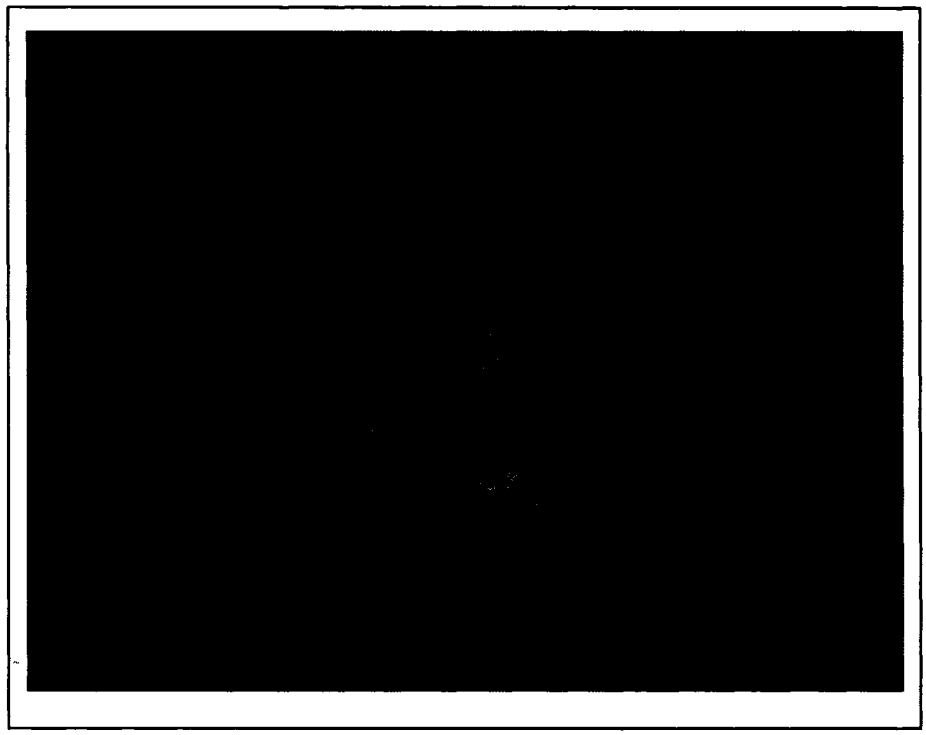

Figure 3.3. Positive dental mold fabricated in stone. The cleft defect has been filled with wax to prevent acrylic from filling the defect during construction of the intraoral splint.

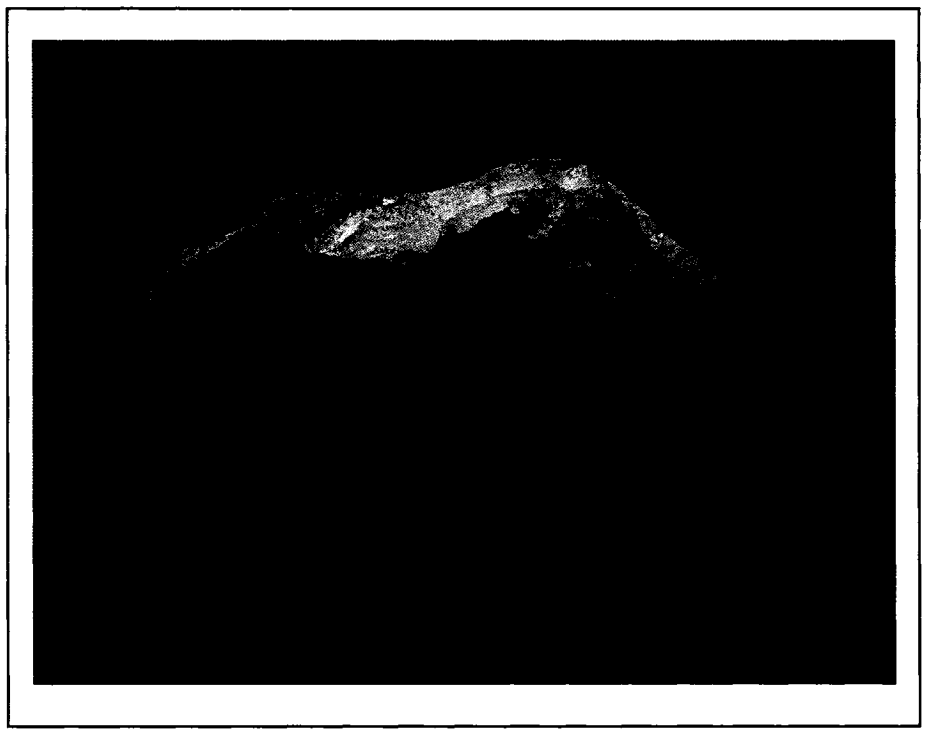

Figure 3.4. Custom-fit intraoral splint fabricated from cold-cure acrylic. A recess was created posteriorly (right) to provide room for the distractor device and monitoring of cleft closure.

3.4 Insertion of the Distractor Device +/- Intraoral Splint (Initiation of SDO)

Ten days post-palate surgery, (allowing sufficient time for the surgical site to heal), the animals in group 2 (distractor only) and group 3 (distractor + intraoral splint) were anaesthetized (same protocol as above). At this time, the distractor device consisting of two nickel-titanium superelastic memory alloy springs (Class One Orthodontics©, Texas, USA), 10 mm long, wired together at their ends with 22g. stainless wire), was inserted by securing the looped ends around the anchoring screws (see Figure 3.5). The springs each exert a contractile force of 175-200N over a range of 1.5x to 5x their original length, for a total of 350-400N (This force was chosen based on the results of Liu et al's study, demonstrating optimal distraction results in this force range[26]). The custom-made prefabricated splints were then inserted in a snap-fit fashion into animals in group 3. A 1mm width, 10 mm long titanium screw was inserted in the midline[1], anterior to the PMS to prevent accidental dislodgement of the splints (see Figure 3.6). The animals were then returned to the recovery room for standard post-operative care.

[1] The fixation screw placed in the midline may penetrate the intermaxillary suture, but since it remains only in place for the period of distraction (i.e. 14 days or less), the effects on growth at this site would be minimal (ie. the amount of growth at the intermaxillary suture over a 14 day period is not significant). Furthermore, the intermaxillary suture has minor growth potential as most palatal growth occurs as a "V" remodeling type.[135] Thus while its possible effect on the intervention should not be ignored, it's midline positioning renders it unlikely to affect the distraction and/or approximation of the cleft edges by SDO.

Figure 3.5. Insertion of the distractor device. Note the healed wound edges at ten days post-cleft induction

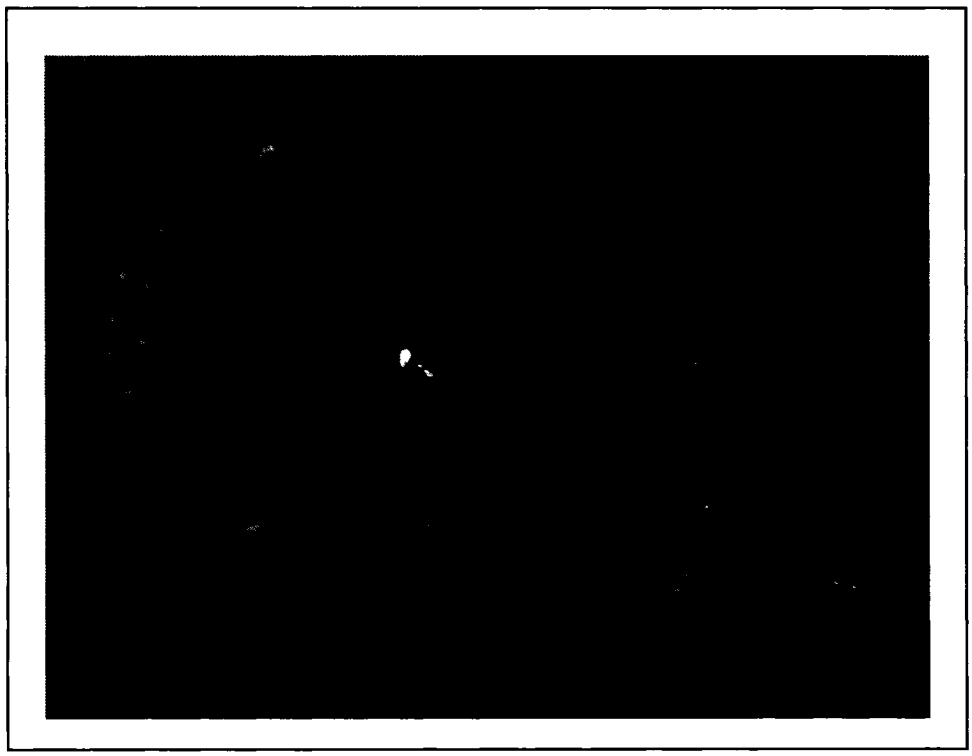

Figure 3.6. Insertion of the intraoral splint. Splints were fabricated the night before insertion to ensure an accurate fit. The recess posteriorly (right) allows for visualization of cleft edge approximation.

3.5 Distraction Phase Data Collection - Intraoral Measurements

Following initiation of the distraction phase (insertion of the distractors +/- splints), **two** sets of intraoral measurements were obtained every two days during distraction:

1) ***Intracuspal measurement***: the distance between the posterolateral cusp of the 2nd molars was obtained as an indicator of the presence/absence of posterior dental arch collapse (see Figure 3.7).

2) ***Intra-screw measurement***: the distance between the anchoring screws (at their point of mucosal penetration) was measured as an indicator of cleft edge approximation (see Figure 3.7). The latter was selected because accurate measurement of cleft width is hindered by variable thicknesses of tissue overlying the bony cleft edges secondary to post-surgical edema (see Figure 3.5).

Animals were examined daily by the investigators until distraction was complete to document healing, proper device positioning, progressive cleft closure, and monitor for surgical site infection.

Figure 3.7. Intraoral measurements. Intracuspal (green) and intra-screw (red).

3.6 Experimental Endpoints

Two animals from each of the three experimental groups (control, distracted, and distracted/splinted) were euthanized with sodium pentothal injection at 4 separate intervals: 1) 7 days post-initiation of distraction, 2) 2 weeks post-initiation of the distraction phase, 3) 6 weeks post-initiation of the distraction phase, and 4) 12 weeks post-initiation of the distraction phase (see figure 3.8). Completion of distraction was based on visual confirmation of cleft edge approximation, ranging from 8-14 days post-distractor insertion.

The sacrifice intervals were selected to satisfy a number of requirements including monitoring maxillary dimensions during the distraction phase, and serial harvesting of bone samples for both histology and histomorphometry/microCT (2 and 12 weeks post-distraction, see below), and for Bone Morphogenetic Protein expression assays[2] (7 days, 2, 6, and 12 weeks post-distraction).To that end, six animals from each of the three experimental groups completed the distraction phase and were used for the intraoral measurement and craniometric analysis (the animals that were sacrificed at 7 days post-initiation of distraction were not used as they had not completed distraction).

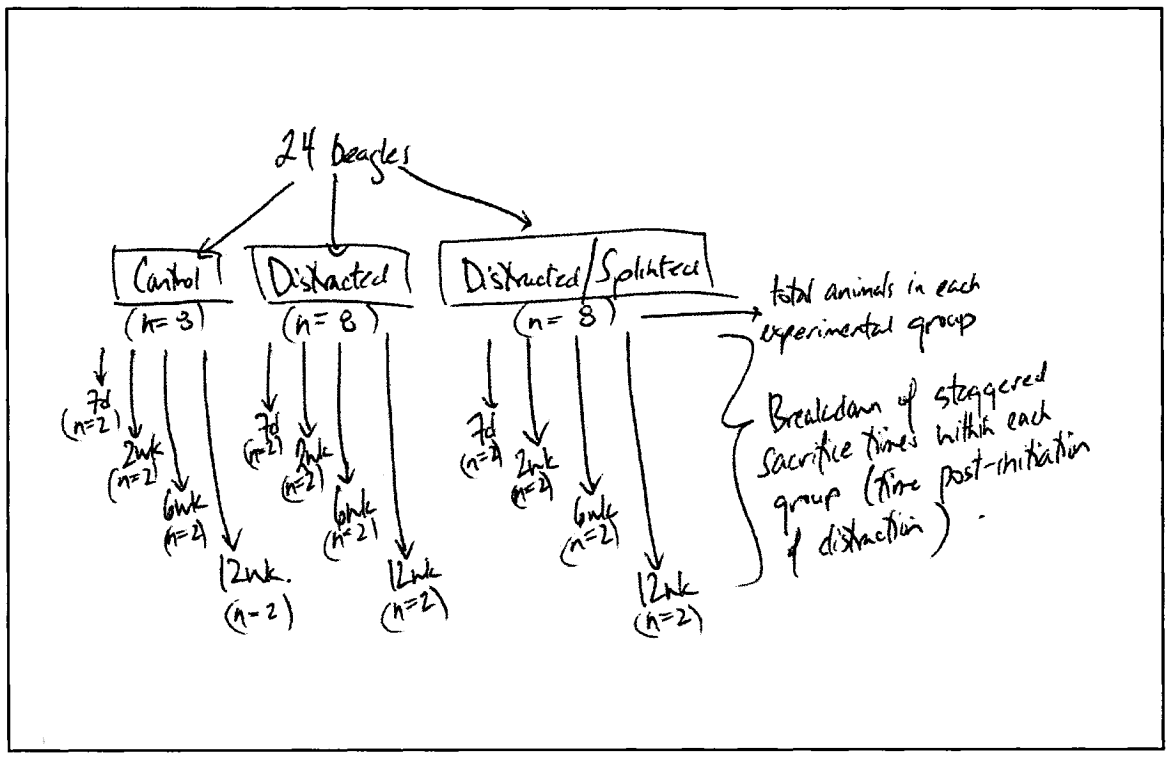

Figure 3.8. Breakdown of experimental groups and timeline for animal sacrifice. Note that "times" are post-initiation of distraction.

[2] The Bone Morphogenetic Protein (BMP) expression assays were integrated into this research protocol to characterize the BMP expression pattern, with the future goal of pharmacologically augmenting the distraction process. This data does not form part of the hypothesis pertaining to this thesis and thus this data is not included in this report. However, its inclusion in the protocol was documented here to explain the need for four sequential sacrifice intervals.

3.7 Histology

Histological analyses were carried out to examine the effects of SDO on the palatal suture. Following animal sacrifice, palatal mucosa was stripped with a periosteal elevator to expose the palatomaxillary suture (see Figure 3.8 below). The palate (containing suture) was harvested bilaterally with a saggital saw and fixed immediately in fresh 4% paraformaldehyde for histological preparation (Figure 3.9 and 3.10). Bone specimens were embedded in polymethylmethacrylate (MMA) and 3-6 μ sections cut on a Leica Polycut SM2500 sledge microtome (Leica Microsystems, Richmond Hill, Ontario). Ten serial sections were cut and mounted on glass slides (see figure 3.12 for the orientation of the sections). Adjacent sections were then stained separately for either vonKossa/toluidine blue or Goldner trichrome. The former stain is a standard stain for distinguishing between mineralized (bone) and non-mineralized tissue (cartilage, suture). The latter stain facilitated visualization of osteoid and osteoblasts for histomorphometric quantification.[136,137] Images for histological analysis were captured from prepared palatal specimens using a Leica DMR microscope equipped with a Retiga 1300 camera (Q imaging, Burnaby, British Columbia).

Figure 3.9. Palate with mucosa stripped at the time of animal sacrifice. Note the position of the anchoring screws medial to the PMS.

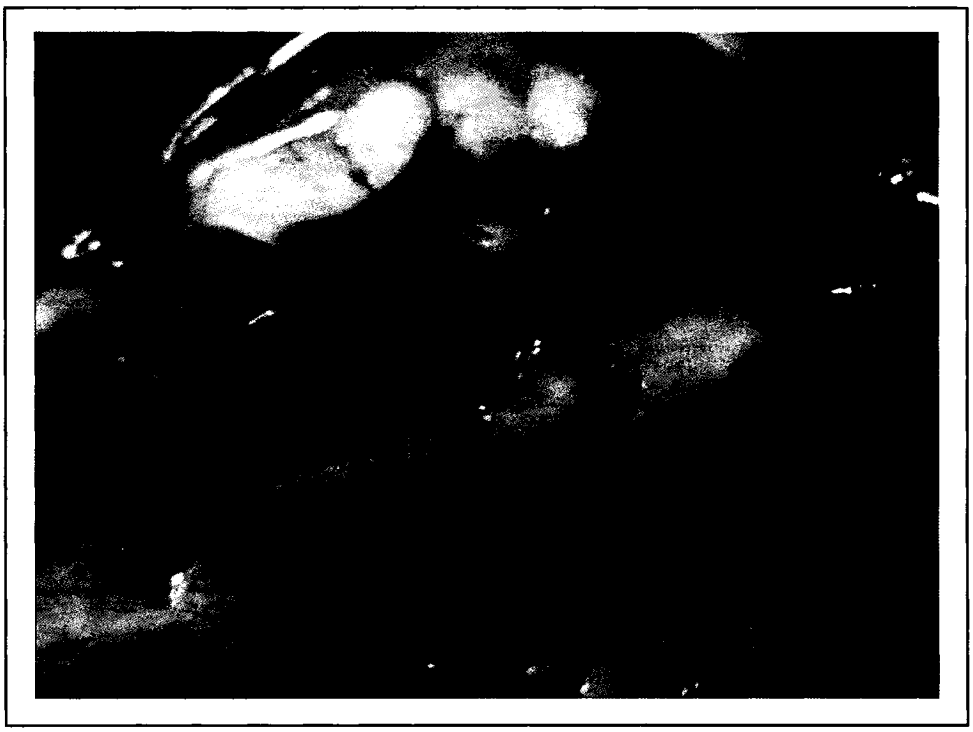

Figure 3.10. Close-up of PMS (anchoring screw removed). Outlined area highlights one side of the PMS and delineates margins for palatal harvest (see figure 3.10).

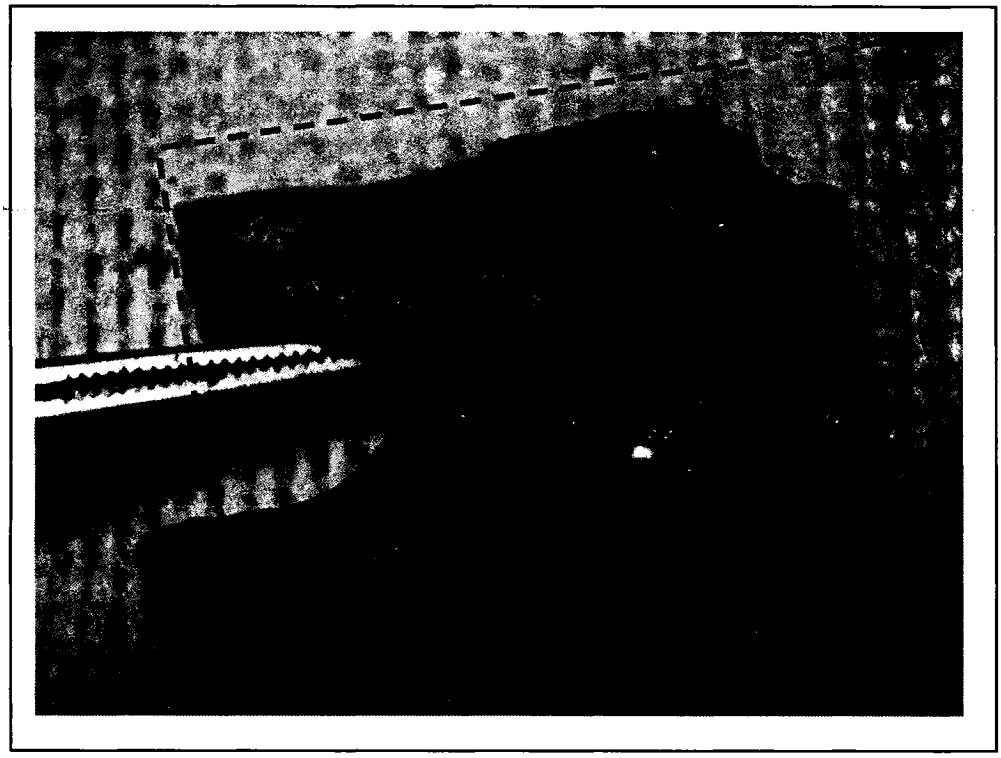

Figure 3.11. Harvested palate containing PMS from both sides of the cleft defect. Screw on the right side was removed for illustration.

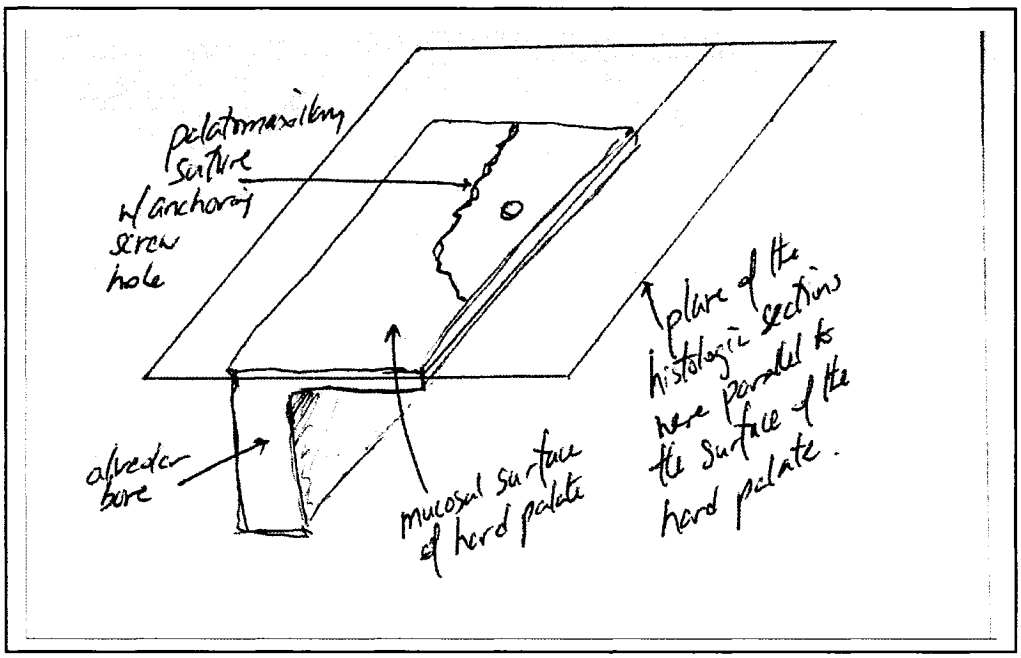

Figure 3.12. Schematic drawing of the orientation of the histologic sections. One hemi-palate is shown here. Sections were cut parallel to the mucosal surface of the hard palate.

3.8 Histomorphometry

Tissues for histomorphometric analysis were prepared as described in section 3.8. Images for histological analysis were captured from prepared palatal specimens using a Leica DMR microscope equipped with a Retiga 1300 camera (Q imaging, Burnaby, British Columbia). Specimens at 2 and 12 weeks post-distraction were analyzed from control and splinted/distracted groups (see Discussion section for a complete explanation regarding specimens selected for analysis). In an attempt to quantify changes in the amount of bone present in the selected samples (i.e. between 2 and 12 weeks for distracted/splinted vs. non-distracted specimens), the histomorphometric parameter *bone percentage composition* was measured within standard size (area) sampling grids by the Bioquant Nova Prime image analysis software (Bioquant Image Analysis Corp, Nashville, Tennessee).

SDO causes bone to be deposited along the edges of the distracted sutures, [26,96,102-104,107] thus in order to quantify any change in bone mass secondary to SDO, the sampling grids were centered along the midline of the suture so as to sample the newly-deposited bone that borders the suture on either side, in addition to the suture tissue itself (see figure 3.13). By sampling multiple sites along the suture in this manner, the *average* percentage bone composition within a standard area containing the newly-deposited bone and the palatomaxillary suture could be determined for a particular specimen, and compared with the average values from other specimens.

Importantly, the interpretation of the data from this analysis (and how it was used to quantify changes in bone deposition) depends on a number of assumptions, in addition to an understanding of the effects of distraction on suture width and the mechanism by which SDO occurs. A complete discussion of the interpretation of the results from the histomorphometric analysis, and its associated sources of error, is found in the discussion (part II).

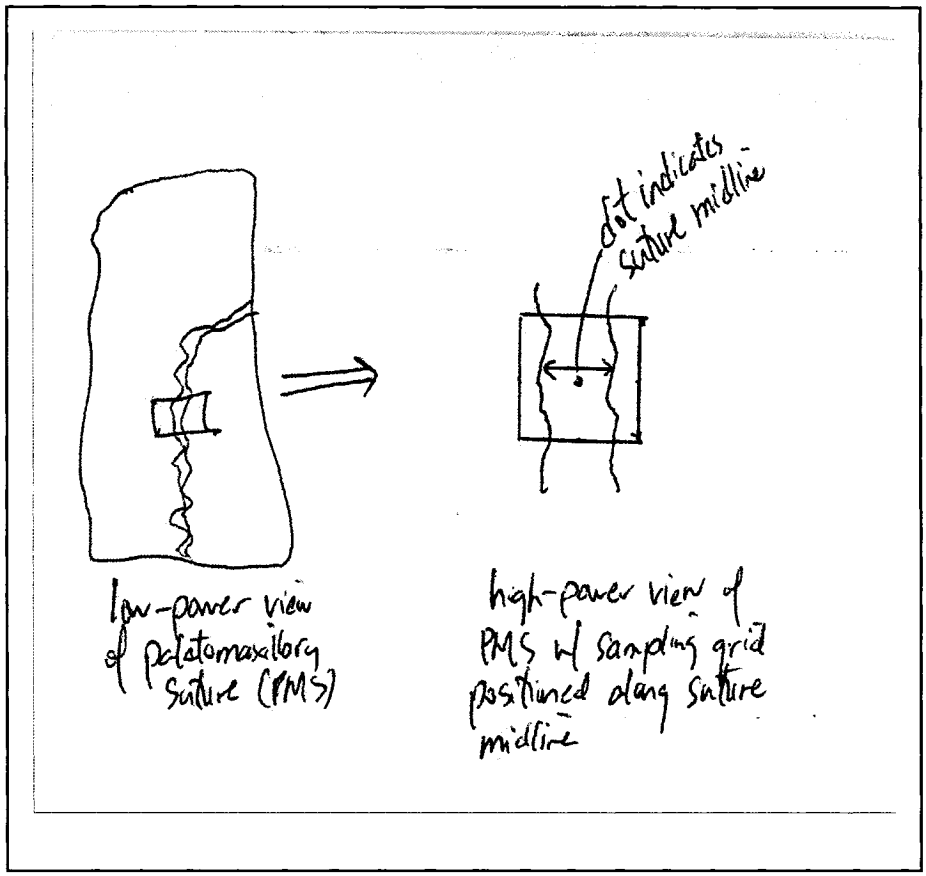

Figure 3.13. Schematic drawing of the technique for positioning the sampling grids along the palatomaxillary suture. Under high power magnification, the square grids were positioned with their centre over the midline of the sutures' width.

3.9 Micro-computed tomography (microCT)

Micro-computed tomography (microCT) was used as a second modality to analyze the percentage bone composition in the same palatal samples studied in section 4.6 Histomorphometry prior to histological fixation for histomorphometry. Palatal specimens from control and distracted/splinted animals at 2 and 12 weeks post-distraction were analyzed (see discussion for a detailed explanation regarding specimen selection.

Scans on samples were performed on a standard desktop micro-CT instrument (Model 1072, Skyscan, Aartselaar, Belgium). This instrument has a 20-100 keV/0-250 μA sealed, air-cooled, microfocus x-ray source with a polychromatic beam derived from a tungsten target and having a spot size of less than 5 μm at 4 W. For these analyses, the x-ray source was operated at 100 kV and at 98 μA (maximum power). Images were captured using a 12-bit, cooled CCD camera (1024 by 1024 pixels) coupled by a fiber optics taper to the scintillator.

Samples were scanned at a magnification resulting in a pixel size of 13.68 μm. Using a rotation step of 0.9 degrees and an exposition time of 2240 ms for each step, a total of 206 images (for each specimen) was generated giving a scanning time of 30 min. The cross-sections along the specimen axis were reconstructed using Cone-Beam Reconstruction Software (SkyScan), with a distance between each cross-section of 27.35 μm. Each cross-section was reduced in half size to facilitate the analysis, giving of a voxel of 27.35 x 27.35 x 27.35 μm^3. 3D Creator and CT-Analyser softwares (both from SkyScan) were used to create 3D renderings and perform analyses, respectively.

From the entire set of cross-sections derived from each palatal specimen, four non-consecutive cross-sections were randomly chosen to perform the analysis of the bone around the suture to sample the suture at various depths (as opposed to histomorphometry which sampled tissue in one cross-section only). The software was then instructed to position the sampling grids (called Regions of Interest or ROI's) in a manner identical to that utilized in the histomorphometric analysis (see figure 3.13 above) – that is, the ROI's were positioned by centering them on the midline of the suture (width), in multiple places along its length, throughout various depths (cross-sections). To determine the percentage composition bone within the ROI's, the global thresholding procedure was used as segmentation method to select the bone from the non-bone. The number of ROI per sample was between 11 and 13. Thus, although the same palatal samples were analyzed with both histomorphometry and microCT using the identical sampling strategy, the microCT sampled different areas at different depths of the specimen. The results was the average percentage composition bone within a standard area containing the newly-deposited bone and the palatomaxillary

suture, determined for a particular specimen, and compared with the average values from other specimens.

Again, a complete discussion of the interpretation of the results from this analysis (and how it was used to quantify changes in bone deposition), and its associated sources of error, is found in the discussion (part II).

3.10 Dual-Energy Xray Absorptiometry (DXA)

Bone mineral density (BMD) measurements provide a noninvasive assessment of bone mineralization and strength during distraction osteogenesis.[46] BMD was measured using DXA in harvested palatal bone specimens to gauge the structural integrity of the palatal bone following SDO using a Lunar PixiMUS 1.46 (GE-Lunar, Madison, Wisconsin). BMD was determined in specimens at 12 weeks post-distraction, and compared with age-matched control (non-distracted) specimens.

3.11 Craniometry

Following harvesting of palatal specimens, skulls were prepared for craniometry using the enzyme-active detergent method.[138] (Figures 3.14, 3.15). Craniometric landmarks to assess maxillary and dental arch width were identified using the technique outlined by Bardach et al.[10-14,73-75,77-79,139] (Figure 3.16)

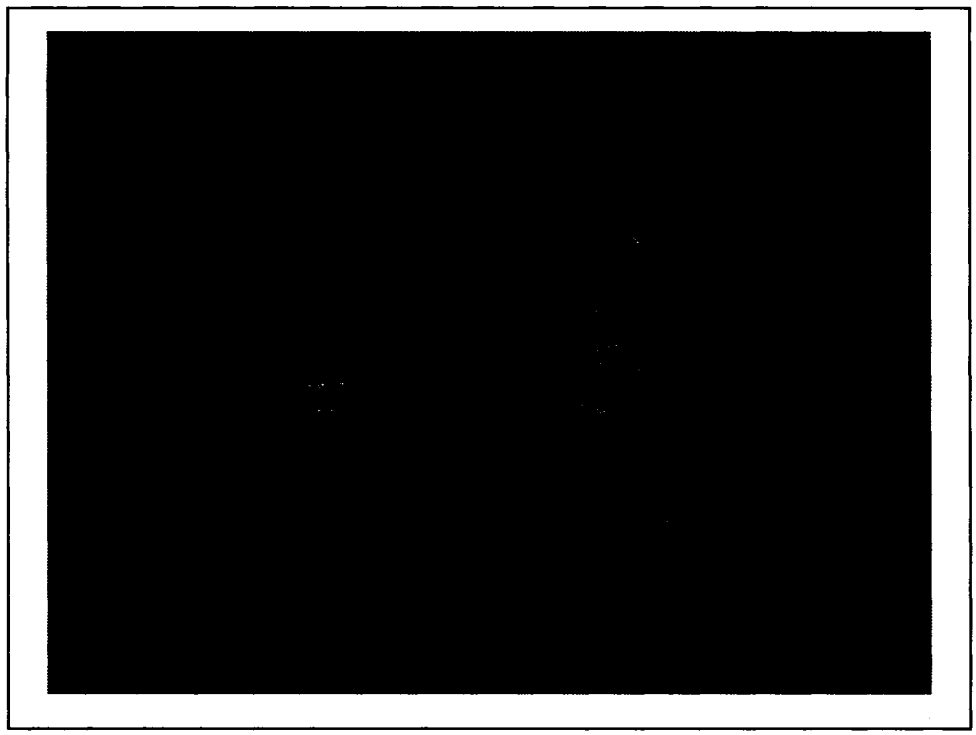

Figure 3.14. Skull with soft-tissues removed. Bird's eye view.

Figure 3.15. Skull with soft-tissues removed. Inferior view. Palate has been harvested bilaterally.

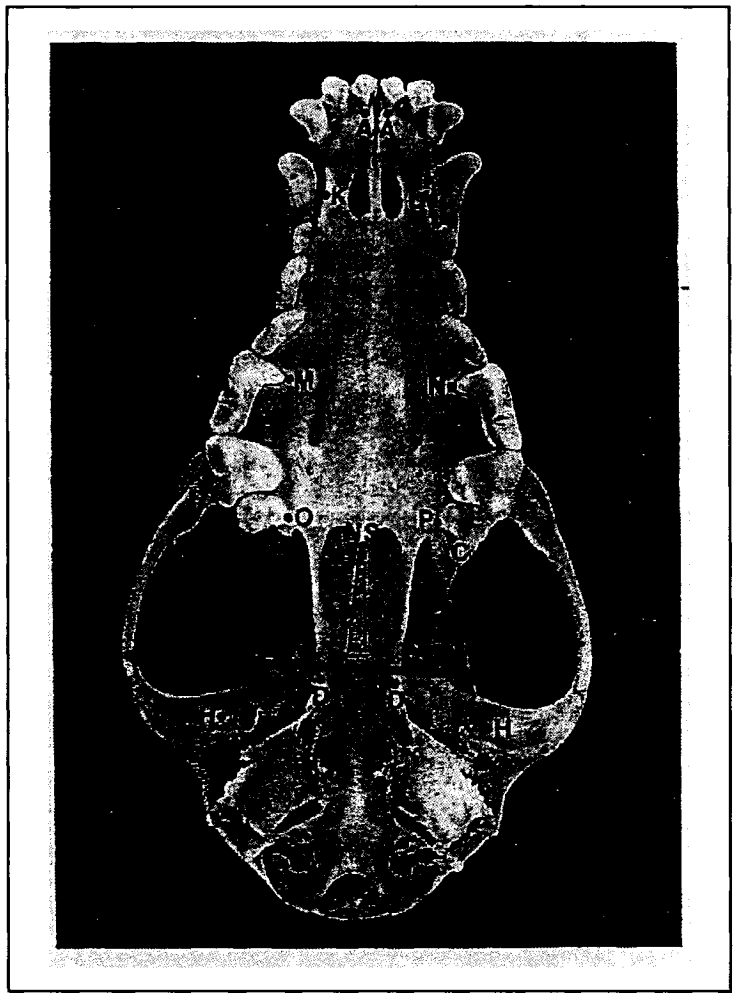

Figure 3.16. Dry-skull with craniometric landmarks labeled. In this study, MN and OP were measured in addition to ZZ (the distance between the zygomatic arches, measured at the zygomatico-temporal suture, highlighted by the red arrow)

CHAPTER 4

Results

4.1 Physical examination

Palate surgery was well-tolerated and by post-operative day 1, all animals were self-grooming and ingesting soft-diets. Only one animal required sacrifice prematurely after an observational period to confirm the diagnosis of distemper virus. There were also two mortalities on induction of anaesthesia (prior to any surgical manipulation). The deceased animals were sent back to the supplier (Marshall Farms©, NY) for autopsy which found the cause of death to have been caused unrecognized cardiac pathology (all animals from this supplier are screened for congenital heart defects prior to shipment). *All animals that died were replaced with newly purchased animals from the same supplier and the experimental protocol repeated to maintain the originally designed study size* (3 groups of eight animals).

Post-implantation of the distractors +/- intraoral splints, there was a brief adjustment period followed by a return to normal eating habits. Accidental dislodgement of an intraoral splint occurred in one dog during feeding but was promptly replaced and secured within hours of displacement. Clefts in the non-splinted group closed at a rate of approximately 1 mm per day and were all closed by 9 days. Clefts in the splinted group closed at a slightly slower rate, with screw approximation maximal* by 14 days.

*Note that maximal approximation of clefts was achieved when the screw heads were touching (i.e. no further approximating force could be applied by the distractor springs). Bony cleft edges were not necessarily completely approximated at this point, as the anchoring screws were set back a few mm's from the bony edge.

4.2 Gross intraoral morphology of the distraction process

4.2.1 Photographic documentation

Photographic images were captured at various stages of the experimental protocol to follow and document intraoral physical findings. Photos before and after distraction (plus or minus splinting) are presented below.

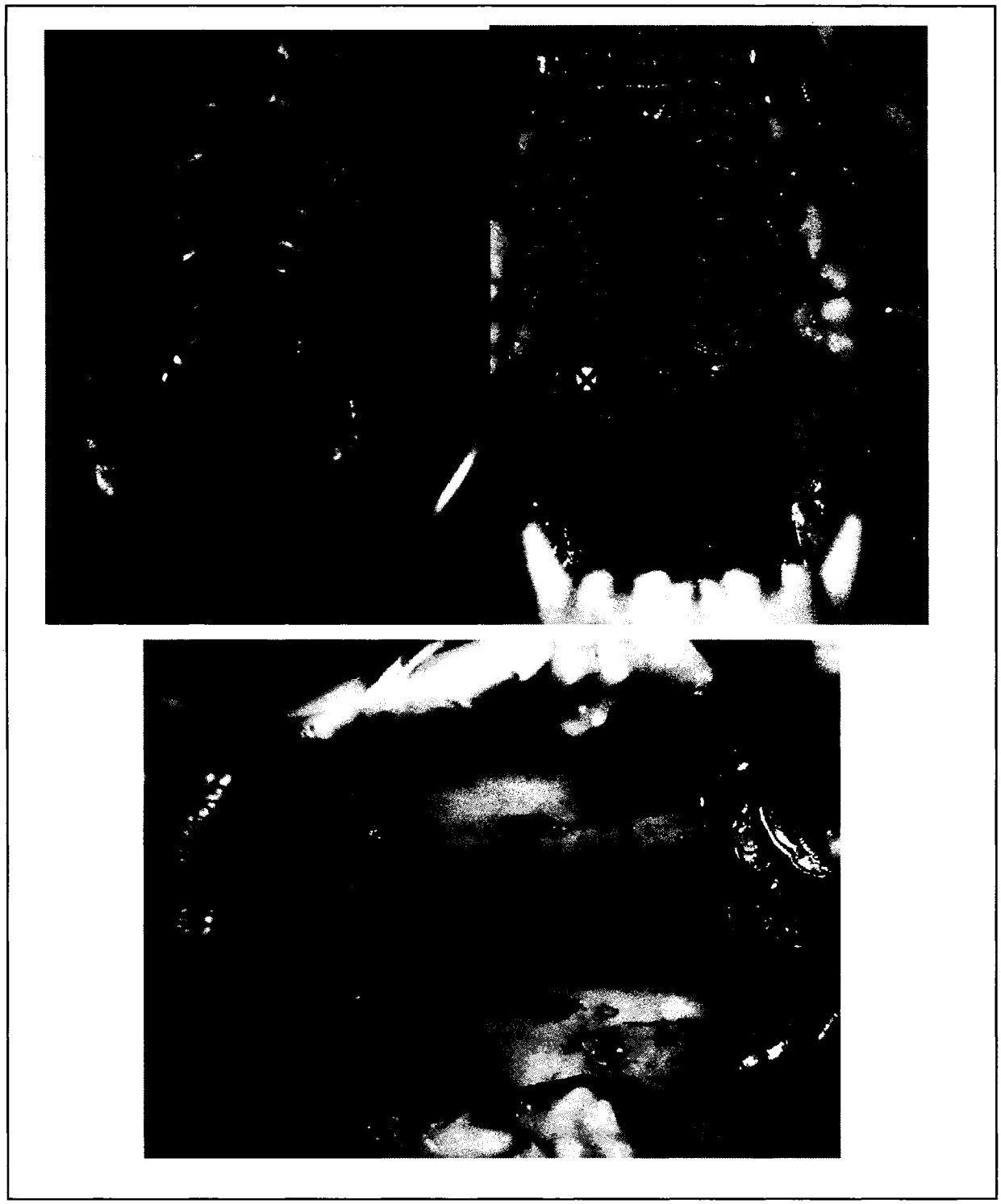

Figure 4.1. Control group (non-distracted). Post-cleft creation (top left), at sacrifice (top right), at sacrifice with mucosa stripped (bottom). Note that the width of the cleft palate defect is unchanged from the time of cleft induction.

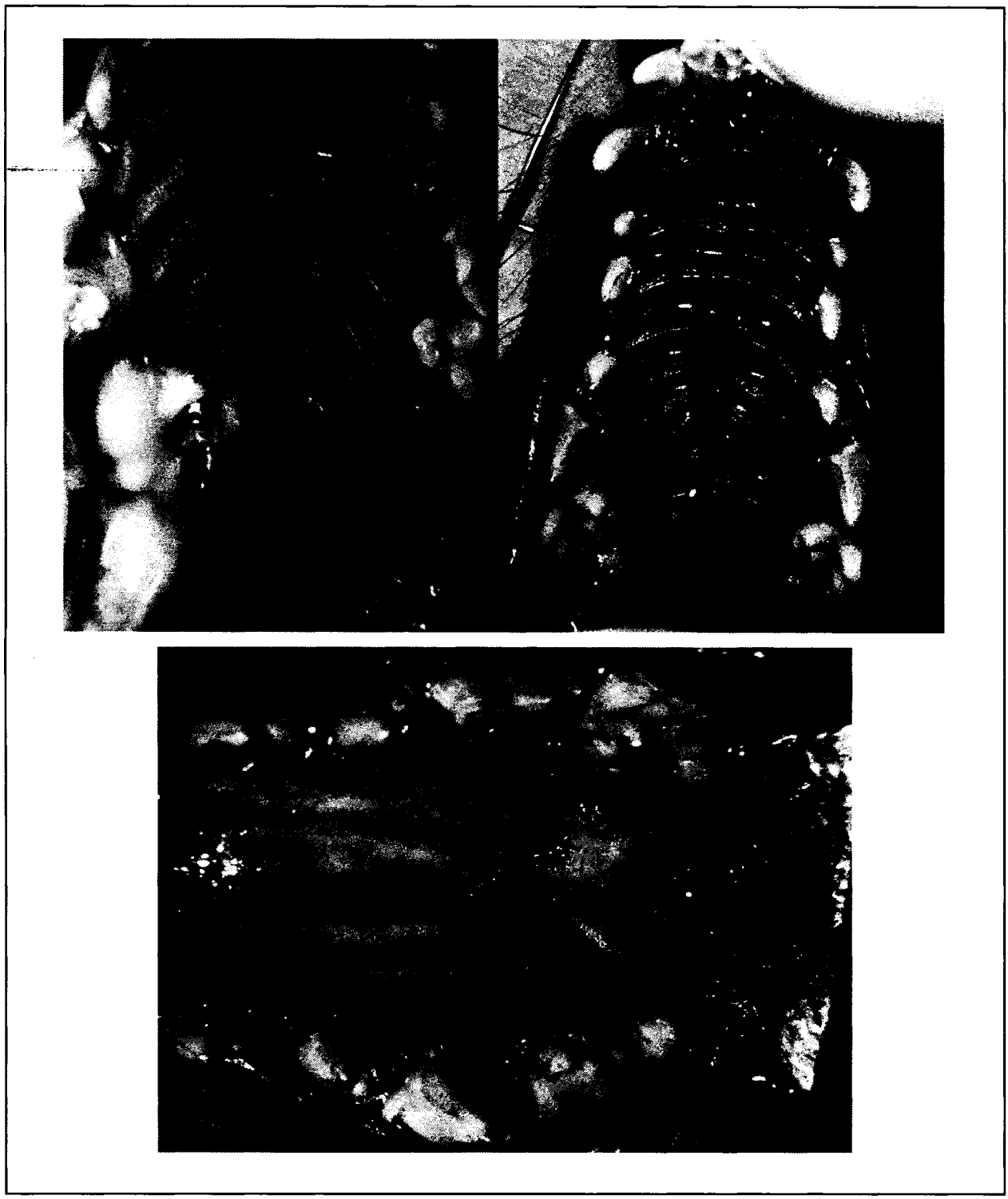

Figure 4.2. Distracted group. Pre-distraction (top left), 2 weeks post-distraction (top right), at sacrifice with mucosa stripped (bottom). Note the approximation of the cleft defect.

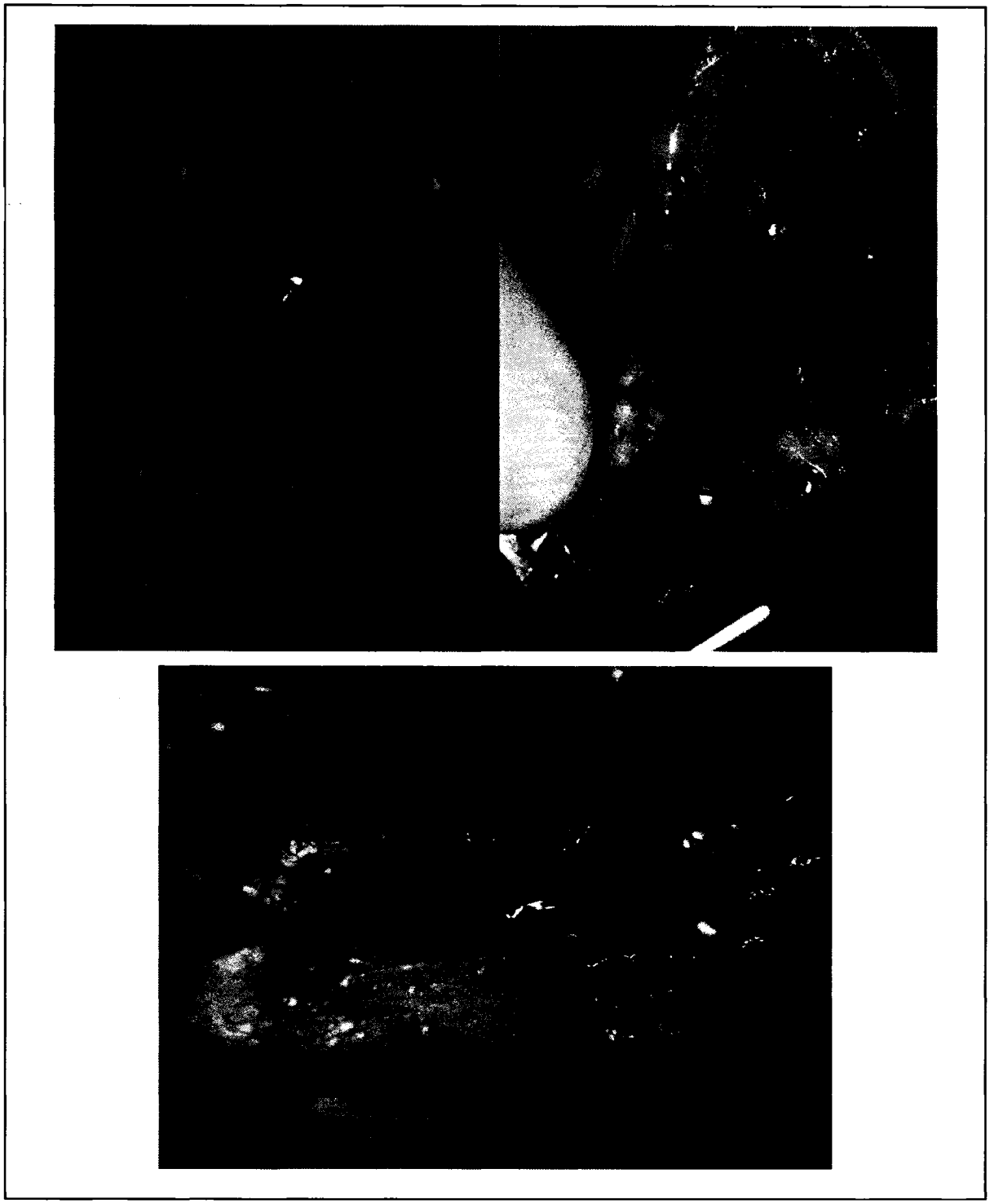

Figure 4.3. Distracted/splinted group. Pre-distraction with splint in place (top left). The cleft defect is visible between the anchoring screws. Two weeks post-distraction (top right). Note the closure of the cleft defect. At sacrifice with mucosa stripped (bottom). Note the approximation of the cleft defect posteriorly at the former location of the distractor device.

4.2.2 Gross intraoral anatomical observations

The width of cleft defects in the control group remained unchanged over time as no intervention (distraction) was applied to this group (other than surgical induction of the cleft palates).

Animals in the distracted group (group 2), had progressive closure of the cleft defects until the cleft edges were approximated. Recall that the anchoring screws were set back from the cleft edges in order to allow for adequate bone purchase. The clefts, therefore, were approximated until the point where the anchoring screws were in contact (i.e. the distractors could no longer exert a medial force). Thus, the cleft defects post-distraction in the distracted and distracted/splinted groups are approximated but not completely closed. Furthermore, as the distractors were situated posteriorly in the hard palate, the cleft edges were frequently approximated completely at this site, whereas a small defect was routinely left anteriorly as no force was applied at this location.

Animals distracted with intraoral splints had similar post-distraction findings with cleft edges that were successfully approximated posteriorly at the sites of the distractor devices. Intraoral splints were all well-positioned and intact at the time of removal.

4.3 Intraoral measurements

Intraoral measurements were gathered on alternate days during the distraction phase and then weekly until animal sacrifice. Two separate measures were obtained as described in *section 3.2.4*. To record the presence or absence of posterior dental arch collapse, ***intracuspal*** distances (between the posterolateral cusps of the 2^{nd} molars) were tabulated. Narrowing of the intracuspal distances suggests medially-directed maxillary/dental arch collapse. The change in distance between the anchoring screws was also measured (referred to as ***intrascrew*** distance) to gauge the status of cleft edge approximation during the distraction process. Intrascrew distance was

selected (as opposed to direct measurement of cleft edges), because bony cleft edges are obscured by varying and inconsistent amounts of overlying soft tissues. These results are presented below in tables 4.1 and 4.2.

Please note that the *mean change* represents the mean difference between **pre-distraction** and **post-distraction day 14** intraoral measurements (a time by which all animals had completed distraction). In addition, note that each group in the table contains 6 animals (n=6) because two animals from each group were sacrificed half-way through distraction (see experimental endpoints, Methods section 3.6) and thus could not be included as they had not completed distraction.

Table 4.1. Intrascrew measurements.

Group	Mean change (mm)	n	SD
Control	n/a	6	n/a
Distracted	-6.667*	6	1.862
Distracted and Splinted	-6.333*	6	0.817

*the negative value signifies that a decrease in width occurred

Table 4.2. Intracuspal measurements

Group	Mean change (mm)	n	SD
Control	-0.333*	6	0.817
Distracted	-5.000*	6	2.367
Distracted and Splinted	0.167	6	1.169

*the negative value signifies that a decrease in width occurred

Initial statistical analysis of the intraoral measurement data with a one-way analysis of variance (ANOVA) did not meet the assumption for "homogeneity of variance" (as reported by the Levene's test). This was expected secondary to the low number of subjects per group (n=6). Thus, non-parametric analysis of the data was carried out.

The Kruskal-Wallis test (a non-parametric equivalent of one-way ANOVA) was utilized and showed a statistically different effect of groups (i.e. distracted or distracted and splinted vs. control) on dental arch width ($H_{(2)}$=12.210; p=0.002). The measurements pre-intervention (i.e. dental arch width prior to distraction) were also tested to make sure that mouths were similar in size. The result of the analysis did not reveal any statistical differences ($H_{(2)}$=0.651; p=0.722) among mouth sizes pre-intervention. Mann-Whitney's U were then used as post-hoc tests to establish where the difference in groups lied (summarized in table 4.3).

Table 4.3. Mann-Whitney test output data for intracuspal measurements

	Distracted – Control	Distracted – Dist/Splint	Control – Dist/Splint
Mann-Whitney U	0.500	0.500	10.500
P-value (2-tailed)	0.002	0.002	0.240

The results revealed that the "distracted" group had a statistically significant decrease in intracuspal distances (arch width) which was not present in either the "control" group or the "distracted + splinted" group. Furthermore, intraoral splinting did not have a statistically significant effect on the change in intrascrew distances (i.e. cleft approximation was similar with and without intraoral splinting). These results are represented graphically in figure 4.4 below.

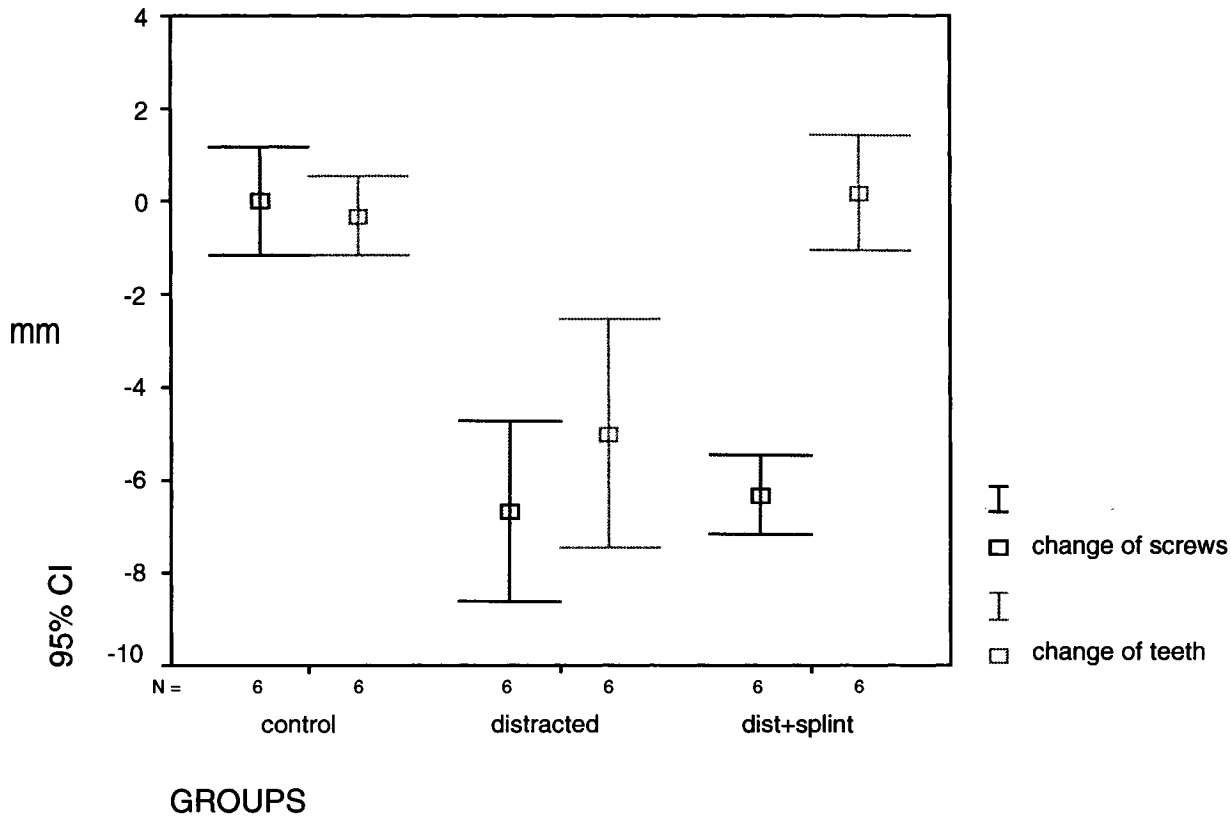

Figure 4.4. Intraoral measurements. The three experimental groups are plotted along the x-axis. The y-axis depicts changes (in mm) in intrascrew and intracuspal measurements. Error bars represent 95% confidence intervals.

From the data in figure 4.4, it is evident that the amount of cleft approximation (approximation of screws) closely resembles the decrease in dental arch width (decrease in intracuspal distance). This finding suggests that cleft closure in animals distracted **without** splints (as described by Liu et al[26]) is achieved for the most part by medial translation of palatal bone secondary to dental arch/maxillary collapse, as opposed to new bone generation secondary to sutural distraction.

In summary, there is statistically significant alveolar arch/maxillary collapse in the animals distracted <u>without</u> intraoral splints. Those animals that were distracted <u>with</u> an intraoral splint achieved comparable cleft approximation without any evidence of alveolar arch/maxillary collapse.

4.4 Craniometry

Various craniometric measurements were made using the method of Bardach[10-15,75,77,79] to directly measure changes to facial/maxillary width following distraction with and without intraoral splints (see figure 3.14 for a schematic of the measured landmarks). Three measurements were taken. MN and OP are both direct measures of posterior maxillary/dental arch width. ZZ represents the distance between the bilateral zygomatic arches, which correlate to midfacial or cheek width. These results are summarized in table 4.4.

Table 4.4. Craniometric data.

Craniometric dimension	Group	Mean (mm)	SD	n
MN	Control	27.50	3.742	8
	Distracted	25.00	2.268	8
	Dist/splint	28.88	2.696	8
OP	Control	54.63	3.068	8
	Distracted	50.38	2.825	8
	Dist/splint	54.38	2.669	8
ZZ	Control	72.63	6.823	8
	Distracted	71.88	6.081	8
	Dist/splint	71.63	5.397	8

Statistical analysis of the data was carried out with a multivariate ANOVA with animal age as a covariate. Levene's Test of Equality of Error Variances output was not significant ($p = 0.093$), therefore permitting the use of an ANOVA.
The measures of OP, MN and ZZ were taken together as a set of dependant variables since they are closely related. The results indicated that there was a statistically significant effect of distraction on measurements MN ($F_{(2,20)} = 5.880$; $p = 0.01$), OP ($F_{(2,20)} = 9.652$; $p = 0.001$) but not ZZ ($F_{(2,20)} = 0.222$; $p = 0.803$). To determine wherein the difference lied, an analysis of contrast was performed. Measurements MN and OP were significantly reduced in animals distracted **without** splints. Alternatively, animals distracted **with** splints did not have any detectable differences in the same measures (MN and OP) when compared to control animals. These results are summarized in table 4.5.

Table 4.5. Analysis of Contrast output data for craniometric data

Craniometric Dimension	Contrast estimate	p-value
MN	-2.500	0.041
OP	-4.250	0.001
ZZ	**-0.750**	**0.637**

The craniometric data (mean values of the labeled craniometric measures) is plotted graphically below in non-linear trends over animal age in weeks (figures 4.5. 4.6, and 4.7). The graphs demonstrate a clear narrowing effect of distraction on posterior dental arch/maxillary width over time for measurements MN and OP. The splinted/distracted animals have arch growth patterns that resemble control (non-distracted) animals.

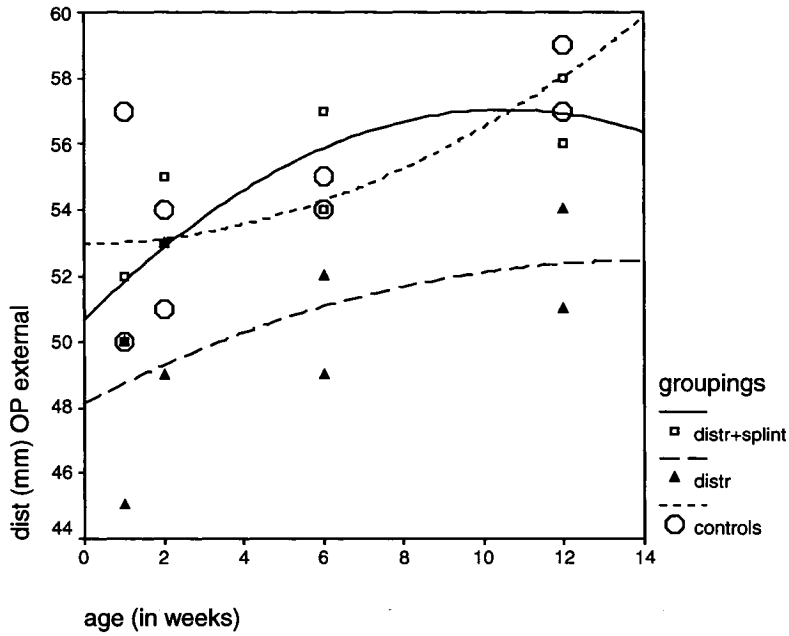

Figure 4.5. Non-linear trends of posterior dental arch width (OP) with age. Note the narrowed OP dimensions in the distracted group across all ages.

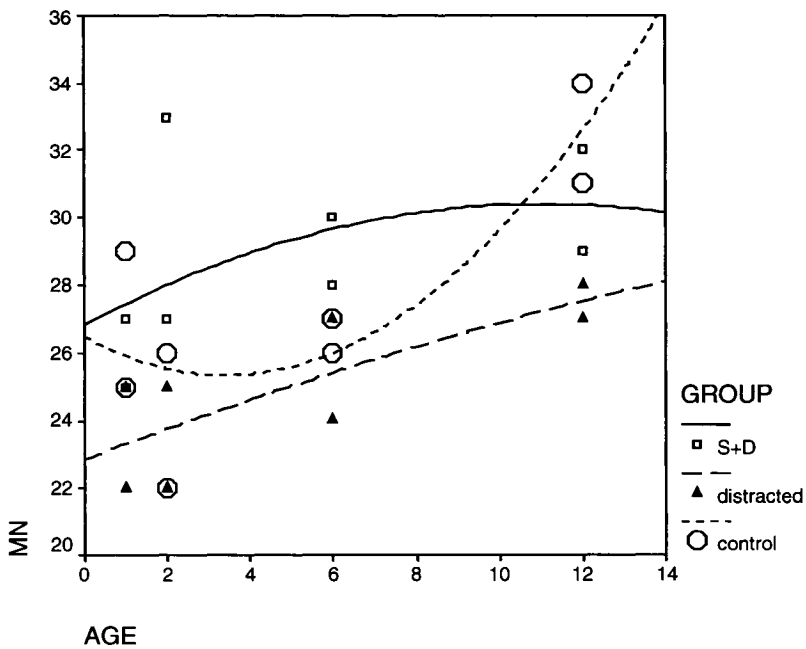

Figure 4.6. Non-linear trends of posterior dental arch width (MN) with age. Note the narrowed MN dimensions in the distracted group across all ages.

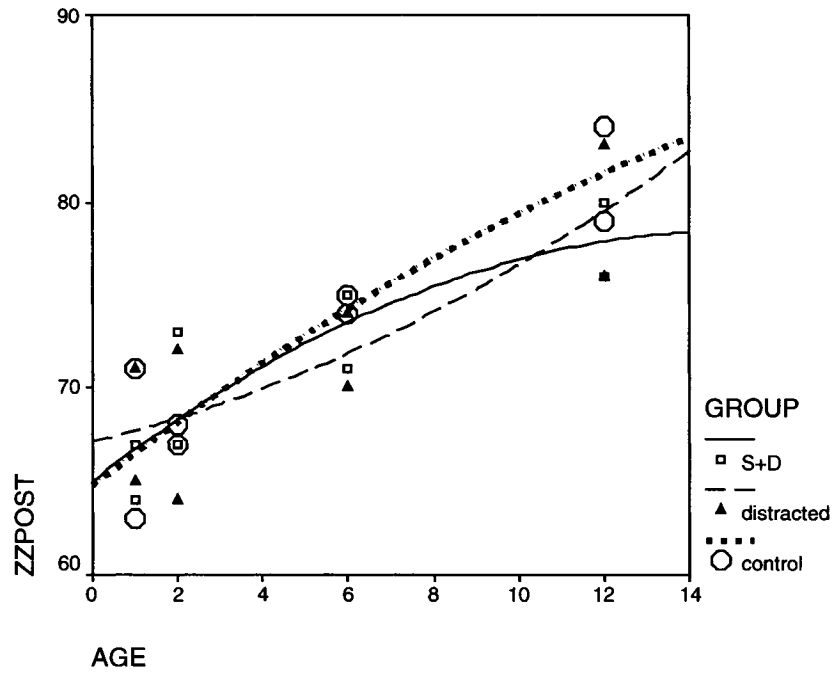

Figure 4.7. Non-linear trends of zygomatic arch width (ZZ) with age. No significant effect of distraction is noted.

4.5 Histology

Histologic examination of the palatal bone specimens was performed to investigate the changes that occurred in palatal bone and palatomaxillary suture architecture as a result of the distraction process. Specimens from animals at 2 and 12 weeks post-distraction in the control (non-distracted) and distracted/splinted animals were analyzed using light microscopy under low and high power (see discussion for an explanation of the selection of specimens examined). The results are presented below.

4.5.1 Low power microscopy

Low power examination of the hemi-palatal specimen, (containing one side of the palate with the longitudinal portion of the palatomaxillary suture, see figure 3.10-3.12 for an explanation of the orientation of the specimens) revealed an acute widening and increasing irregularity of the suture in distracted/splinted specimens at 2 weeks post-distraction (see figure 4.9). Note that the bone in this specimen has a more porous appearance which can be explained by the fact that the specimen was sectioned from a deeper and more spongy portion of the palatal specimens. In addition, as discussed below in the section pertaining to the high power analysis, the sectioning of these specimens was difficult secondary to the density (hardness) of the bone and the required thicknesses (3-5 microns) for high-power analysis. Thus, some of the porosity is also artifact caused by the sectioning procedure (see high power microscopy, below).

Suture morphology at 12 weeks (figure 4.10) had returned to its narrow and smooth (non-distracted, control) appearance. Note that there is also a small amount of artifactual fragmentation of the bone in this specimen due to the sectioning process.

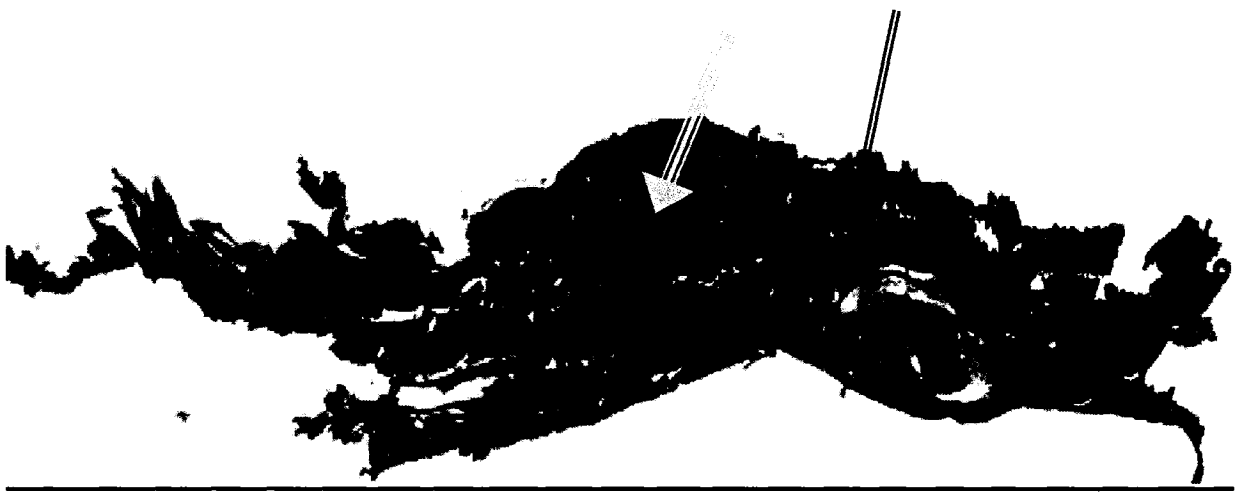

Figure 4.8. Control group (non-distracted) at 12 weeks post-distraction (sham) with von Kossa stain. Low power view of entire hemi-palatal specimen. The cleft edge is superior, the side abutting the alveolar bone is inferior, and the left side is cephalad. The longitudinal portion (red arrow) and transverse portion (short yellow arrow) of the palatomaxillary suture are indicated. Note that the suture is narrow and the edge undulations appear smooth (compare below). The large oval structure is a tooth bud. There is a small amount of bone fragmentation in this specimen which is artifact secondary to the sectioning process.

Figure 4.9. Distracted/splinted group 2 weeks post-distraction with von Kossa stain. Low power view of a section through the entire hemi-palatal specimen. The cleft edge is superior, the side abutting the alveolar bone is inferior, and the left side is cephalad. This photo depicts a section at a deeper level of the palatal bone containing some areas that are more porous. However, it serves well to demonstrate the widened suture with edge undulations that are irregular and shaggy (compared to control and distracted/splinted at 12 weeks post-distraction). The longitudinal portion (red arrow) and transverse portion (short yellow arrow) of the palatomaxillary suture are indicated. The oval structure to the right of the yellow arrow is a tooth bud, while the circular hole between the arrows represents the defect created by the removed anchoring screw. Note that some of the porosity in the bone is also artifact due to the sectioning process, which is present in all specimens.

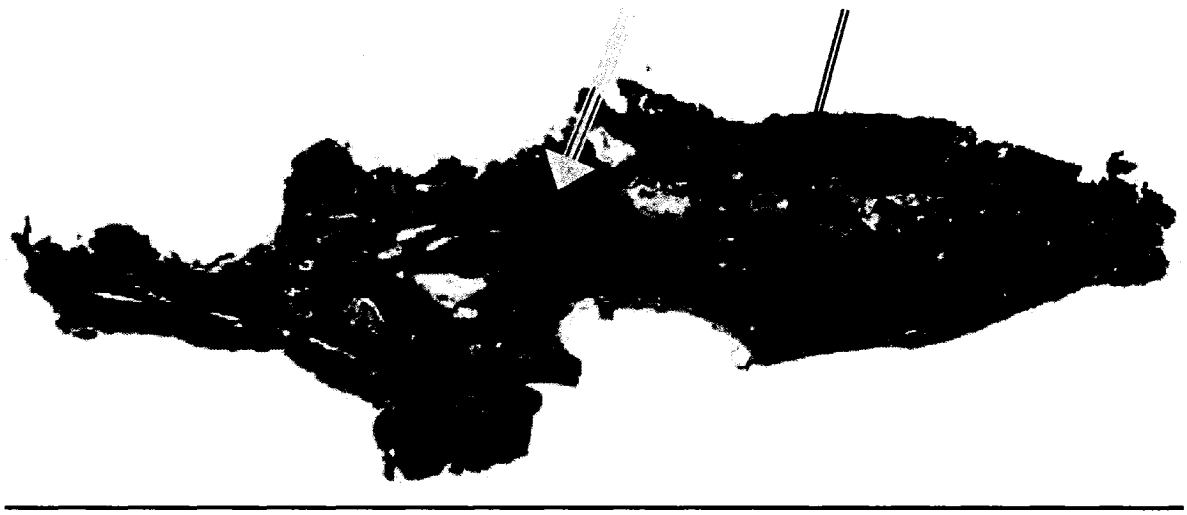

Figure 4.10. Distracted/splinted group 12 weeks post-distraction, with von Kossa stain. Low power view of entire hemi-palatal specimen. The cleft edge is superior, the side abutting the alveolar bone is inferior, and the left side is cephalad. The longitudinal portion (red arrow) and transverse portion (short yellow arrow) of the palatomaxillary suture are indicated. Note that the distracted (longitudinal) portion of the PMS has become narrower again, with suture edge undulations that appear smoother, similar to the morphology in the control group (compare to roughened appearance in figure 4.9, distracted/splinted at 2 weeks post-distraction). The translucent areas are again artifacts caused by the sectioning procedure (the neighboring bone is normal as seen under high power, see section 4.5.2 High power microscopy for an explanation).

4.5.2 High power microscopy

High power examination of the palatal specimens revealed similar patterns of trabeculation and architecture in the palatal bone at 12 weeks in distracted/splinted vs. control samples. The small fragmented areas (seen in all specimens), initially thought to be bone thinning or fenestrations (possibly caused by stretching of the existing bone instead of intended osteogenesis) on low power examination, were found to be surrounded by areas of completely normal bone under high power analysis. In

addition, they were found in all specimens (distracted and non-distracted). Thus, following consultation with our collaborators at the McGill Bone Research Center, these translucent areas were determined to be artifact (fragmentation) caused by the hardness of the palatal bone and the sectioning procedure necessary to achieve 3-5 micron thick slices for high power examination (as true bone thinning would manifest itself more uniformly throughout the bone).

The palatomaxillary suture underwent a subjective increase in width and cellularity at 2 weeks in distracted specimens, returning to its pre-distracted state by 12 weeks. Specifically, osteoblasts in the distracted specimen became enlarged and cuboidal in morphology at 2 weeks, returning to a smaller, rounder shape (like that seen in non-distracted control specimens) by 12 weeks post-distraction. No such changes were appreciated in control specimens. The overall morphology of the suture by 12 weeks following distraction was found to be similar to the non-distracted (control) samples with perhaps slightly increased overall cellularity (figure 4.15).

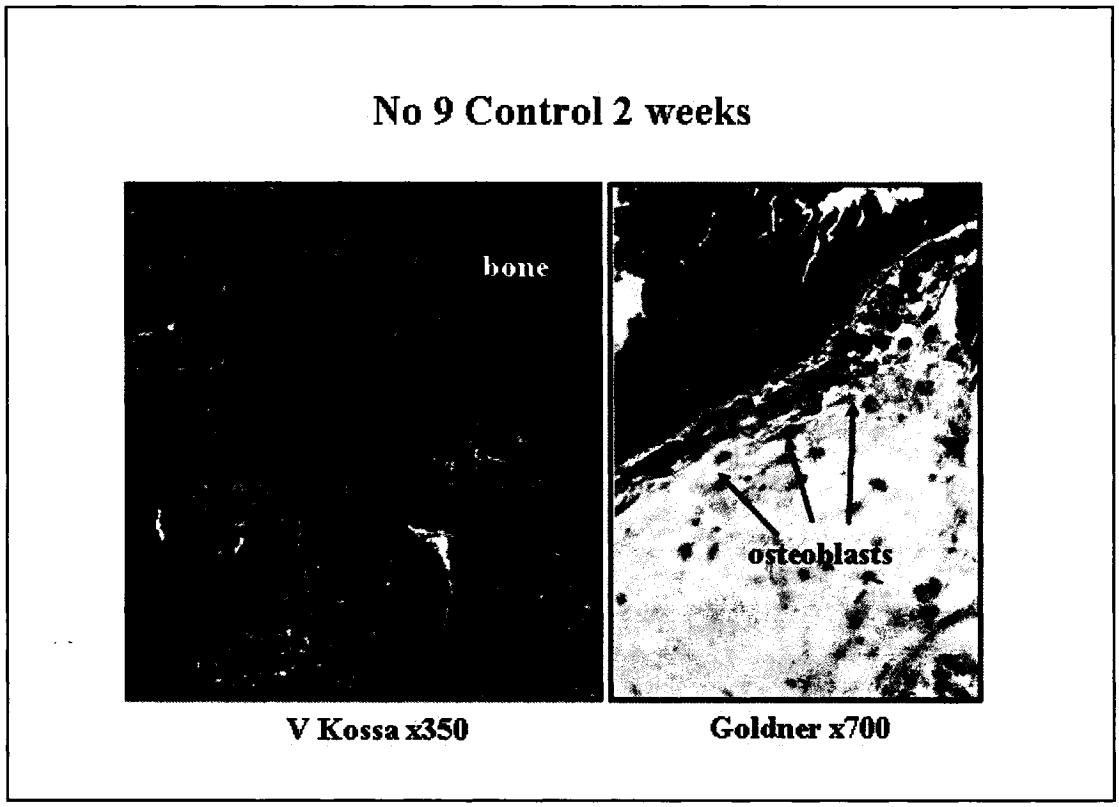

Figure 4.11. Control (non-distracted) group at 2 weeks. High power view of palatomaxillary suture (left). Quiescent appearing osteoblasts lining the suture edge (right). Stains and magnification utilized are indicated under each figure.

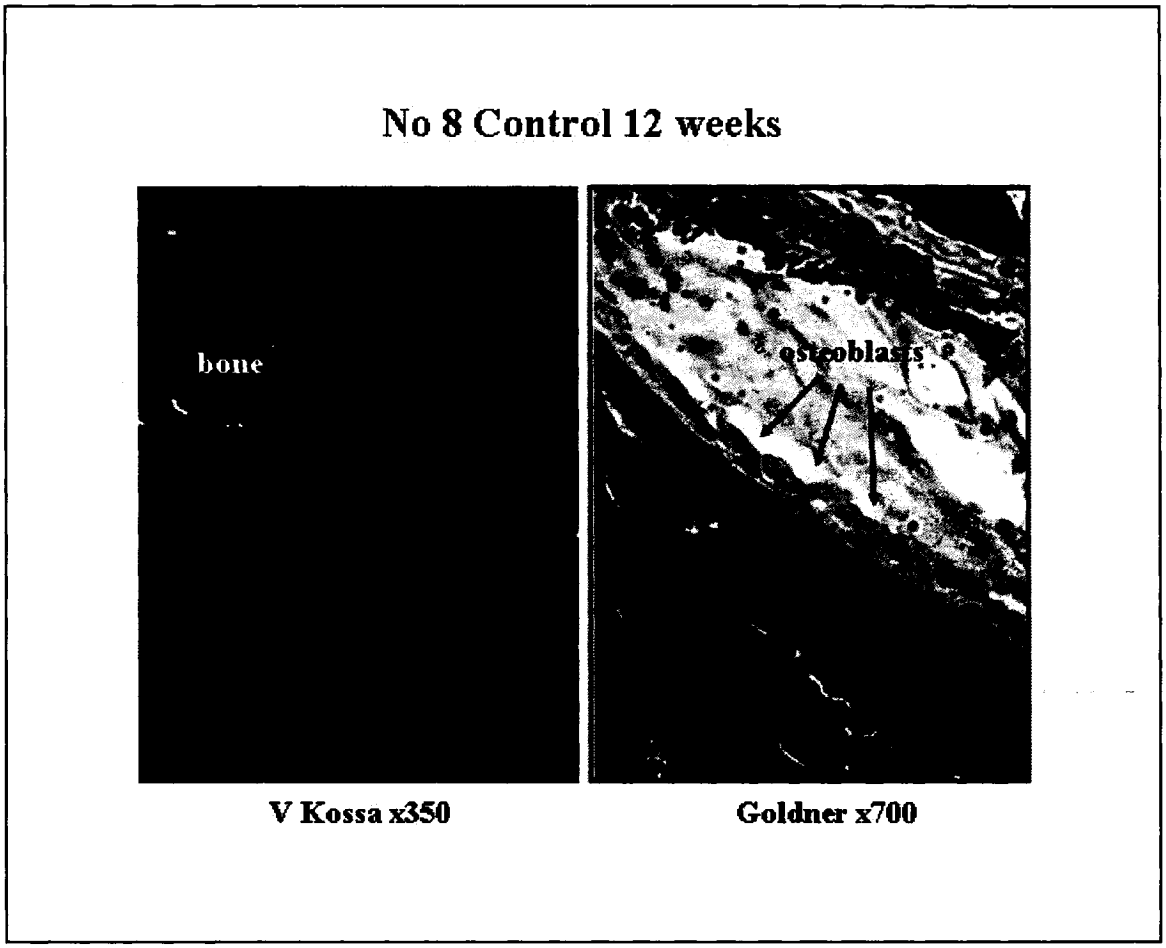

Figure 4.12. Control (non-distracted) group at 12 weeks. High power view of palatomaxillary suture (left). Note the much unchanged morphology of the suture as compared to the control sample at 2 weeks. Again, quiescent appearing osteoblasts lining the suture edge (right). There is no obvious change in the suture cellularity nor the osteoblast morphology. Stains utilized are indicated under each figure.

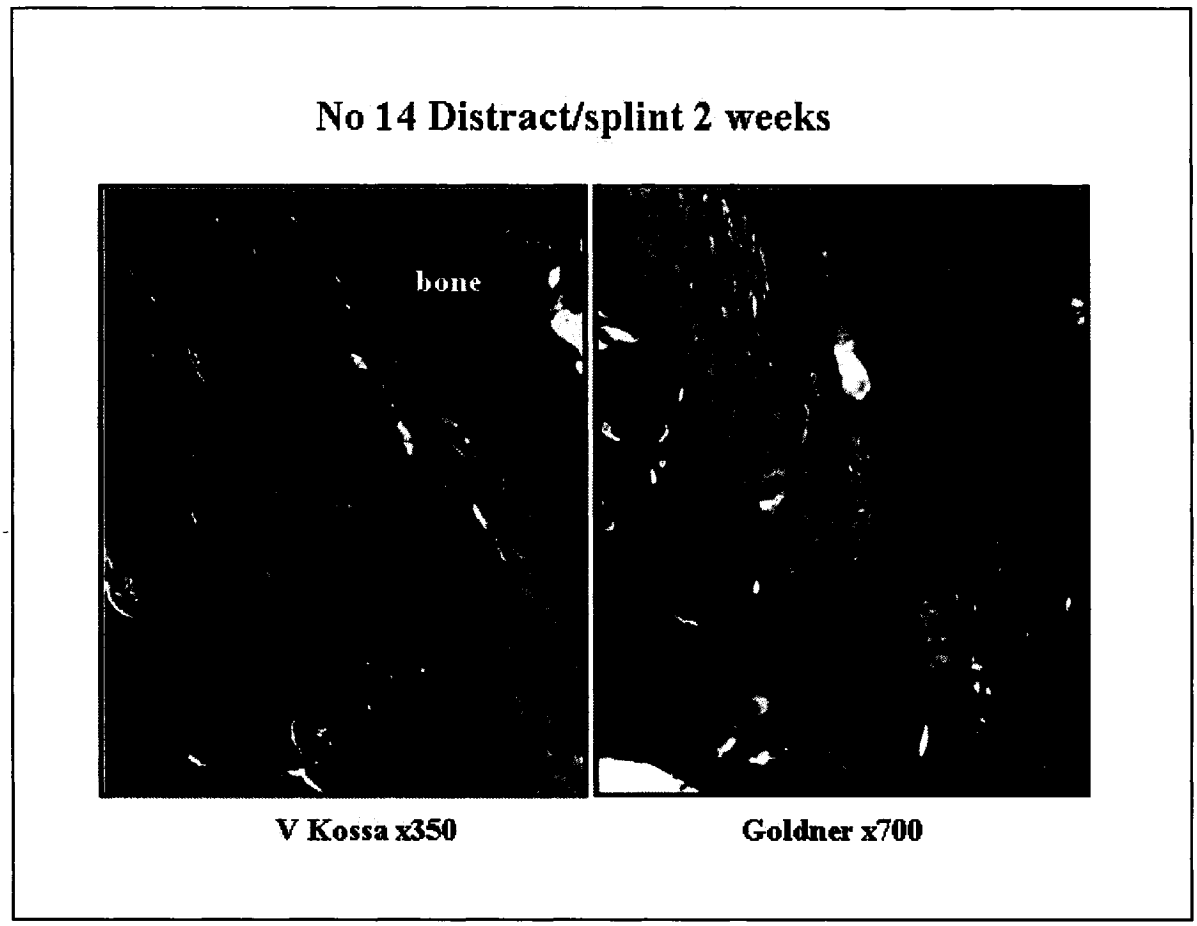

Figure 4.13. Distracted/splinted group at 2 weeks. High power view of palatomaxillary suture (left). Note the enlarged and cuboidal morphology of the osteoblasts (right) compared to the same group at 12 weeks (figure 4.15 below). Stains and magnification utilized are indicated under each figure.

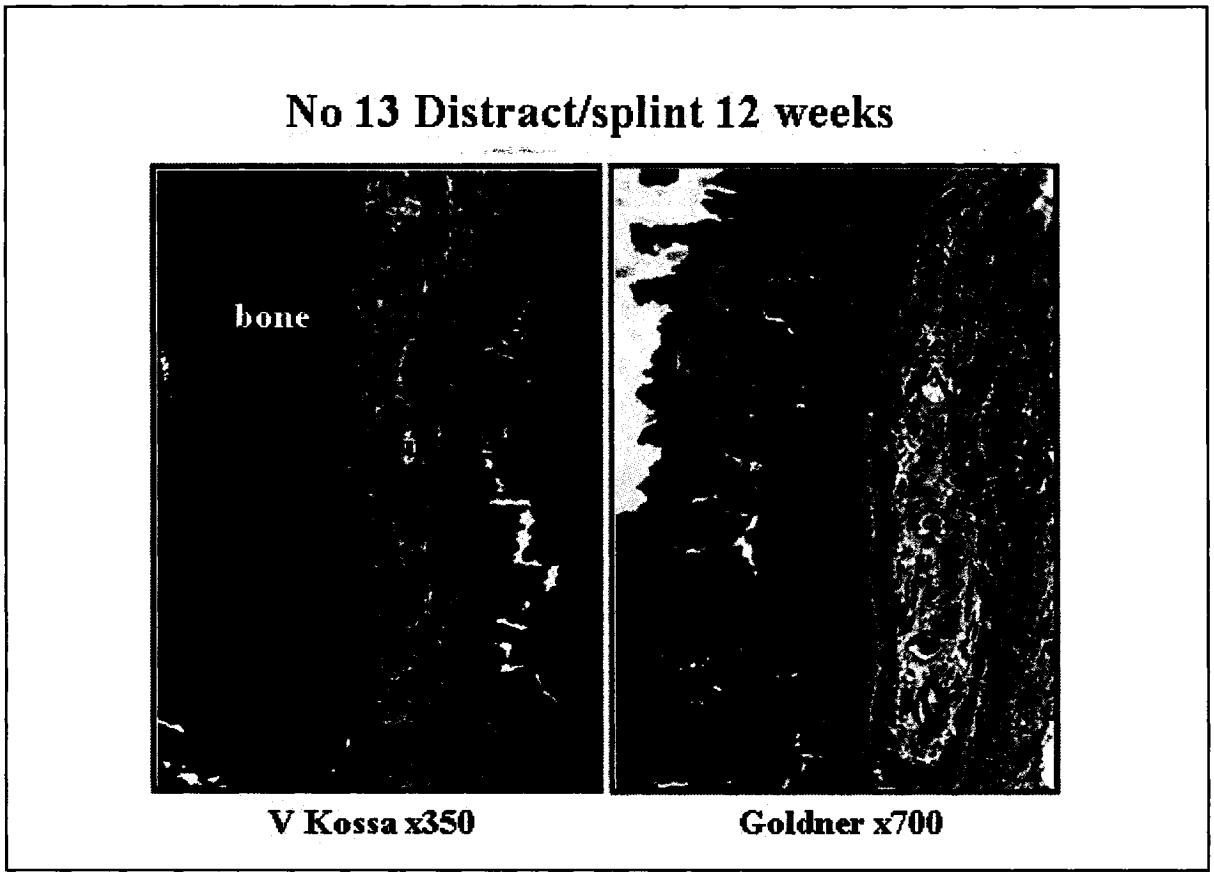

Figure 4.14. Distracted/splinted group at 12 weeks. High power view of palatomaxillary suture (left). Note the return of osteoblasts to their pre-distracted morphology (smaller, rounder) with, however, an apparent overall increase in the final suture cellularity (right). Note the similar density of the bone compared to non-distracted samples (with the exception of the translucent areas of artifact). Stains and magnification utilized are indicated under each figure.

4.6 Histomorphometry

One specimen from each experimental group at two time points (2 weeks and 12 weeks post-distraction) was imaged using the histomorphometric software. Percentage composition bone was measured by the Bioquant Image Analysis software (see methods section) in multiple locations (n) along the palatal suture (as described in Methods section 3.8). The average values of the multiple samples in each group are tabulated in table 4.6. An example of the sampling technique is illustrated in figure 4.15.

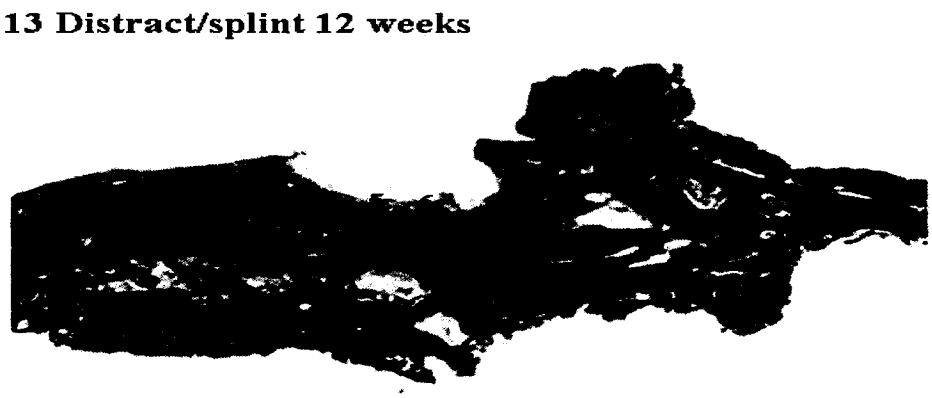

Figure 4.15. Distracted/splinted specimens at 12 weeks post-distraction. The sampling grids (shown for example here only) are positioned in multiple sites along the midline of the PMS, thus sampling both the suture and the bone immediately bordering the suture on either edge.

Table 4.6. Histomorphometry. Average bone percentage composition.

Group	Bone %	SD	n
Control @ 2wks	28.2738	8.02786	8
Splint/Dist @ 2 wks	20.4785	11.53561	13
Control @ 12 wks	42.6233	16.50260	9
Splint/Dist @ 12 wks	52.8580	16.18079	10

SD=standard deviation, n=number of sampling grids

Statistical analysis of this data was performed using a univariate two-way ANOVA with "groups" and "animal growth" as the independent factors. The main effect of the groups (distracted/splinted vs. control) was not statistically significant ($F_{(1,36)} = 0.079$; $p = 0.781$). However, the interaction between animal growth (time) and groups (the distraction/splinting vs. control) was found to be statistically significant ($F_{(1,36)} = 4.304$; $p = 0.045$). In addition, the main effect of animal growth was also independently significant ($F_{(1,36)} = 28.911$; $p < 0.001$). That is, both the control and distracted/splinted specimens had a statistically significant increase in the percentage bone composition around the PMS over time (i.e. between 2 and 12 weeks post-distraction). However, the rate of increase (of percentage bone composition) was greater (statistically significant) in the distracted/splinted group (see figure 4.18).

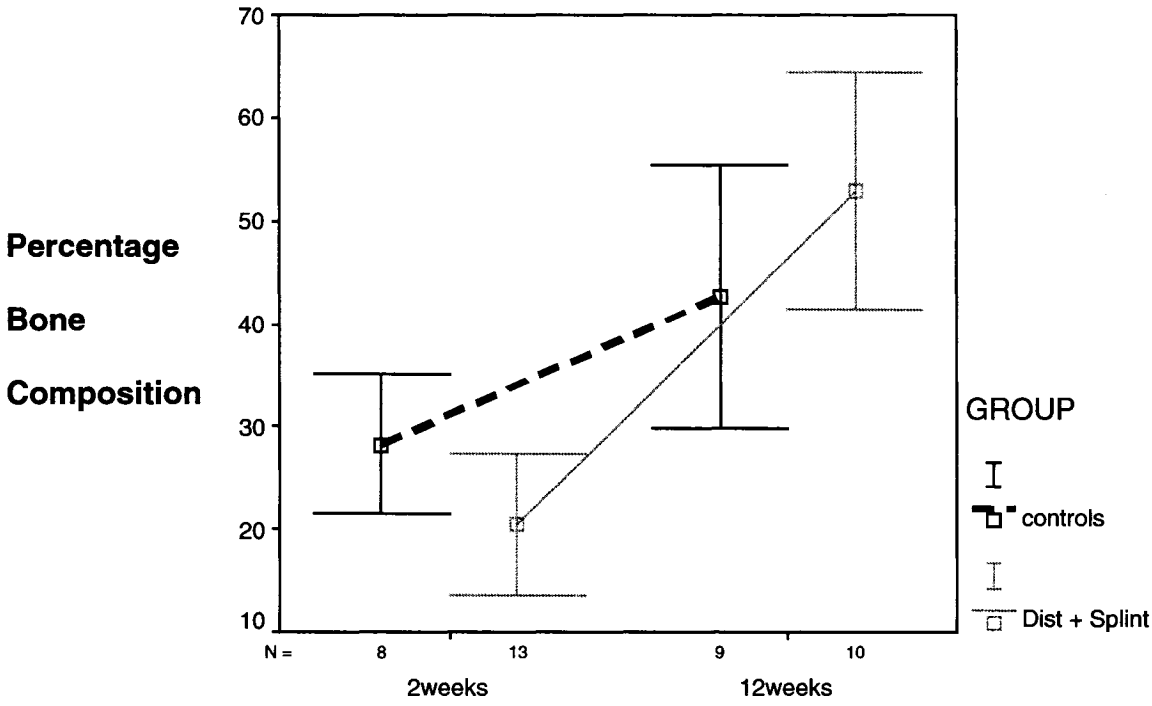

Figure 4.16. Changes in bone percentage composition (y-axis) acquired by histomorphometry between 2 and 12 weeks post-distraction for distracted/splinted vs. control (non-distracted) palatal bone. 95% confidence intervals are identified. Number of samples in each group is also indicated (N=x).

In summary, the increase in percentage composition bone over time was greater in a statistically significant manner in distracted/splinted animals vs. control (non-distracted) animals. The interpretation of this data is found in the discussion.

4.7 Micro CT

Bone percentage composition was determined in the identical palatal samples studied in section 4.6 Histomorphometry (prior to histological fixation for histology/histomorphometry) using MicroCT as a second modality of quantification. However, although ROI's were positioned using the same technique as in the histomorphometric analysis (i.e. along the midline of the suture), different sites along the suture and depths (multiple cross-sections) were sampled using microCT. Examples of the 2D images at different depths, derived from the microCT analysis are shown below with the ROI's in figure 4.17. The average percentage bone compositions from each specimen are tabulated in table 4.7 below.

Figure 4.17. 2D images at differing depths from the microCT analysis. Positioning of the ROI's is demonstrated. Distracted/splinted specimen at 12 weeks, image 180 (left), and distracted/splinted specimen at 12 weeks, image 231 (right).

Table 4.7. Micro CT. Average bone percentage composition.

Group	Bone %	SD	n
Control @ 2wks	66.73	11.23	12
Splint/Dist @ 2 wks	54.93	7.47	11
Control @ 12 wks	69.17	8.90	12
Splint/Dist @ 12 wks	75.13	4.99	13

SD=standard deviation, n=number of ROI's

Statistical analysis of the bone percentage composition acquired using MicroCT was performed using a univariate two-way ANOVA with "groups" and "animal growth" as the independent factors. Levene's Test of Equality of Error Variances output was not significant ($p = 0.162$), therefore permitting the use of an ANOVA.

The main effect of the groups (distracted/splinted vs. control) was not statistically significant ($F_{(1,49)} = 1.483$; $p = 0.229$). However, the interaction between animal growth and groups (the distraction/splinting vs. control) was found to be statistically significant ($F_{(1,49)} = 13.741$; $p = 0.001$). In addition, the main effect of time was also independently significant ($F_{(1,49)} = 22.301$; $p < 0.001$). That is, both the control and distracted/splinted specimens had a statistically significant increase in the percentage bone composition around the PMS over time (i.e. between 2 and 12 weeks post-distraction). However, the rate of increase (of percentage bone composition) was greater (statistically significant) in the distracted/splinted group (see figure 4.18).

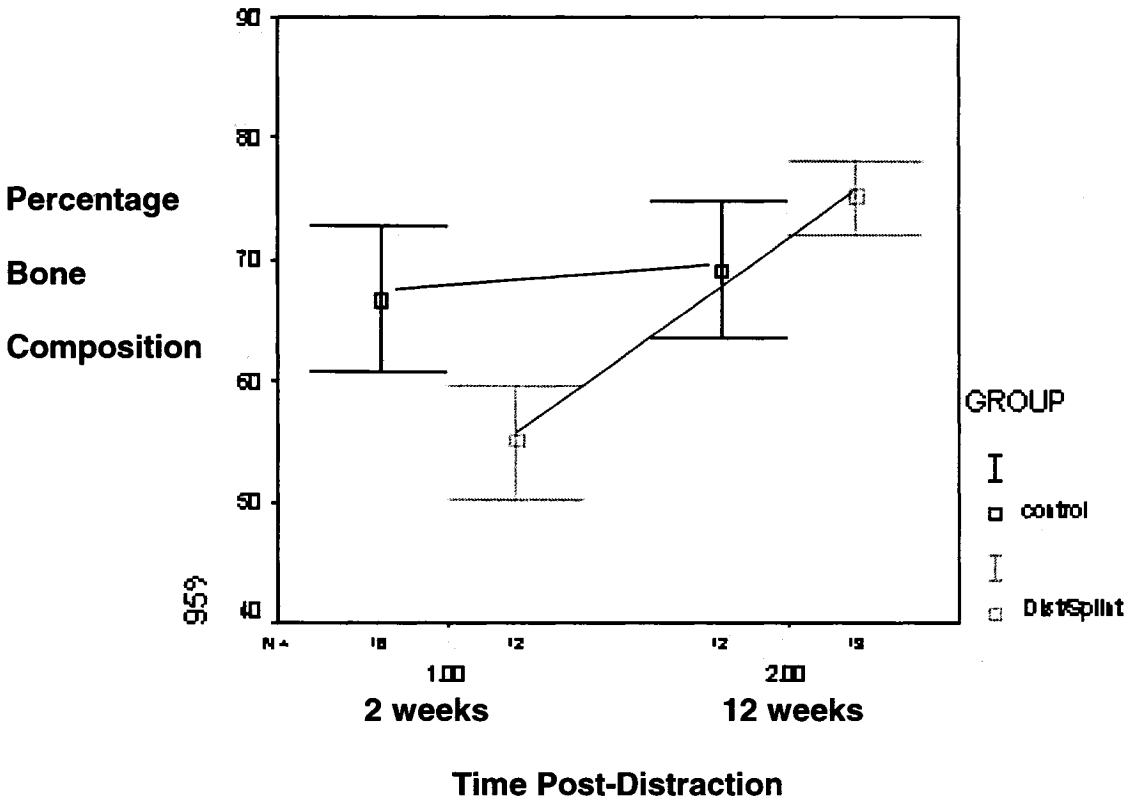

Figure 4.18. Changes in bone percentage composition acquired with MicroCT between 2 and 12 weeks post-distraction for distracted/splinted vs. control (non-distracted) palatal bone. 95% confidence intervals are included.

In summary, the increase in percentage composition bone over time was greater in a statistically significant manner in distracted/splinted animals vs. control (non-distracted) animals. The interpretation of this data is found in the discussion.

4.8 Dual-energy Xray Absorptiometry (Bone Mineral Density)

Bone Mineral Density (BMD) was measured using DXA in harvested palatal bone specimens at 12 weeks post-distraction, and compared with age-matched control (non-distracted) specimens.

The results are as follows:

Table 4.8. DXA results for distracted/splinted vs. control specimens at 12 weeks.

Specimen	**BMD (g/cm^2)**
Control 12 weeks	0.1771
Dist/splint 12 weeks	0.1776

As these are single measures derived from one sample from each group (i.e. not averages), no statistical analysis was undertaken to compare the results.

These results indicate that the BMD is virtually identical for distracted and non-distracted specimens at 12 weeks, suggesting that distraction has little effect on the final BMD, and therefore, strength of the palatal bone.

CHAPTER 5

Discussion

The technique of sutural distraction osteogenesis (SDO), as is its application to the treatment of cleft palate defects is a novel and visionary concept. While most techniques of cleft palate repair rely on the rearrangement of deficient and misaligned soft tissue and bone, SDO involves the production of mechanical strain across a bony suture to stimulate the generation of new bone by osteogenesis and mucosa by soft-tissue expansion. Thus, unlike traditional surgical repair, this technique theoretically achieves closure of both deficient palatal mucosa *and* bone (current surgical therapy addresses only the soft tissue defect). Definitive surgical closure could then be completed with minimal tissue dissection followed by suturing of the approximated cleft edges. The result would be *the potential* to limit facial growth restriction and other complications thought to be associated with extensive tissue mobilization involved in conventional surgical cleft palate repair.

Sutural distraction osteogenesis of cleft palate defects is still, however, in its preliminary stages of development. In 2000, Liu et al[26] published the first attempt at closure of cleft palates (in an animal model) using this technique. In an elegant experiment, the authors demonstrated that successful closure of cleft palates could be achieved with SDO, noting however, that it is associated with a significant reduction in maxillary width ("...significant midfacial underdevelopment"). In spite of the latter observation, the study concluded that clefts had been closed (filled) by new bone generated via bilateral expansion of the palatomaxillary sutures (PMS), supported by subjective evidence of osteogenesis on histologic analysis of distracted palatal bone. In 2003, the same authors published a preliminary clinical report in eight children,[133] and again in the same canine model in 2005 with a re-designed distractor device,[134] reporting similar success in both studies.

While the latter studies provided optimistic evidence that SDO is capable of closing cleft palate defects, the presence of ***unexplained and consistent midfacial underdevelopment,*** however, was never completely or adequately addressed. Since this technique was proposed in an attempt to minimize disturbances in facial growth associated with current gold standard surgical correction, careful re-examination of

this potential complication is, therefore, necessary. To that end, the present study was designed to clarify these questions by accurately monitoring maxillary width following distraction of induced cleft palatal defects. In addition, an intraoral splint was designed and tested for its efficacy in preventing maxillary collapse by providing structural support during the period of force application, and thus facilitating the closure of cleft palatal defects by the intended manner – SDO.

Palatal specimens were harvested after distraction and animal sacrifice to satisfy the secondary aim of this study – gaining a better understanding of what occurs – both structurally and compositionally – to the palatal bone following SDO. The latter is important for a number of reasons. Firstly, to provide further evidence, if possible, that cleft defects were closed by the deposition of new bone stimulated by SDO. And secondly, if the primary aim is accomplished and, therefore, cleft defects are successfully approximated without maxillary collapse, this study would mark the first report of *true* sutural distraction of the palate (Liu et al[26] did not achieve pure distraction, see below in discussion) – and thus the first histological analysis of distracted palatomaxillary suture. In the only two other published reports of midface distraction without osteotomies (zygomaticofrontal and/or nasofrontal suture distraction), only one group studied the histological changes following the intervention.[129] *Thus, little is known about the structural composition and integrity of the distracted bone and the resultant morphology of the distracted palatomaxillary suture following SDO.*

Evidence of permanently altered sutural anatomy (possibly contributing to altered facial growth later in life) or deposition of weak, fibrotic or thinned bone in the palate (recall that unstable dental arches and often fistula formation are problems related to unrepaired bony defects following traditional cleft repair,[18-22,83-85] and that deposition of poor bone would not ameliorate this structural weakness) could all potentially limit the clinical applicability and benefit of this proposed technique of palatal defect closure. Therefore, an analysis of the effect of SDO on bony and sutural anatomy was undertaken using three techniques: classic tissue histomorphometry, microCT, and Dual-energy Xray Absorptiometry. The first two were utilized to *describe* changes in suture architecture and *quantify* bone percentage composition

around the distracted PMS (as a measure of newly deposited bone, see below), while the later technique was used to determine Bone Mineral Density, a direct indicator of bone strength following distraction[46] (a more detailed description of the techniques utilized is provided later in the discussion).

The animal model selected for these experiments was that utilized in Liu et al's study,[26] and is a recurring model published in numerous journals associated with peer-reviewed cleft literature.[10-14,26,73,75-79,139,140] The model consists of a surgically-induced cleft palate, 8 mm wide in the midline, extending posteriorly into the soft-palate. The human correlate is largely spontaneous in etiology with occasional genetic linkage, occurring as a result of incomplete facial development and palatal fusion. Thus, while the selected model does not, of course, mimic the congenital nature of human clefts, which are extremely variable in location, extent of involvement of the palate, and associated defects, it does produce a consistent forum on which to test the effects of an intervention. That is to say, all animals, control and intervention, receive the same surgical procedure and an identical defect. Although this does not completely control for the inflammatory effect of the osteotomy and soft-tissue excision involved in the surgical creation of the cleft palate (and the potential for these effects to stimulate bone/tissue growth and contribute to cleft closure), the standardization of this procedure across all groups helps to unify this effect across all groups (and thus simplify the interpretation of these results).

Other potential cleft palate models were also considered, such as those induced by teratogens, which produce inconsistent clefting with other anatomic and/or systemic aberrations. One such model, for example, is caused by gavaging pregnant goats with *Nicotiana glauca*, causes consistent clefting but is also associated with skull base abnormalities and hypoplastic skulls that have abnormal midfacial growth in the absence of surgical repair.[141] The etiology of teratogenic defects is also not synonymous with developmental failure seen in human clefting and is, thus, arguably no more physiologic than the selected surgical model.

The other important aspect of the selected model was the age of the animals at the time of surgery. The animals were received into our facility between the ages of eight and nine weeks, so as to acclimatize to their new environments prior to

induction of clefts at ten weeks of age. This age was selected to ensure that the animals were in the phase of rapid facial growth, as are human infants when they are born and treated for these malformations. In addition, the species selected, male beagle dogs, are relatively consistent in size with oral apertures conducive to surgical instrumentation and insertion of distractor devices (rodent and rabbit mouths are too small).

Part I – Presence and Prevention of Alveolar Arch/ Maxillary Collapse

Measurements of maxillary width included two intraoral measurements and a post-mortem craniometric analysis. The intraoral measurements results (as summarized in section 4.3) demonstrated that comparable cleft approximation (changes in intrascrew distances) was achieved in both animals distracted *with* and *without* intraoral splints. Importantly, however, the decrease in intracuspal distances (a measure of dental arch/maxillary width) was statistically significant in those animals distracted without splints, whereas those measured in splinted animals were not statistically different from control animals **(research objectives #1 and #2)**. In addition, approximation of the cleft edges (intrascrew distances) in non-splinted subjects paralleled that of the dentomaxillary complex (intracuspal distances), *implying that at least a significant portion of cleft approximation was achieved at the expense of medial collapse of the maxilla* (represented graphically in Figure 4.4).

The most plausible explanation for these observations is that the force required to distract the suture (to stimulate osteogenesis) is greater that what the immature facial bony architecture of the maxilla in the dog can withstand. Thus, implantation of the distractor occurred initiating mechanical strain across the sutures (and the evidence of some amount of sutural distraction osteogenesis seen in Liu's[26] histologic analysis) accompanied shortly by mechanical medial collapse of the maxilla to complete the cleft closure.

As reported in the results section, maxillary collapse during attempted distraction of the longitudinal PMS was prevented with the use of an intraoral splint.

That is to say, a comparable amount of cleft defect approximation was achieved in the splinted group (as the non-splinted group) without any evidence of maxillary collapse. Barring the lateral migration of the teeth within the dentoalveolar arches (the unlikely probability of which is discussed below), *this finding allows us to preliminarily conclude that the cleft defects were approximated by the deposition of new bone via SDO as intended.* Further support for this finding is found in the discussion of the histologic/histomorphometric analysis below. The intraoral splints, thus, achieved the task of anchoring the maxilla/alveolar processes while the force necessary to induce sutural distraction was applied. Not unexpectedly, the time necessary to achieve comparable cleft approximation was lengthier (ranging from 4 to 10 days longer) than in the non-splinted countersubjects, as cleft approximation could only occur, presumably, as a result of SDO.

A second explanation for the observed results, although less likely, must also be considered, and takes into account the possible migration of the measured structures (i.e. teeth and anchoring screws) within the bones due to the force applied. Two such possibilities exist:

1) The teeth were "pushed" outwards within the alveolar bone by the splints in the splinted/distracted group in the face of gross maxillary collapse and cleft approximation (thus maintaining the intracuspal/maxillary distances). Weighing against the latter theory are the principles of dynamic bone biology which dictate the response of the teeth and screws to applied force. Specifically, the periodontal membrane (which envelopes the dental roots) is a tension-adapted and pressure-sensitive membrane. An applied force of 30 g or more exceeds capillary closing pressure causing ischemia in the membrane which, in turn, halts remodeling (drift) of the teeth through the alveolar bone.[142,143] The distractor device utilized in our experiment applied a force of 350-400N, thus essentially limiting the possibility of dental drift (recall that 1kg = 9.8N, thus the distractor applied greater than 40 000g of force). Furthermore, animal and human studies that have examined the histological changes in bone following dental drift (such as in cases of excessive

orthodontic movement) demonstrate fenestrations in the buccal alveolar bone plate.[142,143] Such fenestrations were not appreciated in the distracted/splinted bone specimens in this study, further negating the contribution of dental drift as an explanation for the observed results.

2) The screws migrated medially through the bone in response to the medially-directed distraction force, thus mimicking the approximation of cleft edges (as this was measured using the intrascrew distance) and, of course, preserving maxillary width (as approximation of the screws would have been due their migration through bone and not the medial gross movement of the cleft edges due to SDO). This explanation is also not likely based on the observed and photographically documented gross approximation of the bony cleft edges (see Results section 4.2.1). Furthermore, the migration of the screws from a theoretical perspective is an unlikely occurrence based on the fact that titanium screws lack a "genic" membrane and therefore cannot migrate through bone actively like a tooth surrounded by periodontal ligament.[142] Titanium implants are applied throughout the world as orthodontic anchorage for specifically this reason: they do not move through the bone in response to orthopaedic forces, such as the force applied in this experiment. In addition, the migration of screws through bone due to remodeling, as is described in cranial fixation, occurs from the depository (external) side of the cranium to the resorptive (internal) side of the cranium as the cranial bone is passively remodeled during growth over the long term.[142,143] The latter is not an active process nor is it lateral in direction, and thus, is not likely to be the mechanism responsible for the medial movement of the anchoring screws.

Nevertheless, to confirm the validity of the intraoral measurement results, craniometric analyses were also performed to assess maxillary dimensions directly on the animal skulls. These measurements were acquired using standardized bony (not dental) landmarks[10,12-14,75,77-79] thus circumventing the potential interpretation error

based on dental measurements (discussed above). The prime focus of these measurements was to assess changes to posterior dental arch/maxillary width as a result of the distraction intervention, with or without intraoral splinting. As summarized in section 4.4, maxillary width (landmarks MN and OP) was significantly reduced in distracted animals, whereas the same measures in splinted animals were found to be no different than those in control (non-distracted) animals.

A third landmark ZZ, a measure of the distance between the zygomatic arches (cheeks) was also measured but found not to be significantly altered by the distraction process. The zygomatic arches unite with the superior portion of the maxilla, but are not continuous with its palatal/alveolar extension, and thus, their position was not significantly affected. The collapsing effect of the distraction intervention, therefore, seems to be limited to maxillary width. This result was also in keeping with the craniofacial measurements produced in Liu et al's study, which demonstrated that all facial dimensions were preserved with the exception of midfacial width, following the distraction intervention.[26]

Part II – Sutural Distraction Osteogenesis – A Qualitative and Quantitative Analysis of Palatal Bone and the Palatomaxillary Suture

The data presented to this point has demonstrated that cleft palatal defects can be approximated without associated maxillary/alveolar arch collapse, gauged via gross anatomic measurements (intraoral measurements and craniometrics). As alluded to earlier in this discussion, *these results **suggest** that the cleft defects were closed with new bone generated secondary to SDO.* The aim of the second portion of this experiment was to attempt to provide further evidence that this was indeed the manner by which the cleft defects were narrowed (i.e. to demonstrate new bone was deposited). Perhaps an equally important task of this post-mortem tissue analysis was to investigate the effect of distraction on the morphology of the palatomaxillary suture and distracted palatal bone – a clinically relevant and as yet unstudied aspect of palatomaxillary suture distraction. Recall that the impetus for studying this novel

technique was to circumvent the problems of unstable dental arch form and fistulization (both often directly related to the lack of bony repair in a conventional surgical cleft palate repair), and to avoid facial growth complications thought to be due to scarring along dissected tissue planes. Thus, a histological analysis of the distracted specimens, examining for evidence of structural compromise to the palatal bone or permanently altered suture morphology, is important, as such findings could indicate structural compromise that could affect future growth or stability of distracted palates.

To that end, palatal bone was harvested from the animals at the time of sacrifice, and prepared as outlined in Methods section 3.7 for histologic analysis. The selection of the timing of the specimens for the analysis (i.e. at which age to sacrifice the animals) was based, in part, on the results of the histologic analysis performed by Liu et al, in their first study.[26] Although it has been shown in this report that the animals in Liu's study did not undergo "pure" distraction (but instead a combination of distraction and alveolar collapse), the histologic results were nonetheless useful as a guideline to streamline the selection of the timing for our analysis. In his analysis, Liu noted that between seven and fourteen days into distraction, bone precursors, such as highly proliferated osteoblasts, were beginning to appear, signaling the beginning of the bone deposition cascade. In addition, an acute widening of the suture occurred, likely representing the anatomical response to the application of the distraction force. Over the following 6 weeks, remodeling of newly deposited bone, in addition to a gradual maturation of the distracted suture.[26]

Based on these results, we selected our first of two sampling times from animals sacrificed at two weeks following the initiation of distraction **(referred to herein as "2 weeks post-distraction")**, under the pretense that this safely marked an early point of the stimulation of bone deposition. That is, early enough to capture the baseline bone composition of the palate and far enough into distraction to consistently capture the acute "suture-deforming" stage. The other option for early sampling in our protocol (animals sacrificed at mid-distraction or 7 days post-distraction) seemed to be less consistent, with some of the specimens displaying significantly widened sutures and others still early in the deformation cascade (unlike samples at 2 weeks

that were uniformly deformed, unpublished data). In addition, although a small difference in baseline bone composition possibly existed between samples at 7 days and 2 weeks post-distraction, the latter is likely small or even negligible, especially in light of the fact that these specimens were to be compared with those harvested at 12 weeks post-distraction (see below).

The second sampling time was selected in order to have allowed sufficient time for calcified bone (the type which is quantified using the two techniques described) to be deposited following SDO, thus allowing for a useful comparison with our early (baseline) measurement. Liu et al[26] did not document the exact timing of the completion of this process as the last histological sample was from an animal at 56 days (roughly 6 weeks post-distraction) which demonstrated the maturation of the tissues was well-advanced but not complete).[26] Taking into account that the specimens Liu et al analyzed underwent a combination of SDO and alveolar collapse, while the specimens in this study were believed to undergo "pure" SDO, the second sample for analysis was harvested from animals 12 weeks following initiation of distraction **(referred to herein as "12 weeks post-distraction"),** to ensure that maturation and calcification of new bone was complete. In addition, because both the control and experimental groups were identical in age and were statistically similar in size to begin with (see results section 4.3) the effects of growth during the experimental period could be identified using statistical analyses (see sections 4.6 and 4.7) to yield the effects of SDO (vs. no intervention) on percentage bone composition alone.

Of note, the analysis was only carried out as a comparison between two experimental groups – control (non-distracted) and distracted/splinted. The reasoning behind this was based on our data presented in *section 4.2 Intraoral measurements* which quite clearly demonstrated the presence of significant maxillary collapse in the "distracted" group. And although examining whether the distracted/splinted group (recall: no maxillary collapse) displayed greater bone deposition than the distracted (no splint) group would have been interesting on an academic level, the focus of this project was to further knowledge in potentially clinically applicable avenues, for

which the group distracted without splints will likely not qualify secondary to the detrimental effects of associated maxillary collapse.

Thus, a histologic comparison between control and distracted specimens, at 2 and 12 weeks post-distraction was undertaken. The results of the low-power microscopy revealed, similar to Liu et al's findings, an acute widening and increasing irregularity of the suture in the acute phase (2 weeks post-distraction, see section 4.5 Histology), thought to be induced by the sudden initiation of traction across the PMS. Suture morphology at 12 weeks had returned to its narrow and smooth (non-distracted) appearance. There was also no subjective difference in the architecture of palatal bone at 12 weeks when comparing distracted/splinted and non-distracted (control) specimens, nor evidence or bone thinning or fenestration (caused by migration of the teeth within the bone), either under low- or high-power examination. The histologic (and histomorphometric) analysis was complicated, however, by the presence of artifact caused by the sectioning process and the hardness of the palatal bone. However, high power analysis confirmed that the fragmentation was indeed artifact, as these areas were surrounded by normal bone, a finding uncharacteristic of thinned or weakened bone.[136] High power analysis also revealed that osteoblasts were enlarged and cuboidal in morphology at 2 weeks, returning to a smaller, rounder morphology (like that seen in non-distracted control specimens) by 12 weeks post-distraction. The only noted change in distracted suture at 12 weeks was a slightly more cellular appearing suture, although this was not quantitated. The latter change in osteoblast morphology often represents a transition to an "activated" state,[136] and may well indicate that these cells were stimulated to deposit bone in response to the distraction of the suture.

Thus, judging from the histological examination of the suture at 12 weeks post-distraction, it appears that animals that underwent SDO of the palatomaxillary suture had no permanent alterations in suture architecture, other than subjective evidence of mildly increased cellularity (**research objective #5**). Clinically, this is relevant because evidence of irreversible changes to suture morphology could conceivably precipitate aberrations in normal facial growth – a clearly undesired complication.

The preceding pattern of widening of the distracted segment is consistent with other forms of distraction osteogenesis where an initial widening of the bone osteotomy (or suture in this case) induces cellular proliferation and deposition of new bone which eventually remodels to fill the "widened gap".[91,93-95] Thus, *we questioned whether the acute widening followed by narrowing of the suture we observed at 12 weeks represent an overall increase in bone mass with new bone deposited to reconstitute the pre-distracted (narrow) suture morphology (i.e. sutural distraction osteogenesis)?*

To address this question, a quantitative analysis of the changes in bone mass or volume was performed using traditional tissue histomorphometry, a validated and quantitative technique of microscopic tissue analysis.[144-147] and microCT, a technique that analyzes specified parameters (such as percentage bone composition) simultaneously in numerous 2D sections throughout the samples. This novel tissue imaging technology is gradually replacing "classic" static bone histomorphometry secondary to the quality of the results, improved affordability, and rapid multiplanar analysis capabilities.[148,149]

These techniques were selected following a literature review and discussion with senior investigators at the McGill Bone Research Center. One technique which was discussed and discarded was Tetracycline labeling, which involves feeding or intravenously administering animals with tetracycline, an antibiotic which stains newly-deposited bone and can be visualized under fluorescent lighting. The concensus was that because our model employed young, actively growing animals (and actively depositing and remodeling bone), tetracycline would be deposited intensely throughout the palate, rendering the task of quantitatively detecting subtle changes in intensity (due to bone deposition stimulated secondary to SDO vs. that caused by active growth alone) difficult or insensitive secondary to the confluent background staining.

The decision was thus made to utilize microCT and histomorphometry, both of which are capable of quantifying differences in measured parameters, such as percentage bone composition. Although these techniques cannot readily decipher the difference between "new" bone and "old" bone (without tetracycline, whose

disadvantages have already been discussed), they are able, however, to accurately measure changes in a specified parameter within sampled areas, thus providing a sensitive measure of small changes in palatal bone that might be expected from SDO.

Thus, our focus turned to fine-tuning the sampling techniques to be utilized for histomorphometry and microCT, as both rely on the analysis of tissue composition within positioned sampling grids, aimed at targeting areas of interest within the bony samples. The results of our histologic analysis (Results section 4.5), however, demonstrated a significant amount of heterogeneity in the palatal bone (including tooth buds, screw holes, artifact, etc.) with the exception of the bone immediately bordering the suture. The latter is important because the accurate sampling of the parameter "bone percentage composition" (explained in further detail below) was essential to the attempted quantification of SDO. Thus, to avoid sampling percentage bone composition in heterogeneous areas of the palatal bone (that would skew the analysis), another strategy to sample the specimens was designed, based on an understanding of the effects of distraction on suture width and the mechanism by which SDO occurs.

The deposition of new bone secondary to SDO, *the entity which we are attempting to quantify*, occurs along the edge of the distracted suture[26,96,102-104,107]. In addition, recall that at the first sampling time (2 weeks post-distraction), there was an acute widening (or distraction) of the PMS, which had narrowed down to its pre-distracted width (as in the control animals) by 12 weeks. In the absence of medial shift of the bone *lateral* to the suture (which was shown *not* to occur in either control or distracted/splinted animals, with both intraoral measurements and craniometry clearly demonstrating the preservation of alveolar arch width), nor the lateral shift (retrusion) of the bone *medial* to the PMS (the bone which is being medially pulled by the distractor), the only way for the suture to reconstitute its narrow morphology was via the deposition or remodeling of bone to "fill the gap" – the central premise of SDO (see figure 4.19 below). Thus, if a standard size sampling grid is centered over the midline of the widened suture (see Methods section 3.8), an increase in the percentage bone composition (i.e. a higher percentage of tissue within the fixed area of the grid is bone) would thus reflect a quantitated measure of newly-deposited (or

remodeled) bone. If multiple grids were to be positioned over the length of the suture and the resultant bone percentage compositions averaged, and compared between different palatal samples, one would theoretically quantitate the "average" increase or decrease in bone deposition.

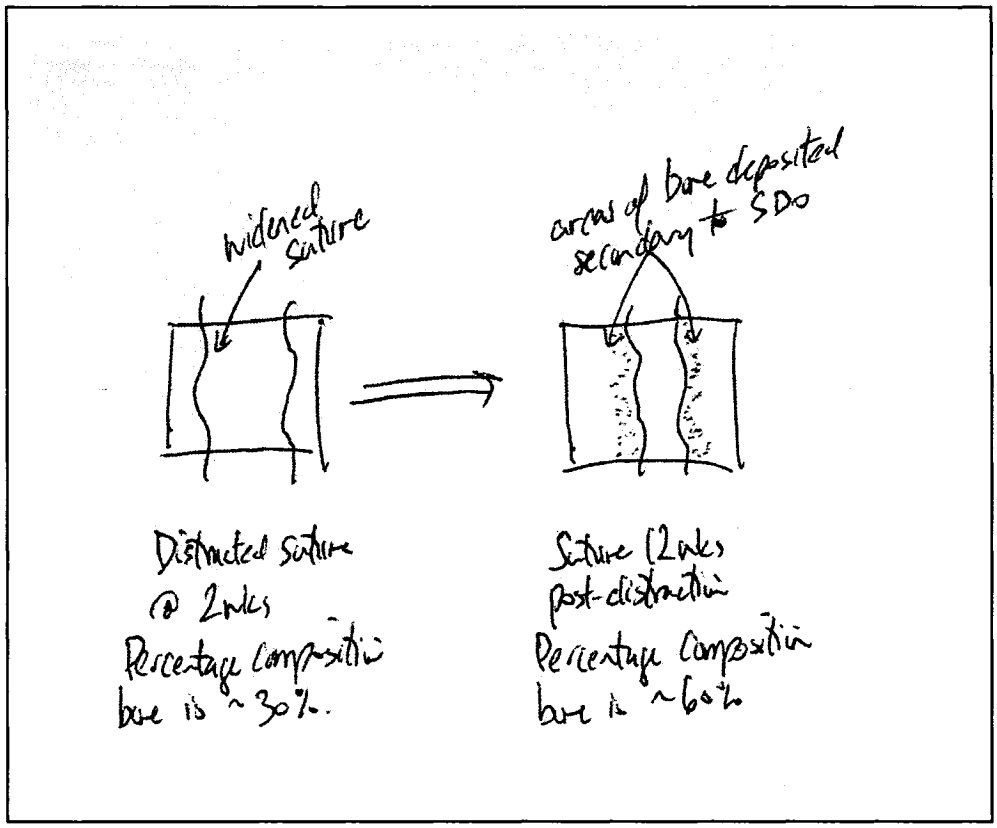

Figure 5.1. Schematic drawing representing the change in suture morphology secondary to distraction (acute widening, shown left), followed by the reconstitution of its narrower form by the deposition of new bone (stippling, shown right) via SDO – and the effect of these changes on the measured percentage bone composition within a sampling grid.

Using this technique, the results of the histomorphometric analysis demonstrated a statistically significant increase in the percentage bone composition between two and 12 weeks post-distraction in both the control and distracted/splinted groups. *Again, comparing equal areas of sampled bone (standard grid sizes), in the absence of physical movement of either the palatal bone medial or lateral to the PMS, this result* ***suggests*** *an increase in the overall bone mass around the PMS.* However,

while both groups demonstrated an increase in bone mass over time, an effect thought to be related to the actively growing status of the animals as it was present in both distracted and non-distracted specimens, the increase bone mass (i.e. the slope of the trace between the two time-points) was statistically greater in the distracted/splinted group (see Results section 4.6). *That is, the results of the quantitative histomorphometric analysis* ***suggest*** *that distraction/splinting caused a statistically significant increase in the rate of bone deposition in the palatal samples (specifically near the distracted PMS); an effect that could not be accounted for solely by the growth of facial bones (time)* **(research objective #3).** This measured increase in overall bone mass in the distracted palate may account for the mechanism by which the cleft defects in splinted animals are approximated in the absence of medial maxillary/dental arch collapse (i.e. new bone is deposited via SDO).

Histomorphometry, along with the described sampling technique is, by no means, impervious to sampling or measurement error and, of course, relies on the assumption, in this case, that the bone segments were not migrating. Histomorphometry is further plagued by another type of sampling error, based on the fact that it measures selected parameters on a single "slice" or depth of a tissue sample. One must thus assume that the analyzed section is representative of the whole specimen. With these sources of error in mind, the samples were also analyzed using a second modality called microCT. This technique is superior in that it allows for the simultaneous analysis of specified parameters in numerous 2D sections throughout the sample. In addition, as this technique does not require histological preparation and sectioning, the artifacts encountered by these techniques in the histomorphometric analysis could be avoided.

The results of the microCT analysis, summarized in the results section, paralleled those from the histomorphometric analysis, demonstrating again a statistically significant increase in percentage bone composition, and thus bone deposition, in the area surrounding the distracted/splinted palatomaxillary suture (see Results section 4.7). The effects of time (possibly reflecting the growth of the animals between two and 12 week samples) on percentage bone composition was also significant (as it was in the histomorphometric), but was independent of the

significantly greater effect of the intervention (explained in the statistical analysis, section 4.6 and 4.7).

In reviewing the results of the two quantitative analyses, one can note that while the increasing trend of percentage composition bone was consistent, the absolute values measured by microCT were higher than those obtained using histomorphometry. Although sampling error is possible, the same sample was used for both analyses with the absolute values being derived from the mathematical average of multiple "random" samples along the suture. Thus, inter-sample variability is a less likely explanation. However, the difference can be better explained by the fact that computer software programs employed by each modality were different, and were programmed with individual thresholds to differentiate between bone and "non-bone" tissues (i.e. the software has an established threshold above or below which tissues are deemed "bone" or "soft-tissue"). This threshold discrepancy could possibly account for the consistent trend results, in light of the differing absolute values.

It is equally important to note that one possible explanation for the similar result trends from both quantitative analyses may stem from the fact that both techniques utilized the same sampling technique (i.e. centering the grids over the suture). However, the microCT software positioned sampling grids in different locations (and at different suture depths) than those measured with histomorphometry - yielding identical trends. While this is, by no means, proof that the results of either modality are accurate, the complementary results are somewhat reassuring.

An important source of error to be considered that pertains to both quantitative modalities concerns the fact that the results for each group were measured from a single animal from each of the four compared groups (control and distracted/splinted at two and 12 weeks post-distraction). The main contributor to this decision was financial, as both histomorphometry (secondary to the time-consuming nature of the tissue preparation and analysis) and microCT (new and expensive technology) are very costly to perform. Statistically, however, one can make a case for the significant findings presented, in light of the low sample numbers (i.e. n=1), based on the

generalizability of the data. That is, there is no reason to believe that the specimens are derived from animals that differ from the rest of the population, and thus, the unit of analysis is truly the number of measures taken (i.e. multiple samples from each tissue specimen), as opposed to the actual number of animals in each group. However, because the specimens are derived from different animals (for example, two weeks vs. 12 weeks post-distraction), one must make the assumption that they are developmentally similar (again, there is no reason to believe otherwise).

Thus, although the results from the two "quantitative" analyses supported the same conclusion, the *significant* sources of error and assumptions necessary to make these preliminary conclusions necessitate *cautious* interpretation of these results. In addition, the quantitative tissue analysis portion of this study was *secondary* to the prime focus of this thesis – that is, demonstrating the ability to close cleft palatal defects by distraction of the palatomaxillary suture, without causing maxillary collapse. With the latter in mind, *the results from both the microCT and histomorphometry suggest that animals distracted with splints had a statistically significant greater increase in bone deposition around the PMS than non-distracted (control) animals; a finding that supports our hypothesis that cleft palatal defects in our canine model were approximated (in the absence of maxillary collapse) by the deposition of bone stimulated via SDO* **(research objective #3).**

Having demonstrated that SDO is capable of closing cleft palatal defects in a canine model without maxillary collapse in part I, the final portion of this experiment was aimed at analyzing the compositional quality and stability of the distracted palatal bone. The clinical relevance in the latter lies in the impetus for studying this novel technique – that is, achieving cleft palate closure while minimizing facial growth restriction and achieving bony palatal stability via repair of the bony defect. Thus, demonstrating that SDO generated structurally sound bone, capable of stabilizing the alveolar arches and decreasing the rate of fistula formation, would be contributory.

The latter was achieved using two methods. First, a subjective histological analysis (performed as part of the histomorphometric analysis), which confirmed

there was no visible difference in the architecture of palatal bone at 12 weeks post-distraction between distracted and non-distracted samples, nor evidence of bone thinning or fenestration (caused by migration of the teeth within the bone), either under low- or high-power examination. Thus, histologically there was no evidence of structural compromise in the distracted palates.

The second tool utilized was Dual-energy Xray Absorptiometry (DXA), an accurate and quantitative technique of measuring mineral bone density (BMD). The latter has been suggested to be a reliable, non-invasive indicator of bone strength during osteodistraction.[46] Thus, BMD was compared between the non-distracted and distracted animals at 12 weeks post-distraction to delineate any differences in bone composition that may have occurred secondary to distraction of the PMS. The results demonstrated virtually identical BMD between the two experimental groups.

The results of the DXA and histological analysis, demonstrating unaltered BMD and sutural morphology 12 weeks post-distraction, respectively, suggest that there were no permanent compositional or structural changes in the palatal bone secondary to distraction of the palatomaxillary suture **(research objective #4)**. Although mechanical testing of the palatal bone would have supplied further useful data, the sampling of the palatal bone for histomorphometric and microCT analyses (and the need for unadulterated bone specimens) precluded the use of this modality.

In conclusion, this study has demonstrated that closure of surgically-induced cleft palate defects in a *canine* model can be successfully achieved without associated maxillary collapse using the technique of SDO. The clinical applicability of this technique to *human* infants is still, however, unclear. The latter is based largely on the extrapolation of the results from this canine model to the human cleft palate patient[150] – an issue that was partially addressed early in the discussion, and more recently in a anatomic study in neonate cadavers.[151] Following various dissections, the latter investigators questioned the presence of a human equivalent to the PMS in canines, as they appreciated a lateral palatine suture only posteriorly, in continuity with the greater palatine foramen.[151] This can be explained by the fact that the horizontal plate

of the palatine bone makes up less of the anteroposterior dimension of the human infant palate than in dogs. That is to say, a lateral palatine suture that would allow distraction in the medial direction is present in human infants (by definition, the horizontal plate of the palatine bone requires its presence), however, its position would allow distraction of the posterior hard palate only.

Recalling Enlow's[142,143] two main categories of development (displacement and remodeling), this issue of distracting only the posterior palate is, likely, of little consequence for the following reason. *Displacement* development (i.e. growth at sutures) occurs as a direct effect of our traction force across the suture. Instead, *remodeling* development must occur to, at least, an equal amount (to fill in the displacement gap) in this particular case (popularly referred to as SDO). However, remodeling can be expected to extend outside the suture interface as the mucoperiosteum is pulled medially, exerting its tension through Sharpey's fibers to the medial edges of the palate. Such narrowing of the bony defect following closure of the soft palate is observed clinically following two-stage palatal repairs using the method of Schweckendiek.[1,152] This effect was also observed in our study where medialized palatal bone blends with the palatal process of the maxilla (see Results figure 3.8). Thus, one could predict that in spite of some anatomical differences between the PMS in humans and dogs, this technique may well have applicability to the human cleft palate patient. Liu et al's recent preliminary clinical study in eight human cleft palate patients, although lacking proper monitoring of maxillary dimensions nor any form of alveolar anchorage, further supports the feasibility of this novel technique.[133]

Finally, this project has served to uncover areas of necessary further investigation necessary to bring this technique to clinical fruition. These include modifications to technical parameters in the distraction protocol (such as employing multiple distractors along the cleft to more evenly disperse force to achieve uniform cleft approximation anteroposteriorly), and long-term follow-up to study the effects of distraction with intraoral splinting on facial maturation. As this technique is being developed to circumvent some of the complications of conventional surgical repair, improved outcomes (i.e. decreased facial growth restriction) and decreased morbidity

(i.e. uniformly approximated cleft palate defects that simplify and facilitate definitive surgical closure) must be ensured.

CHAPTER 6

Summary of Results and Conclusions

1) Attempted *sutural distraction osteogenesis* for cleft palate closure ***without*** intraoral splinting causes statistically significant medial collapse of the maxilla/alveolar arches.

2) Maxillary collapse during approximation of cleft palatal defects using SDO can be successfully prevented with the use of a newly-designed intraoral splint.

3) The results of the quantitative histomorphometry and microCT analysis ***suggest*** that there is a statistically significant greater amount of bone deposition in distracted/splinted animals compared to non-distracted (control) animals. This effect is independent from the effects of growth (time) on bone deposition and *may* explain the mechanism by which cleft palatal defects in this canine model were closed in the absence of maxillary collapse (in splinted animals)

4) The results of the gross histological analysis reveal no evidence of structural changes to the palatal bone, nor permanent alterations to the palatomaxillary suture morphology in palatal bone samples analyzed at 12 weeks following distraction.

5) Bone mineral density (as measured by DXA) is not different between distracted/splinted and non-distracted (control) animals in samples analyzed 12 weeks post-distraction.

6) The results of this study suggest that cleft palate defects in a canine model can be successfully approximated without maxillary collapse by using an intraoral splint to provide structural support during the distraction

process. In addition, there appear to be no structural or compositional changes to the distracted palatal bone or palatomaxillary suture that would have the potential to alter the growth or stability of cleft palates repaired using SDO.

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